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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: NOVEL ACETYL CoA CARBOXYLASES

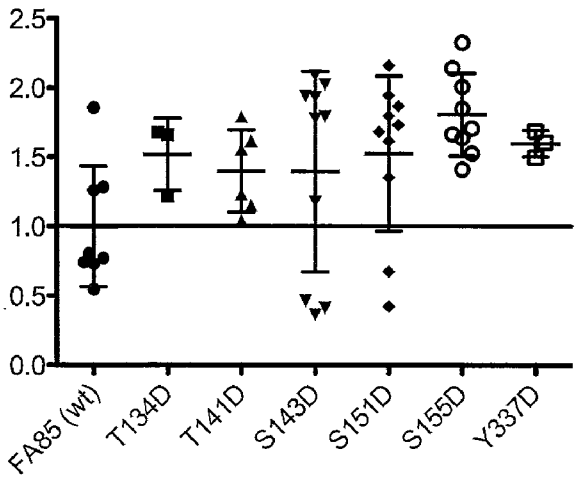

FIG. 1

(57) Abstract: Provided herein are novel ACCases and nucleotides encoding the same, that when introduced into a cell or organism results in an increase and/or accumulation of fatty acids, glycerol lipids, and/or oils in the cell or organism, and/or a change in the types of fatty acids, glycerol lipids, and/or oils that are normally present in the cell or organism. Also provided herein are organisms transformed with the novel ACCases.

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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— *with sequence listing part of description (Rule 5.2(a))*

NOVEL ACETYL CoA CARBOXYLASES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Provisional Application Number 61/242,489, filed September 15, 2009, the entire contents of which are incorporated by reference for all purposes.

INCORPORATION BY REFERENCE

[0002] 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.

BACKGROUND

[0003] Acetyl Coenzyme A carboxylase (ACCase) is the rate-limiting enzyme in the fatty acid biosynthesis pathway in plant, animal, yeast, and bacterial cells. Structurally, ACCases are biotinylated and are large enzymes consisting of two or more subunits. For example, most ACCases of animals, the cytoplasmic version in plants, and yeast are dimers of 420 to 700 kD native MW and contain subunits of 200 to 280 kD. Higher plant and algal plastid, and bacterial ACCases are 700 to 740 kD complexes 20 to 180 kD subunits.

[0004] Acetyl CoA Carboxylase (ACCase) catalyzes the formation of malonyl-CoA from acetyl-CoA and bicarbonate in animal, plant, and bacterial cells. Malonyl-CoA is an essential substrate for (i) de novo fatty acid (FA) synthesis, (ii) fatty acid elongation, (iii) synthesis of secondary metabolites such as flavonoids and anthocyanins, and (iv) malonylation of some amino acids and secondary metabolites. Synthesis of malonyl-CoA is the first committed step of flavonoid and fatty acid synthesis and current evidence suggests that ACCase catalyzes the rate-limiting step of fatty acid synthesis. Formation of malonyl-CoA by ACCase occurs via two partial reactions and requires a biotin prosthetic group:

(i) Enzyme-biotin+ATP+HCO_3 -> Enzyme-biotin-CO_2 +ADP+Pi

(ii) Enzyme-biotin-CO_2 +Acetyl-CoA ->Enzyme-biotin+malonyl CoA

The net reaction is:

Acetyl CoA+ATP+HCO_3 -> malonyl-CoA+ADP+Pi

[0005] In *E. coli*, these reactions are catalyzed by three distinct components; biotin carboxylase, biotin-acetyl CoA transcarboxylase, and biotin carboxyl carrier protein, which can be separated and yet retain partial activity. Plant and animal cytoplasmic ACCases contain all three activities on a single polypeptide.

[0006] Two different forms of the ACCase complex exist in plants (as described, for example, in Sasaki, Y. and Nagano, Y. (2004) *Biosci. Biotechnol. Biochem.* 68(6):1175-1184); the cytoplasmic enzyme, consisting of a very large single polypeptide chain, and the plastidic ACCase complex. The plastidic complex is a multi-enzyme complex

composed of biotin carboxyl carrier protein (BCCP), biotin carboxylase, and a carboxyltransferase complex made up of two pairs of α and β subunits.

[0007] Several pieces of evidence indicate that, at least in higher plants, the chloroplast ACCase complex is subject to control via post-translational modification. Kozaki and Sasaki Biochem J., 339:541 (1999) describe light levels and the addition of reducing agent (dithiothreitol) as being able to increase chloroplast ACCase activity, while the amount of ACCase protein remained roughly unchanged.

[0008] Savage and Ohlrogge, Plant J., 18:521 (1999) described purification of pea chloroplast ACCase complex, and showed that the β-subunit of the complex was phosphorylated in vivo. Removal of the phosphates by phosphatase treatment dramatically reduced the ACCase activity in the sample.

[0009] Under certain physiological conditions, mammalian ACC activity is rapidly regulated by reversible phosphorylation (for example, as described in Kim, K.-H. (1983) *Curr. Top. Cell Regul.*, 22, 143-176; and Kim, K. -H., *et al.*, *FASEB J.* (1989) 3, 2250-2256) which involves specific protein kinases that phosphorylate and inactivate ACC (for example, as described in Kim, K. -H., *et al.*, *FASEB J.* (1989) 3, 2250-2256), and phosphatases that dephosphorylate and activate the enzyme.

[0010] Ha, J. *et al.* (The J. of Biol. Chem. (1994) 269 (35) pp. 22162-22168) created and expressed a cDNA of the entire coding region of the rat Acetyl-CoA carboxylase and identified eight different phosphorlyation sites on the carboxylase molecule. The sites were identified by comparing phosphopeptide sequences and the deduced amino acid sequences from rat ACC cDNA (for example, as described in Lopez-Casillas, F., *et al.* (1988) *Proc. Natl. Acad. Sci. U. S. A.*, 85, 5784-5788; Munday, M. R., *et al.* (1988) *Eur. J. Biochem.*, 175, 331-338; Haystead, T. A. J. and Hardie, D. G. (1988) *Eur. J. Biochem.*, 175, 339-345; and Haystead, T. A. J. *et al.* (1988) *Eur. J. Biochem.*, 175, 347-354). The identified sites are Ser 23, 25, 29, 77, 79, 95, 1200, and 1215. The roles of these phosphorylation sites on the activation of ACCase are not well understood.

[0011] Increasing the amount of ACCase activity in the cell has been proposed as a mechanism to increase the lipid content (for example, TAG, DAG, and other acyl lipids) in algae, higher plants, yeast, and mammals. Attempts have been made to increase ACCase activity by increasing the amount of protein present via upregulation of a native ACCase gene or by introduction of a transgene under a stronger promoter. These efforts have produced increased levels of ACCase protein in the target organisms, but have not significantly altered lipid level (for example, as described in Hu et al., The Plant J.,54:621 (2008)).

[0012] In order to increase fatty acid synthesis in a cell, what is needed is not simply to increase production of an ACCase protein, but rather to increase the level of ACCase activity in the cell, resulting in an increase in lipid production. The present disclosure meets that need.

SUMMARY

[0013] Provided herein are novel ACCases, and nucleotides encoding the same, that when introduced into a cell or organism result in an increase and/or accumulation of fatty acids, glycerol lipids, and/or oils. Also, provided herein are novel ACCases, and nucleotides encoding the same, that when introduced into a cell or organism result in a change in the types of fatty acids, glycerol lipids, and/or oils that are normally present in the cell or organism.

[0014] 1. An isolated polynucleotide capable of transforming a photosynthetic organism comprising a nucleic acid sequence encoding an acetyl CoA carboxylase, wherein the acetyl CoA carboxylase comprises: 1) an amino acid sequence of SEQ ID NO: 157; or 2) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

[0015] 2. An acetyl CoA carboxylase present in a photosynthetic organism comprising: 1) an amino acid sequence of SEQ ID NO: 157; or 2) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

[0016] 3. A nucleotide sequence encoding an acetyl CoA carboxylase wherein the nucleotide sequence comprises: 1) a nucleic acid sequence of SEQ ID NO: 114 or SEQ ID NO: 155; or 2) a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 114 or SEQ ID NO: 115, wherein the nucleotide sequence is capable of transforming a photosynthetic organism.

[0017] 4. A vector comprising a nucleotide sequence encoding an acetyl CoA carboxylase, wherein the acetyl CoA carboxylase comprises: 1) an amino acid sequence of SEQ ID NO: 157; or 2) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157, wherein the vector is used to transform a photosynthetic organism. 5. The vector of claim 4, wherein the vector is an expression vector. 6. The vector of claim 4 or 5, wherein the vector further comprises a 5' regulatory region. 7. The vector of claim 6, wherein the 5' regulatory region further comprises a promoter. 8. The vector of claim 7, wherein the promoter is a constitutive promoter. 9. The vector of claim 7, wherein the promoter is an inducible promoter. 10. The vector of claim 9, wherein the inducible promoter is a light inducible promoter, a nitrate inducible promoter, or a heat responsive promoter. 11. The vector of any one of claims 4 to 10, further comprising a 3' regulatory region.

[0018] 12. A method for increasing production of malonyl CoA in a photosynthetic organism, comprising transforming the photosynthetic organism with a polynucleotide encoding an ACCase comprising an amino acid sequence of SEQ ID NO: 157, or with a polynucleotide encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157. 13. The method of claim 12, wherein the photosynthetic organism is a prokaryote. 14. The method of claim 13, wherein the prokaryote is a cyanobacterium. 15. The method of claim 12, wherein the photosynthetic organism is a eukaryote. 16. The method of claim 15, wherein the eukaryote is a vascular plant. 17. The method of claim 15, wherein the eukaryote is a non-vascular photosynthetic organism. 18. The method of claim 17, wherein the non-vascular photosynthetic organism is an alga. 19. The method of claim of any one of claims 12 to 18, further comprising transforming a plastid with the polynucleotide. 20. The method of claim 19, wherein the plastid is a chloroplast.

[0019] 21. A method for increasing fatty acid synthesis in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding an ACCase comprising an amino acid sequence of SEQ ID NO: 157, or with a polynucleotide encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at

least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157. 22. The method of claim 21, wherein the photosynthetic organism is a prokaryote. 23. The method of claim 22, wherein the prokaryote is a cyanobacterium. 24. The method of claim 21, wherein the organism is a eukaryote. 25. The method of claim 24, wherein the eukaryote is a vascular plant. 26. The method of claim 24, wherein the eukaryote is a non-vascular photosynthetic organism. 27. The method of claim 21, wherein the photosynthetic organism is an alga. 28. The method of claim of any one of claims 21 to 27, further comprising transforming a plastid with the polynucleotide. 29. The method of claim 28, wherein the plastid is a chloroplast.

[0020] 30. A transgenic host cell comprising a nucleotide sequence encoding an amino acid sequence of SEQ ID NO: 157, or comprising a nucleotide sequence encoding an ACCase comprising an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157. 31. The transgenic host cell of claim 30, wherein the host cell is a prokaryote. 32. The transgenic host cell of claim 31, wherein the prokaryote is a cyanobacterium. 33. The transgenic host cell of claim 30, wherein the host cell is a plant cell. 34. The transgenic host cell of claim 33, wherein the plant cell is from a vascular plant. 35. The transgenic host cell of claim 33, wherein the plant cell is from an alga. 36. The transgenic host cell of claim 35, wherein the alga is a green alga. 37. The transgenic host cell of claim 36, wherein the green alga is a *Chlorophycean*.

[0021] 38. A transgenic plastid comprising a polynucleotide encoding an acetyl CoA carboxylase comprising an amino acid sequence of SEQ ID NO: 157, or encoding an acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157. 39. The transgenic plastid of claim 38, wherein the plastid is a chloroplast. 40. A host cell comprising the transgenic plastid of claim 38 or claim 39. 41. The host cell of claim 40, wherein the host cell is a prokaryote. 42. The host cell of claim 41, wherein the host cell is a cyanobacterium. 43. The host cell of claim 40, wherein the host cell is a plant cell. 44. The host cell of claim 43, wherein the plant cell is from a vascular plant. 45. The host cell of claim 40, wherein the plant cell is an alga. 46. The transgenic host cell of 45, wherein the alga is a green alga. 47. The transgenic host cell of claim 46, wherein the green alga is a *Chlorophycean*.

[0022] 48. An acetyl CoA carboxylase present in a photosynthetic organism comprising: an amino acid sequence of a mammalian acetyl CoA carboxylase. 49. The acetyl CoA carboxylase of claim 48, wherein the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 157, or an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

[0023] 50. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO:

22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

[0024] 51. An acetyl CoA carboxylase comprising an amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

[0025] 52. A nucleotide sequence encoding a beta subunit of an acetyl CoA carboxylase wherein the nucleotide sequence comprises: 1) a nucleic acid sequence of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO:160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 168, or SEQ ID NO: 169; or 2) a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO:160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 168, or SEQ ID NO: 169.

[0026] 53. A vector comprising a nucleotide sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167. 54. The vector of claim 53, wherein the vector is an expression vector. 55. The vector of claim 53 or claim 54, wherein the vector further comprises a 5' regulatory region. 56. The vector of claim 55, wherein the 5' regulatory region further comprises a promoter. 57. The vector of claim 56, wherein the promoter is a constitutive promoter. 58. The vector of claim 56, wherein the promoter is an inducible promoter. 59. The vector of claim 58, wherein the inducible promoter is a light inducible promoter, a nitrate inducible promoter, or a heat responsive promoter. 60. The vector of any one of claims 53 to 59, further comprising a 3' regulatory region.

[0027] 61. A method for increasing production of malonyl CoA in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding a beta subunit of an ACCase, wherein the beta subunit of the ACCase comprises the amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at

least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167. 62. The method of claim 61, wherein the photosynthetic organism is a prokaryote. 63. The method of claim 62, wherein the prokaryote is a cyanobacterium. 64. The method of claim 61, wherein the organism is a eukaryote. 65. The method of claim 64, wherein the eukaryote is a vascular plant. 66. The method of claim 64, wherein the eukaryote is a non-vascular photosynthetic organism. 67. The method of claim 66, wherein the non-vascular photosynthetic organism is an alga. 68. The method of claim of any one of claims 61 to 67, further comprising transforming a plastid with the polynucleotide. 69. The method of claim 68, wherein the plastid is a chloroplast.

[0028] 70. A method for increasing fatty acid synthesis in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding an ACCase comprising an amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167. 71. The method of claim 70, wherein the photosynthetic organism is a prokaryote. 72. The method of claim 71, wherein the prokaryote is a cyanobacterium. 73. The method of claim 70, wherein the organism is a eukaryote. 74. The method of claim 73, wherein the eukaryote is a vascular plant. 75. The method of claim 70, wherein the eukaryote is a non-vascular photosynthetic organism. 76. The method of claim 75, wherein the non-vascular photosynthetic organism is an alga. 77. The method of claim of any one of claims 70 to 76, further comprising transforming a plastid with the polynucleotide. 78. The method of claim 77, wherein the plastid is a chloroplast.

[0029] 79. A transgenic host cell comprising a nucleotide sequence encoding an ACCase comprising an amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acids sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167. 80. The transgenic host cell of claim 79, wherein the host cell is a prokaryote. 81. The transgenic host cell of claim 80, wherein the prokaryote is a cyanobacterium. 82. The transgenic host cell of any one of claims 79 to 81, wherein the host cell is a plant cell. 83. The transgenic host cell of claim 82, wherein the plant cell is from a vascular plant. 84. The transgenic host cell of claim 82, wherein the plant cell is from an alga. 85. The transgenic host cell of claim 84, wherein the alga is a green alga. 86. The transgenic host cell of claim 85, wherein the green alga is a *Chlorophycean*.

[0030] 87. A transgenic plastid comprising a polynucleotide encoding a beta subunit of an acetyl CoA carboxylase comprising an amino acid sequence of: a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID

NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or b) comprising an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acids sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167. 88. The transgenic plastid of claim 87, wherein the plastid is a chloroplast. 89. A host cell comprising the transgenic plastid of claim 87 or claim 88. 90. The host cell of claim 89, wherein the host cell is a prokaryote. 91. The host cell of claim 90, wherein the prokaryote is a cyanobacterium. 92. The host cell of 89, wherein the host cell is a plant cell. 93. The host cell of claim 92, wherein the plant cell is from a vascular plant. 94. The host cell of claim 92, wherein the plant cell is an alga. 95. The host cell of 94, wherein the alga is a green alga 96. The host cell of claim 95, wherein the green alga is a *Chlorophycean.*

[0031] 97. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 15; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15.

[0032] 98. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 16; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 16.

[0033] 99. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 17; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 17.

[0034] 100. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 18; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 18.

[0035] 101. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 19; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 19.

[0036] 102. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 20; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 20.

[0037] 103. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO:

21; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 21.

[0038] 104. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 22; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 22.

[0039] 105. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 23; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 23.

[0040] 106. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 24; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 24.

[0041] 107. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 163; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 163.

[0042] 108. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 164; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 164.

[0043] 109. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 165; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 165.

[0044] 110. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 166; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 166.

[0045] 111. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 167; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 167.

[0046] 112. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 158; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 158.

[0047] 113. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 159; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 159.

[0048] 114. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 160; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 160.

[0049] 115. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 161; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 161.

[0050] 116. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 162; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 162.

[0051] 117. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 168; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 168.

[0052] 118. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 169; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 169.

[0053] 119. An isolated polynucleotide comprising a sequence encoding an acetyl CoA carboxylase comprising an amino acid sequence of:

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA
LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X$_1$ is T or D or E or N or H or Q or K; X$_2$ is T or D or E or N or H or Q or K; X$_3$ is S or D or E or N or H or Q or K; X$_4$ is S or D or E or N or H or Q or K; X$_5$ is S or D or E or N or H or Q or K; X$_6$ is C or D or E or N or H or Q or K; X$_7$ is Y or D or E or N or H or Q or K; provided, however, that the combination of X$_1$, X$_2$, X$_3$, X$_4$, X$_5$, X$_6$ and X$_7$ is not T, T, S, S, S, C, Y, respectively. 120. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is S, X$_6$ is C and X$_7$ is Y. 121. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is D, X$_6$ is C and X$_7$ is Y. 122. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is D and X$_7$ is Y. 123. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is C and X$_7$ is D. 124. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is Y. 125. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is S, X$_6$ is D and X$_7$ is Y. 126. The isolated polynucleotide of claim 119, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is D.

[0054] 127. An acetyl CoA carboxylase comprising an amino acid sequence of:

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA

LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X_1 is T or D or E or N or H or Q or K; X_2 is T or D or E or N or H or Q or K; X_3 is S or D or E or N or H or Q or K; X_4 is S or D or E or N or H or Q or K; X_5 is S or D or E or N or H or Q or K; X_6 is C or D or E or N or H or Q or K; X_7 is Y or D or E or N or H or Q or K; provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively. 128. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y. 129. The acetyl CoA carboxylase of claim 127 wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y. 130. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y. 131. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D. 132. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y. 133. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is D and X_7 is Y. 134. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

[0055] 135. A vector comprising a nucleotide sequence encoding an acetyl CoA carboxylase comprising an amino acid sequence of:

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X_1 is T or D or E or N or H or Q or K; X_2 is T or D or E or N or H or Q or K; X_3 is S or D or E or N or H or Q or K; X_4 is S or D or E or N or H or Q or K; X_5 is S or D or E or N or H or Q or K; X_6 is C or D or E or N or H or Q or K; X_7 is Y or D or E or N or H or Q or K; provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively. 136. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y. 137. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y. 138. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y. 139. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D. 140. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y. 141. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y. 142. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D. 143. The vector of any one of claims 135 to 142, wherein the vector is an expression vector. 144. The vector of any one of claims 135 to 143, wherein the vector further comprises a 5' regulatory region. 145. The vector of claim 144, wherein the 5' regulatory region further comprises a promoter. 146. The vector of claim 145, wherein the promoter is a constitutive promoter. 147. The vector of claim 145, wherein the promoter is an inducible promoter. 148. The vector of claim 147, wherein the inducible promoter is a light inducible promoter, nitrate inducible promoter or a heat responsive promoter. 149. The vector of any one of claims 135 to 148, further comprising a 3' regulatory region.

[0056] 150. A method for increasing production of malonyl CoA in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY

HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X$_1$ is T or D or E or N or H or Q or K; X$_2$ is T or D or E or N or H or Q or K; X$_3$ is S or D or E or N or H or Q or K; X$_4$ is S or D or E or N or H or Q or K; X$_5$ is S or D or E or N or H or Q or K; X$_6$ is C or D or E or N or H or Q or K; X$_7$ is Y or D or E or N or H or Q or K; provided, however, that the combination of X$_1$, X$_2$, X$_3$, X$_4$, X$_5$, X$_6$ and X$_7$ is not T, T, S, S, S, C, Y, respectively. 151. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is S, X$_6$ is C and X$_7$ is Y. 152. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is D, X$_6$ is C and X$_7$ is Y. 153. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is D and X$_7$ is Y. 154. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is C and X$_7$ is D. 155. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is Y. 156. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is D and X$_7$ is Y. 157. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is D. 158. The method of any one of claims 150 to 157, wherein the photosynthetic organism is a prokaryote. 159. The method of claim 158, wherein the prokaryote is a cyanobacterium. 160. The method of claim 150, wherein the organism is a eukaryote. 161. The method of claim 160, wherein the eukaryote is a vascular plant. 162. The method of claim 160, wherein the eukaryote is a non-vascular photosynthetic organism. 163. The method of claim 162, wherein the non-vascular photosynthetic organism is an alga. 164. The method any one of claims 150 to 163, further comprising transforming a plastid with the polynucleotide. 165. The method of claim 164, wherein the plastid is a chloroplast.

[0057] 166. A method for increasing fatty acid synthesis in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X$_1$ is T or D or E or N or H or Q or K; X$_2$ is T or D or E or N or H or Q or K; X$_3$ is S or D or E or N or H or Q or K; X$_4$ is S or D or E or N or H or Q or K; X$_5$ is S or D or E or N or H or Q or K; X$_6$ is C or D or E or N or H or Q or K; X$_7$ is Y or D or E or N or H or Q or K; provided, however, that the combination of X$_1$, X$_2$, X$_3$, X$_4$, X$_5$, X$_6$ and X$_7$ is not T, T, S, S, S, C, Y, respectively. 167. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is S, X$_6$ is C and X$_7$ is Y. 168. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is D, X$_6$ is C and X$_7$ is Y. 169. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is D and X$_7$ is Y. 170. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is C and X$_7$ is D. 171. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is Y. 172. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is D and X$_7$ is Y. 173. The method of claim 166, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is D. 174. The method of any one of claims 166 to 173, wherein the photosynthetic organism is a prokaryote. 175. The method of claim 174, wherein the prokaryote is a cyanobacterium. 176. The method of claim 166, wherein the organism is a eukaryote. 177. The method of claim 176, wherein the eukaryote is a vascular plant. 178. The method of claim 176,

wherein the eukaryote is a non-vascular photosynthetic organism. 179. The method of claim 178, wherein the non-vascular photosynthetic organism is an alga. 180. The method of any one of claims 166 to 179, further comprising transforming a plastid with the polynucleotide. 181. The method of claim 180, wherein the plastid is a chloroplast.

[0058] 182. A transgenic host cell comprising a nucleotide sequence encoding AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X_1 is T or D or E or N or H or Q or K; X_2 is T or D or E or N or H or Q or K; X_3 is S or D or E or N or H or Q or K; X_4 is S or D or E or N or H or Q or K; X_5 is S or D or E or N or H or Q or K; X_6 is C or D or E or N or H or Q or K; X_7 is Y or D or E or N or H or Q or K; provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively. 183. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y. 184. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y. 185. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y. 186. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D. 187. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y. 188. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y. 189. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D. 190. The transgenic host cell of any one of claims 182 to 189, wherein the host cell is a prokaryote. 191. The transgenic host cell of claim 190, wherein the host cell is a cyanobacterium. 192. The transgenic host cell of claim 182, wherein the host cell is a plant cell. 193. The transgenic host cell of claim 192, wherein the plant cell is from a vascular plant. 194. The transgenic host cell of claim 182, wherein the plant cell is from an alga. 195. The transgenic host cell of claim 194, wherein the alga is a green alga. 196. The transgenic host cell of claim 195, wherein the green alga is a *Chlorophycean*.

[0059] 197. A transgenic plastid comprising a polynucleotide encoding an acetyl CoA carboxylase comprising an amino acid sequence of: AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11) wherein X_1 is T or D or E or N or H or Q or K; X_2 is T or D or E or N or H or Q or K; X_3 is S or D or E or N or H or Q or K; X_4 is S or D or E or N or H or Q or K; X_5 is S or D or E or N or H or Q or K; X_6 is C or D or E or N or H or Q or K; X_7 is Y or D or E or N or H or Q or K; provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively. 198. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y. 199. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y. 200. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y. 201. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D. 202. The

transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y. 203. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y. 204. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D. 205. The transgenic plastid of any one of claims 197 to 204, wherein the plastid is a chloroplast. 206. A host cell comprising the transgenic plastid of any one of claims 197 to 205. 207. The host cell of claim 206, wherein the host cell is a prokaryote. 208. The host cell of claim 207, wherein the prokaryote is a cyanobacterium. 209. The host cell of claim 206, wherein the host cell is a plant cell. 210. The host cell of claim 209, wherein the plant cell is from a vascular plant. 211. The host cell of claim 209, wherein the plant cell is an alga. 212. The transgenic host cell of 211, wherein the alga is a green alga. 213. The transgenic host cell of claim 212, wherein the green alga is a *Chlorophycean*.

[0060] 214. The ACCase of claim 48, wherein the mammalian ACCase comprises the amino acid sequence of mouse (Mus Musculus: NM_133360.2 Identity: 99%); cattle (Bos Taurus: NM_174224.2. Identity: 97%); dog (Canis Lupus: XM_862501.1. Identity: 96%); chicken (Gallus gallus: NM_205505.1. Identity: 92%); or goat (Capra hircus: DQ370054.1. Identity: 98%).

[0061] 215. The isolated polypeptide of claim 1, wherein the photosynthetic organism is *Chlamydomonas reinhardtii*.

[0062] Some of the novel ACCases comprise the following amino acid sequence:

[0063] AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHIC FGCNYHLKMSSMERINHLIDAGX1WRPLDEX2LX3PVDPLEFX4DLKX5YTDRIKEAQEKTGLQDGVRTGTGL LHGIPVALGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHV HQNX6ANLLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLV VPRSFLKGALX7EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKVTG (SEQ ID NO: 2) wherein X1 is T or D or E or N or H or Q or K; X2 is T or D or E or N or H or Q or K; X3 is S or D or E or N or H or Q or K; X4 is S or D or E or N or H or Q or K; X5 is S or D or E or N or H or Q or K; X6 is C or D or E or N or H or Q or K; and X7 is Y or D or E or N or H or Q or K. Specifically excluded is the amino acid sequence of the wild type ACCase of SEQ ID NO: 1, that is, that the combination of X1, X2, X3, X4, X5, X6 and X7 is not T, T, S, S, S, C, Y, respectively, is expressly excluded from the scope of the present disclosure.

[0064] The present disclosure encompasses any polypeptide which has one of the possible amino acid sequences allowed by SEQ ID NO: 2. For example, and without limitation, in certain embodiments, X1 is T, X2 is T, X3 is S, X4 is D, X5 is S, X6 is C and X7 is Y. In other embodiments, X1 is T, X2 is T, X3 is S, X4 is S, X5 is D, X6 is C and X7 is Y. In further embodiments X1 is T, X2 is T, X3 is S, X4 is S, X5 is S, X6 is D and X7 is Y. In still further embodiments X1 is T, X2 is T, X3 is S, X4 is S, X5 is S, X6 is C and X7 is D. In other embodiments X1 is T, X2 is T, X3 is S, X4 is D, X5 is D, X6 is C and X7 is Y. In additional embodiments X1 is T, X2 is T, X3 is S, X4 is D, X5 is S, X6 is D and X7 is Y. While in yet another exemplary embodiment X1 is T, X2 is T, X3 is S, X4 is D, X5 is D, X6 is C and X7 is D.

[0065] Also provided are polypeptides and polynucleotides consisting of or consisting essentially of any of the amino acid sequences described herein. The above polypeptides and polynucleotides may be provided in an isolated, purified or substantially purified form.

[0066] Also provided are vectors comprising polynucleotides encoding any of the novel ACCases described by SEQ ID NO: 2, provided that the polynucleotide does not encode for a polypeptide of SEQ ID NO: 2, wherein is the

combination of X1, X2, X3, X4, X5, X6 and X7 is T, T, S, S, S, C, Y, respectively. The vector may be a cloning vector or an expression vector. In the case of an expression vector, the vector may further comprise a 5' regulatory region, a 3' regulatory region, or both. In certain embodiments, the 5' regulatory region contains a promoter, which may be a constitutive promoter or an inducible promoter. Also provided are vectors consisting of a polynucleotide encoding a polypeptide of SEQ ID NO: 2 and vectors consisting essentially of a polynucleotide encoding a polypeptide of SEQ ID NO: 2.

[0067] One aspect provides a method for increasing the production of malonyl CoA in a photosynthetic cell or organism by transforming said cell or organism with a polynucleotide encoding any of the novel ACCase polypeptides of SEQ ID NO: 2. The cell or organism may be a prokaryote or a eukaryote. In one embodiment, the cell or organism is a cyanobacterium. In another embodiment, the photosynthetic organisms is a vascular plant, while in other embodiments the cell or organism is a non-vascular photosynthetic eukaryote such as an alga. In one embodiment, the method further comprises transforming a plastid of the photosynthetic cell or organism with a polynucleotide encoding a polypeptide of SEQ ID NO: 2. Any plastid may be transformed, for example a chloroplast, a chloroplast or a leucoplast.

[0068] Another aspect provides a method for increasing fatty acid synthesis in a photosynthetic cell or organism comprising transforming said cell or organism with a polynucleotide encoding any of the novel ACCase polypeptides of SEQ ID NO: 2. The photosynthetic cell or organism may be a prokaryote or a eukaryote. In the case of prokaryote, the photosynthetic cell or organism may be a cyanobacterium. In the case of a eukaryote, the photosynthetic cell or organism may be a vascular plant or a non-vascular photosynthetic organism, such as an alga. In certain embodiments, the method further comprises transforming the polynucleotide encoding the novel ACCase into a plastid of the photosynthetic cell or organism. The plastid may be, but is not limited to, a chloroplast, a chloroplast, or a leucoplast.

[0069] Yet another aspect provides a transgenic host cell comprising a polynucleotide encoding a polypeptide of SEQ ID NO: 2. The transgenic host cell may be a prokaryote or a eukaryote. The host cell may be a single cell organism or a part of a multicellular organism. In one embodiment, the host cell is a bacterium, for example a cyanobacterium. In other embodiments, the cell is a plant cell. The plant cell may be from a vascular plant or from a non-vascular plant, such as an alga.

[0070] Still another aspect provides a transgenic plastid comprising a polynucleotide encoding a polypeptide of SEQ ID NO: 2. The plastid may be a chloroplast, a chloroplast or a leucoplast. In a further embodiment, the transgenic plastid is contained in a plant cell. In one embodiment, the plant cell is from or is part of a vascular plant. In other embodiments, the plant cell is an algal cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, appended claims and accompanying figures where:

[0072] **Figure 1** shows BODIPY staining by Guava of algae transformed with mutated ACCases described herein. The fold change in population median fluorescence as compared to the wild-type organism (FA85) is shown for six mutant ACCase overexpression strains (T134D, T141D, S143D, S151D, S155D, and Y337D).

[0073] **Figure 2** shows the distribution of algae containing mutated ACCase polynucleotides (S151D, S155D, T134D, C255S, S143D, and Y337D) in pre- and post- sort populations as compared to the wild-type algae (WT).

[0074] **Figure 3** shows the change in the proportion of ACCase genotypes form pre-sort to post-sort populations.

Figure 4 shows a schematic of an exemplary expression vector pSE-3HB-K-tD2.

[0075] **Figure 5** shows a plasmicity screen using PCR.

[0076] **Figure 6** shows a gene specific screen using PCR.

[0077] **Figure 7** shows a schematic of an exemplary expression vector pJ201-FA85.

[0078] **Figures 8A** and **8B** show PCR screening results for D2Rn transgenic cell lines.

[0079] **Figure 9** shows an exemplary expression vector PO4_SDACC1.

[0080] **Figure 10** shows an exemplary expression vector PO4_SDACC2.

[0081] **Figure 11** shows an exemplary expression vector D2RnACC.

[0082] **Figure 12** shows a Western blot with a clear band of approximately 266KDa indicating the presence of the RnACC protein in high salt media (HSM) media, and a faint band of approximately 266 KDa indicating the presence of the RnACC protein in TAP media.

[0083] **Figure 13** shows the lipid oil content of three ACCase transgenic *Chlamydomonas reinhardtii* cell lines (D2Rn5, D2Rn15, and D2Rn16) grown in TAP media. The y-axis is lipid content (% MTBE extractable) and the x-axis represents the three clones compared to a wild-type untransformed *Chlamydomonas reinhardtii* cell line.

[0084] **Figure 14** shows the lipid oil content of three ACCase transgenic *Chlamydomonas reinhardtii* cell lines (D2Rn5, D2Rn15, and D2Rn16) grown in HSM media. The y-axis is lipid content (% MTBE extractable) and the x-axis represents the three clones compared to a wild-type untransformed *Chlamydomonas reinhardtii* cell line.

[0085] **Figure 15** shows the compartmentation of the two forms of ACCase in plants.

[0086] **Figure 16** is a schematic depiction of the fatty acid biosynthesis pathway in plants.

[0087] **Figure 17** shows a schematic of the ACCase protein found in eukaryotes, mammals, and yeast. The size of the protein ranges from approximately 2200 to 2500 amino acids.

[0088] **Figure 18** shows the open reading frame of the first transcript of the novel ACCase β-subunit of *Scenedesmus dimorphus*. A putative chloroplast targeting transit peptide is underlined.

[0089] **Figure 19** shows the open reading frame of the second transcript of the novel ACCase β-subunit of *Scenedesmus dimorphus*. A putative chloroplast targeting transit peptide is underlined.

[0090] **Figure 20** shows the open reading frame of the third transcript of the novel ACCase β-subunit of *Scenedesmus dimorphus*. A putative chloroplast targeting transit peptide is underlined.

[0091] **Figure 21** shows the open reading frame of the fourth transcript of the novel ACCase β-subunit of *Scenedesmus dimorphus*. A putative chloroplast targeting transit peptide is underlined.

[0092] **Figure 22** shows the open reading frame of the fifth transcript of the novel ACCase β-subunit of *Scenedesmus dimorphus*. A putative chloroplast targeting transit peptide is underlined.

[0093] **Figure 23** shows an alignment of all five transcripts of the novel Scenedesmus dimorphus ACCase β-subunit.

[0094] **Figure 24** shows an alignment of the coded proteins of the five transcripts of the novel Scenedesmus dimorphus ACCase β-subunit.

DETAILED DESCRIPTION

[0095] The following detailed description is provided to aid those skilled in the art in practicing the present disclosure. Even so, this detailed description should not be construed to unduly limit the present disclosure as modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present inventive discovery.

[0096] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise

[0097] **Endogenous**

[0098] An endogenous nucleic acid, nucleotide, polypeptide, or protein as described herein is defined in relationship to the host organism. An endogenous nucleic acid, nucleotide, polypeptide, or protein is one that naturally occurs in the host organism.

[0099] **Exogenous**

[00100] An exogenous nucleic acid, nucleotide, polypeptide, or protein as described herein is defined in relationship to the host organism. An exogenous nucleic acid, nucleotide, polypeptide, or protein is one that does not naturally occur in the host organism or is a different location in the host organism.

[00101] The following (SEQ ID NOs: 1 to 55) are amino acid and nucleotide sequences for the β-subunit of acetyl-CoA carboxylase from *Chlamydomonas reinhardtii* (CC-503 cw92 mt+) that are useful in the embodiments disclosed herein.

[00102] If a stop codon is not present at the end of a coding sequence, one of skill in the art would know to insert nucleotides encoding for a stop codon (TAA, TAG, or TGA) at the end of the nucleotide sequence. If an initial start codon (Met) is not present from the amino acid sequence, one of skill in the art would be able to include, at the nucleotide level, an initial ATG, so that the translated polypeptide would have the initial Met.

[00103] Also listed below are primer sequences and affinity tags useful in the embodiments disclosed herein.

[00104] For SEQ ID NOs: 1-10, the last two amino acids, Thr and Gly, are not part of the protein sequence.

[00105] **SEQ ID NO: 1** is an open reading frame for the β-subunit of acetyl-CoA carboxylase. The first 43 amino acids are a probable transit peptide.

[00106] **SEQ ID NO: 2** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met.

[00107] **SEQ ID NO: 3** is a Strep affinity tag (positions 1 to 13) and the protein sequence for the β-subunit of acetyl-CoA carboxylase.

[00108] **SEQ ID NO: 4** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Thr to Asp mutation at position 91.

[00109] **SEQ ID NO: 5** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Thr to Asp mutation at position 98.

[00110] **SEQ ID NO: 6** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Ser to Asp mutation at position 100.

[00111] **SEQ ID NO: 7** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Ser to Asp mutation at position 108.

[00112] **SEQ ID NO: 8** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Ser to Asp mutation at position 112.

[00113] **SEQ ID NO: 9** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Tyr to Asp mutation at position 294.

[00114] **SEQ ID NO: 10** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without an initial Met and with a Cys to Ser mutation at position 212.

[00115] **SEQ ID NO: 11** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with variable amino acids at X_1, X_2, X_3, X_4, X_5, X_6, and X_7.

[00116] **SEQ ID NO: 12** is a protein sequence for the β-subunit of acetyl-CoA carboxylase without variable amino acids at X_1, X_2, X_3, X_4, X_5, X_6, and X_7.

[00117] **SEQ ID NO: 13** is an open reading frame for the β-subunit of acetyl-CoA carboxylase. The first 43 amino acids are a probable transit peptide.

[00118] **SEQ ID NO: 14** is a Strep affinity tag (positions 1 to 13) and the protein sequence for the β-subunit of acetyl-CoA carboxylase.

[00119] **SEQ ID NO: 15** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Thr to Asp mutation at position 92.

[00120] **SEQ ID NO: 16** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Thr to Asp mutation at position 99.

[00121] **SEQ ID NO: 17** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Ser to Asp mutation at position 101.

[00122] **SEQ ID NO: 18** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Ser to Asp mutation at position 109.

[00123] **SEQ ID NO: 19** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Ser to Asp mutation at position 113.

[00124] **SEQ ID NO: 20** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Cys to Ser mutation at position 213.

[00125] **SEQ ID NO: 21** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Tyr to Asp mutation at position 295.

[00126] **SEQ ID NO: 22** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Ser to Asp mutation at 109 and a Ser to Asp mutation at 113.

[00127] **SEQ ID NO: 23** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Ser to Asp mutation at 109, a Ser to Asp mutation at 113, and a Cys to Ser mutation at 213.

[00128] **SEQ ID NO: 24** is a protein sequence for the β-subunit of acetyl-CoA carboxylase with a Ser to Asp mutation at 109, a Ser to Asp mutation at 113, and a Tyr to Asp mutation at 295.

[00129] **SEQ ID NO: 25** is a nucleotide sequence of a Strep affinity tag codon optimized for the chloroplast genome of *C. reinhardtii*.

[00130] **SEQ ID NO: 26** is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase.

[00131] SEQ ID NO: 27 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a C213S mutation, according to the numbering of SEQ ID NO: 26.

[00132] SEQ ID NO: 28 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a S101D mutation, according to the numbering of SEQ ID NO: 26.

[00133] SEQ ID NO: 29 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a S109D mutation, according to the numbering of SEQ ID NO: 26.

[00134] SEQ ID NO: 30 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a S113D mutation, according to the numbering of SEQ ID NO: 26.

[00135] SEQ ID NO: 31 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a T92D mutation, according to the numbering of SEQ ID NO: 26.

[00136] SEQ ID NO: 32 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a T99D mutation, according to the numbering of SEQ ID NO: 26.

[00137] SEQ ID NO: 33 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a Y295D mutation, according to the numbering of SEQ ID NO: 26).

[00138] SEQ ID NO: 34 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a S109D and a S113D mutation, according to the numbering of SEQ ID NO: 26).

[00139] SEQ ID NO: 35 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a S109D, a S113D, and a Y295D mutation, according to the numbering of SEQ ID NO: 26).

[00140] SEQ ID NO: 36 is a codon optimized (for the chloroplast genome of *C. reinhardtii*) nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase with a S109D mutation, a S113D mutation, and a C213S mutation, according to the numbering of SEQ ID NO: 26).

[00141] SEQ ID NO: 37 is a non codon optimized nucleic acid sequence encoding the β-subunit of acetyl-CoA carboxylase without a stop codon.

[00142] SEQ ID NO: 38 is an amino acid sequence of a Strep affinity tag.

[00143] SEQ ID NO: 39 is an amino acid sequence of the probable transit peptide for the β-subunit of acetyl-CoA carboxylase.

[00144] SEQ ID NO: 40 is a PCR primer.

[00145] SEQ ID NO: 41 is a PCR primer.

[00146] SEQ ID NO: 42 is a PCR primer.

[00147] **SEQ ID NO: 43** is a PCR primer.

[00148] **SEQ ID NO: 44** is a PCR primer.

[00149] **SEQ ID NO: 45** is a PCR primer.

[00150] **SEQ ID NO: 46** is a PCR primer.

[00151] **SEQ ID NO: 47** is a PCR primer.

[00152] **SEQ ID NO: 48** is a PCR primer.

[00153] **SEQ ID NO: 49** is a PCR primer.

[00154] **SEQ ID NO: 50** is a PCR primer.

[00155] **SEQ ID NO: 51** is a PCR primer.

[00156] **SEQ ID NO: 52** is a PCR primer.

[00157] **SEQ ID NO: 53** is a PCR primer.

[00158] **SEQ ID NO: 54** is a PCR primer.

[00159] **SEQ ID NO: 55** is a PCR primer.

[00160] The following are amino acid and nucleotide sequences (SEQ ID NOs: 56 to 113) of five transcripts of a newly cloned acetyl-CoA carboxylase β-subunit from *Scenedesmus dimorphus* that are useful in the embodiments disclosed herein. If a stop codon is not present at the end of a coding sequence, one of skill in the art would know to insert nucleotides encoding for a stop codon (TAA, TAG, or TGA) at the end of the nucleotide sequence. If an initial start codon (Met) is not present from the amino acid sequence, one of skill in the art would be able to include, at the nucleotide level, an initial ATG, so that the translated polypeptide would have the initial Met. Also listed below are primer sequences, conserved motifs, and affinity tags useful in the embodiments disclosed herein.

[00161] A transcript is an unique mRNA encoding an unique protein sequence that may have been produced by alternative splicing from one gene.

[00162] **SEQ ID NO: 56** is a conserved amino acid motif found in a diverse range of ACCase protein sequences.

[00163] **SEQ ID NO: 57** is a conserved amino acid motif found in a diverse range of ACCase protein sequences.

[00164] **SEQ ID NO: 58** is a conserved amino acid motif found in a diverse range of ACCase protein sequences.

[00165] **SEQ ID NO: 59** is a conserved amino acid motif found in a diverse range of ACCase protein sequences.

[00166] **SEQ ID NO: 60** is a conserved amino acid motif found in a diverse range of ACCase protein sequences.

[00167] **SEQ ID NO: 61** is a conserved amino acid motif found in a diverse range of ACCase protein sequences.

[00168] **SEQ ID NO: 62** is a PCR primer.

[00169] **SEQ ID NO: 63** is a PCR primer.

[00170] **SEQ ID NO: 64** is a PCR primer.

[00171] **SEQ ID NO: 65** is a PCR primer.

[00172] **SEQ ID NO: 66** is a PCR primer.

[00173] **SEQ ID NO: 67** is a PCR primer.

[00174] **SEQ ID NO: 68** is a PCR primer.

[00175] **SEQ ID NO: 69** is an oligo (dT) PCR primer.

[00176] **SEQ ID NO: 70** is a putative ACC fragment.

[00177] **SEQ ID NO: 71** is a putative ACC fragment.

[00178] **SEQ ID NO: 72** is a putative ACC fragment.

[00179] **SEQ ID NO: 73** is a putative ACC fragment.

[00180] **SEQ ID NO: 74** is a nucleotide sequence of the first transcript of the novel acetyl-CoA carboxylase β-subunit from *Scenedesmus dimorphus* (SDACC1). The open reading frame includes a putative chloroplast targeting transit peptide sequence.

[00181] **SEQ ID NO: 75** is a nucleotide sequence encoding for the first novel ACCase β-subunit protein. **SEQ ID NO: 75** does not include a nucleic acid encoding for a putative chloroplast targeting transit peptide.

[00182] **SEQ ID NO: 76** is a nucleotide sequence encoding for a putative chloroplast targeting transit peptide. This nucleotide sequence was found at the 5' end of each of the five transcripts encoding for all of the five novel ACCase β-subunit proteins.

[00183] **SEQ ID NO: 77** is the translated amino acid sequence of **SEQ ID NO: 75**.

[00184] **SEQ ID NO: 78** is the translated amino acid sequence of **SEQ ID NO: 74**.

[00185] **SEQ ID NO: 79** is the translated amino acid sequence of **SEQ ID NO: 76**.

[00186] **SEQ ID NO: 80** is a nucleotide sequence of the second transcript of the novel acetyl-CoA carboxylase β-subunit from *Scenedesmus dimorphus* (SDACC2). The open reading frame includes a putative chloroplast targeting transit peptide sequence.

[00187] **SEQ ID NO: 81** is the translated amino acid sequence of **SEQ ID NO: 80**.

[00188] **SEQ ID NO: 82** is a nucleotide sequence encoding for the second novel ACCase β-subunit protein. **SEQ ID NO: 82** does not include a nucleic acid encoding for a putative chloroplast targeting transit peptide.

[00189] **SEQ ID NO: 83** is the translated amino acid sequence of **SEQ ID NO: 82**.

[00190] **SEQ ID NO: 84** is a nucleotide sequence of the third transcript of the newly cloned acetyl-CoA carboxylase β-subunit from *Scenedesmus dimorphus* (SDACC3). The open reading frame includes a putative chloroplast targeting transit peptide sequence.

[00191] **SEQ ID NO: 85** is the translated amino acid sequence of **SEQ ID NO: 84**.

[00192] **SEQ ID NO: 86** is a nucleotide sequence encoding for the third novel ACCase β-subunit protein. **SEQ ID NO: 86** does not include a nucleic acid encoding for a putative chloroplast targeting transit peptide.

[00193] **SEQ ID NO: 87** is the translated amino acid sequence of **SEQ ID NO: 86**.

[00194] **SEQ ID NO: 88** is a nucleotide sequence of the fourth transcript of the newly cloned acetyl-CoA carboxylase β-subunit from *Scenedesmus dimorphus* (SDACC4). The open reading frame includes a putative chloroplast targeting transit peptide sequence.

[00195] **SEQ ID NO: 89** is the translated amino acid sequence of **SEQ ID NO: 88**.

[00196] **SEQ ID NO: 90** is a nucleotide sequence encoding for the fourth novel ACCase β-subunit protein. **SEQ ID NO: 90** does not include a nucleic acid encoding for a putative chloroplast targeting transit peptide.

[00197] **SEQ ID NO: 91** is the translated amino acid sequence of **SEQ ID NO: 90**.

[00198] **SEQ ID NO: 92** is a nucleotide sequence of the fifth transcript of the newly cloned acetyl-CoA carboxylase β-subunit from *Scenedesmus dimorphus* (SDACC5). The open reading frame includes a putative chloroplast targeting transit peptide sequence.

[00199] **SEQ ID NO: 93** is a nucleotide sequence encoding for the fifth novel ACCase β-subunit protein. **SEQ ID NO: 93** does not include a nucleic acid encoding for a putative chloroplast targeting transit peptide.

[00200] **SEQ ID NO: 94** is the translated amino acid sequence of **SEQ ID NO: 92**.

[00201] **SEQ ID NO: 95** is the translated amino acid sequence of **SEQ ID NO: 93**.

[00202] **SEQ ID NO: 96** is the genomic sequence encoding for the second novel ACCase β-subunit protein (SDACC2). The last 81 nucleotides were not resolved because of a lack of sequencing information.

[00203] **SEQ ID NO: 97** is the nucleotide sequence of **SEQ ID NO: 75**, codon optimized for expression in the chloroplast of *Scenedesmus dimorphus* based on the *Chlamydomonas reinhardtii* tRNA codon usage table. In addition, a Flag tag has been attached to the 5' prime end of the nucleotide sequence after the initial ATG. The Flag tag was also codon optimized for expression in the chloroplast of *Scenedesmus dimorphus* based on the *Chlamydomonas reinhardtii* tRNA codon usage table.

[00204] **SEQ ID NO: 98** is the sequence of **SEQ ID NO: 97** without the Flag tag.

[00205] **SEQ ID NO: 99** is the nucleotide sequence of **SEQ ID NO: 82**, codon optimized for expression in the chloroplast of *Scenedesmus dimorphus* based on the *Chlamydomonas reinhardtii* tRNA codon usage table.

[00206] **SEQ ID NO: 100** is the amino acid sequence of **SEQ ID NO: 77** with Ser 91 mutated to Asp.

[00207] **SEQ ID NO: 101** is the amino acid sequence of **SEQ ID NO: 77** with Thr 98 mutated to Asp.

[00208] **SEQ ID NO: 102** is the amino acid sequence of **SEQ ID NO: 77** with Ser 100 mutated to Asp.

[00209] **SEQ ID NO: 103** is the amino acid sequence of **SEQ ID NO: 77** with Val 108 mutated to Asp.

[00210] **SEQ ID NO: 104** is the amino acid sequence of **SEQ ID NO: 77** with Pro 112 mutated to Asp.

[00211] **SEQ ID NO: 105** is the amino acid sequence of **SEQ ID NO: 77** with Ser 120 mutated to Asp.

[00212] **SEQ ID NO: 106** is the amino acid sequence of **SEQ ID NO: 77** with Thr 259 mutated to Asp.

[00213] **SEQ ID NO: 107** is the amino acid sequence of **SEQ ID NO: 83** with Ser 91 mutated to Asp.

[00214] **SEQ ID NO: 108** is the amino acid sequence of **SEQ ID NO: 83** with Thr 98 mutated to Asp.

[00215] **SEQ ID NO: 109** is the amino acid sequence of **SEQ ID NO: 83** with Ser 100 mutated to Asp.

[00216] **SEQ ID NO: 110** is the amino acid sequence of **SEQ ID NO: 83** with Val 108 mutated to Asp.

[00217] **SEQ ID NO: 111** is the amino acid sequence of **SEQ ID NO: 83** with Pro 112 mutated to Asp.

[00218] **SEQ ID NO: 112** is the amino acid sequence of **SEQ ID NO: 83** with Ser 120 mutated to Asp.

[00219] **SEQ ID NO: 113** is the amino acid sequence of **SEQ ID NO: 83** with Thr 259 mutated to Asp.

[00220] The following are additional amino acid and nucleotide sequences that are useful in the embodiments disclosed herein. If a stop codon is not present at the end of a coding sequence, one of skill in the art would know to insert nucleotides encoding for a stop codon (TAA, TAG, or TGA) at the end of the nucleotide sequence. If an initial start codon (Met) is not present from the amino acid sequence, one of skill in the art would be able to include, at the nucleotide level, an initial ATG, so that the translated polypeptide would have the initial Met.

[00221] **SEQ ID NO: 114** is the nucleotide sequence of the Rat ACCase gene codon optimized for expression in the chloroplast genome of *Chlamydomonas reinhardtii*.

[00222] **SEQ ID NO: 115** is the nucleotide sequence of the Rat ACCase gene. This gene is not codon optimized.

[00223] SEQ ID NO: 116 is the nucleotide sequence of the Flag tag that was attached to the 3'end of the codon-optimized nucleotide sequence of the Rat ACCase gene (SEQ ID NO: 114). The Flag tag was codon optimized for the chloroplast genome of *C. reinhardtii*.

[00224] SEQ ID NO: 117 is the nucleotide sequence of the Flag tag that was attached to the 5'end of the sequence of SEQ ID NO: 98 after the initial "ATG", and SEQ ID NO: 99 after the initial "ATG". The Flag tag was codon optimized for the chloroplast genome of *Scenedesmus dimorphus* based on the *Chlamydomonas reinhardtii* tRNA codon usage table.

[00225] SEQ ID NO: 118 is the translated amino acid sequence of **SEQ ID NO: 116.**

[00226] SEQ ID NO: 119 is a PCR primer.

[00227] SEQ ID NO: 120 is a PCR primer.

[00228] SEQ ID NO: 121 is a PCR primer.

[00229] SEQ ID NO: 122 is a PCR primer.

[00230] SEQ ID NO: 123 is a PCR primer.

[00231] SEQ ID NO: 124 is a PCR primer.

[00232] SEQ ID NO: 125 is a PCR primer.

[00233] SEQ ID NO: 126 is a PCR primer.

[00234] SEQ ID NO: 127 is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence (SEQ ID NO: 99) of SDACC2 with a Flag tag (SEQ ID NO: 117).

[00235] SEQ ID NO: 128 is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence (SEQ ID NO: 98) of SDACC1 with a Flag tag (SEQ ID NO: 117), and a mutation changing the nucleotides at positions 295-297 from TCA to GAT.

[00236] SEQ ID NO: 129 is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC1 with a Flag tag, and a mutation changing the nucleotides at positions 316-318 from ACA to GAT.

[00237] SEQ ID NO: 130 is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC1 with a Flag tag, and a mutation changing the nucleotides at positions 322-324 from TCA to GAT.

[00238] SEQ ID NO: 131 is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC1 with a Flag tag, and a mutation changing the nucleotides at positions 346-348 from GTA to GAT.

[00239] SEQ ID NO: 132 is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC1 with a Flag tag, and a mutation changing the nucleotides at positions 358-360 from CCA to GAT.

[00240] SEQ ID NO: 133 is the codon-optimized sequence (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) of SDACC1 with a Flag tag, and a mutation changing the nucleotides at positions 382-384 from TCA to GAT.

[00241] **SEQ ID NO: 134** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC1 with a Flag tag, and a mutation changing the nucleotides at positions 799-801 from ACA to GAT.

[00242] **SEQ ID NO: 135** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 295-297 from TCA to GAT.

[00243] **SEQ ID NO: 136** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 316-318 from ACA to GAT.

[00244] **SEQ ID NO: 137** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 322-324 from TCA to GAT.

[00245] **SEQ ID NO: 138** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 346-348 from GTA to GAT.

[00246] **SEQ ID NO: 139** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 358-360 from CCA to GAT.

[00247] **SEQ ID NO: 140** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 382-384 from TCA to GAT.

[00248] **SEQ ID NO: 141** is the codon-optimized (for the chloroplast genome of *Scenedesmus dimorphus* based on the *C. reinhardtii* tRNA codon usage table) sequence of SDACC2 with a Flag tag, and a mutation changing the nucleotides at positions 799-801 from ACA to GAT.

[00249] **SEQ ID NO: 142** is a PCR primer.

[00250] **SEQ ID NO: 143** is a PCR primer.

[00251] **SEQ ID NO: 144** is a PCR primer.

[00252] **SEQ ID NO: 145** is a PCR primer.

[00253] **SEQ ID NO: 146** is a PCR primer.

[00254] **SEQ ID NO: 147** is a PCR primer.

[00255] **SEQ ID NO: 148** is a PCR primer.

[00256] **SEQ ID NO: 149** is a PCR primer.

[00257] **SEQ ID NO: 150** is a PCR primer.

[00258] **SEQ ID NO: 151** is a PCR primer.

[00259] **SEQ ID NO: 152** is a PCR primer.

[00260] **SEQ ID NO: 153** is a PCR primer.

[00261] **SEQ ID NO: 154** is a PCR primer.

[00262] **SEQ ID NO: 155** is a PCR primer.

[00263] **SEQ ID NO: 156** is the codon-optimized sequence of the rat ACCase gene (SEQ ID NO: 114) with a 3' Flag tag (SEQ ID NO: 116) inserted prior to the stop codon (TAA).

[00264] **SEQ ID NO: 157** is the protein sequence of the rat ACCase gene.

[00265] **SEQ ID NO: 158** is the nucleotide sequence of SEQ ID NO: 75, without the initial "ATG".

[00266] **SEQ ID NO: 159** is the nucleotide sequence of SEQ ID NO: 82, without the initial "ATG".

[00267] **SEQ ID NO: 160** the nucleotide sequence of SEQ ID NO: 86, without the initial "ATG".

[00268] **SEQ ID NO: 161** the nucleotide sequence of SEQ ID NO: 90, without the initial "ATG".

[00269] **SEQ ID NO: 162** the nucleotide sequence of SEQ ID NO: 93, without the initial "ATG".

[00270] **SEQ ID NO: 163** is the translated sequence of **SEQ ID NO: 158**.

[00271] **SEQ ID NO: 164** is the translated sequence of **SEQ ID NO: 159**.

[00272] **SEQ ID NO: 165** is the translated sequence of **SEQ ID NO: 160**.

[00273] **SEQ ID NO: 166** is the translated sequence of **SEQ ID NO: 161**.

[00274] **SEQ ID NO: 167** is the translated sequence of **SEQ ID NO: 162**.

[00275] **SEQ ID NO: 168** is the sequence of **SEQ ID NO: 98** without the initial "ATG".

[00276] **SEQ ID NO: 169** is the sequence of **SEQ ID NO: 99** without the initial "ATG".

[00277] The present disclosure relates to novel ACCases having an improved activity (for example, being constitutively active) that are useful in increasing the production and/or accumulation of malonyl-CoA, fatty acids, glycerol lipids, and/or oils, in an organism, for example, a photosynthetic organism. Also provided are nucleic acids encoding the novel ACCases disclosed herein.

[00278] Provided herein are novel ACCases comprising SEQ ID NO: 2, wherein the amino acids at position X3, X4 and X5 may be serine or aspartic acid or glutamic acid or asparagine or histidine or glutamine or lysine; the amino acids at position X6 may be cysteine or aspartic acid or glutamic acid or asparagine or histidine or glutamine or lysine; and the amino acids at X7 may be tyrosine or aspartic acid or glutamic acid or asparagine or histidine or glutamine or lysine, provided, however, that the combination of X1, X2, X3, X4, X5, X6, and X7 is not threonine, threonine, serine, serine, cysteine, and tyrosine, respectively (wild type, SEQ ID NO: 1). In certain embodiments, the amino acid at X4 is aspartic acid and X1, X2, X3, X5, X6, and X7 are threonine, threonine, serine, serine, cysteine and tyrosine, respectively. In other embodiments, the amino acid at X5 is aspartic acid and X1, X2, X3, X4, X6, and X7 are threonine, threonine, serine, serine, cysteine and tyrosine, respectively. In other embodiments, the amino acid X6 is aspartic acid and X1, X2, X3, X4, X5, and X7 are threonine, threonine, serine, serine, serine and tyrosine, respectively. In still other embodiments, X7 is aspartic acid and X1, X2, X3, X4, X5, and X6 are threonine, threonine, serine, serine, serine and cysteine, respectively. In still other embodiments, X4 and X5 are aspartic acid and X1, X2, X3, X6 and X7 are threonine, threonine, serine, cysteine and tyrosine, respectively. In other embodiments, X4, X5, and X6 are aspartic acid and X1, X2, X3, X7 are threonine, threonine, serine, and tyrosine, respectively; while in still other embodiments, X4, X5 and X7 are aspartic acid and X1, X2, X3, X6 are threonine, threonine, serine, and cysteine, respectively.

[00279] Also provided herein is a method for increasing the production of malonyl-CoA in a photosynthetic organism. Malonyl-CoA is created by the carboxylation of Acetyl-CoA and is the committed step in fatty acid synthesis. Malonyl-CoA is the central carbon donor in fatty acid synthesis and is thought to be rate limiting. In fatty acid synthesis, the malonyl group is transferred from CoA to a protein co-factor on the acyl carrier protein (ACP). Malonyl-ACP then

undergoes a series of condensation reactions with acyl-ACP. The first of these reactions catalyzed by the condensing enzyme 3-ketoacyl ACP synthase III (KASIII) forms a four-carbon product. Another enzyme KASI is involved in producing products of varying lengths. Additional reactions take place to produce either saturated or unsaturated fatty acids. The reactions proceed resulting in an increase of the precursor fatty acid by 2 carbons at a time. The elongation is halted when either the acyl group is removed from the ACP by an acyl-ACP thioesterase or acyltransferases in the chloroplast transfer the fatty acid from ACP to glycerol-3-phosphate or monoacylglycerol-3 phosphate. The final fatty acid chain length is determined by the activities of the enzymes present. Thus, the novel ACCases disclosed herein may be introduced into a host cell or organelle to increase the production of malonyl-CoA, which in turn results in increased fatty acid synthesis.

[00280] **Acetyl-coenzyme A carboxylase (ACCase)**

[00281] Acetyl-coenzyme A carboxylase (ACCase) has been described, for example, in Roesssler, P.G. and Ohlrogge, J.B., *J. Biol. Chem.* (1993) 268 (26):19254-19259. ACCase is a biotin-containing enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA. This reaction is believed to be a key regulatory step in fatty acid biosynthesis in animals, bacteria, yeast, and plants (for example, as described in Kim, K.-H., et al. (1989) *FASEB J.* 3, 2250-2256; Jackowski, S., et al. (1991) *Biochemistry of Lipids, Lipoproteins and Membranes* (Vance, D. E., and Vance, J., eds) pp. 43-85, Elsevier Science Publishers, Amsterdam; Post-Beittenmiller, D., et al. (1991) *J. Biol.Chem.* 266, 1858-1865; and Post-Beittenmiller, D., et al. (1992) *Plant Physiol.* 100, 923-930). Two partial reactions are involved in this process: 1) carboxylation of an enzyme-bound biotin molecule, and 2) transfer of the carboxyl group to acetyl-CoA. **Figure 16** is a schematic depiction of the fatty acid biosynthesis pathway in plants.

[00282] ACCase from the bacterium *Escherichia coli* consists of four distinct, separable protein components: 1) biotin carboxyl carrier protein, 2) biotin carboxylase, and 3) α and β subunits of carboxyltransferase.

[00283] In eukaryotes, these entities are located on a single, multifunctional polypeptide typically having a molecular mass exceeding 200 kDa (for example, as described in Samols, D., et al. (1988) *J. Biol. Chem.* 263, 6461-6464) and as shown in **Figure 17**. The functional ACCase enzyme in eukaryotes is composed of multimers of this large polypeptide. In animals, ACCase has been shown to be a highly regulated enzyme that catalyzes the rate-limiting step in fatty acid biosynthesis (for example, as described in Kim, K.-H., et al. (1989) *FASEB J.* 3, 2250-2256 and Lane, M.D., et al. (1974) *Current Topics in Cellular Recognition* (Horecker, B. L., and Stadtman, E. R., eds) Vol. 8, pp.139-195, Academic Press, New York).

[00284] ACCase has been purified from several higher plants and algae (for example, as described in Roessler, P. G. (1990) *Plant Physiol.* 92, 73-78; Egli, M. A., et al. (1993) *Plant Physiol.* 101, 499-506; Livne, A. and Sukenik, A. (1990) *Plant Physiol.* 31, 851-858; Charles, D. J. and Cherry, J. H. (1986) *Phytochemistry* 25, 1067-1071; Slabas, A. R. and Hellyer, A. (1985) *Plant Sci.* 39, 177-182; Nikolau, B. J. and Hawke, J. C. (1984) *Arch. Biochem. Biophys.* 228, 86-96; Egin-Buhler, B. and Ebel, J. (1983) *Eur. J. Biochem.* 133, 335-339; and Finlayson, S. A. and Dennis, D. T. (1983) *Arch. Biochem. Biophys.* 225, 576-585).

[00285] There have only been a few publications describing the isolation of an ACCase-encoding gene from a photosynthetic organism (for example, as described in Roesssler, P.G. and Ohlrogge, J.B., *J. Biol. Chem.* (1993) 268(26):19254-19259).

[00286] As discussed in Hu, Q., *et al.* (The Plant Journal (2008) 54:621-639) ACCases have been purified and kinetically characterized from two unicellular algae, the diatom *Cyclotella cryptic* (Roessler, P.G. (1990) *Plant Physiol.* 92, 73–78) and the prymnesiophyte *Isochrysis galbana* (Livne, A. and Sukenik, A. (1990) *Plant Cell Physiol.* 31, 851–858). Native ACCase isolated from *C. cryptica* has a molecular mass of approximately 740 kDa and appears to be composed of four identical biotin containing subunits. The molecular mass of the native ACCase from *I. galbana* was estimated at 700 kDa. This suggests that ACCases from algae and the majority of ACCases from higher plants are similar in that they are composed of multiple identical subunits, each of which are multi-functional peptides containing domains responsible for both biotin carboxylation and subsequent carboxyl transfer to acetyl CoA (Roessler, P.G. (1990) *Plant Physiol.* 92, 73–78).

[00287] The gene that encodes ACCase in *Cyclotella cryptica* has been isolated, cloned, and characterized (Roessler, P.G. and Ohlrogge, J.B. (1993) *J. Biol. Chem.* 268, 19254-19259; and Roessler, P. G., *et al.*, *Ann. N. Y. Acad. Sci.* (1994) 721:250-256). The gene was shown to encode a polypeptide composed of 2089 amino acids, with a molecular mass of 230 kDa. The deduced amino acid sequence exhibited strong similarity to the sequences of animal and yeast ACCases in the biotin carboxylase and carboxyltransferase domains. Less sequence similarity was observed in the biotin carboxyl carrier protein domain, although the highly conserved Met-Lys-Met sequence of the biotin binding site was present. The N-terminus of the predicted ACCase sequence has characteristics of a signal sequence, indicating that the enzyme may be imported into chloroplasts via the endoplasmic reticulum.

[00288] Roessler, P. G., *et al.* (Applied Biochemistry and Biotechnology (1996) 57/58:223-231) has introduced additional copies of the ACCase gene (*acc1*) into *C. cryptica* T13L and *N. saprophila* by cotransforming these organisms with pACC1, which contains the genomic ACCase gene, and pACCNpt5.1. Preliminary results showed that for *C. cryptica* introducing additional copies of the ACCase gene may result in the enhanced activity of the enzyme.

[00289] ACCase genes have been isolated from three nonphotosynthetic eukaryotes: rat (Lopez-Casillas, et al. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 5784-5788), chicken (Takai, T. et al. (1988) *J. Biol. Chem.* 263, 2651-2657), and yeast (20 Al-Feel, W., et al. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 4534-4538). In addition, the genes encoding the individual polypeptides comprising the different subunits of ACCase in *E. coli* have been cloned and sequenced (Li, S. –J. and Cronan J. E. Jr., (1992) *J. Biol. Chem.* 267, 855-863; Li, S. –J. and Cronan J. E. Jr., (1992) *J. Biol. Chem.* 267, 16841-16847; Kondo, H., et al. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 9730-9733; and Alix, J. –H. (1989) *DNA (NY)* 8, 779-789).

[00290] Differences in the rates of fatty acid synthesis in plants may be attributable to changes in ACCase activity (for example, as described in Post-Beittenmiller, D., et al. (1991) *J. Biol.Chem.* 266, 1858-1865 and Post-Beittenmiller, D., et al. (1992) *Plant Physiol.* 100, 923-930). Increased ACCase activity also appears to play a role in environmentally induced triacylglycerol accumulation in the diatom Cyclotella cryptica (for example, as described in Roessler, P. G. (1988) *Arch. Biochem. Biophys.* 267, 521-528). Several allosteric effectors of plant and algal ACCases have been identified that may contribute to the regulation of ACCase activity *in vivo* (as reviewed in Roessler, P. G. (1990) *Plant Physiol.* 92, 73-78). However, little is known about the regulation of ACCase gene expression in photosynthetic organisms.

[00291] The level of ACCase gene expression has clearly been shown to be an important determinant of fatty acid biosynthetic rates in animals (for example, as described in Katsurada, A., et al. (1990) *Eur. J. Biochem.* 190, 435-441 and Pape, M. E., et al. (1988) *Arch. Biochem. Biophys.*267, 104-109).

[00292] In plants, most ACCase activity is located in plastids of green and non-green plant tissues including leaves and oil seeds. Leaf ACCase activity is primarily located in mesophyll cells, but lesser amounts have been found in C-4 bundle sheath cells and in epidermal cells. The subcellular location of ACCase activity in epidermal cells is unknown, but since synthesis of very long-chain fatty acids (VLCFA) for formation of waxes, cutin, and suberin occurs on the endoplasmic reticulum (ER), malonyl-CoA might also be derived from a cytosolic ACCase. **Figure 15** shows the compartmentation of the two forms of ACCase in plants.

[00293] In contrast, rat ACCase is primarily cytosolic or associated with the outer mitochondrial membrane.

[00294] De novo fatty acid synthesis in chloroplasts involves successive 2-carbon additions to acetate, using malonate as the 2-C donor. All intermediates are attached to acyl carrier protein (ACP). Synthesis in plastids resembles that in *E. coli* in that the fatty acid synthesis complex can be dissociated into separate enzymes: β-ketoacyl-ACP synthase (KAS), P-ketoacyl-ACP reductase, β-hydroxyl-ACP dehydratase, and enoyl-ACP reductase, acetyl-CoA:ACP transacylase, and malonyl-CoAACP transacylase. A highly active KASIII isozyme catalyzes the condensation of acetyl-CoA and malonyl-ACP. Successive additions of malonyl-CoA to acy-1-ACPs catalyzed by KAS I form C16 acyl-ACP, some of which is converted to C18 acyl-ACP by KAS II and then to C18: 1-ACP. Fatty acid metabolism then diverges; de-esterification allows movement to the cytoplasm (eukaryotic path) where fatty acids may be further unsaturated and/or elongated by additions of malonyl-CoA in the ER. Alternatively, fatty acids are linked to glycerol-3-phosphate (prokaryotic path), further unsaturated, and used for synthesis of chloroplast lipids. A portion of cytoplasmic lipids returns to the chloroplast. The relative contributions of these two paths are species-specific but appear to be relatively flexible in mutants blocked in either path. In oil-storing organs such as cotyledons and monocot embryos the triacylglycerides are stored in cytoplasmic oil bodies surrounded by a single unit membrane.

[00295] Condensation of malonyl-CoA with phenylpropionyl-CoAs or acetyl-CoA leads to synthesis of flavonoids, anthocyanins, or to polyacetates. Condensation is increased by light, elicitors, or pathogens and may be the rate-limiting step in synthesis of some phytoalexins. In addition to the secondary metabolites derived by de novo synthesis, malonyl conjugates of flavonoid glycosides, formed by malonylCoA: flavonoid glycoside malonyltransferase, D-amino acids and 1-amino-carboxyl-cyclopropane (ethylene precursor) are found in plants. Malonylated compounds accumulate in vacuoles, probably after synthesis in the cytoplasm.

[00296] Regulation of plant ACCase by reversible protein phosphorylation has not been studied extensively. Protein phosphorylation is involved in the regulation of many other pathways in plants where complex biochemical controls and light dependence are coordinated (as reviewed in Bennett, J. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42, 281-311 and Huber, S.C., et al. (1994) *Int. Rev. Cytology*, 149, 47-98). In many of these cases, light-and MgATP-dependence, such as that observed for ACCase, are factors involved in the control of the respective protein kinases and protein phosphatases. It has been observed that when fatty acid synthase (FAS) in isolated chloroplasts is inhibited by the addition of photosynthetic inhibitors such as 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the inhibition cannot be reversed by supplying the products of photosynthesis, i.e. ATP or NADPH alone (Nakamura, Y. and Yamada, M. (1975) *Plant Cell Physiol.* 1, 163-174 and Roughan, P.G., et al. (1980) *Plant Sci. Lett.* 18, 221-228). Therefore,

some intermediary method of control such as protein phosphorylation, instead of a direct dependence on photophosphorylation, has been suggested. Additionally, in yeast and mammals, ACCase is regulated by reversible protein phosphorylation (Kim, K.-H. (1997) *Annu. Rev. Nutr.* 17, 77-99), suggesting the possibility that this method of regulation may also occur in plants.

[00297] Plastid fatty acid synthesis is believed to be tightly regulated and under the control of a number of factors including metabolite pools and feedback inhibition (reviewed in Ohlrogge, J.B. and Jaworski, J. (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 4, 109-136). In all organisms examined so far, ACCase has been found to have a regulatory role over the flux of fatty acid synthesis. Light is one factor long known to regulate the flux of plastid fatty acid synthesis and its effect has largely been attributed to the production of co-factors and alterations of the stromal environment (for example, as described in Hunter, S.C. and Ohlrogge, J.B. (1998) *Arch. Biochem. Biophys.* 359, 170-178). The possibility that some other factor is involved in light activation of FAS in chloroplasts besides photosynthesis and resulting metabolite pools was first proposed by Nakamura, Y. and Yamada, M. (1975) *Plant Cell Physiol.* 1, 163-174, who observed that light-dependent fatty acid synthesis in isolated spinach chloroplasts was not dependent on ATP from photophosphorylation. More recent work has also revealed that ACCase activity from lysates of dark-incubated chloroplasts is low but increases to the levels of light-incubated chloroplast lysates within minutes (Hunter, S.C. and Ohlrogge, J.B. (1998) *Arch. Biochem. Biophys.* 359, 170-178). Since the dark-induced difference could not be attributed to metabolite levels in the diluted extracts, it was therefore speculated that during dark incubation some unknown inhibition or inactivation occurs. Savage, L. J. and Ohlrogge, J. B. (1999) *The Plant Journal*, 18(5), 521-527, set out to determine whether chloroplast ACCase was post-translationally modified by phosphorylation. Based on this work, the β-CT of ACCase is a phosphoprotein. Antibodies to pea β-CT, but not pre-immune serum, immunoprecipitate a protein labeled with [γ-33P]-ATP from pea chloroplasts which co-migrates precisely with endogenous pea β -CT. In addition, *E. coli*-expressed β -CT competes directly for specific antibody binding sites with this labeled protein in immunoprecipitation assays.

[00298] **Host Cells or Host Organisms**

[00299] Malonyl-CoA and fatty acid production can be increased by introducing polynucleotides encoding the present novel ACCases in any suitable host cell or organism.

[00300] A host cell can contain a polynucleotide encoding a polypeptide of the present disclosure. In some embodiments, a host cell is part of a multicellular organism. In other embodiments, a host cell is cultured as a unicellular organism.

[00301] Host organisms can include any suitable host, for example, a microorganism. Microorganisms which are useful for the methods described herein include, for example, photosynthetic bacteria (e.g., cyanobacteria), non-photosynthetic bacteria (e.g., *E. coli*), yeast (e.g., *Saccharomyces cerevisiae*), and algae (e. g., microalgae such as *Chlamydomonas reinhardtii*).

[00302] Examples of host organisms that can be transformed with a polynucleotide of interest include vascular and non-vascular organisms. The organism can be prokaryotic or eukaryotic. The organism can be unicellular or multicellular. A host organism is an organism comprising a host cell. In other embodiments, the host organism is photosynthetic. A photosynthetic organism is one that naturally photosynthesizes (e.g., an alga) or that is genetically engineered or otherwise modified to be photosynthetic. In some instances, a photosynthetic organism may be

transformed with a construct or vector of the disclosure which renders all or part of the photosynthetic apparatus inoperable.

[00303] By way of example, a non-vascular photosynthetic microalga species (for example, *C. reinhardtii*, *Nannochloropsis oceania*, *N. salina*, *D. salina*, *H. pluvalis*, *S. dimorphus*, *D. viridis*, *Chlorella sp.*, and *D. tertiolecta*) can be genetically engineered to produce a polypeptide of interest, for example an ACCase. Production of an ACCase in these microalgae can be achieved by engineering the microalgae to express an ACCase in the algal chloroplast or nucleus.

[00304] In other embodiments the host organism is a vascular plant. Non-limiting examples of such plants include various monocots and dicots, including high oil seed plants such as high oil seed *Brassica* (e.g., *Brassica nigra*, *Brassica napus*, *Brassica hirta*, *Brassica rapa*, *Brassica campestris*, *Brassica carinata*, and *Brassica juncea*), soybean (*Glycine max*), castor bean (*Ricinus communis*), cotton, safflower (*Carthamus tinctorius*), sunflower (*Helianthus annuus*), flax (*Linum usitatissimum*), corn (*Zea mays*), coconut (*Cocos nucifera*), palm (*Elaeis guineensis*), oil nut trees such as olive (*Olea europaea*), sesame, and peanut (*Arachis hypogaea*), as well as *Arabidopsis*, tobacco, wheat, barley, oats, amaranth, potato, rice, tomato, and legumes (e.g., peas, beans, lentils, alfalfa, etc.).

[00305] The host cell can be prokaryotic. Examples of some prokaryotic organisms of the present disclosure include, but are not limited to, cyanobacteria (e.g., *Synechococcus*, *Synechocystis*, *Athrospira*, *Gleocapsa*, *Oscillatoria*, and, *Pseudoanabaena*). Suitable prokaryotic cells include, but are not limited to, any of a variety of laboratory strains of Escherichia coli, Lactobacillus sp., Salmonella sp., and Shigella sp. (for example, as described in Carrier et al. (1992) J. Immunol. 148:1176-1181; U.S. Pat. No. 6,447,784; and Sizemore et al. (1995) Science 270:299-302). Examples of Salmonella strains which can be employed in the present disclosure include, but are not limited to, Salmonella typhi and S. typhimurium. Suitable Shigella strains include, but are not limited to, Shigella flexneri, Shigella sonnei, and Shigella disenteriae. Typically, the laboratory strain is one that is non-pathogenic. Non-limiting examples of other suitable bacteria include, but are not limited to, Pseudomonas pudita, Pseudomonas aeruginosa, Pseudomonas mevalonii, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum, and Rhodococcus sp..

[00306] In some embodiments, the host organism is eukaryotic (e.g. green algae, red algae, brown algae). In some embodiments, the algae is a green algae, for example, a Chlorophycean. The algae can be unicellular or multicellular. Suitable eukaryotic host cells include, but are not limited to, yeast cells, insect cells, plant cells, fungal cells, and algal cells. Suitable eukaryotic host cells include, but are not limited to, Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia sp., Saccharomyces cerevisiae, Saccharomyces sp., Hansenula polymorpha, Kluyveromyces sp., Kluyveromyces lactis, Candida albicans, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum, Neurospora crassa, and Chlamydomonas reinhardtii. In other embodiments, the host cell is a microalga (e.g., *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Haematococcus pluvialis*, *Nannochloropsis oceania*, *N. salina*, *Scenedesmus dimorphus*, *Chlorella spp.*, *D. viridis*, or *D. tertiolecta*).

[00307] In some instances the organism is a rhodophyte, chlorophyte, heterokontophyte, tribophyte, glaucophyte, chlorarachniophyte, euglenoid, haptophyte, cryptomonad, dinoflagellum, or phytoplankton.

[00308] In some instances a host organism is vascular and photosynthetic. Examples of vascular plants include, but are not limited to, angiosperms, gymnosperms, rhyniophytes, or other tracheophytes.

[00309] In some instances a host organism is non-vascular and photosynthetic. As used herein, the term "non-vascular photosynthetic organism," refers to any macroscopic or microscopic organism, including, but not limited to, algae, cyanobacteria and photosynthetic bacteria, which does not have a vascular system such as that found in vascular plants. Examples of non-vascular photosynthetic organisms include bryophtyes, such as marchantiophytes or anthocerotophytes. In some instances the organism is a cyanobacteria. In some instances, the organism is algae (e.g., macroalgae or microalgae). The algae can be unicellular or multicellular algae. For example, the microalgae *Chlamydomonas reinhardtii* may be transformed with a vector, or a linearized portion thereof, encoding one or more proteins of interest (e.g., an ACCase).

[00310] Methods for algal transformation are described in U.S. Provisional Patent Application No. 60/142,091. The methods of the present disclosure can be carried out using algae, for example, the microalga, *C. reinhardtii*. The use of microalgae to express a polypeptide or protein complex according to a method of the disclosure provides the advantage that large populations of the microalgae can be grown, including commercially (Cyanotech Corp.; Kailua-Kona HI), thus allowing for production and, if desired, isolation of large amounts of a desired product.

[00311] The vectors of the present disclosure may be capable of stable or transient transformation of multiple photosynthetic organisms, including, but not limited to, photosynthetic bacteria (including cyanobacteria), cyanophyta, prochlorophyta, rhodophyta, chlorophyta, heterokontophyta, tribophyta, glaucophyta, chlorarachniophytes, euglenophyta, euglenoids, haptophyta, chrysophyta, cryptophyta, cryptomonads, dinophyta, dinoflagellata, pyrmnesiophyta, bacillariophyta, xanthophyta, eustigmatophyta, raphidophyta, phaeophyta, and phytoplankton. Other vectors of the present disclosure are capable of stable or transient transformation of, for example, *C. reinhardtii*, *N. oceania, N. salina, D. salina, H. pluvalis*, *S. dimorphus*, *D. viridis*, or *D. tertiolecta.*

[00312] Examples of appropriate hosts, include but are not limited to: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells, such as Drosophila S2 and Spodoptera Sf9; animal cells, such as CHO, COS or Bowes melanoma; adenoviruses; and plant cells. The selection of an appropriate host is deemed to be within the scope of those skilled in the art.

[00313] Polynucleotides selected and isolated as described herein are introduced into a suitable host cell. A suitable host cell is any cell which is capable of promoting recombination and/or reductive reassortment. The selected polynucleotides can be, for example, in a vector which includes appropriate control sequences. The host cell can be, for example, a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of a construct (vector) into the host cell can be effected by, for example, calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation.

[00314] Recombinant polypeptides, including protein complexes, can be expressed in plants, allowing for the production of crops of such plants and, therefore, the ability to conveniently produce large amounts of a desired product. Accordingly, the methods of the disclosure can be practiced using any plant, including, for example, microalga and macroalgae, (such as marine algae and seaweeds), as well as plants that grow in soil.

[00315] In one embodiment, the host cell is a plant. The term "plant" is used broadly herein to refer to a eukaryotic organism containing plastids, such as chloroplasts, and includes any such organism at any stage of development, or to

part of a plant, including a plant cutting, a plant cell, a plant cell culture, a plant organ, a plant seed, and a plantlet. A plant cell is the structural and physiological unit of the plant, comprising a protoplast and a cell wall. A plant cell can be in the form of an isolated single cell or a cultured cell, or can be part of higher organized unit, for example, a plant tissue, plant organ, or plant. Thus, a plant cell can be a protoplast, a gamete producing cell, or a cell or collection of cells that can regenerate into a whole plant. As such, a seed, which comprises multiple plant cells and is capable of regenerating into a whole plant, is considered plant cell for purposes of this disclosure. A plant tissue or plant organ can be a seed, protoplast, callus, or any other groups of plant cells that is organized into a structural or functional unit. Particularly useful parts of a plant include harvestable parts and parts useful for propagation of progeny plants. A harvestable part of a plant can be any useful part of a plant, for example, flowers, pollen, seedlings, tubers, leaves, stems, fruit, seeds, and roots. A part of a plant useful for propagation includes, for example, seeds, fruits, cuttings, seedlings, tubers, and rootstocks.

[00316] A method of the disclosure can generate a plant containing genomic DNA (for example, a nuclear and/or plastid genomic DNA) that is genetically modified to contain a stably integrated polynucleotide (for example, as described in Hager and Bock, *Appl. Microbiol. Biotechnol.* 54:302-310, 2000). Accordingly, the present disclosure further provides a transgenic plant, e.g. *C. reinhardtii*, which comprises one or more chloroplasts containing a polynucleotide encoding one or more exogenous or endogenous polypeptides, including polypeptides that can allow for secretion of fuel products and/or fuel product precursors (e.g., isoprenoids, fatty acids, lipids, triglycerides). A photosynthetic organism of the present disclosure comprises at least one host cell that is modified to generate, for example, a fuel product or a fuel product precursor.

[00317] Some of the host organisms useful in the disclosed embodiments are, for example, are extremophiles, such as hyperthermophiles, psychrophiles, psychrotrophs, halophiles, barophiles and acidophiles. Some of the host organisms which may be used to practice the present disclosure are halophilic (e.g., *Dunaliella salina*, *D. viridis*, or *D. tertiolecta*). For example, *D. salina* can grow in ocean water and salt lakes (for example, salinity from 30-300 parts per thousand) and high salinity media (e.g., artificial seawater medium, seawater nutrient agar, brackish water medium, and seawater medium). In some embodiments of the disclosure, a host cell expressing a protein of the present disclosure can be grown in a liquid environment which is, for example, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2,1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 31., 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3 molar or higher concentrations of sodium chloride. One of skill in the art will recognize that other salts (sodium salts, calcium salts, potassium salts, or other salts) may also be present in the liquid environments.

[00318] Where a halophilic organism is utilized for the present disclosure, it may be transformed with any of the vectors described herein. For example, *D. salina* may be transformed with a vector which is capable of insertion into the chloroplast or nuclear genome and which contains nucleic acids which encode a protein (e.g., an ACCase). Transformed halophilic organisms may then be grown in high-saline environments (e.g., salt lakes, salt ponds, and high-saline media) to produce the products (e.g., lipids) of interest. Isolation of the products may involve removing a transformed organism from a high-saline environment prior to extracting the product from the organism. In instances where the product is secreted into the surrounding environment, it may be necessary to desalinate the liquid environment prior to any further processing of the product.

[00319] The present disclosure further provides compositions comprising a genetically modified host cell. A composition comprises a genetically modified host cell; and will in some embodiments comprise one or more further components, which components are selected based in part on the intended use of the genetically modified host cell. Suitable components include, but are not limited to, salts; buffers; stabilizers; protease-inhibiting agents; cell membrane- and/or cell wall-preserving compounds, e.g., glycerol and dimethylsulfoxide; and nutritional media appropriate to the cell.

[00320] For the production of a protein, for example, an isoprenoid or isoprenoid precursor compound, a host cell can be, for example, one that produces, or has been genetically modified to produce, one or more enzymes in a prenyl transferase pathway and/or a mevalonate pathway and/or an isoprenoid biosynthetic pathway. In some embodiments, the host cell is one that produces a substrate of a prenyl transferase, isoprenoid synthase or mevalonate pathway enzyme.

[00321] In some embodiments, a genetically modified host cell is a host cell that comprises an endogenous mevalonate pathway and/or isoprenoid biosynthetic pathway and/or prenyl transferase pathway. In other embodiments, a genetically modified host cell is a host cell that does not normally produce mevalonate or IPP via a mevalonate pathway, or FPP, GPP or GGPP via a prenyl transferase pathway, but has been genetically modified with one or more polynucleotides comprising nucleotide sequences encoding one or more mevalonate pathway, isoprenoid synthase pathway or prenyl transferase pathway enzymes (for example, as described in U.S. Patent Publication No. 2004/005678; U.S. Patent Publication No. 2003/0148479; and Martin et al. (2003) Nat. Biotech. 21(7):796-802).

[00322] **Culturing of Cells or Organisms**

[00323] An organism may be grown under conditions which permit photosynthesis, however, this is not a requirement (e.g., a host organism may be grown in the absence of light). In some instances, the host organism may be genetically modified in such a way that its photosynthetic capability is diminished or destroyed. In growth conditions where a host organism is not capable of photosynthesis (e.g., because of the absence of light and/or genetic modification), typically, the organism will be provided with the necessary nutrients to support growth in the absence of photosynthesis. For example, a culture medium in (or on) which an organism is grown, may be supplemented with any required nutrient, including an organic carbon source, nitrogen source, phosphorous source, vitamins, metals, lipids, nucleic acids, micronutrients, and/or an organism-specific requirement. Organic carbon sources include any source of carbon which the host organism is able to metabolize including, but not limited to, acetate, simple carbohydrates (e.g., glucose, sucrose, and lactose), complex carbohydrates (e.g., starch and glycogen), proteins, and lipids. One of skill in the art will recognize that not all organisms will be able to sufficiently metabolize a particular nutrient and that nutrient mixtures may need to be modified from one organism to another in order to provide the appropriate nutrient mix.

[00324] Optimal growth of organisms occurs usually at a temperature of about 20°C to about 25 °C, although some organisms can still grow at a temperature of up to about 35 °C. Active growth is typically performed in liquid culture. If the organisms are grown in a liquid medium and are shaken or mixed, the density of the cells can be anywhere from about 1 to 5 x 10^8cells/ml at the stationary phase. For example, the density of the cells at the stationary phase for Chlamydomonas sp. can be about 1 to 5 x 10^7cells/ml; the density of the cells at the stationary phase for Nannochloropsis sp. can be about 1 to 5 x 10^8cells/ml; the density of the cells at the stationary phase for Scenedesmus sp. can be about 1 to 5 x 10^7cells/ml; and the density of the cells at the stationary phase for Chlorella sp. can be about 1

to 5 x 10^8cells/ml. Exemplary cell densities at the stationary phase are as follows: Chlamydomonas sp. can be about 1 x 10^7cells/ml; Nannochloropsis sp. can be about 1 x 10^8cells/ml; Scenedesmus sp. can be about 1 x 10^7cells/ml; and Chlorella sp. can be about 1 x 10^8cells/ml. An exemplary growth rate may yield, for example, a two to four fold increase in cells per day, depending on the growth conditions. In addition, doubling times for organisms can be, for example, 5 hours to 30 hours. The organism can also be grown on solid media, for example, media containing about 1.5% agar, in plates or in slants.

[00325] One source of energy is fluorescent light that can be placed, for example, at a distance of about 1 inch to about two feet from the organism. Examples of types of fluorescent lights includes, for example, cool white and daylight. Bubbling with air or CO_2 improves the growth rate of the organism. Bubbling with CO_2 can be, for example, at 1% to 5% CO_2. If the lights are turned on and off at regular intervals (for example, 12:12 or 14:10 hours of light:dark) the cells of some organisms will become synchronized.

[00326] Long term storage of organisms can be achieved by streaking them onto plates, sealing the plates with, for example, Parafilm™, and placing them in dim light at about 10 °C to about 18 °C. Alternatively, organisms may be grown as streaks or stabs into agar tubes, capped, and stored at about 10 °C to about 18 °C. Both methods allow for the storage of the organisms for several months.

[00327] For longer storage, the organisms can be grown in liquid culture to mid to late log phase and then supplemented with a penetrating cryoprotective agent like DMSO or MeOH, and stored at less than -130 °C. An exemplary range of DMSO concentrations that can be used is 5 to 8%. An exemplary range of MeOH concentrations that can be used is 3 to 9% .

[00328] Organisms can be grown on a defined minimal medium (for example, high salt medium (HSM), modified artificial sea water medium (MASM), or F/2 medium) with light as the sole energy source. In other instances, the organism can be grown in a medium (for example, tris acetate phosphate (TAP) medium), and supplemented with an organic carbon source.

[00329] Organisms, such as algae, can grow naturally in fresh water or marine water. Culture media for freshwater algae can be, for example, synthetic media, enriched media, soil water media, and solidified media, such as agar. Various culture media have been developed and used for the isolation and cultivation of fresh water algae and are described in Watanabe, M.W. (2005). Freshwater Culture Media. In R.A. Andersen (Ed.), Algal Culturing Techniques (pp. 13-20). Elsevier Academic Press. Culture media for marine algae can be, for example, artificial seawater media or natural seawater media. Guidelines for the preparation of media are described in Harrison, P.J. and Berges, J.A. (2005). Marine Culture Media. In R.A. Andersen (Ed.), Algal Culturing Techniques (pp. 21-33). Elsevier Academic Press.

[00330] Organisms may be grown in outdoor open water, such as ponds, the ocean, seas, rivers, waterbeds, marshes, shallow pools, lakes, aqueducts, and reservoirs. When grown in water, the organism can be contained in a halo-like object comprised of lego-like particles. The halo-like object encircles the organism and allows it to retain nutrients from the water beneath while keeping it in open sunlight.

[00331] In some instances, organisms can be grown in containers wherein each container comprises one or two organisms, or a plurality of organisms. The containers can be configured to float on water. For example, a container can be filled by a combination of air and water to make the container and the organism(s) in it buoyant. An organism that is

adapted to grow in fresh water can thus be grown in salt water (i.e., the ocean) and vice versa. This mechanism allows for automatic death of the organism if there is any damage to the container.

[00332] Culturing techniques for algae are well know to one of skill in the art and are described, for example, in Freshwater Culture Media. In R.A. Andersen (Ed.), Algal Culturing Techniques. Elsevier Academic Press.

[00333] Because photosynthetic organisms, for example, algae, require sunlight, CO_2 and water for growth, they can be cultivated in, for example, open ponds and lakes. However, these open systems are more vulnerable to contamination than a closed system. One challenge with using an open system is that the organism of interest may not grow as quickly as a potential invader. This becomes a problem when another organism invades the liquid environment in which the organism of interest is growing, and the invading organism has a faster growth rate and takes over the system.

[00334] In addition, in open systems there is less control over water temperature, CO_2 concentration, and lighting conditions. The growing season of the organism is largely dependent on location and, aside from tropical areas, is limited to the warmer months of the year. In addition, in an open system, the number of different organisms that can be grown is limited to those that are able to survive in the chosen location. An open system, however, is cheaper to set up and/or maintain than a closed system.

[00335] Another approach to growing an organism is to use a semi-closed system, such as covering the pond or pool with a structure, for example, a "greenhouse-type" structure. While this can result in a smaller system, it addresses many of the problems associated with an open system. The advantages of a semi-closed system are that it can allow for a greater number of different organisms to be grown, it can allow for an organism to be dominant over an invading organism by allowing the organism of interest to out compete the invading organism for nutrients required for its growth, and it can extend the growing season for the organism. For example, if the system is heated, the organism can grow year round.

[00336] A variation of the pond system is an artificial pond, for example, a raceway pond. In these ponds, the organism, water, and nutrients circulate around a "racetrack." Paddlewheels provide constant motion to the liquid in the racetrack, allowing for the organism to be circulated back to the surface of the liquid at a chosen frequency. Paddlewheels also provide a source of agitation and oxygenate the system. These raceway ponds can be enclosed, for example, in a building or a greenhouse, or can be located outdoors.

[00337] Raceway ponds are usually kept shallow because the organism needs to be exposed to sunlight, and sunlight can only penetrate the pond water to a limited depth. The depth of a raceway pond can be, for example, about 4 to about 12 inches. In addition, the volume of liquid that can be contained in a raceway pond can be, for example, about 200 liters to about 600,000 liters.

[00338] The raceway ponds can be operated in a continuous manner, with, for example, CO_2 and nutrients being constantly fed to the ponds, while water containing the organism is removed at the other end.

[00339] If the raceway pond is placed outdoors, there are several different ways to address the invasion of an unwanted organism. For example, the pH or salinity of the liquid in which the desired organism is in can be such that the invading organism either slows down its growth or dies.

[00340] Also, chemicals can be added to the liquid, such as bleach, or a pesticide can be added to the liquid, such as glyphosate. In addition, the organism of interest can be genetically modified such that it is better suited to survive in the

liquid environment. Any one or more of the above strategies can be used to address the invasion of an unwanted organism.

[00341] Alternatively, organisms, such as algae, can be grown in closed structures such as photobioreactors, where the environment is under stricter control than in open systems or semi-closed systems. A photobioreactor is a bioreactor which incorporates some type of light source to provide photonic energy input into the reactor. The term photobioreactor can refer to a system closed to the environment and having no direct exchange of gases and contaminants with the environment. A photobioreactor can be described as an enclosed, illuminated culture vessel designed for controlled biomass production of phototrophic liquid cell suspension cultures. Examples of photobioreactors include, for example, glass containers, plastic tubes, tanks, plastic sleeves, and bags. Examples of light sources that can be used to provide the energy required to sustain photosynthesis include, for example, fluorescent bulbs, LEDs, and natural sunlight. Because these systems are closed everything that the organism needs to grow (for example, carbon dioxide, nutrients, water, and light) must be introduced into the bioreactor.

[00342] Photobioreactors, despite the costs to set up and maintain them, have several advantages over open systems, they can, for example, prevent or minimize contamination, permit axenic organism cultivation of monocultures (a culture consisting of only one species of organism), offer better control over the culture conditions (for example, pH, light, carbon dioxide, and temperature), prevent water evaporation, lower carbon dioxide losses due to out gassing, and permit higher cell concentrations.

[00343] On the other hand, certain requirements of photobioreactors, such as cooling, mixing, control of oxygen accumulation and biofouling, make these systems more expensive to build and operate than open systems or semi-closed systems.

[00344] Photobioreactors can be set up to be continually harvested (as is with the majority of the larger volume cultivation systems), or harvested one batch at a time (for example, as with polyethlyene bag cultivation). A batch photobioreactor is set up with, for example, nutrients, an organism (for example, algae), and water, and the organism is allowed to grow until the batch is harvested. A continuous photobioreactor can be harvested, for example, either continually, daily, or at fixed time intervals.

[00345] High density photobioreactors are described in, for example, Lee, et al., Biotech. Bioengineering 44:1161-1167, 1994. Other types of bioreactors, such as those for sewage and waste water treatments, are described in, Sawayama, et al., Appl. Micro. Biotech., 41:729-731, 1994. Additional examples of photobioreactors are described in, U.S. Appl. Publ. No. 2005/0260553, U.S. Pat. No. 5,958,761, and U.S. Pat. No. 6,083,740. Also, organisms, such as algae may be mass-cultured for the removal of heavy metals (for example, as described in Wilkinson, Biotech. Letters, 11:861-864, 1989), hydrogen (for example, as described in U.S. Patent Application Publication No. 2003/0162273), and pharmaceutical compounds from a water, soil, or other source or sample. Organisms can also be cultured in conventional fermentation bioreactors, which include, but are not limited to, batch, fed-batch, cell recycle, and continuous fermentors. Additional methods of culturing organisms and variations of the methods described herein are known to one of skill in the art.

[00346] Organisms can also be grown near ethanol production plants or other facilities or regions (e.g., cities and highways) generating CO_2. As such, the methods herein contemplate business methods for selling carbon credits to

ethanol plants or other facilities or regions generating CO_2 while making fuels or fuel products by growing one or more of the organisms described herein near the ethanol production plant, facility, or region.

[00347] The organism of interest, grown in any of the systems described herein, can be, for example, continually harvested, or harvested one batch at a time.

[00348] CO_2 can be delivered to any of the systems described herein, for example, by bubbling in CO_2 from under the surface of the liquid containing the organism. Also, sparges can be used to inject CO_2 into the liquid. Spargers are, for example, porous disc or tube assemblies that are also referred to as Bubblers, Carbonators, Aerators, Porous Stones and Diffusers.

[00349] Nutrients that can be used in the systems described herein include, for example, nitrogen (in the form of NO_3^- or NH_4^+), phosphorus, and trace metals (Fe, Mg, K, Ca, Co, Cu, Mn, Mo, Zn, V, and B). The nutrients can come, for example, in a solid form or in a liquid form. If the nutrients are in a solid form they can be mixed with, for example, fresh or salt water prior to being delivered to the liquid containing the organism, or prior to being delivered to a photobioreactor.

[00350] Organisms can be grown in cultures, for example large scale cultures, where large scale cultures refers to growth of cultures in volumes of greater than about 6 liters, or greater than about 10 liters, or greater than about 20 liters. Large scale growth can also be growth of cultures in volumes of 50 liters or more, 100 liters or more, or 200 liters or more. Large scale growth can be growth of cultures in, for example, ponds, containers, vessels, or other areas, where the pond, container, vessel, or area that contains the culture is for example, at lease 5 square meters, at least 10 square meters, at least 200 square meters, at least 500 square meters, at least 1,500 square meters, at least 2,500 square meters, in area, or greater.

[00351] *Chlamydomonas sp., Nannochloropsis sp., Scenedesmus sp.,* and *Chlorella sp.* are exemplary algae that can be cultured as described herein and can grow under a wide array of conditions.

[00352] One organism that can be cultured as described herein is a commonly used laboratory species C. reinhardtii. Cells of this species are haploid, and can grow on a simple medium of inorganic salts, using photosynthesis to provide energy. This organism can also grow in total darkness if acetate is provided as a carbon source. C. reinhardtii can be readily grown at room temperature under standard fluorescent lights. In addition, the cells can be synchronized by placing them on a light-dark cycle. Other methods of culturing C. reinhardtii cells are known to one of skill in the art.

[00353] **Polynucleotides and Polypeptides**

[00354] Also provided are isolated polynucleotides encoding a protein, for example, an ACCase described herein. As used herein "isolated polynucleotide" means a polynucleotide that is free of one or both of the nucleotide sequences which flank the polynucleotide in the naturally-occurring genome of the organism from which the polynucleotide is derived. The term includes, for example, a polynucleotide or fragment thereof that is incorporated into a vector or expression cassette; into an autonomously replicating plasmid or virus; into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule independent of other polynucleotides. It also includes a recombinant polynucleotide that is part of a hybrid polynucleotide, for example, one encoding a polypeptide sequence.

[00355] The novel ACCases of the present disclosure can be made by any method known in the art. The protein may be synthesized using either solid-phase peptide synthesis or by classical solution peptide synthesis also known as liquid-phase peptide synthesis. Using Val-Pro-Pro, Enalapril and Lisinopril as starting templates, several series of peptide

analogs such as X-Pro-Pro, X-Ala-Pro, and X-Lys-Pro, wherein X represents any amino acid residue, may be synthesized using solid-phase or liquid-phase peptide synthesis. Methods for carrying out liquid phase synthesis of libraries of peptides and oliqonucleotides coupled to a soluble oligomeric support have also been described. Bayer, Ernst and Mutter, Manfred, Nature 237:512-513 (1972) ; Bayer, Ernst, et al., J. Am. Chem. Soc. 96:7333-7336 (1974); Bonora, Gian Maria, et al., Nucleic Acids Res. 18:3155-3159 (1990). Liquid phase synthetic methods have the advantage over solid phase synthetic methods in that liquid phase synthesis methods do not require a structure present on a first reactant which is suitable for attaching the reactant to the solid phase. Also, liquid phase synthesis methods do not require avoiding chemical conditions which may cleave the bond between the solid phase and the first reactant (or intermediate product). In addition, reactions in a homogeneous solution may give better yields and more complete reactions than those obtained in heterogeneous solid phase/liquid phase systems such as those present in solid phase synthesis.

[00356] In oligomer-supported liquid phase synthesis the growing product is attached to a large soluble polymeric group. The product from each step of the synthesis can then be separated from unreacted reactants based on the large difference in size between the relatively large polymer-attached product and the unreacted reactants. This permits reactions to take place in homogeneous solutions, and eliminates tedious purification steps associated with traditional liquid phase synthesis. Oligomer-supported liquid phase synthesis has also been adapted to automatic liquid phase synthesis of peptides. Bayer, Ernst, et al., Peptides: Chemistry, Structure, Biology, 426-432.

[00357] For solid-phase peptide synthesis, the procedure entails the sequential assembly of the appropriate amino acids into a peptide of a desired sequence while the end of the growing peptide is linked to an insoluble support. Usually, the carboxyl terminus of the peptide is linked to a polymer from which it can be liberated upon treatment with a cleavage reagent. In a common method, an amino acid is bound to a resin particle, and the peptide generated in a stepwise manner by successive additions of protected amino acids to produce a chain of amino acids. Modifications of the technique described by Merrifield are commonly used. See, e.g., Merrifield, J. Am. Chem. Soc. 96: 2989-93 (1964). In an automated solid-phase method, peptides are synthesized by loading the carboxy-terminal amino acid onto an organic linker (e.g., PAM, 4-oxymethylphenylacetamidomethyl), which is covalently attached to an insoluble polystyrene resin cross-linked with divinyl benzene. The terminal amine may be protected by blocking with t-butyloxycarbonyl. Hydroxyl- and carboxyl- groups are commonly protected by blocking with O-benzyl groups. Synthesis is accomplished in an automated peptide synthesizer, such as that available from Applied Biosystems (Foster City, California). Following synthesis, the product may be removed from the resin. The blocking groups are removed by using hydrofluoric acid or trifluoromethyl sulfonic acid according to established methods. A routine synthesis may produce 0.5 mmole of peptide resin. Following cleavage and purification, a yield of approximately 60 to 70% is typically produced. Purification of the product peptides is accomplished by, for example, crystallizing the peptide from an organic solvent such as methyl-butyl ether, then dissolving in distilled water, and using dialysis (if the molecular weight of the subject peptide is greater than about 500 daltons) or reverse high pressure liquid chromatography (e.g., using a C^{18} column with 0.1% trifluoroacetic acid and acetonitrile as solvents) if the molecular weight of the peptide is less than 500 daltons. Purified peptide may be lyophilized and stored in a dry state until use. Analysis of the resulting peptides may be accomplished using the common methods of analytical high pressure liquid chromatography (HPLC) and electrospray mass spectrometry (ES-MS).

[00358] In other cases, a protein, for example, an ACCase is produced by recombinant methods. For production of any of the proteins described herein, host cells transformed with an expression vector containing the polynucleotide encoding such a protein can be used. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell such as a yeast or algal cell, or the host can be a prokaryotic cell such as a bacterial cell. Introduction of the expression vector into the host cell can be accomplished by a variety of methods including calcium phosphate transfection, DEAE-dextran mediated transfection, polybrene, protoplast fusion, liposomes, direct microinjection into the nuclei, scrape loading, biolistic transformation and electroporation. Large scale production of proteins from recombinant organisms is a well established process practiced on a commercial scale and well within the capabilities of one skilled in the art.

[00359] In some embodiments, the novel ACCases are provided in a substantially pure or substantially purified form. "Substantially pure" or "substantially purified" means that the substance is free from other contaminating proteins, nucleic acids, and other biologicals derived from the source organism. Purity may be assayed by standard methods, and will ordinarily be at least about 40% pure, at least about 50% pure, at least about 60% pure, at least about 70% pure, least about 75% pure, at least about 80% pure, at least about 85% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least 99% pure. The analysis may be weight or molar percentages, evaluated, e.g., by gel staining, spectrophotometry, terminus labeling, etc.

[00360] It should be recognized that the present disclosure is not limited to transgenic cells, organisms, and plastids containing a protein or proteins as disclosed herein, but also encompasses such cells, organisms, and plastids transformed with additional nucleotide sequences encoding enzymes involved in fatty acid synthesis. Thus, some embodiments involve the introduction of one or more sequences encoding proteins involved in fatty acid synthesis in addition to a protein disclosed herein. For example, several enzymes in a fatty acid production pathway may be linked, either directly or indirectly, such that products produced by one enzyme in the pathway, once produced, are in close proximity to the next enzyme in the pathway. These additional sequences may be contained in a single vector either operatively linked to a single promoter or linked to multiple promoters, e.g. one promoter for each sequence. Alternatively, the additional coding sequences may be contained in a plurality of additional vectors. When a plurality of vectors are used, they can be introduced into the host cell or organism simultaneously or sequentially.

[00361] Additional embodiments provide a plastid, and in particular a chloroplast, transformed with a polynucleotide encoding a protein of the present disclosure. The protein may be introduced into the genome of the plastid using any of the methods described herein or otherwise known in the art. The plastid may be contained in the organism in which it naturally occurs. Alternatively, the plastid may be an isolated plastid, that is, a plastid that has been removed from the cell in which it normally occurs. Methods for the isolation of plastids are known in the art and can be found, for example, in Maliga et al., *Methods in Plant Molecular Biology*, Cold Spring Harbor Laboratory Press, 1995; Gupta and Singh, *J. Biosci.*, 21:819 (1996); and Camara et al., *Plant Physiol.*, 73:94 (1983). The isolated plastid transformed with a protein of the present disclosure can be introduced into a host cell. The host cell can be one that naturally contains the plastid or one in which the plastid is not naturally found.

[00362] Also within the scope of the present disclosure are artificial plastid genomes, for example chloroplast genomes, that contain nucleotide sequences encoding any one or more of the proteins of the present disclosure. Methods for the assembly of artificial plastid genomes can be found in co-pending U.S. Patent Application serial number 12/287,230

filed October 6, 2008, published as U.S. Publication No. 2009/0123977 on May 14, 2009, and U.S. Patent Application serial number 12/384,893 filed April 8, 2009, published as U.S. Publication No. 2009/0269816 on October 29, 2009, each of which is incorporated by reference in its entirety.

[00363] Introduction of Polynucleotide into a Host Organism or Cell

[00364] To generate a genetically modified host cell, a polynucleotide, or a polynucleotide cloned into a vector, is introduced stably or transiently into a host cell, using established techniques, including, but not limited to, electroporation, calcium phosphate precipitation, DEAE-dextran mediated transfection, and liposome-mediated transfection. For transformation, a polynucleotide of the present disclosure will generally further include a selectable marker, e.g., any of several well-known selectable markers such as neomycin resistance, ampicillin resistance, tetracycline resistance, chloramphenicol resistance, and kanamycin resistance.

[00365] A polynucleotide or recombinant nucleic acid molecule described herein, can be introduced into a cell (e.g., alga cell) using any method known in the art. A polynucleotide can be introduced into a cell by a variety of methods, which are well known in the art and selected, in part, based on the particular host cell. For example, the polynucleotide can be introduced into a cell using a direct gene transfer method such as electroporation or microprojectile mediated (biolistic) transformation using a particle gun, or the "glass bead method," or by pollen-mediated transformation, liposome-mediated transformation, transformation using wounded or enzyme-degraded immature embryos, or wounded or enzyme-degraded embryogenic callus (for example, as described in Potrykus, *Ann. Rev. Plant. Physiol. Plant Mol. Biol.* 42:205-225, 1991).

[00366] As discussed above, microprojectile mediated transformation can be used to introduce a polynucleotide into a cell (for example, as described in Klein et al., *Nature* 327:70-73, 1987). This method utilizes microprojectiles such as gold or tungsten, which are coated with the desired polynucleotide by precipitation with calcium chloride, spermidine or polyethylene glycol. The microprojectile particles are accelerated at high speed into a cell using a device such as the BIOLISTIC PD-1000 particle gun (BioRad; Hercules Calif.). Methods for the transformation using biolistic methods are well known in the art (for example, as described in Christou, *Trends in Plant Science* 1:423-431, 1996). Microprojectile mediated transformation has been used, for example, to generate a variety of transgenic plant species, including cotton, tobacco, corn, hybrid poplar and papaya. Important cereal crops such as wheat, oat, barley, sorghum and rice also have been transformed using microprojectile mediated delivery (for example, as described in Duan et al., *Nature Biotech.* 14:494-498, 1996; and Shimamoto, *Curr. Opin. Biotech.* 5:158-162, 1994). The transformation of most dicotyledonous plants is possible with the methods described above. Transformation of monocotyledonous plants also can be transformed using, for example, biolistic methods as described above, protoplast transformation, electroporation of partially permeabilized cells, introduction of DNA using glass fibers, and the glass bead agitation method.

[00367] The basic techniques used for transformation and expression in photosynthetic microorganisms are similar to those commonly used for *E. coli, Saccharomyces cerevisiae* and other species. Transformation methods customized for a photosynthetic microorganisms, e.g., the chloroplast of a strain of algae, are known in the art. These methods have been described in a number of texts for standard molecular biological manipulation (see Packer & Glaser, 1988, "Cyanobacteria", Meth. Enzymol., Vol. 167; Weissbach & Weissbach, 1988, "Methods for plant molecular biology," Academic Press, New York, Sambrook, Fritsch & Maniatis, 1989, "Molecular Cloning: A laboratory manual," 2nd edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; and Clark M S, 1997, Plant Molecular

Biology, Springer, N.Y.). These methods include, for example, biolistic devices (See, for example, Sanford, Trends In Biotech. (1988) 6: 299-302, U.S. Pat. No. 4,945,050; electroporation (Fromm et al., Proc. Nat'l. Acad. Sci. (USA) (1985) 82: 5824-5828); use of a laser beam, electroporation, microinjection or any other method capable of introducing DNA into a host cell.

[00368] Plastid transformation is a routine and well known method for introducing a polynucleotide into a plant cell chloroplast (see U.S. Pat. Nos. 5,451,513, 5,545,817, and 5,545,818; WO 95/16783; McBride et al., *Proc. Natl. Acad. Sci.*, USA 91:7301-7305, 1994). In some embodiments, chloroplast transformation involves introducing regions of chloroplast DNA flanking a desired nucleotide sequence, allowing for homologous recombination of the exogenous DNA into the target chloroplast genome. In some instances one to 1.5 kb flanking nucleotide sequences of chloroplast genomic DNA may be used. Using this method, point mutations in the chloroplast 16S rRNA and rps12 genes, which confer resistance to spectinomycin and streptomycin, can be utilized as selectable markers for transformation (Svab et al., *Proc. Natl. Acad. Sci.*, USA 87:8526-8530, 1990), and can result in stable homoplasmic transformants, at a frequency of approximately one per 100 bombardments of target leaves.

[00369] A further refinement in chloroplast transformation/expression technology that facilitates control over the timing and tissue pattern of expression of introduced DNA coding sequences in plant plastid genomes has been described in PCT International Publication WO 95/16783 and U.S. Patent 5,576,198. This method involves the introduction into plant cells of constructs for nuclear transformation that provide for the expression of a viral single subunit RNA polymerase and targeting of this polymerase into the plastids via fusion to a plastid transit peptide. Transformation of plastids with DNA constructs comprising a viral single subunit RNA polymerase-specific promoter specific to the RNA polymerase expressed from the nuclear expression constructs operably linked to DNA coding sequences of interest permits control of the plastid expression constructs in a tissue and/or developmental specific manner in plants comprising both the nuclear polymerase construct and the plastid expression constructs. Expression of the nuclear RNA polymerase coding sequence can be placed under the control of either a constitutive promoter, or a tissue-or developmental stage-specific promoter, thereby extending this control to the plastid expression construct responsive to the plastid-targeted, nuclear-encoded viral RNA polymerase.

[00370] When nuclear transformation is utilized, the protein can be modified for plastid targeting by employing plant cell nuclear transformation constructs wherein DNA coding sequences of interest are fused to any of the available transit peptide sequences capable of facilitating transport of the encoded enzymes into plant plastids, and driving expression by employing an appropriate promoter. Targeting of the protein can be achieved by fusing DNA encoding plastid, e.g., chloroplast, leucoplast, amyloplast, etc., transit peptide sequences to the 5' end of DNAs encoding the enzymes. The sequences that encode a transit peptide region can be obtained, for example, from plant nuclear-encoded plastid proteins, such as the small subunit (SSU) of ribulose bisphosphate carboxylase, EPSP synthase, plant fatty acid biosynthesis related genes including fatty acyl-ACP thioesterases, acyl carrier protein (ACP), stearoyl-ACP desaturase, β-ketoacyl-ACP synthase and acyl-ACP thioesterase, or LHCPII genes, etc. Plastid transit peptide sequences can also be obtained from nucleic acid sequences encoding carotenoid biosynthetic enzymes, such as GGPP synthase, phytoene synthase, and phytoene desaturase. Other transit peptide sequences are disclosed in Von Heijne et al. (1991) *Plant Mol. Biol. Rep.* 9: 104; Clark et al. (1989) *J. Biol. Chem.* 264: 17544; della-Cioppa et al. (1987) *Plant Physiol.* 84: 965; Romer et al. (1993) *Biochem. Biophys. Res. Commun.* 196: 1414; and Shah et al. (1986) *Science* 233: 478. Another

transit peptide sequence is that of the intact ACCase from Chlamydomonas (genbank EDO96563, amino acids 1-33). The encoding sequence for a transit peptide effective in transport to plastids can include all or a portion of the encoding sequence for a particular transit peptide, and may also contain portions of the mature protein encoding sequence associated with a particular transit peptide. Numerous examples of transit peptides that can be used to deliver target proteins into plastids exist, and the particular transit peptide encoding sequences useful in the present disclosure are not critical as long as delivery into a plastid is obtained. Proteolytic processing within the plastid then produces the mature enzyme. This technique has proven successful with enzymes involved in polyhydroxyalkanoate biosynthesis (Nawrath et al. (1994) *Proc. Natl. Acad. Sci. USA* 91: 12760), and neomycin phosphotransferase II (NPT-II) and CP4 EPSPS (Padgette et al. (1995) *Crop Sci.* 35: 1451), for example.

[00371] Of interest are transit peptide sequences derived from enzymes known to be imported into the leucoplasts of seeds. Examples of enzymes containing useful transit peptides include those related to lipid biosynthesis (e.g., subunits of the plastid-targeted dicot acetyl-CoA carboxylase, biotin carboxylase, biotin carboxyl carrier protein, α-carboxy-transferase, and plastid-targeted monocot multifunctional acetyl-CoA carboxylase (Mw, 220,000); plastidic subunits of the fatty acid synthase complex (e.g., acyl carrier protein (ACP), malonyl-ACP synthase, KASI, KASII, and KASIII); steroyl-ACP desaturase; thioesterases (specific for short, medium, and long chain acyl ACP); plastid-targeted acyl transferases (e.g., glycerol-3-phosphate and acyl transferase); enzymes involved in the biosynthesis of aspartate family amino acids; phytoene synthase; gibberellic acid biosynthesis (e.g., *ent*-kaurene synthases 1 and 2); and carotenoid biosynthesis (e.g., lycopene synthase).

[00372] In some embodiments, an alga is transformed with a nucleic acid which encodes a protein of interest, for example, an ACCase, a prenyl transferase, an isoprenoid synthase, or an enzyme capable of converting a precursor into a fuel product or a precursor of a fuel product (e.g., an isoprenoid or fatty acid).

[00373] In one embodiment, a transformation may introduce a nucleic acid into a plastid of the host alga (e.g., chloroplast). In another embodiments a transformation may introduce a nucleic acid into the nuclear genome of the host alga. In still another embodiment, a transformation may introduce nucleic acids into both the nuclear genome and into a plastid.

[00374] Transformed cells can be plated on selective media following introduction of exogenous nucleic acids. This method may also comprise several steps for screening. A screen of primary transformants can be conducted to determine which clones have proper insertion of the exogenous nucleic acids. Clones which show the proper integration may be propagated and re-screened to ensure genetic stability. Such methodology ensures that the transformants contain the genes of interest. In many instances, such screening is performed by polymerase chain reaction (PCR); however, any other appropriate technique known in the art may be utilized. Many different methods of PCR are known in the art (e.g., nested PCR, real time PCR). For any given screen, one of skill in the art will recognize that PCR components may be varied to achieve optimal screening results. For example, magnesium concentration may need to be adjusted upwards when PCR is performed on disrupted alga cells to which (which chelates magnesium) is added to chelate toxic metals. Following the screening for clones with the proper integration of exogenous nucleic acids, clones can be screened for the presence of the encoded protein(s) and/or products. Protein expression screening can be performed by Western blot analysis and/or enzyme activity assays. Transporter and/or product screening may be

performed by any method known in the art, for example ATP turnover assay, substrate transport assay, HPLC or gas chromatography.

[00375] The expression of the protein or enzyme can be accomplished by inserting a polynucleotide sequence (gene) encoding the protein or enzyme into the chloroplast or nuclear genome of a microalgae. The modified strain of microalgae can be made homoplasmic to ensure that the polynucleotide will be stably maintained in the chloroplast genome of all descendents. A microalga is homoplasmic for a gene when the inserted gene is present in all copies of the chloroplast genome, for example. It is apparent to one of skill in the art that a chloroplast may contain multiple copies of its genome, and therefore, the term "homoplasmic" or "homoplasmy" refers to the state where all copies of a particular locus of interest are substantially identical. Plastid expression, in which genes are inserted by homologous recombination into all of the several thousand copies of the circular plastid genome present in each plant cell, takes advantage of the enormous copy number advantage over nuclear-expressed genes to permit expression levels that can readily exceed 10% or more of the total soluble plant protein. The process of determining the plasmic state of an organism of the present disclosure involves screening transformants for the presence of exogenous nucleic acids and the absence of wild-type nucleic acids at a given locus of interest.

[00376] **Vectors**

[00377] Construct, vector and plasmid are used interchangeably throughout the disclosure. Nucleic acids encoding the novel ACCases can be contained in vectors, including cloning and expression vectors. A cloning vector is a self-replicating DNA molecule that serves to transfer a DNA segment into a host cell. Three common types of cloning vectors are bacterial plasmids, phages, and other viruses. An expression vector is a cloning vector designed so that a coding sequence inserted at a particular site will be transcribed and translated into a protein. Both cloning and expression vectors can contain nucleotide sequences that allow the vectors to replicate in one or more suitable host cells. In cloning vectors, this sequence is generally one that enables the vector to replicate independently of the host cell chromosomes, and also includes either origins of replication or autonomously replicating sequences.

[00378] In some embodiments, a polynucleotide of the present disclosure is cloned or inserted into an expression vector using cloning techniques know to one of skill in the art. The nucleotide sequences may be inserted into a vector by a variety of methods. In the most common method the sequences are inserted into an appropriate restriction endonuclease site(s) using procedures commonly known to those skilled in the art and detailed in, for example, Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, (1989) and Ausubel et al., Short Protocols in Molecular Biology, 2nd Ed., John Wiley & Sons (1992).

[00379] Suitable expression vectors include, but are not limited to, baculovirus vectors, bacteriophage vectors, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral vectors (e.g. viral vectors based on vaccinia virus, poliovirus, adenovirus, adeno-associated virus, SV40, and herpes simplex virus), PI-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as *E. coli* and yeast). Thus, for example, a polynucleotide encoding an ACCase can be inserted into any one of a variety of expression vectors that are capable of expressing the enzyme. Such vectors can include, for example, chromosomal, nonchromosomal and synthetic DNA sequences.

[00380] Suitable expression vectors include chromosomal, non-chromosomal and synthetic DNA sequences, for example, SV 40 derivatives; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from

combinations of plasmids and phage DNA; and viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. In addition, any other vector that is replicable and viable in the host may be used. For example, vectors such as Ble2A, Arg7/2A, and SEnuc357 can be used for the expression of a protein.

[00381] Numerous suitable expression vectors are known to those of skill in the art. The following vectors are provided by way of example; for bacterial host cells: pQE vectors (Qiagen), pBluescript plasmids, pNH vectors, lambda-ZAP vectors (Stratagene), pTrc99a, pKK223-3, pDR540, and pRIT2T (Pharmacia); for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, pET21a-d(+) vectors (Novagen), and pSVLSV40 (Pharmacia). However, any other plasmid or other vector may be used so long as it is compatible with the host cell.

[00382] The expression vector, or a linearized portion thereof, can encode one or more exogenous or endogenous nucleotide sequences. Examples of exogenous nucleotide sequences that can be transformed into a host include genes from bacteria, fungi, plants, photosynthetic bacteria or other algae. Examples of other types of nucleotide sequences that can be transformed into a host, include, but are not limited to, transporter genes, isoprenoid producing genes, genes which encode for proteins which produce isoprenoids with two phosphates (e.g., GPP synthase and/or FPP synthase), genes which encode for proteins which produce fatty acids, lipids, or triglycerides, for example, ACCases, endogenous promoters, and 5' UTRs from the psbA, atpA, or rbcL genes. In some instances, an exogenous sequence is flanked by two homologous sequences.

[00383] Homologous sequences are, for example, those that have at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to a reference amino acid sequence or nucleotide sequence, for example, the amino acid sequence or nucleotide sequence that is found naturally in the host cell. The first and second homologous sequences enable recombination of the exogenous or endogenous sequence into the genome of the host organism. The first and second homologous sequences can be at least 100, at least 200, at least 300, at least 400, at least 500, or at least 1500 nucleotides in length.

[00384] The polynucleotide sequence may comprise nucleotide sequences that are codon biased for expression in the organism being transformed. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Without being bound by theory, by using a host cell's preferred codons, the rate of translation may be greater. Therefore, when synthesizing a gene for improved expression in a host cell, it may be desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell. In some organisms, codon bias differs between the nuclear genome and organelle genomes, thus, codon optimization or biasing may be performed for the target genome (e.g., nuclear codon biased or chloroplast codon biased). In some embodiments, codon biasing occurs before mutagenesis to generate a polypeptide. In other embodiments, codon biasing occurs after mutagenesis to generate a polynucleotide. In yet other embodiments, codon biasing occurs before mutagenesis as well as after mutagenesis. Codon bias is described in detail herein.

[00385] In some embodiments, a vector comprises a polynucleotide operably linked to one or more control elements, such as a promoter and/or a transcription terminator. A nucleic acid sequence is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operatively linked to DNA for a polypeptide if it is expressed as a preprotein which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it affects the transcription of the sequence; or a

ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, operably linked sequences are contiguous and, in the case of a secretory leader, contiguous and in reading phase. Linking is achieved by ligation at restriction enzyme sites. If suitable restriction sites are not available, then synthetic oligonucleotide adapters or linkers can be used as is known to those skilled in the art. Sambrook et al., *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press, (1989) and Ausubel et al., *Short Protocols in Molecular Biology*, 2nd Ed., John Wiley & Sons (1992).

[00386] A vector in some embodiments provides for amplification of the copy number of a polynucleotide. A vector can be, for example, an expression vector that provides for expression of an ACCase, a prenyl transferase, an isoprenoid synthase, or a mevalonate synthesis enzyme in a host cell, e.g., a prokaryotic host cell or a eukaryotic host cell.

[00387] A polynucleotide or polynucleotides can be contained in a vector or vectors. For example, where a second (or more) nucleic acid molecule is desired, the second nucleic acid molecule can be contained in a vector, which can, but need not be, the same vector as that containing the first nucleic acid molecule. The vector can be any vector useful for introducing a polynucleotide into a genome and can include a nucleotide sequence of genomic DNA (e.g., nuclear or plastid) that is sufficient to undergo homologous recombination with genomic DNA, for example, a nucleotide sequence comprising about 400 to about 1500 or more substantially contiguous nucleotides of genomic DNA.

[00388] A regulatory or control element, as the term is used herein, broadly refers to a nucleotide sequence that regulates the transcription or translation of a polynucleotide or the localization of a polypeptide to which it is operatively linked. Examples include, but are not limited to, an RBS, a promoter, enhancer, transcription terminator, an initiation (start) codon, a splicing signal for intron excision and maintenance of a correct reading frame, a STOP codon, an amber or ochre codon, and an IRES. A regulatory element can include a promoter and transcriptional and translational stop signals. Elements may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of a nucleotide sequence encoding a polypeptide. Additionally, a sequence comprising a cell compartmentalization signal (i.e., a sequence that targets a polypeptide to the cytosol, nucleus, chloroplast membrane or cell membrane) can be attached to the polynucleotide encoding a protein of interest. Such signals are well known in the art and have been widely reported (see, e.g., U.S. Pat. No. 5,776,689).

[00389] Promoters are untranslated sequences located generally 100 to 1000 base pairs (bp) upstream from the start codon of a structural gene that regulate the transcription and translation of nucleic acid sequences under their control.

[00390] Promoters useful for the present disclosure may come from any source (e.g., viral, bacterial, fungal, protist, and animal). The promoters contemplated herein can be specific to photosynthetic organisms, non-vascular photosynthetic organisms, and vascular photosynthetic organisms (e.g., algae, flowering plants). In some instances, the nucleic acids above are inserted into a vector that comprises a promoter of a photosynthetic organism, e.g., algae. The promoter can be a constitutive promoter or an inducible promoter. A promoter typically includes necessary nucleic acid sequences near the start site of transcription, (e.g., a TATA element). Common promoters used in expression vectors include, but are not limited to, LTR or SV40 promoter, the E. coli lac or trp promoters, and the phage lambda PL promoter. Other promoters known to control the expression of genes in prokaryotic or eukaryotic cells can be used and are known to those skilled in the art. Expression vectors may also contain a ribosome binding site for translation initiation, and a transcription terminator. The vector may also contain sequences useful for the amplification of gene expression.

[00391] A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under controllable environmental or developmental conditions. Examples of inducible promoters/regulatory elements include, for example, a nitrate-inducible promoter (for example, as described in Bock et al, *Plant Mol. Biol.* 17:9 (1991)), or a light-inducible promoter, (for example, as described in Feinbaum et al, Mol Gen. Genet. 226:449 (1991); and Lam and Chua, Science 248:471 (1990)), or a heat responsive promoter (for example, as described in Muller et al., Gene 111: 165-73 (1992)).

[00392] In many embodiments, a polynucleotide of the present disclosure includes a nucleotide sequence encoding a protein or enzyme of the present disclosure, where the nucleotide sequence encoding the polypeptide is operably linked to an inducible promoter. Inducible promoters are well known in the art. Suitable inducible promoters include, but are not limited to, the pL of bacteriophage λ; Placo; Ptrp; Ptac (Ptrp-lac hybrid promoter); an isopropyl-beta-D-thiogalactopyranoside (IPTG)-inducible promoter, e.g., a lacZ promoter; a tetracycline-inducible promoter; an arabinose inducible promoter, e.g., P_{BAD} (for example, as described in Guzman et al. (1995) J. Bacteriol. 177:4121-4130); a xylose-inducible promoter, e.g., Pxyl (for example, as described in Kim et al. (1996) Gene 181:71-76); a GAL1 promoter; a tryptophan promoter; a lac promoter; an alcohol-inducible promoter, e.g., a methanol-inducible promoter, an ethanol-inducible promoter; a raffinose-inducible promoter; and a heat-inducible promoter, e.g., heat inducible lambda P_L promoter and a promoter controlled by a heat-sensitive repressor (e.g., C1857-repressed lambda-based expression vectors; for example, as described in Hoffmann et al. (1999) FEMS Microbiol Lett. 177(2):327-34).

[00393] In many embodiments, a polynucleotide of the present disclosure includes a nucleotide sequence encoding a protein or enzyme of the present disclosure, where the nucleotide sequence encoding the polypeptide is operably linked to a constitutive promoter. Suitable constitutive promoters for use in prokaryotic cells are known in the art and include, but are not limited to, a sigma70 promoter, and a consensus sigma70 promoter.

[00394] Suitable promoters for use in prokaryotic host cells include, but are not limited to, a bacteriophage T7 RNA polymerase promoter; a trp promoter; a lac operon promoter; a hybrid promoter, e.g., a lac/tac hybrid promoter, a tac/trc hybrid promoter, a trp/lac promoter, a T7/lac promoter; a trc promoter; a tac promoter; an araBAD promoter; in vivo regulated promoters, such as an ssaG promoter or a related promoter (for example, as described in U.S. Patent Publication No. 20040131637), a pagC promoter (for example, as described in Pulkkinen and Miller, J. Bacteriol., 1991: 173(1): 86-93; and Alpuche-Aranda et al., PNAS, 1992; 89(21): 10079-83), a nirB promoter (for example, as described in Harborne et al. (1992) Mol. Micro. 6:2805-2813; Dunstan et al. (1999) Infect. Immun. 67:5133-5141; McKelvie et al. (2004) Vaccine 22:3243-3255; and Chatfield et al. (1992) Biotechnol. 10:888-892); a sigma70 promoter, e.g., a consensus sigma70 promoter (for example, GenBank Accession Nos. AX798980, AX798961, and AX798183); a stationary phase promoter, e.g., a dps promoter, a spv promoter; a promoter derived from the pathogenicity island SPI-2 (for example, as described in WO96/17951); an actA promoter (for example, as described in Shetron-Rama et al. (2002) Infect. Immun. 70:1087-1096); an rpsM promoter (for example, as described in Valdivia and Falkow (1996). Mol. Microbiol. 22:367-378); a tet promoter (for example, as described in Hillen, W. and Wissmann, A. (1989) In Saenger, W. and Heinemann, U. (eds), Topics in Molecular and Structural Biology, Protein-Nucleic Acid Interaction. Macmillan, London, UK, Vol. 10, pp. 143-162); and an SP6 promoter (for example, as described in Melton et al. (1984) Nucl. Acids Res. 12:7035-7056).

[00395] In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review of such vectors see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel, et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant, et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 31987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast Saccharomyces, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II. A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL may be used (for example, as described in Cloning in Yeast, Ch. 3, R. Rothstein In: DNA Cloning Vol. 11, A Practical Approach, Ed. DM Glover, 1986, IRL Press, Wash., D.C.). Alternatively, vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

[00396] Non-limiting examples of suitable eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression.

[00397] A vector utilized in the practice of the disclosure also can contain one or more additional nucleotide sequences that confer desirable characteristics on the vector, including, for example, sequences such as cloning sites that facilitate manipulation of the vector, regulatory elements that direct replication of the vector or transcription of nucleotide sequences contain therein, and sequences that encode a selectable marker. As such, the vector can contain, for example, one or more cloning sites such as a multiple cloning site, which can, but need not, be positioned such that a exogenous or endogenous polynucleotide can be inserted into the vector and operatively linked to a desired element.

[00398] The vector also can contain a prokaryote origin of replication (ori), for example, an E. coli ori or a cosmid ori, thus allowing passage of the vector into a prokaryote host cell, as well as into a plant chloroplast. Various bacterial and viral origins of replication are well known to those skilled in the art and include, but are not limited to the pBR322 plasmid origin, the 2u plasmid origin, and the SV40, polyoma, adenovirus, VSV, and BPV viral origins.

[00399] A regulatory or control element, as the term is used herein, broadly refers to a nucleotide sequence that regulates the transcription or translation of a polynucleotide or the localization of a polypeptide to which it is operatively linked. Examples include, but are not limited to, an RBS, a promoter, enhancer, transcription terminator, an initiation (start) codon, a splicing signal for intron excision and maintenance of a correct reading frame, a STOP codon, an amber or ochre codon, an IRES. Additionally, an element can be a cell compartmentalization signal (i.e., a sequence that targets a polypeptide to the cytosol, nucleus, chloroplast membrane or cell membrane). In some aspects of the present disclosure, a cell compartmentalization signal (e.g., a cell membrane targeting sequence) may be ligated to a gene and/or transcript, such that translation of the gene occurs in the chloroplast. In other aspects, a cell compartmentalization signal may be ligated to a gene such that, following translation of the gene, the protein is transported to the cell membrane. Cell compartmentalization signals are well known in the art and have been widely reported (see, e.g., U.S. Pat. No. 5,776,689).

[00400] A vector, or a linearized portion thereof, may include a nucleotide sequence encoding a reporter polypeptide or other selectable marker. The term "reporter" or "selectable marker" refers to a polynucleotide (or encoded polypeptide)

that confers a detectable phenotype. A reporter generally encodes a detectable polypeptide, for example, a green fluorescent protein or an enzyme such as luciferase, which, when contacted with an appropriate agent (a particular wavelength of light or luciferin, respectively) generates a signal that can be detected by eye or using appropriate instrumentation (for example, as described in Giacomin, *Plant Sci.* 116:59-72, 1996; Scikantha, *J. Bacteriol.* 178:121, 1996; Gerdes, *FEBS Lett.* 389:44-47, 1996; and Jefferson, *EMBO J.* 6:3901-3907, 1997, fl-glucuronidase). A selectable marker generally is a molecule that, when present or expressed in a cell, provides a selective advantage (or disadvantage) to the cell containing the marker, for example, the ability to grow in the presence of an agent that otherwise would kill the cell.

[00401] A selectable marker can provide a means to obtain, for example, prokaryotic cells, eukaryotic cells, and/or plant cells that express the marker and, therefore, can be useful as a component of a vector of the disclosure. The selection gene or marker can encode for a protein necessary for the survival or growth of the host cell transformed with the vector. One class of selectable markers are native or modified genes which restore a biological or physiological function to a host cell (e.g., restores photosynthetic capability or restores a metabolic pathway). Other examples of selectable markers include, but are not limited to, those that confer antimetabolite resistance, for example, dihydrofolate reductase, which confers resistance to methotrexate (for example, as described in Reiss, *Plant Physiol.* (*Life Sci. Adv.*) 13:143-149, 1994); neomycin phosphotransferase, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (for example, as described in Herrera-Estrella, *EMBO J.* 2:987-995, 1983), hygro, which confers resistance to hygromycin (for example, as described in Marsh, *Gene* 32:481-485, 1984), trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (for example, as described in Hartman, *Proc. Natl. Acad. Sci.*, USA 85:8047, 1988); mannose-6-phosphate isomerase which allows cells to utilize mannose (for example, as described in PCT Publication Application No. WO 94/20627); ornithine decarboxylase, which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine (DFMO; for example, as described in McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.); and deaminase from *Aspergillus terreus*, which confers resistance to Blasticidin S (for example, as described in Tamura, *Biosci. Biotechnol. Biochem.* 59:2336-2338, 1995). Additional selectable markers include those that confer herbicide resistance, for example, phosphinothricin acetyltransferase gene, which confers resistance to phosphinothricin (for example, as described in White et al., *Nucl. Acids Res.* 18:1062, 1990; and Spencer et al., *Theor. Appl. Genet.* 79:625-631, 1990), a mutant EPSPV-synthase, which confers glyphosate resistance (for example, as described in Hinchee et al., *BioTechnology* 91:915-922, 1998), a mutant acetolactate synthase, which confers imidazolione or sulfonylurea resistance (for example, as described in Lee et al., *EMBO J.* 7:1241-1248, 1988), a mutant psbA, which confers resistance to atrazine (for example, as described in Smeda et al., *Plant Physiol.* 103:911-917, 1993), or a mutant protoporphyrinogen oxidase (for example, as described in U.S. Pat. No. 5,767,373), or other markers conferring resistance to an herbicide such as glufosinate. Selectable markers include polynucleotides that confer dihydrofolate reductase (DHFR) or neomycin resistance for eukaryotic cells; tetramycin or ampicillin resistance for prokaryotes such as *E. coli*; and bleomycin, gentamycin, glyphosate, hygromycin, kanamycin, methotrexate, phleomycin, phosphinotricin, spectinomycin, dtreptomycin, streptomycin, sulfonamide and sulfonylurea resistance in plants (for example, as described in Maliga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Laboratory

Press, 1995, page 39). The selection marker can have its own promoter or its expression can be driven by a promoter driving the expression of a polypeptide of interest.

[00402] Reporter genes greatly enhance the ability to monitor gene expression in a number of biological organisms. Reporter genes have been successfully used in chloroplasts of higher plants, and high levels of recombinant protein expression have been reported. In addition, reporter genes have been used in the chloroplast of *C. reinhardtii*. In chloroplasts of higher plants, β-glucuronidase (uidA, for example, as described in Staub and Maliga, *EMBO J.* 12:601-606, 1993), neomycin phosphotransferase (nptII, for example, as described in Carrer et al., *Mol. Gen. Genet.* 241:49-56, 1993), adenosyl-3-adenyltransf- erase (aadA, for example, as described in Svab and Maliga, *Proc. Natl. Acad. Sci.*, USA 90:913-917, 1993), and the *Aequorea victoria* GFP (for example, as described in Sidorov et al., *Plant J.* 19:209-216, 1999) have been used as reporter genes (for example, as described in Heifetz, *Biochemie* 82:655-666, 2000). Each of these genes has attributes that make them useful reporters of chloroplast gene expression, such as ease of analysis, sensitivity, or the ability to examine expression in situ. Based upon these studies, other exogenous proteins have been expressed in the chloroplasts of higher plants such as *Bacillus thuringiensis* Cry toxins, conferring resistance to insect herbivores (for example, as described in Kota et al., *Proc. Natl. Acad. Sci., USA* 96:1840-1845, 1999), or human somatotropin (for example, as described in Staub et al., *Nat. Biotechnol.* 18:333-338, 2000), a potential biopharmaceutical. Several reporter genes have been expressed in the chloroplast of the eukaryotic green alga, *C. reinhardtii*, including aadA (for example, as described in Goldschmidt-Clermont, *Nucl. Acids Res.* 19:4083-4089 1991; and Zerges and Rochaix, *Mol. Cell Biol.* 14:5268-5277, 1994), uidA (for example, as described in Sakamoto et al., *Proc. Natl. Acad. Sci.*, USA 90:477-501, 1993; and Ishikura et al., *J. Biosci. Bioeng.* 87:307-314 1999), Renilla luciferase (for example, as described in Minko et al., *Mol. Gen. Genet.* 262:421-425, 1999) and the amino glycoside phosphotransferase from *Acinetobacter baumanii*, aphA6 (for example, as described in Bateman and Purton, *Mol. Gen. Genet* 263:404-410, 2000). In one embodiment the protein described herein is modified by the addition of an N-terminal strep tag epitope to add in the detection of protein expression. In one embodiment the ACCases described herein are modified by the addition of an N-terminal strep tag epitope to add in detection of ACCase expression.

[00403] In some instances, the vectors of the present disclosure will contain elements such as an *E. coli* or *S. cerevisiae* origin of replication. Such features, combined with appropriate selectable markers, allows for the vector to be "shuttled" between the target host cell and a bacterial and/or yeast cell. The ability to passage a shuttle vector of the disclosure in a secondary host may allow for more convenient manipulation of the features of the vector. For example, a reaction mixture containing the vector and inserted polynucleotide(s) of interest can be transformed into prokaryote host cells such as *E. coli*, amplified and collected using routine methods, and examined to identify vectors containing an insert or construct of interest. If desired, the vector can be further manipulated, for example, by performing site directed mutagenesis of the inserted polynucleotide, then again amplifying and selecting vectors having a mutated polynucleotide of interest. A shuttle vector then can be introduced into plant cell chloroplasts, wherein a polypeptide of interest can be expressed and, if desired, isolated according to a method of the disclosure.

[00404] Knowledge of the chloroplast or nuclear genome of the host organism, for example, *C. reinhardtii*, is useful in the construction of vectors for use in the disclosed embodiments. Chloroplast vectors and methods for selecting regions of a chloroplast genome for use as a vector are well known (see, for example, Bock, J. Mol. Biol. 312:425-438, 2001; Staub and Maliga, *Plant Cell* 4:39-45, 1992; and Kavanagh et al., *Genetics* 152:1111-1122, 1999, each of which is

incorporated herein by reference). The entire chloroplast genome of *C. reinhardtii* is available to the public on the world wide web, at the URL "biology.duke.edu/chlamy_genome/- chloro.html" (see "view complete genome as text file" link and "maps of the chloroplast genome" link; J. Maul, J. W. Lilly, and D. B. Stern, unpublished results; revised Jan. 28, 2002; to be published as GenBank Acc. No. AF396929; and Maul, J. E., et al. (2002) The Plant Cell, Vol. 14 (2659-2679)). Generally, the nucleotide sequence of the chloroplast genomic DNA that is selected for use is not a portion of a gene, including a regulatory sequence or coding sequence. For example, the selected sequence is not a gene that if disrupted, due to the homologous recombination event, would produce a deleterious effect with respect to the chloroplast. For example, a deleterious effect on the replication of the chloroplast genome or to a plant cell containing the chloroplast. In this respect, the website containing the *C. reinhardtii* chloroplast genome sequence also provides maps showing coding and non-coding regions of the chloroplast genome, thus facilitating selection of a sequence useful for constructing a vector (also described in Maul, J. E., et al. (2002) The Plant Cell, Vol. 14 (2659-2679)). For example, the chloroplast vector, p322, is a clone extending from the Eco (Eco RI) site at about position 143.1 kb to the Xho (Xho I) site at about position 148.5 kb (see, world wide web, at the URL "biology.duke.edu/chlamy_genome/chloro.html", and clicking on "maps of the chloroplast genome" link, and "140-150 kb" link; also accessible directly on world wide web at URL "biology.duke.edu/chlam- y/chloro/chlorol40.html").

[00405] In addition, the entire nuclear genome of *C. reinhardtii* is described in Merchant, S. S., et al., Science (2007), 318(5848):245-250, thus facilitating one of skill in the art to select a sequence or sequences useful for constructing a vector.

[00406] For expression of the polypeptide in a host, an expression cassette or vector may be employed. The expression vector will provide a transcriptional and translational initiation region, which may be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. These control regions may be native to the gene, or may be derived from an exogenous source. Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding exogenous or endogenous proteins. A selectable marker operative in the expression host may be present.

[00407] The nucleotide sequences may be inserted into a vector by a variety of methods. In the most common method the sequences are inserted into an appropriate restriction endonuclease site(s) using procedures commonly known to those skilled in the art and detailed in, for example, Sambrook et al., *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press, (1989) and Ausubel et al., *Short Protocols in Molecular Biology*, 2nd Ed., John Wiley & Sons (1992).

[00408] The description herein provides that host cells may be transformed with vectors. One of skill in the art will recognize that such transformation includes transformation with circular or linearized vectors, or linearized portions of a vector. Thus, a host cell comprising a vector may contain the entire vector in the cell (in either circular or linear form), or may contain a linearized portion of a vector of the present disclosure. In some instances 0.5 to 1.5 kb flanking nucleotide sequences of chloroplast genomic DNA may be used. In some instances 0.5 to 1.5 kb flanking nucleotide sequences of nuclear genomic DNA may be used, or 2.0 to 5.0 kb may be used.

[00409] **Codon Optimization**

[00410] As discussed above, one or more codons of an encoding polynucleotide can be "biased" or "optimized" to reflect the codon usage of the host organism. For example, one or more codons of an encoding polynucleotide can be "biased" or "optimized" to reflect chloroplast codon usage (**Table A**) or nuclear codon usage (**Table B**). Most amino acids are encoded by two or more different (degenerate) codons, and it is well recognized that various organisms utilize certain codons in preference to others. "Biased" or codon "optimized" can be used interchangeably throughout the specification. Codon bias can be variously skewed in different plants, including, for example, in alga as compared to tobacco. Generally, the codon bias selected reflects codon usage of the plant (or organelle therein) which is being transformed with the nucleic acids of the present disclosure.

[00411] A polynucleotide that is biased for a particular codon usage can be synthesized de novo, or can be genetically modified using routine recombinant DNA techniques, for example, by a site directed mutagenesis method, to change one or more codons such that they are biased for chloroplast codon usage.

[00412] Such preferential codon usage, which is utilized in chloroplasts, is referred to herein as "chloroplast codon usage." **Table A** (below) shows the chloroplast codon usage for *C. reinhardtii* (see U.S. Patent Application Publication No.: 2004/0014174, published January 22, 2004).

[00413] **Table A**

Chloroplast Codon Usage in Chlamydomonas reinhardtii			
UUU 34.1*(348**)	UCU 19.4(198)	UAU 23.7(242)	UGU 8.5(87)
UUC 14.2(145)	UCC 4.9(50)	UAC 10.4(106)	UGC 2.6(27)
UUA 72.8(742)	UCA 20.4(208)	UAA 2.7(28)	UGA 0.1(1)
UUG 5.6(57)	UCG 5.2(53)	UAG 0.7(7)	UGG 13.7(140)
CUU 14.8(151)	CCU 14.9(152)	CAU 11.1(113)	CGU 25.5(260)
CUC 1.0(10)	CCC 5.4(55)	CAC 8.4(86)	CGC 5.1(52)
CUA 6.8(69)	CCA 19.3(197)	CAA 34.8(355)	CGA 3.8(39)
CUG 7.2(73)	CCG 3.0(31)	CAG 5.4(55)	CGG 0.5(5)
AUU 44.6(455)	ACU 23.3(237)	AAU 44.0(449)	AGU 16.9(172)
AUC 9.7(99)	ACC 7.8(80)	AAC 19.7(201)	AGC 6.7(68)
AUA 8.2(84)	ACA 29.3(299)	AAA 61.5(627)	AGA 5.0(51)
AUG 23.3(238)	ACG 4.2(43)	AAG 11.0(112)	AGG 1.5(15)
GUU 27.5(280)	GCU 30.6(312)	GAU 23.8(243)	GGU 40.0(408)
GUC 4.6(47)	GCC 11.1(113)	GAC 11.6(118)	GGC 8.7(89)
GUA 26.4(269)	GCA 19.9(203)	GAA 40.3(411)	GGA 9.6(98)
GUG 7.1(72)	GCG 4.3(44)	GAG 6.9(70)	GGG 4.3(44)

[00414] * -Frequency of codon usage per 1,000 codons. ** - Number of times observed in 36 chloroplast coding sequences (10,193 codons).

[00415] The chloroplast codon bias can, but need not, be selected based on a particular organism in which a synthetic polynucleotide is to be expressed. The manipulation can be a change to a codon, for example, by a method such as site directed mutagenesis, by a method such as PCR using a primer that is mismatched for the nucleotide(s) to be changed such that the amplification product is biased to reflect chloroplast codon usage, or can be the de novo synthesis of polynucleotide sequence such that the change (bias) is introduced as a consequence of the synthesis procedure.

[00416] In addition to utilizing chloroplast codon bias as a means to provide efficient translation of a polypeptide, it will be recognized that an alternative means for obtaining efficient translation of a polypeptide in a chloroplast is to re-engineer the chloroplast genome (e.g., a *C. reinhardtii* chloroplast genome) for the expression of tRNAs not otherwise expressed in the chloroplast genome. Such an engineered algae expressing one or more exogenous tRNA

molecules provides the advantage that it would obviate a requirement to modify every polynucleotide of interest that is to be introduced into and expressed from a chloroplast genome; instead, algae such as *C. reinhardtii* that comprise a genetically modified chloroplast genome can be provided and utilized for efficient translation of a polypeptide according to any method of the disclosure. Correlations between tRNA abundance and codon usage in highly expressed genes is well known (for example, as described in Franklin et al., Plant J. 30:733-744, 2002; Dong et al., J. Mol. Biol. 260:649-663, 1996; Duret, Trends Genet. 16:287-289, 2000; Goldman et. al., J. Mol. Biol. 245:467-473, 1995; and Komar et. al., Biol. Chem. 379:1295-1300, 1998). In *E. coli*, for example, re-engineering of strains to express underutilized tRNAs resulted in enhanced expression of genes which utilize these codons (see Novy et al., in Novations 12:1-3, 2001). Utilizing endogenous tRNA genes, site directed mutagenesis can be used to make a synthetic tRNA gene, which can be introduced into chloroplasts to complement rare or unused tRNA genes in a chloroplast genome, such as a *C. reinhardtii* chloroplast genome.

[00417] Generally, the chloroplast codon bias selected for purposes of the present disclosure, including, for example, in preparing a synthetic polynucleotide as disclosed herein reflects chloroplast codon usage of a plant chloroplast, and includes a codon bias that, with respect to the third position of a codon, is skewed towards A/T, for example, where the third position has greater than about 66% AT bias, or greater than about 70% AT bias. In one embodiment, the chloroplast codon usage is biased to reflect alga chloroplast codon usage, for example, *C. reinhardtii*, which has about 74.6% AT bias in the third codon position. Preferred codon usage in the chloroplasts of algae has been described in US 2004/0014174.

[00418] **Table B** exemplifies codons that are preferentially used in algal nuclear genes. The nuclear codon bias can, but need not, be selected based on a particular organism in which a synthetic polynucleotide is to be expressed. The manipulation can be a change to a codon, for example, by a method such as site directed mutagenesis, by a method such as PCR using a primer that is mismatched for the nucleotide(s) to be changed such that the amplification product is biased to reflect nuclear codon usage, or can be the de novo synthesis of polynucleotide sequence such that the change (bias) is introduced as a consequence of the synthesis procedure.

[00419] In addition to utilizing nuclear codon bias as a means to provide efficient translation of a polypeptide, it will be recognized that an alternative means for obtaining efficient translation of a polypeptide in a nucleus is to re-engineer the nuclear genome (e.g., a *C. reinhardtii* nuclear genome) for the expression of tRNAs not otherwise expressed in the nuclear genome. Such an engineered algae expressing one or more exogenous tRNA molecules provides the advantage that it would obviate a requirement to modify every polynucleotide of interest that is to be introduced into and expressed from a nuclear genome; instead, algae such as *C. reinhardtii* that comprise a genetically modified nuclear genome can be provided and utilized for efficient translation of a polypeptide according to any method of the disclosure. Correlations between tRNA abundance and codon usage in highly expressed genes is well known (for example, as described in Franklin et al., Plant J. 30:733-744, 2002; Dong et al., J. Mol. Biol. 260:649-663, 1996; Duret, Trends Genet. 16:287-289, 2000; Goldman et. Al., J. Mol. Biol. 245:467-473, 1995; and Komar et. Al., Biol. Chem. 379:1295-1300, 1998). In *E. coli*, for example, re-engineering of strains to express underutilized tRNAs resulted in enhanced expression of genes which utilize these codons (see Novy et al., in Novations 12:1-3, 2001). Utilizing endogenous tRNA genes, site directed mutagenesis can be used to make a synthetic tRNA gene, which can be

introduced into the nucleus to complement rare or unused tRNA genes in a nuclear genome, such as a *C. reinhardtii* nuclear genome.

[00420] Generally, the nuclear codon bias selected for purposes of the present disclosure, including, for example, in preparing a synthetic polynucleotide as disclosed herein, can reflect nuclear codon usage of an algal nucleus and includes a codon bias that results in the coding sequence containing greater than 60% G/C content.

[00421] **Table B**

[00422] fields: [triplet] [frequency: **per thousand**] ([number])

[00423] Coding GC 66.30% 1st letter GC 64.80% 2nd letter GC 47.90% 3rd letter GC 86.21%

Nuclear Codon Usage in Chlamydomonas reinhardtii			
UUU 5.0 (2110)	UCU 4.7 (1992)	UAU 2.6 (1085)	UGU 1.4 (601)
UUC 27.1 (11411)	UCC 16.1 (6782)	UAC 22.8 (9579)	UGC 13.1 (5498)
UUA 0.6 (247)	UCA 3.2 (1348)	UAA 1.0 (441)	UGA 0.5 (227)
UUG 4.0 (1673)	UCG 16.1 (6763)	UAG 0.4 (183)	UGG 13.2 (5559)
CUU 4.4 (1869)	CCU 8.1 (3416)	CAU 2.2 (919)	CGU 4.9 (2071)
CUC 13.0 (5480)	CCC 29.5 (12409)	CAC 17.2 (7252)	CGC 34.9 (14676)
CUA 2.6 (1086)	CCA 5.1 (2124)	CAA 4.2 (1780)	CGA 2.0 (841)
CUG 65.2 (27420)	CCG 20.7 (8684)	CAG 36.3 (15283)	CGG 11.2 (4711)
AUU 8.0 (3360)	ACU 5.2 (2171)	AAU 2.8 (1157)	AGU 2.6 (1089)
AUC 26.6 (11200)	ACC 27.7 (11663)	AAC 28.5 (11977)	AGC 22.8 (9590)
AUA 1.1 (443)	ACA 4.1 (1713)	AAA 2.4 (1028)	AGA 0.7 (287)
0AUG 25.7 (10796)	ACG 15.9 (6684)	AAG 43.3 (18212)	AGG 2.7 (1150)
GUU 5.1 (2158)	GCU 16.7 (7030)	GAU 6.7 (2805)	GGU 9.5 (3984)
GUC 15.4 (6496)	GCC 54.6 (22960)	GAC 41.7 (17519)	GGC 62.0 (26064)
GUA 2.0 (857)	GCA 10.6 (4467)	GAA 2.8 (1172)	GGA 5.0 (2084)
GUG 46.5 (19558)	GCG 44.4 (18688)	GAG 53.5 (22486)	GGG 9.7 (4087)

[00424] **Table C** lists the codon selected at each position for backtranslating the protein to a DNA sequence for synthesis. The selected codon is the sequence recognized by the tRNA encoded in the chloroplast genome when present; the stop codon (TAA) is the codon most frequently present in the chloroplast encoded genes. If an undesired restriction site is created, the next best choice according to the regular Chlamydomonas chloroplast usage table that eliminates the restriction site is selected.

[0001] **Table C**

Amino acid	Codon utilized
F	TTC
L	TTA
I	ATC
V	GTA
S	TCA
P	CCA
T	ACA
A	GCA
Y	TAC
H	CAC
Q	CAA
N	AAC
K	AAA
D	GAC
E	GAA

C	TGC
R	CGT
G	GGC
W	TGG
M	ATG
STOP	TAA

[00425] Percent Sequence Identity

[00426] One example of an algorithm that is suitable for determining percent sequence identity or sequence similarity between nucleic acid or polypeptide sequences is the BLAST algorithm, which is described, e.g., in Altschul *et al., J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (as described, for example, in Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA*, 89:10915). In addition to calculating percent sequence identity, the BLAST algorithm also can perform a statistical analysis of the similarity between two sequences (for example, as described in Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA*, 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, less than about 0.01, or less than about 0.001.

[00427] Fatty Acids and Glycerol Lipids

[00428] The present disclosure describes host cells capable of making polypeptides that contribute to the accumulation and/or secretion of fatty acids, glycerol lipids, or oils, by transforming host cells (e.g., alga cells such as *C. reinhardtii, D. salina, H. pluvalis*, and cyanobacterial cells) with nucleic acids encoding one or more different enzymes. Examples of such enzymes include acetyl-CoA carboxylase, ketoreductase, thioesterase, malonyltransferase, dehydratase, acyl-CoA ligase, ketoacylsynthase, enoylreductase, and desaturase. The enzymes can be, for example, catabolic or biodegrading enzymes.

[00429] In some instances, the host cell will naturally produce the fatty acid, glycerol lipid, triglyceride, or oil of interest. Therefore, transformation of the host cell with a polynucleotide encoding an enzyme, for example an ACCase, will allow for the increased activity of the enzyme and/or increased accumulation and/or secretion of a molecule of interest (e.g., a lipid) in the cell.

[00430] A change in the accumulation and/or secretion of a desired product, for example, fatty acids, glycerol lipids, or oils, by a transformed host cell can include, for example, a change in the total lipid content over that normally present in the cell, or a change in the type of lipids that are normally present in the cell.

[00431] Increased malonyl CoA production is required for increased fatty acid biosynthesis. Increased fatty acid biosynthesis is required for increased accumulation of fatty acid based lipids. An increase in fatty acid based lipids can be measured by methyl tert-butyl ether (MTBE) extraction.

[00432] Some host cells may be transformed with multiple genes encoding one or more enzymes. For example, a single transformed cell may contain exogenous nucleic acids encoding enzymes that make up an entire glycerolipid synthesis pathway. One example of a pathway might include genes encoding an acetyl CoA carboxylase, a malonyltransferase, a ketoacylsynthase, and a thioesterase. Cells transformed with an entire pathway and/or enzymes extracted from those cells, can synthesize, for example, complete fatty acids or intermediates of the fatty acid synthesis pathway. Constructs may contain, for example, multiple copies of the same gene, multiple genes encoding the same enzyme from different organisms, and/or multiple genes with one or more mutations in the coding sequence(s).

[00433] The enzyme(s) produced by the modified cells may result in the production of fatty acids, glycerol lipids, triglycerides, or oils that may be collected from the cells and/or the surrounding environment (e.g., bioreactor or growth medium). In some embodiments, the collection of the fatty acids, glycerol lipids, triglycerides, or oils is performed after the product is secreted from the cell via a cell membrane transporter.

[00434] Examples of candidate *Chlamydomonas* genes encoding enzymes of glycerolipid metabolism that can be used in the described embodiments are described in The Chlamydomonas Sourcebook Second Edition, Organellar and Metabolic Processes, Vol. 2, pp. 41-68, David B. Stern (Ed.), (2009), Elsevier Academic Press.

[00435] For example, enzymes involved in plastid, mitochondrial, and cytosolic pathways, along with plastidic and cytosolic isoforms of fatty acid desaturases, and triglyceride synthesis enzymes are described (and their accession numbers provided). An exemplary chart of some of the genes described is provided below:

Acyl-ACP thioesterase	FAT1	EDP08596
Long-chain acyl-CoA synthetase	LCS1	EDO96800
CDP-DAG: Inositol phosphotransferase	PIS1	EDP06395
Acyl-CoA: Diacylglycerol acyltransferase	DGA1	EDO96893
Phospholipid: Diacylglycerol acyltransferase	LRO1(LCA1)	EDP07444

[00436] Examples of the types of fatty acids and/or glycerol lipids that a host cell or organism can produce, are described below.

[00437] Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.

[00438] Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups. Lipids may be divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits). For this disclosure, saccharolipids will not be discussed.

[00439] Fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol. Humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids.

[00440] Fatty Acyls

[00441] Fatty acyls, a generic term for describing fatty acids, their conjugates and derivatives, are a diverse group of molecules synthesized by chain-elongation of an acetyl-CoA primer with malonyl-CoA or methylmalonyl-CoA groups in a process called fatty acid synthesis. A fatty acid is any of the aliphatic monocarboxylic acids that can be liberated by hydrolysis from naturally occurring fats and oils. They are made of a hydrocarbon chain that terminates with a carboxylic acid group; this arrangement confers the molecule with a polar, hydrophilic end, and a nonpolar, hydrophobic end that is insoluble in water. The fatty acid structure is one of the most fundamental categories of biological lipids, and is commonly used as a building block of more structurally complex lipids. The carbon chain, typically between four to 24 carbons long, may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen and sulfur; branched fatty acids and hydroxyl fatty acids also occur, and very long chain acids of over 30 carbons are found in waxes. Where a double bond exists, there is the possibility of either a cis or trans geometric isomerism, which significantly affects the molecule's molecular configuration. Cis-double bonds cause the fatty acid chain to bend, an effect that is more pronounced the more double bonds there are in a chain. This in turn plays an important role in the structure and function of cell membranes. Most naturally occurring fatty acids are of the cis configuration, although the trans form does exist in some natural and partially hydrogenated fats and oils.

[00442] Examples of biologically important fatty acids are the eicosanoids, derived primarily from arachidonic acid and eicosapentaenoic acid, which include prostaglandins, leukotrienes, and thromboxanes. Other major lipid classes in the fatty acid category are the fatty esters and fatty amides. Fatty esters include important biochemical intermediates such as wax esters, fatty acid thioester coenzyme A derivatives, fatty acid thioester ACP derivatives and fatty acid carnitines. The fatty amides include N-acyl ethanolamines.

[00443] Glycerolipids

[00444] Glycerolipids are composed mainly of mono-, di- and tri-substituted glycerols, the most well-known being the fatty acid esters of glycerol (triacylglycerols), also known as triglycerides. In these compounds, the three hydroxyl groups of glycerol are each esterified, usually by different fatty acids. Because they function as a food store, these lipids comprise the bulk of storage fat in animal tissues. The hydrolysis of the ester bonds of triacylglycerols and the release of glycerol and fatty acids from adipose tissue is called fat mobilization.

[00445] Additional subclasses of glycerolipids are represented by glycosylglycerols, which are characterized by the presence of one or more sugar residues attached to glycerol via a glycosidic linkage. An example of a structure in this category is the digalactosyldiacylglycerols found in plant membranes.

[00446] Exemplary *Chlamydomonas* glycerolipids include: DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl-N, N, N-trimethylhomoserine; MGDG, monogalactosyldiacylglycerol; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PtdIns, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; and TAG, triacylglycerol.

[00447] Glycerophospholipids

[00448] Glycerophospholipids are any derivative of glycerophosphoric acid that contains at least one *O*-acyl, *O*-alkyl, or *O*-alkenyl group attached to the glycerol residue. The common glycerophospholipids are named as derivatives of phosphatidic acid (phosphatidyl choline, phosphatidyl serine, and phosphatidyl ethanolamine).

[00449] Glycerophospholipids, also referred to as phospholipids, are ubiquitous in nature and are key components of the lipid bilayer of cells, as well as being involved in metabolism and cell signaling. Glycerophospholipids may be

subdivided into distinct classes, based on the nature of the polar headgroup at the *sn-3* position of the glycerol backbone in eukaryotes and eubacteria, or the *sn-1* position in the case of archaebacteria.

[00450] Examples of glycerophospholipids found in biological membranes are phosphatidylcholine (also known as PC, GPCho or lecithin), phosphatidylethanolamine (PE or GPEtn) and phosphatidylserine (PS or GPSer). In addition to serving as a primary component of cellular membranes and binding sites for intra- and intercellular proteins, some glycerophospholipids in eukaryotic cells, such as phosphatidylinositols and phosphatidic acids are either precursors of, or are themselves, membrane-derived second messengers. Typically, one or both of these hydroxyl groups are acylated with long-chain fatty acids, but there are also alkyl-linked and 1Z-alkenyl-linked (plasmalogen) glycerophospholipids, as well as dialkylether variants in archaebacteria.

[00451] Sphingolipids

[00452] Sphingolipids are any of class of lipids containing the long-chain amino diol, sphingosine, or a closely related base (i.e. a sphingoid). A fatty acid is bound in an amide linkage to the amino group and the terminal hydroxyl may be linked to a number of residues such as a phosphate ester or a carbohydrate. The predominant base in animals is sphingosine while in plants it is phytosphingosine.

[00453] The main classes are: (1) phosphosphigolipids (also known as sphingophospholipids), of which the main representative is sphingomyelin; and (2) glycosphingolipids, which contain at least one monosaccharide and a sphingoid, and include the cerebrosides and gangliosides. Sphingolipids play an important structural role in cell membranes and may be involved in the regulation of protein kinase C.

[00454] As mentioned above, sphingolipids are a complex family of compounds that share a common structural feature, a sphingoid base backbone, and are synthesized de novo from the amino acid serine and a long-chain fatty acyl CoA, that are then converted into ceramides, phosphosphingolipids, glycosphingolipids and other compounds. The major sphingoid base of mammals is commonly referred to as sphingosine. Ceramides (N-acyl-sphingoid bases) are a major subclass of sphingoid base derivatives with an amide-linked fatty acid. The fatty acids are typically saturated or mono-unsaturated with chain lengths from 16 to 26 carbon atoms.

[00455] The major phosphosphingolipids of mammals are sphingomyelins (ceramide phosphocholines), whereas insects contain mainly ceramide phosphoethanolamines, and fungi have phytoceramide phosphoinositols and mannose-containing head groups. The glycosphingolipids are a diverse family of molecules composed of one or more sugar residues linked via a glycosidic bond to the sphingoid base. Examples of these are the simple and complex glycosphingolipids such as cerebrosides and gangliosides.

[00456] Sterol Lipids

[00457] Sterol lipids, such as cholesterol and its derivatives, are an important component of membrane lipids, along with the glycerophospholipids and sphingomyelins. The steroids, all derived from the same fused four-ring core structure, have different biological roles as hormones and signaling molecules. The eighteen-carbon (C18) steroids include the estrogen family whereas the C19 steroids comprise the androgens such as testosterone and androsterone. The C21 subclass includes the progestogens as well as the glucocorticoids and mineralocorticoids. The secosteroids, comprising various forms of vitamin D, are characterized by cleavage of the B ring of the core structure. Other examples of sterols are the bile acids and their conjugates, which in mammals are oxidized derivatives of cholesterol and are synthesized in the liver. The plant equivalents are the phytosterols, such as β-sitosterol, stigmasterol, and

brassicasterol; the latter compound is also used as a biomarker for algal growth. The predominant sterol in fungal cell membranes is ergosterol.

[00458] Prenol Lipids

[00459] Prenol lipids are synthesized from the 5-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate that are produced mainly via the mevalonic acid (MVA) pathway. The simple isoprenoids (for example, linear alcohols and diphosphates) are formed by the successive addition of C5 units, and are classified according to the number of these terpene units. Structures containing greater than 40 carbons are known as polyterpenes. Carotenoids are important simple isoprenoids that function as antioxidants and as precursors of vitamin A. Another biologically important class of molecules is exemplified by the quinones and hydroquinones, which contain an isoprenoid tail attached to a quinonoid core of non-isoprenoid origin. Prokaryotes synthesize polyprenols (called bactoprenols) in which the terminal isoprenoid unit attached to oxygen remains unsaturated, whereas in animal polyprenols (dolichols) the terminal isoprenoid is reduced.

[00460] Polyketides

[00461] Polyketides or sometimes acetogenin are any of a diverse group of natural products synthesized via linear poly-β-ketones, which are themselves formed by repetitive head-to-tail addition of acetyl (or substituted acetyl) units indirectly derived from acetate (or a substituted acetate) by a mechanism similar to that for fatty-acid biosynthesis but without the intermediate reductive steps. In many case, acetyl-CoA functions as the starter unit and malonyl-CoA as the extending unit. Various molecules other than acetyl-CoA may be used as starter, often with methoylmalonyl-CoA as the extending unit. The poly-β-ketones so formed may undergo a variety of further types of reactions, which include alkylation, cyclization, glycosylation, oxidation, and reduction. The classes of product formed – and their corresponding starter substances – comprise *inter alia*: coniine (of hemlock) and orsellinate (of lichens) – acetyl-CoA; flavanoids and stilbenes – cinnamoyl-CoA; tetracyclines – amide of malonyl-CoA; urushiols (of poison ivy) – palmitoleoyl-CoA; and erythonolides – propionyl-CoA and methyl-malonyl-CoA as extender.

[00462] Polyketides comprise a large number of secondary metabolites and natural products from animal, plant, bacterial, fungal and marine sources, and have great structural diversity. Many polyketides are cyclic molecules whose backbones are often further modified by glycosylation, methylation, hydroxylation, oxidation, and/or other processes. Many commonly used anti-microbial, anti-parasitic, and anti-cancer agents are polyketides or polyketide derivatives, such as erythromycins, tetracyclines, avermectins, and antitumor epothilones

EXAMPLES

[00463] The following examples are intended to provide illustrations of the application of the present disclosure. The following examples are not intended to completely define or otherwise limit the scope of the disclosure.

[00464] One of skill in the art will appreciate that many other methods known in the art may be substituted in lieu of the ones specifically described or referenced herein.

[00465] Example 1. Analyses of the *Chlamydomonas reinhardtii* plastid β-ACCase gene.

[00466] The Chlamydomonas plastid β-ACCase gene was examined (SEQ ID NO: 1; genbank EDO96563). All amino acid position numbers refer to SEQ ID NO: 1 unless otherwise noted.

[00467] Annotation of this gene describes a chloroplast transit peptide as expected; this gene is present in the nuclear genome but active in the chloroplast. The mature gene sequence was submitted to the Swiss-Model server to produce a homology model based on the crystal structure of the β-subunit of the Staphylococcus aureus ACCase (PDB structure 2F9I). From examination of this structure, it was apparent that residue 255 (cysteine 255) would be across the heterotetramer axis from the other β-subunit, and conceivably could form a disulfide bond under oxidizing conditions. Another cysteine residue would be predicted to be buried within the protein, and probably not under redox control. Finally, four cysteine residues were predicted to form a zinc-binding cluster at the n-terminus of the protein. While this could form a locus for redox control, no modification was conceived of that could produce a "constitutively reduced" state for this site. Therefore, the mutation Cys255Ser was hypothesized as a potential constitutively activating mutation.

[00468] For prediction of potential phosphorylation sites, a number of methods have been used. The simplest method is by utilization of an artificial neural network trained on known phosphorylation sites in eukaryotic proteins to predict potential sites in a new eukaryotic protein. One publicly available tool is NetPhos 2.0 (as described, for example, in Blom, N., *et al.* (1999) J. Mol. Biol. 294:1351-1362; and http://www.cbs.dtu.dk/services/NetPhos/). Analysis of the Chlamydomonas ACCase sequence with this server predicted 19 potential phosphorylation sites. **Table 1** lists the 19 sites.

[00469] **Table 1**

T6D	S36D	S38D	S50D	S62D
S64D	S78D	S121D	S122D	T134D
T141D	S143D	S151D	S155D	C255S
T269D	T302D	Y337D	T365D	

[00470] After examining these residues on the homology model, six residues appeared to be present on the surface of the protein, present in loop structures, and therefore, both accessible to an activating kinase and capable of altering local structure or interactions with other subunits of the complex. These residues were Threonine 134, Threonine 141, Serine 143, Serine 151, Serine 155, and Tyrosine 337.

[00471] Example 2. Mutagenesis of the gene encoding the wild-type *Chlamydomonas reinhardtii* ACCase β-subunit.

[00472] To determine if a mutation of one or more of the above residues to aspartic acid (or serine, if the native amino acid is cysteine) would produce an ACCase β-subunit that would make a constitutively active complex with the endogenous alpha and biotin domain proteins, a gene encoding the wild-type Chlamydomonas ACCase β-subunit open reading frame, codon optimized for chloroplast expression and containing an N-terminal Strept tag epitope (ATGGGTTCTGCTTGGTCTCATCCACAATTTGAAAAACAT; SEQ ID NO: 25), was synthesized and cloned into the pSE-3HB-K-tD2 Chlamydomonas plastid expression vector downstream of a D2 promoter (**Figure 4**). The vector was then transformed into both 137c and 1690 background Chlamydomonas.

[00473] In parallel, seven pairs of oligonucleotides (SEQ ID NOs: 40 to 53) encoding the proposed activating mutations were designed (C255S, T134D, T141D, S143D, S151D, S155D, and Y337D) and used to mutagenize the wild-type gene to produce the desired point mutants.

[00474] **Table 2** shows the seven pairs of oligonucleotides used to create the seven mutants; "F" is the forward primer and "R" is the reverse primer. The nucleotides that encode for the mutated amino acids are underlined and bolded.

[00475] **Table 2**

T134D-F	TTAATTGATGCTGGT**GAT**TGGCGTCCACTTGAT (SEQ ID NO: 40)
T134D-R	ATCAAGTGGACGCCA**ATC**ACCAGCATCAATTAA (SEQ ID NO: 41)
T141D-F	CGTCCACTTGATGAA**GAT**CTTTCTCCAGTAGAT (SEQ ID NO: 42)
T141D-R	ATCTACTGGAGAAAG**ATC**TTCATCAAGTGGACG (SEQ ID NO: 43)
T143D-F	CTTGATGAAACTCTT**GAT**CCAGTAGATCCTTTA (SEQ ID NO: 44)
T143D-R	TAAAGGATCTACTGG**ATC**AAGAGTTTCATCAAG (SEQ ID NO: 45)
S151D-F	GATCCTTTAGAATTT**GAT**GACTTAAAATCTTAT (SEQ ID NO: 46)
S151D-R	ATAAGATTTTAAGTC**ATC**AAATTCTAAAGGATC (SEQ ID NO: 47)
S155D-F	TTTTCTGACTTAAAA**GAT**TATACTGATCGTATT (SEQ ID NO: 48)
S155D-R	AATACGATCAGTATA**ATC**TTTTAAGTCAGAAAA (SEQ ID NO: 49)
C255S-F	CATGTACATCAAAAC**TCA**GCTAATCTTTTATAC (SEQ ID NO: 50)
C255S-R	GTATAAAAGATTAGC**TGA**GTTTTGATGTACATG (SEQ ID NO: 51)
Y337D-F	CTTAAAGGTGCATTA**GAT**GAAATCATTGACTTT (SEQ ID NO: 52)
Y337D-R	AAAGTCAATGATTTC**ATC**TAATGCACCTTTAAG (SEQ ID NO: 53)

[00476] **Table 3** shows the PCR reaction parameters that were used to create the point mutations.

[00477] **Table 3**

50 µl QuikChange PCR Master Mix			Cycling Parameters		
		µl			
1	Buffer, 10X	5	1	95 C	2 min
2	$MgSO_4$, 25 mM	3	2	95 C	20 sec
3	dNTPs 10X	5	3	55 C	15 sec
4	Oligo-f (10 µM)	1.5	4	70 C	2.5 min
5	Oligo-r (10 µM)	1.5	5	Go to step 2, 24 cycles	
6	Polymerase (KOD, 1.0 U/µl)	1	6	70 C	5 min
7	DNA	1	7	4 C	Forever
8	H_2O	32			
	Total volume	50			

[00478] After the PCR reactions were run, 1 µl of DpnI was added to each of the PCR tubes to digest the template DNA. The DpnI reaction was incubated for 1 hour at 37 °C.

[00479] 50 µl of Top10 competent cells (Invitrogen, U.S.A.) were transformed with 3 µl of DpnI treated reaction mixture and plated onto LB Amp (100 µg/ml) plates. Individual colonies were picked and grown up overnight in LB Amp (100 µg/ml) media. After overnight growth, plasmid DNA was prepared.

[00480] Plasmid DNA was sequence verified and DNA containing each of the seven mutations were selected for subcloning into the plastid transformation vector (**Figure 4**).

[00481] The wild-type gene and each of the seven plasmids containing the desired mutation were digested with both NdeI and XbaI. Each of the NdeI – XbaI inserts, each of which include at the 5' end epitope tag (SEQ ID NO: 25), were subcloned into *Chlamydomonas reinhardtii* chloroplast transformation vector pSE-3HB-K-tD2 (**Figure 4**).

[00482] Individual plasmids containing either the wild-type gene or the desired mutation were transformed into *Chlamydomonas reinhardtii* (1690 and 137C) using a microprojectile mediated (biolistic) particle gun (Biorad).

[00483] Chlamydomonas expression vector pSE-3HB-K-tD2 (**Figure 4**) contains a Kanamycin resistance gene driven by the *Chlamydomonas* atpA promoter, and the gene of interest ("FA85") is flanked by two homologous regions to drive integration into the *Chlamydomonas* chloroplast genome 3HB site. The wild type or a mutated ACCase β-subunit is driven by the psbD promoter (a truncated Chlamydomonas D2 promoter-accurate). FA85 is the gene encoding wild-type C. reinhardtii ACCase β-subunit.

[00484] Example 3. Creation of multiple mutations in the gene encoding the *Chlamydomonas reinhardtii* ACCase β-subunit.

[00485] In addition to the seven single mutations that were made in the ACCase gene, several combinations of the seven single mutations were also made in the ACCase gene. Specifically, S151D + S155D; S151D + S155D + Y337D; and S151D + S155D + C255S.

[00486] The forward and reverse primers that were used to create the S151D + S155D double mutant are listed below. The nucleotides that encode for the mutated amino acids are underlined and bolded.

[00487] S151D/S155D-forward ATCCTTTAGAATTT**GAT**GACTTAAAA**GAT**TATACTGATCGTATT (SEQ ID NO: 54)

[00488] S151D/S155D-reverse AATACGATCAGTAT**AAT**CTTTTAAGT**CAT**CAAATTCTAAAGGATC (SEQ ID NO: 55)

[00489] The triple mutant S151D + S155D + Y337D was made by using the PCR product of the double mutant (S151D + S155D) as template DNA and using the forward and reverse primers listed above (SEQ ID NOs: 52 and 53) that were used for the single mutant Y337D.

[00490] The triple mutant S151D + S155D + C255S was made by using the PCR product of the double mutant (S151D + S155D) as template DNA and using the forward and reverse primers listed above (SEQ ID NOs: 50 and 51) that were used for the single mutant C255S.

[00491] **Table 3** above shows the PCR reaction parameters that were used to create the double mutant and the two triple mutants.

[00492] After the PCR reactions were run, 1 µl of DpnI was added to each of the PCR tubes to digest the template DNA. The DpnI reaction was incubated for 1 hour at 37 °C.

[00493] 50 µl of Top10 competent cells (Invitrogen, U.S.A.) were transformed with 3 µl of DpnI treated reaction mixture and plated onto LB Amp (100 µg/ml) plates. Individual colonies were picked and grown up overnight in LB Amp (100 µg/ml) media. After overnight growth, plasmid DNA was prepared.

[00494] Plasmid DNA was sequence verified and DNA containing the double and triple mutants were selected for subcloning into the plastid transformation vector (**Figure 4**).

[00495] The plasmids containing the double or triple mutants were digested with both NdeI and XbaI. Each of the NdeI – XbaI inserts, each of which include at the 5' end an epitope tag (SEQ ID NO: 25), were subcloned into *Chlamydomonas reinhardtii* chloroplast transformation vector pSE-3HB-K-tD2 (**Figure 4**).

[00496] Individual plasmids containing the desired double or triple mutations were transformed into *Chlamydomonas reinhardtii* (1690 and 137C) using a microprojectile mediated (biolistic) particle gun (Biorad).

[00497] Chlamydomonas expression vector pSE-3HB-K-tD2 (**Figure 4**) contains a Kanamycin resistance gene driven by the *Chlamydomonas* atpA promoter, and the gene of interest ("FA85") is flanked by two homologous regions to drive integration into the *Chlamydomonas* chloroplast genome 3HB site. The double or triple mutant ACCase β-subunit is driven by the psbD (a truncated Chlamydomonas D2 promoter).

[00498] Example 4. FA85 plasmicity screen by PCR.

[00499] In order to determine whether all copies of the chloroplast genome were successfully transformed with the target gene a plamicity screen was conducted by PCR. The PCR reaction conditions are provided below in **Table 4**.

[00500] **Table 4**

25 µl multi screen PCR master mix			**# rxns**	**cycling parameters**		
		µl	100			
1	Buffer, 10x	2.5	275	1	95 °C	2 min
2	2.5 mM dNTPs, 10x	0.5	55	2	95 °C	30 sec
3	$MgCl_2$ (12.5 mM)	1	110	3	55 °C	30 sec
4	primer 79 (SEQ ID NO: 123) (10 µM)	1.25	137.5	4	72 °C	30 sec
5	primer 80 (SEQ ID NO: 124) (10 µM)	1.25	137.5	5	go to step 2	39 cycles
6	primer 1995 (SEQ ID NO: 121) (10 µM)	1.25	137.5	6	72 °C	2 min
7	primer 1996 (SEQ ID NO: 122) (10 µM)	1.25	137.5	7	4 °C	Forever
8	polymerase (Taq, 5.0 U/µl)	0.4	44			
	DNA	2	220			
	H_2O	13.6	1496			
	total volume	25				

[00501] The presence of a single PCR band indicates homoplasmicity, and the presence of two PCR bands indicates heteroplasmicity. Primers 1995, 1996, 79, and 80 (SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, and SEQ ID NO:124, respectively), were used in the PCR reaction.

[00502] 1) Reverse primer, 100216-DM-**1995**: TGTTTGTTAAGGCTAGCTGC (SEQ ID NO: 121). 3HB-D2 multi screen primer shows a band of 212 base pairs if no insert is present.

[00503] 2) Forward primer, 100216-DM-**1996**: CGCCACTGTCATCCTTTAAGT (SEQ ID NO: 122). 3HB-D2 multi screen primer shows a band of 212 base pairs if no insert is present.

[00504] 3) Reverse primer, 100216-DM-**79**: CCGAACTGAGGTTGGGTTTA (SEQ ID NO: 123) (tD2-3HB multi-screen primer).

[00505] 4) Forward primer, 100216-DM-**80**: GGGGGAGCGAATAGGATTAG (SEQ ID NO: 124) (tD2-3HB multi-screen primer).

[00506] Primer pair 79 and 80 was used as a control PCR for amplification of the chloroplast genome. The use of primers 79 and 80 in a PCR reaction will result in the amplification of an approximately 513 bp fragment. Use of primers 1995 and 1996 will result in a 212 bp amplicon if the integration cassette, which includes the target gene, is not integrated into the chloroplast genome. If the integration cassette which includes the target gene is integrated into the genome, use of primer pair 1995 and 1996 in theory, should result in a PCR product of about 7 kb. However, an extension time (as described above) of 72 °C for 30 seconds will not allow for a 7 kb fragment to be made, a longer extension time is required.

[00507] A lack of a 212 bp amplicon indicates homoplasmity. **Figure 5** shows that wild-type, single, double, and triple mutants are all homoplasmic for the desired gene. **Table 5** below is a key to **Figure 5.**

[00508] **Table 5**

Column	Gene Description
1	FA85-wild type
2-4	FA85-T134D
5-12	FA85-T141D
13-20	FA85-S143D
14	Blank
21-28	FA85-S151D
29-31	FA85-S155D
32-35	FA85-Y337D
36-37	Untransformed *Chlamydomonas reinhardtii* (1690)
38-39	negative control (water)
40-51	FA85-S151D, S155D
52-58	FA85-S151D, S155D, C255S

59-63	FA85-S151D, S155D, Y337D

[00509] Example 5. Gene specific screen by PCR.

[00510] In order to ensure that the desired gene is integrated into the chloroplast genome a PCR gene screen was conducted. A gene specific primer #4764 (SEQ ID NO: 126) was designed to be specific for the codon-optimized gene of interest and will not bind to the endogenous ACCase β-subunit gene sequence. The gene specific primer was used with an integration vector specific primer #270 (SEQ ID NO: 125). The vector specific primer sequence is not homologous to any portion of the *C. reinhardtii* chloroplast genome. The presence of the wild-type, single, double, and triple mutants were confirmed by the PCR gene screen. **Table 6** below is a key to **Figure 6.**

[00511] **Table 6**

Column	Gene Description
1	FA85-wild type
2-4	FA85-T134D
5-12	FA85-T141D
13-20	FA85-S143D
14	Blank
21-28	FA85-S151D
29-31	FA85-S155D
32-35	FA85-Y337D
36-37	untransformed *C. reinhardtii* (1690)
38-39	positive control (wild-type FA85 plasmid DNA)
40-51	FA85-S151D, S155D
52-58	FA85-S151D, S155D, C255S
59-63	FA85-S151D, S155D, Y337D

[00512] Example 6. BODIPY staining of ACCase mutants by Guava.

[00513] To determine the initial phenotype of the wild-type and single mutant ACCases, two experiments were conducted. In the first experiment, cells of biological replicate strains containing the various versions of the ACCase gene were grown in liquid culture, stained with one of three lipid dyes (BODIPY, Nile red, and Lipitox green), and analyzed for fluorescence using Guava Easycyte cytometer. Between three and ten biological replicate strains were isolated for each ACCase variant. The fold change in the population median fluorescence signal was plotted against that of the FA85 wild type transgenic population median fluorescence signal. Staining with nile red and lipitox green were inconclusive, but staining with BODIPY showed that several of the mutants have increased staining. In particular, cells containing the S155D transgene has significantly higher fluorescence than those containing the wild-type transgene (**Figure 1**). The y-axis of **Figure 1** is relative fluorescence and the x-axis represents the various mutants and the wild type transgene. Error bars at +/-1 standard deviation. S155D is significantly different from wild type ($p \leq 0.05$).

[00514] Example 7. Distribution of engineered ACCase genes in the pre- and post-sort populations.

[00515] The second experiment consisted of growing all of the strains carrying the single-mutant ACCase transgene (except for T141D), along with cells overexpressing the wild-type non-mutated gene (WT), and non-transformed genetic background cells in liquid culture. The cultures were mixed to produce a heterogeneous population of cells containing non-transformed cells of *C. reinhardtii* strain 1690 background, cells overexpressing the wild-type non-mutated gene, and the six single-mutant transgenic versions of ACCase. This population was plated to isolate clonal colonies, and 288 colonies were picked from this pre-sorting population. The mixed population of cells were subjected to sequential staining and fluorescence-gated cell sorting with the various lipid dyes to isolate strongly stained cells; thus the population was selected for those showing the strongest fluorescence from the three lipid staining dyes. This post-sort population was plated out, and 864 colonies were selected and grown. Once colonies of the pre- and post- sort populations were obtained, all were analyzed by PCR amplification of the ACCase transgene cassette to determine whether or not the colony carried the engineered ACCase transgene. For those colonies that did carry a transgene (for example, S151D), the PCR amplicon was sequenced to determine which version of the engineered ACCase gene was carried by that colony. The distribution of engineered ACCase genes in the pre- and post-sort populations are shown in **Figure 2**. The y-axis represents the fraction of the engineered population and the x-axis represents the clones tested (wild type or mutant). The pre-sort population is shown by an empty bar and the post-sort population is shown by a cross-hatched bar.

[00516] Example 8. Change in proportion of ACCase genotypes from pre-sort to post-sort populations.

[00517] If the introduced ACCase transgene had no effect on the response to lipid specific staining, or if all of the various single mutants and wild-type transgene had the same impact on response to staining, it would be expected that the distribution of the various versions of the gene after sorting would resemble the distribution present pre-sorting. Instead, a significant change in the distribution of the genotype is observed. **Figure 3** shows the change in the proportion of the observed genotypes from the pre-sort to post- sort populations. As is clear in the figure, the sorting strongly selected for the presence of the S151D mutant ACCase, at the expense of the other genotypes. The y-axis represents the change in fraction of population from pre- to post-sort and the x-axis represents the clones tested (wild type or single mutant).

[00518] Example 9. Cloning of five novel transcripts of an ACCase□-subunit from *Scenedesmus dimorphus* UTEX 1237.

[00519] 29 Acetyl-CoA carboxylase (ACCase) □-subunit protein sequences from diverse organisms (e.g. plant and algae) were aligned and six conserved amino acid regions (motifs) were identified (**Table 7**). Motif 5 is FAGK(R)RVIEQTL and is written below as Motifs 5 and 6.

[00520] **Table 7**

Motif 1	MGGSMGSVVGEK (SEQ ID NO: 56)
Motif 2	SGGARMQEG (SEQ ID NO: 57)
Motif 3	SLMQMAKI (SEQ ID NO: 58)
Motif 4	PTTGGVTASF (SEQ ID NO: 59)
Motif 5	FAGKRVIEQTL (SEQ ID NO: 60)

Motif 6	FAGRRVIEQTL (SEQ ID NO: 61)

[00521] The organisms that were compared are provided below in **Table 8** along with their GenBank accession numbers.

[00522] **Table 8**

YP 001518184: *Acaryochloris marina*
YP 001687225: *Aneura mirabilis*
YP 001023710: *Angiopteris evecta*
NP 777422: *Anthoceros formosae*
ACS14664: *Camellia oleifera*
YP 001671692: *Carica papaya*
YP 635724: *Chara vulgaris*
XP 001703187: *Chlamydomons reinhartdtii*
NP 045833: *Chlorella vulgaris*
YP 817491: *Coffea arabica*
YP 002370462.1: *Cyanothece sp.* PCC 8801
YP 001312211.1: *Cycas taitungensis*
ABO33321.1: *Dunaliella salina*
ACF33357.1: *Gonystylus bancanus*
YP 209520.1: *Huperzia lucidula*
ACP52212.1: *Larix occidentalis*
YP 001595517.1: *Lemna minor*
YP 001718446.1: *Manihot esculenta*
CA 087376.1: *Microcystis aeruginosa*
YP 358685.1: *Nicotiana sylvestris*
NP 054508.1: *Nicotiana tabacum*
YP 001866275.1 : *Nostoc punctiforme*
NP 904193.1 : *Physcomitrella patens*
ACP51846.1 : *Pinus canariensis*
ACP51156.1 : *Pinus taeda*
NP 053808.1 : *Porphyra purpurea*
YP 536879.1 : *Porphyra yezoensis*
NP 569638.1 : *Psilotum nudum*
ABY85555.1 : *Silene sorensensis*
YP 514861.1 : *Solanum lycopersicum*
YP 635648.1 : *Solanum tuberosum*
YP 636397.1 : *Staurastrum punctulatum*
YP 002586927.1 : *Syntrichia ruralis*
BAG50119.1 : *Takakia lepidozioides*
NP 682433.1 : *Thermosynechococcus elongatus*
YP 722346.1 : *Trichodesmium erythraeum*
YP 636510.1 : *Zygnema circumcarinatum*

[00523] Degenerate primers were then designed from the identified motif regions. The degenerate primers and the motifs that they target are provided below in **Table 9**. The standard MixBase definitions are provided in **Table 10**, also below.

[00524] **Table 9**

SdACC195-dF	ATGGGNGGNWSNATGGGNWSNGTNGTNGG (SEQ ID NO: 62)	Motif #1

SdACC196-dF	GGNWSNATGGGNWSNGTNGTNGGNGARAA (SEQ ID NO: 63)	Motif #1
SdACC226-dF	WSNGGNGGNGCNMGNATGCARGARGG (SEQ ID NO: 64)	Motif #2
SdACC237-dF	WSNYTNATGCARATGGCNAARAT (SEQ ID NO: 65)	Motif #3
ScdCC244-dR	DATYTTNGCCATYTGCATNAR (SEQ ID NO: 66)	Motif #3
SdACC275-dR	AANSWNGCNGTNACNCCNCCNGTNGTNGG (SEQ ID NO: 67)	Motif #4
SdACC302-dR	GTYTGYTCDATNACNCKNYKNCCNGCRAA (SEQ ID NO: 68)	Motif #5

[00525] **Table 10**

R is A or G **K** is G or T **H** is A, C or T **D** is A, G or T
Y is C or T **S** is C or G **B** is C, G or T **N** is A, C, G, or T
M is A or C **W** is A or T **V** is A, C or G

[00526] Total RNA was isolated from *Scenedesmus dimorphus* UTEX 1237. The mRNA was purified from total RNA by using Qiagen mRNA purification kit (QIAGEN, U.S.A.). First strand cDNA was prepared from mRNA with oligo(dT) primer (ATTCTAGAGGCCGAGGCGGCCGACTATGTTTTTTTTTTTTTTTT) (SEQ ID NO: 69), following the manufacture's protocol.

[00527] The putative conservative ACC β-subunit fragment was PCR amplified by using various degenerate primer combinations. The PCR reaction conditions utilized for each of the four fragments are provided in **Table 11** below. Four putative ACC fragments SEQ ID NOs: 70-73) were obtained.

[00528] **Table 11**

Reaction component	Volume (µl)	Cycling Parameters		
10x EX Taq Buffer	5	95°C	1 min	
2.5 mM dNTP mixture	4	95°C	30 sec	35 cycles
Forward (F) Primer 10 µM	2.5	55 °C	30 sec	
Reverse (R) Primer 10 µM	2.5	72 °C	1 min	
cDNA	2			
Ex Taq	0.4			
Distilled Water	33.6			

[00529] Primer combinations were as follows: for dg12 (SEQ ID NO: 70), 196dF (SEQ ID NO: 63)/244dR (SEQ ID NO: 66); for dg15 (SEQ ID NO: 71), 195dF (SEQ ID NO: 62)/244dR (SEQ ID NO: 66); for dg61 (SEQ ID NO:72), 237dF(SEQ ID NO: 65)/275dR (SEQ ID NO: 67); and for dg62 (SEQ ID NO: 73), 196dF(SEQ ID NO: 63)/275dR (SEQ ID NO:67).

[00530] dg12 (SEQ ID NO: 70) – targeting motifs 1 and 3

[00531] ATGGGGTCGGTCGTCGGAGAGAAGCTGACGCGCCTGATTGAGTACGCCACGCAGGAGGGGCTCA CGCTGCTGGTGGTGTGCACCAGCGGAGGCGCGCGCATGCAGGAGGGCATCATGAGCCTAATGCAGATGG CCAAGATTAAG

[00532] dg15 (SEQ ID NO: 71) - targeting motifs 1 and 3

[00533] ATGGGGTCGGTCGTGGGAGAGAAAATTACGCGCCTTTTTGAGTATGCCAGAGAAGAACGATTAC CTGTTGTCATTTTCACGGCATCAGGAGGAGCTCGTATGCAAGAAGGTATCATGAGCTTTATGCAAATGGC CAAAATC

[00534] dg61 (SEQ ID NO: 72) - targeting motifs 3 and 4

[00535] AGGTTAATGCAGATGGCCAAAATTTCTGCTGCTGTAAAGCGACATTCTAATGCTGGACTTTTTTAT CTCACCGTATTGACCGACCCCACAACTGGTGGCGTAACCGCCTGGTTA

[00536] dg62 (SEQ ID NO: 73) - targeting motifs 3 and 4

[00537] TTGACTGATGCAAATGGCGAAGATCAGCGGCGCGCTGCACGTGCACCAGAATGAGGCCAACCTG CTGTACATCTCCATCCTGACCAGCCCTACCACAGGTGGCGTCACCGCCTGGTT

[00538] The Rapid Amplification of cDNA Ends (RACE) method (as described for example in Frohman, M.A., et al.(1988) Proc Natl Acad Sci USA 85: 8998–9002) was used to extend these four putative ACC fragments. A total of five putative *Scenedesmus dimorphus* ACCase β-subunit (SDACC1-5) transcripts were obtained. The open reading frames of the five sequences are listed as SEQ ID NO: 74 (**Figure 18**), SEQ ID NO: 80 (**Figure 19**), SEQ ID NO: 84 (**Figure 20**), SEQ ID NO: 88 (**Figure 21**), and SEQ ID NO: 92 (**Figure 22**). All five gene transcripts are conserved at both the nucleic acid level (at the 5' end) and at the protein level (see **Figures 23 and 24**), but have diverse 3' ends and carboxy terminal regions. All five gene transcripts also comprise a 5'-terminal sequence encoding for a putative chloroplast targeting transit peptide (SEQ ID NO: 76). SDACC 1 and 2 were the two longest full-length transcripts and were used for further overexpresion experiments.

[00539] Example 10. Obtaining the genomic sequence for SDACC2.

[00540] The genomic sequence (SEQ ID NO: 96) was obtained for SDACC2 described above (SEQ ID NO: 80). The upstream region was successfully obtained by using Genome Walker Universal Kit (Clontech, U.S.A.) according to manufacturer's instructions. A total of 6986 bp of DNA sequence were obtained. The last 81 nucleotides (of which the sequence of the cDNA has been determined (SEQ ID NO: 80 and SEQ ID NO: 82) were not resolved because of a lack of sequencing information.

[00541] Example 11. Codon optimization of novel ACCase β-subunit of *Scenedesmus dimorphus*.

[00542] A polynucleotide sequence comprising SDACC1 (SEQ ID NO: 75) was codon optimized (SEQ ID NO: 98) for expression in the chloroplast of *Scenedesmus dimorphus* based on the *Chlamydomonas reinhardtii* tRNA codon usage table. A flag tag (SEQ ID NO: 117), that was also codon optimized, was added to the 5' end of SEQ ID NO: 98 after the initial "ATG". The resulting construct is shown in SEQ ID NO: 97.

[00543] In addition, a polynucleotide sequence comprising SDACC2 (SEQ ID NO: 82) was codon optimized (SEQ ID NO: 99) for expression in the chloroplast of *Scenedesmus dimorphus* based on the *Chlamydomonas reinhardtii* tRNA codon usage table. A flag tag (SEQ ID NO: 117), that was also codon optimized, was added to the 5' end of SEQ ID NO: 99 after the initial "ATG". The resulting construct is shown in SEQ ID NO: 127.

[00544] Since the first 43 amino acids were predicted as a putative chloroplast targeting transit peptide in both SDACC1 (SEQ ID NO: 74) and SDACC2 (SEQ ID NO: 80) by PSORT program prediction (for example, as described in Nakai, K. and Horton, P., *Trends Biochem. Sci*, 24(1)34-35(1999) and Nakai, K. and Kanehisa, M., *Genomics*, 14, 897-911(1992)), these regions were eliminated except that a start codon sequence (ATG) was retained for proper protein translation initiation.

[00545] Example 12. Mutation of novel ACCase β-subunits of *Scenedesmus dimorphus.*

[00546] The protein sequences of SDACC1 (SEQ ID NO: 78) and SDACC2 (SEQ ID NO: 81) were submitted to the Swiss-Model server to produce a homology model based on the crystal structure of the β-subunit of the *Staphylococcus aureus* ACCase (PDB structure 2F9I).

[00547] For prediction of potential phosphorylation sites, a number of methods can been used. One method is by utilization of an artificial neural network trained on known phosphorylation sites in eukaryotic proteins to predict potential sites in a new eukaryotic protein. One publicly available tool is NetPhos 2.0 server (as described, for example, in Blom, N., *et al.* (1999) J. Mol. Biol. 294:1351-1362; and http://www.cbs.dtu.dk/services/NetPhos/). By comparison to the identified *Chlamydomonas reinhardtii* ACCase β-subunit, seven potential phosphorylation sites were identified in both SDACC1 (SEQ ID NO: 78) and SDACC2 (SEQ ID NO: 81).

[00548] **Table 12** and **Table 13**, respectively, list the 7 sites that were targeted for mutation in SDACC1 (SEQ ID NO: 78) and SDACC2 (SEQ ID NO: 81). The numbering of the amino acids below relate to the numbering of the amino acids in SEQ ID NO: 78 and SEQ ID NO: 81.

[00549] **Table 12: SDACC1**

Ser (S) 133 to Asp (D)
Thr (T) 140 to Asp (D)
Ser (S) 142 to Asp (D)
Val (V) 150 to Asp (D)
Pro (P) 154 to Asp (D)
Ser (S) 162 to Asp (D)
Thr (T) 301 to Asp (D)

[00550] **Table 13: SDACC2**

Ser (S) 133 to Asp (D)
Thr (T) 140 to Asp (D)
Ser (S) 142 to Asp (D)
Val (V) 150 to Asp (D)

Pro (P) 154 to Asp (D)
Ser (S) 162 to Asp (D)
Thr (T) 301 to Asp (D)

[00551] Each of the codon-optimized nucleotide sequences SDACC1 (SEQ ID NO: 97) and SDACC2 (SEQ ID NO: 127) were mutated by mutagenesis to create the seven mutations listed above.

[00552] Example 13. Cloning of mutant and non-mutagenized codon optimized ACCase β-subunits of *Scenedesmus dimorphus.*

[00553] The two non-mutagenized codon-optimized SDACC1 (SEQ ID NO: 97) and SDACC2 (SEQ ID NO: 127) sequences were each ligated into P04 vector (**Figure 9** and **Figure 10**, respectively) between the NdeI and XbaI sites.

[00554] As mentioned above, site-directed mutagenesis was applied to SEQ ID NO: 97 to generate each of the seven mutations. In addition, site-directed mutagenesis was also applied to SEQ ID NO: 127 to generate each of the seven mutations. Nucleotides encoding the 14 mutants (SEQ ID NOs: 128-141) were each ligated into the P04 vector between the NdeI and XbaI sites.

[00555] The P04 vector comprises a constitutive PsbD promoter that drives the expression of the target gene. In addition, P04 comprises a sequence encoding for a chloramphenicol resistance gene which was used for selection of desired clones.

[00556] The expressed proteins will comprise the amino acid sequence of each of the 14 mutated proteins (SEQ ID NOs: 100 to 113), along with a Flag tag (SEQ ID NO: 118) inserted after the initial Met.

[00557] Individual plasmids containing the mutations were transformed into *Scenedesmus dimorphus* using a microprojectile mediated (biolistic) particle gun (Biorad). The range of psi was 500 to 700. Individual clones were picked and grown up in selection media (TAP) comprising a concentration of 34 ng/ul chloramphenicol.

[00558] Example 14. Expression of a eukaryotic Rat ACCase in *Chlamydomonas reinhardtii.*

[00559] The rat ACCase sequence (SEQ ID NO: 115) (NM 022193) was codon-optimized for integration into the chloroplast genome of *Chlamydomonas reinhardtii*. The codon-optimized nucleotide sequence is shown in SEQ ID NO: 114. A Flag tag, also codon-optimized for integration into the chloroplast genome of *Chlamydomonas reinhardtii* (SEQ ID NO: 116), was added to the 3'-end of the codon-optimized gene sequence in front of the stop codon (SEQ ID NO: 156), and cloned into expression vector D2RnACC (**Figure 11**) using restriction sites NdeI and XbaI. D2RnACC comprises a PsbD promoter to drive expression of the Rat ACCase protein. SEQ ID NO: 157 is the amino acid sequence of the protein without the carboxy-terminal Flag tag. The expressed protein has the sequence of SEQ ID NO: 157 with the amino acid sequence of the tag (SEQ ID NO: 118) at the carboxy terminus of the protein. Expression of the kanamycin resistance protein is under the control of the PatpA promoter.

[00560] The plasmid (D2RnACC) comprising the nucleotide sequence of SEQ ID NO: 156 was transformed into the chloroplast genome of *Chlamydomonas reinhardtii* (1690 and 137C) using a microprojectile mediated (biolistic) particle gun (Biorad). The transformation product was spread on TAP plates with 100 ng/μl kanamycin.

[00561] **Figure 8A** is a screen for the presence of the gene encoding the recombinant Rat ACCase in the chloroplast genome using gene specific primers D2Rv (GGACGTCCTGCCAACTGCCTATGGTAGC) (SEQ ID NO: 119) and Rn

Fw (GTTGAGGGCACAGTGAAAGCATACGTTTGGG) (SEQ ID NO: 120). Colonies 4, 5, 6, 15, and 16, amongst others, were positive for the presence of the gene.

[00562] In order to determine whether all copies of the chloroplast genome were successfully transformed with the target gene a plasmicity screen was conducted by PCR. The PCR reaction conditions are provided below in **Table 14**.

[00563] **Table 14**

25 µl multi screen PCR master mix			**cycling parameters**		
		µl			
1	Ex taq Buffer, 10x	2.5	1	95 °C	2 min
2	2.5 mM dNTPs	2.0	2	95 °C	30 sec
3			3	55 °C	30 sec
4	primer 79 (SEQ ID NO: 123) (10 µM)	1.25	4	72 °C	30 sec
5	primer 80 (SEQ ID NO: 124) (10 µM)	1.25	5	go to step 2	39 cycles
6	primer 1995 (SEQ ID NO: 121) (10 µM)	1.25	6	72 °C	2 min
7	primer 1996 (SEQ ID NO: 122) (10 µM)	1.25	7	4 °C	Forever
8	polymerase (Ex Taq, 5.0 U/µl)	0.4			
	DNA	2			
	H_2O	13.1			
	total volume	25			

[00564] The presence of a single PCR band indicates homoplasmicity, and the presence of two PCR bands indicates heteroplasmicity. All of the primers 1995, 1996, 79, and 80 (SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, and SEQ ID NO:124, respectively), were used in the PCR reaction.

[00565] 1) Reverse primer, 100216-DM-**1995**: TGTTTGTTAAGGCTAGCTGC (SEQ ID NO: 121). 3HB-D2 multi screen primer shows a band of 212 base pairs if no insert is present.

[00566] 2) Forward primer, 100216-DM-**1996**: CGCCACTGTCATCCTTTAAGT (SEQ ID NO: 122). 3HB-D2 multi screen primer shows a band of 212 base pairs if no insert is present.

[00567] 3) Reverse primer, 100216-DM-**79**: CCGAACTGAGGTTGGGTTTA (SEQ ID NO: 123) (tD2-3HB multi-screen primer).

[00568] 4) Forward primer, 100216-DM-**80**: GGGGGAGCGAATAGGATTAG (SEQ ID NO: 124) (tD2-3HB multi-screen primer).

[00569] Primer pair 79 and 80 was used as a control PCR for amplification of the chloroplast genome. The use of primers 79 and 80 in a PCR reaction will result in the amplification of an approximately 513 bp fragment. Use of primers 1995 and 1996 will result in a 212 bp amplicon if the integration cassette, which includes the target gene, is not integrated into the chloroplast genome. If the integration cassette which includes the target gene is integrated into the genome, use of primer pair 1995 and 1996 in theory, should result in a PCR product of about 9750 bp. However, an extension time (as described above) of 72 °C for 30 seconds will not allow for a 9750 bp fragment to be made, a longer extension time is required.

[00570] A lack of a 212 bp band indicates homoplasmity. **Figure 8B** shows the results of the screen. Colonies 4, 5, 6, 15, and 16, amongst others, were homoplasmic for the gene.

[00571] The total protein size of RnACC was estimated to be about 266KDa (as shown in **Figure 12** with a "*"). First, a Western screen with an anti-Flag antibody was conducted; this experiment did not give a clear band. This result is not surprising because of the large size of the protein. It is also possible that the C-terminal fusion Flag tag was contained inside of the protein making it impossible for detection.

[00572] Since RnACC is a fully functional anti-biotinylated enzyme, it allows the use of an anti-biotin antibody for screening. First, 50 mls of culture (TAP, and HSM with a CO_2 supply) were collected for the crude protein extraction. Then, an anti-biotin resin was subsequently used to partially purify the protein. The partially purified protein was used for the Western screening.

[00573] As shown in **Figure 12**, a clear band in the expected size was detected in cells grown in HSM (as shown with a "*"), but not in the wild type cells (untransformed *Chlamydomonas reinhardtii*). Transformed cells grown in TAP showed a very faint band.

[00574] Example 15. Lipid accumulation in RnACC expressing cell lines.

[00575] The RnACC transgenic lines (D2Rn5, D2Rn15, and D2Rn16) along with the wild-type cells were grown in TAP media in an air environment under constant light, until cells reached late log phase. Separately, the same cells were grown in HSM media in a 5% carbon dioxide in an air environment under constant light, until cells reached late log phase. The cells were harvested by centrifugation and analyzed for total gravimetric lipids by methanol/methyl-tert-butyl ether extraction according to a modified Bligh Dyer method (as described in Matyash V., *et al.* (2008) Journal of Lipid Research 49:1137-1146).

[00576] Specifically, biomass was pelleted and excess water removed. After the addition of methanol, samples were vortexed vigorously to lyse cells. MTBE was added and samples were vortexed again for an extended period of time (approximately 1 hr). Addition of water to samples after vortexing gave a ratio of 4:1.2:1; MTBE:MeOH:water respectively. Samples were centrifuged to aid in phase separation. The organic layer was removed and the process repeated a second time. Samples were extracted a third time adding only MTBE; the samples were vortexed, centrifuged, and phase separated as described above. The organic layers were combined, dried with magnesium sulfate, filtered and concentrated into tared vials. The percent extractables was calculated using the ash free dry weight of the sample.

[00577] The measurement of the total gravimetric lipid content in several transgenic cell lines is shown in **Figure 13** and **Figure 14**. D2Rn5, D2Rn15, and D2Rn16 are the individual clones shown in **Figures 8A** and **8B**. The Y axis shows the lipid content as a percent of the ash-free dry weight of the culture. The X axis shows the strain of algae

analyzed. All extractions were conducted in triplicate with error bars showing the standard deviation of the percent extractable.

[00578] In TAP growth media, the lipid accumulation in RnACC expressing cell lines can be as high as 27.84% of ash-free dry weight (D2Rn16) compared to 25.12% (WT), an 11% increase (**Figure 13**).

[00579] In HSM growth media, the lipid accumulation in RnACC overexpressing cell lines can be as high as 26.16% of ash-free dry weight (D2Rn16) compared to 23.08% (WT), a 13.3% increase (**Figure 14**).

[00580] While certain embodiments 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 disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

What is claimed is:

1. An isolated polynucleotide capable of transforming a photosynthetic organism comprising a nucleic acid sequence encoding an acetyl CoA carboxylase, wherein the acetyl CoA carboxylase comprises:

1) an amino acid sequence of SEQ ID NO: 157; or

2) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

2. An acetyl CoA carboxylase present in a photosynthetic organism comprising:

1) an amino acid sequence of SEQ ID NO: 157; or

2) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

3. A nucleotide sequence encoding an acetyl CoA carboxylase wherein the nucleotide sequence comprises:

1) a nucleic acid sequence of SEQ ID NO: 114 or SEQ ID NO: 155; or

2) a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 114 or SEQ ID NO: 115, wherein the nucleotide sequence is capable of transforming a photosynthetic organism.

4. A vector comprising a nucleotide sequence encoding an acetyl CoA carboxylase, wherein t he acetyl CoA carboxylase comprises:

1) an amino acid sequence of SEQ ID NO: 157; or

2) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157, wherein the vector is used to transform a photosynthetic organism.

5. The vector of claim 4, wherein the vector is an expression vector.

6. The vector of claim 4 or 5, wherein the vector further comprises a 5' regulatory region.

7. The vector of claim 6, wherein the 5' regulatory region further comprises a promoter.

8. The vector of claim 7, wherein the promoter is a constitutive promoter.

9. The vector of claim 7, wherein the promoter is an inducible promoter.

10. The vector of claim 9, wherein the inducible promoter is a light inducible promoter, a nitrate inducible promoter, or a heat responsive promoter.

11. The vector of any one of claims 4 to 10, further comprising a 3' regulatory region.

12. A method for increasing production of malonyl CoA in a photosynthetic organism, comprising transforming the photosynthetic organism with a polynucleotide encoding an ACCase comprising an amino acid sequence of SEQ ID NO: 157, or with a polynucleotide encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

13. The method of claim 12, wherein the photosynthetic organism is a prokaryote.

14. The method of claim 13, wherein the prokaryote is a cyanobacterium.

15. The method of claim 12, wherein the photosynthetic organism is a eukaryote.

16. The method of claim 15, wherein the eukaryote is a vascular plant.

17. The method of claim 15, wherein the eukaryote is a non-vascular photosynthetic organism.

18. The method of claim 17, wherein the non-vascular photosynthetic organism is an alga.

19. The method of claim of any one of claims 12 to 18, further comprising transforming a plastid with the polynucleotide.

20. The method of claim 19, wherein the plastid is a chloroplast.

21. A method for increasing fatty acid synthesis in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding an ACCase comprising an amino acid sequence of SEQ ID NO: 157, or with a polynucleotide encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

22. The method of claim 21, wherein the photosynthetic organism is a prokaryote.

23. The method of claim 22, wherein the prokaryote is a cyanobacterium.

24. The method of claim 21, wherein the organism is a eukaryote.

25. The method of claim 24, wherein the eukaryote is a vascular plant.

26. The method of claim 24, wherein the eukaryote is a non-vascular photosynthetic organism.

27. The method of claim 21, wherein the photosynthetic organism is an alga.

28. The method of claim of any one of claims 21 to 27, further comprising transforming a plastid with the polynucleotide.

29. The method of claim 28, wherein the plastid is a chloroplast.

30. A transgenic host cell comprising a nucleotide sequence encoding an ACCase comprising an amino acid sequence of SEQ ID NO: 157, or comprising a nucleotide sequence encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

31. The transgenic host cell of claim 30, wherein the host cell is a prokaryote.

32. The transgenic host cell of claim 31, wherein the prokaryote is a cyanobacterium.

33. The transgenic host cell of claim 30, wherein the host cell is a plant cell.

34. The transgenic host cell of claim 33, wherein the plant cell is from a vascular plant.

35. The transgenic host cell of claim 33, wherein the plant cell is from an alga.

36. The transgenic host cell of claim 35, wherein the alga is a green alga.

37. The transgenic host cell of claim 36, wherein the green alga is a *Chlorophycean*.

38. A transgenic plastid comprising a polynucleotide encoding an acetyl CoA carboxylase comprising an amino acid sequence of SEQ ID NO: 157, or encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

39. The transgenic plastid of claim 38, wherein the plastid is a chloroplast.

40. A host cell comprising the transgenic plastid of claim 38 or claim 39.

41. The host cell of claim 40, wherein the host cell is a prokaryote.

42. The host cell of claim 41, wherein the host cell is a cyanobacterium.

43. The host cell of claim 40, wherein the host cell is a plant cell.

44. The host cell of claim 43, wherein the plant cell is from a vascular plant.

45. The host cell of claim 40, wherein the plant cell is an alga.

46. The transgenic host cell of 45, wherein the alga is a green alga

47. The transgenic host cell of claim 46, wherein the green alga is a *Chlorophycean*.

48. An acetyl CoA carboxylase present in a photosynthetic organism comprising:
an amino acid sequence of a mammalian acetyl CoA carboxylase.

49. The acetyl CoA carboxylase of claim 48, wherein the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 157, or an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 157.

50. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID

NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

51. An acetyl CoA carboxylase comprising an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

52. A nucleotide sequence encoding a beta subunit of an acetyl CoA carboxylase wherein the nucleotide sequence comprises:

1) a nucleic acid sequence of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO:160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 168, or SEQ ID NO: 169; or

2) a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO:160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 168, or SEQ ID NO: 169.

53. A vector comprising a nucleotide sequence encoding a beta subunit of an acetyl CoA carboxylase wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

54. The vector of claim 53, wherein the vector is an expression vector.

55. The vector of claim 53 or claim 54, wherein the vector further comprises a 5' regulatory region.

56. The vector of claim 55, wherein the 5' regulatory region further comprises a promoter.

57. The vector of claim 56, wherein the promoter is a constitutive promoter.

58. The vector of claim 56, wherein the promoter is an inducible promoter.

59. The vector of claim 58, wherein the inducible promoter is a light inducible promoter, a nitrate inducible promoter, or a heat responsive promoter.

60. The vector of any one of claims 53 to 59, further comprising a 3' regulatory region.

61. A method for increasing production of malonyl CoA in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding a beta subunit of an ACCase, wherein the beta subunit of the ACCase comprises an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

62. The method of claim 61, wherein the photosynthetic organism is a prokaryote.

63. The method of claim 62, wherein the prokaryote is a cyanobacterium.

64. The method of claim 61, wherein the organism is a eukaryote.

65. The method of claim 64, wherein the eukaryote is a vascular plant.

66. The method of claim 64, wherein the eukaryote is a non-vascular photosynthetic organism.

67. The method of claim 66, wherein the non-vascular photosynthetic organism is an alga.

68. The method of claim of any one of claims 61 to 67, further comprising transforming a plastid with the polynucleotide.

69. The method of claim 68, wherein the plastid is a chloroplast.

70. A method for increasing fatty acid synthesis in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding an ACCase comprising an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

71. The method of claim 70, wherein the photosynthetic organism is a prokaryote.

72. The method of claim 71, wherein the prokaryote is a cyanobacterium.

73. The method of claim 70, wherein the organism is a eukaryote.

74. The method of claim 73, wherein the eukaryote is a vascular plant.

75. The method of claim 70, wherein the eukaryote is a non-vascular photosynthetic organism.

76. The method of claim 75, wherein the non-vascular photosynthetic organism is an alga.

77. The method of claim of any one of claims 70 to 76, further comprising transforming a plastid with the polynucleotide.

78. The method of claim 77, wherein the plastid is a chloroplast.

79. A transgenic host cell comprising a nucleotide sequence encoding an ACCase comprising an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) encoding an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acids sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

80. The transgenic host cell of claim 79, wherein the host cell is a prokaryote.

81. The transgenic host cell of claim 80, wherein the prokaryote is a cyanobacterium.

82. The transgenic host cell of any one of claims 79 to 81, wherein the host cell is a plant cell.

83. The transgenic host cell of claim 82, wherein the plant cell is from a vascular plant.

84. The transgenic host cell of claim 82, wherein the plant cell is from an alga.

85. The transgenic host cell of claim 84, wherein the alga is a green alga.

86. The transgenic host cell of claim 85, wherein the green alga is a *Chlorophycean*.

87. A transgenic plastid comprising a polynucleotide encoding a beta subunit of an acetyl CoA carboxylase comprising an amino acid sequence of:

a) SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167; or

b) comprising an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acids sequence of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, or SEQ ID NO: 167.

88. The transgenic plastid of claim 87, wherein the plastid is a chloroplast.

89. A host cell comprising the transgenic plastid of claim 87 or claim 88.

90. The host cell of claim 89, wherein the host cell is a prokaryote.

91. The host cell of claim 90, wherein the prokaryote is a cyanobacterium.

92. The host cell of 89, wherein the host cell is a plant cell.

93. The host cell of claim 92, wherein the plant cell is from a vascular plant.

94. The host cell of claim 92, wherein the plant cell is an alga.

95. The host cell of 94, wherein the alga is a green alga

96. The host cell of claim 95, wherein the green alga is a *Chlorophycean*.

97. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 15; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15.

98. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 16; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 16.

99. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 17; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 17.

100. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 18; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 18.

101. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 19; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 19.

102. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO:

20; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 20.

103. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 21; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 21.

104. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 22; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 22.

105. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 23; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 23.

106. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 24; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 24.

107. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 163; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%; at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 163.

108. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 164; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 164.

109. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 165; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 165.

110. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 166; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 166.

111. An isolated polynucleotide comprising a nucleic acid sequence encoding a beta subunit of an acetyl CoA carboxylase, wherein the beta subunit of the acetyl CoA carboxylase comprises an amino acid sequence of SEQ ID NO: 167; or comprises an amino acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 167.

112. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 158; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 158.

113. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 159; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 159.

114. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 160; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 160.

115. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 161; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 161.

116. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 162; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 162.

117. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 168; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 168.

118. An isolated polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 169; or comprising a nucleic acid sequence that has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least at least 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 169.

119. An isolated polynucleotide comprising a sequence encoding an acetyl CoA carboxylase comprising an amino acid sequence of:

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)

wherein

X_1 is T or D or E or N or H or Q or K;

X_2 is T or D or E or N or H or Q or K;

X_3 is S or D or E or N or H or Q or K;

X_4 is S or D or E or N or H or Q or K;

X_5 is S or D or E or N or H or Q or K;

X_6 is C or D or E or N or H or Q or K;

X_7 is Y or D or E or N or H or Q or K;

provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively.

120. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y.

121. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y.

122. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y.

123. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D.

124. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y.

125. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is D and X_7 is Y.

126. The isolated polynucleotide of claim 119, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

127. An acetyl CoA carboxylase comprising an amino acid sequence of:
AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA
LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)
wherein
X_1 is T or D or E or N or H or Q or K;
X_2 is T or D or E or N or H or Q or K;
X_3 is S or D or E or N or H or Q or K;
X_4 is S or D or E or N or H or Q or K;
X_5 is S or D or E or N or H or Q or K;
X_6 is C or D or E or N or H or Q or K;
X_7 is Y or D or E or N or H or Q or K;
provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively.

128. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y.

129. The acetyl CoA carboxylase of claim 127 wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y.

130. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y.

131. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D.

132. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y.

133. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is D and X_7 is Y.

134. The acetyl CoA carboxylase of claim 127, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

135. A vector comprising a nucleotide sequence encoding an acetyl CoA carboxylase comprising an amino acid sequence of:

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA
LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)

wherein

X_1 is T or D or E or N or H or Q or K;

X_2 is T or D or E or N or H or Q or K;

X_3 is S or D or E or N or H or Q or K;

X_4 is S or D or E or N or H or Q or K;

X_5 is S or D or E or N or H or Q or K;

X_6 is C or D or E or N or H or Q or K;

X_7 is Y or D or E or N or H or Q or K;

provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively.

136. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y.

137. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y.

138. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y.

139. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D.

140. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y.

141. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y.

142. The vector of claim 135, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

143. The vector of any one of claims 135 to 142, wherein the vector is an expression vector.

144. The vector of any one of claims 135 to 143, wherein the vector further comprises a 5' regulatory region.

145. The vector of claim 144, wherein the 5' regulatory region further comprises a promoter.

146. The vector of claim 145, wherein the promoter is a constitutive promoter.

147. The vector of claim 145, wherein the promoter is an inducible promoter.

148. The vector of claim 147, wherein the inducible promoter is a light inducible promoter, nitrate inducible promoter or a heat responsive promoter.

149. The vector of any one of claims 135 to 148, further comprising a 3' regulatory region.

150. A method for increasing production of malonyl CoA in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding
AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA
LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)
wherein
X$_1$ is T or D or E or N or H or Q or K;
X$_2$ is T or D or E or N or H or Q or K;
X$_3$ is S or D or E or N or H or Q or K;
X$_4$ is S or D or E or N or H or Q or K;
X$_5$ is S or D or E or N or H or Q or K;
X$_6$ is C or D or E or N or H or Q or K;
X$_7$ is Y or D or E or N or H or Q or K;
provided, however, that the combination of X$_1$, X$_2$, X$_3$, X$_4$, X$_5$, X$_6$ and X$_7$ is not T, T, S, S, S, C, Y, respectively.

151. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is S, X$_6$ is C and X$_7$ is Y.

152. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is D, X$_6$ is C and X$_7$ is Y.

153. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is D and X$_7$ is Y.

154. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is S, X$_5$ is S, X$_6$ is C and X$_7$ is D.

155. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is Y.

156. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is D and X$_7$ is Y.

157. The method of claim 150, wherein X$_1$ is T, X$_2$ is T, X$_3$ is S, X$_4$ is D, X$_5$ is D, X$_6$ is C and X$_7$ is D.

158. The method of any one of claims 150 to 157, wherein the photosynthetic organism is a prokaryote.

159. The method of claim 158, wherein the prokaryote is a cyanobacterium.

160. The method of claim 150, wherein the organism is a eukaryote.

161. The method of claim 160, wherein the eukaryote is a vascular plant.

162. The method of claim 160, wherein the eukaryote is a non-vascular photosynthetic organism.

163. The method of claim 162, wherein the non-vascular photosynthetic organism is an alga.

164. The method of any one of claims 150 to 163, further comprising transforming a plastid with the polynucleotide.

165. The method of claim 164, wherein the plastid is a chloroplast.

166. A method for increasing fatty acid synthesis in a photosynthetic organism comprising transforming the photosynthetic organism with a polynucleotide encoding

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA
LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)

wherein

X_1 is T or D or E or N or H or Q or K;

X_2 is T or D or E or N or H or Q or K;

X_3 is S or D or E or N or H or Q or K;

X_4 is S or D or E or N or H or Q or K;

X_5 is S or D or E or N or H or Q or K;

X_6 is C or D or E or N or H or Q or K;

X_7 is Y or D or E or N or H or Q or K;

provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively.

167. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y.

168. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y.

169. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y.

170. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D.

171. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y.

172. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y.

173. The method of claim 166, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

174. The method of any one of claims 166 to 173, wherein the photosynthetic organism is a prokaryote.

175. The method of claim 174, wherein the prokaryote is a cyanobacterium.

176. The method of claim 166, wherein the organism is a eukaryote.

177. The method of claim 176, wherein the eukaryote is a vascular plant.

178. The method of claim 176, wherein the eukaryote is a non-vascular photosynthetic organism.

179. The method of claim 178, wherein the non-vascular photosynthetic organism is an alga.

180. The method of any one of claims 166 to 179, further comprising transforming a plastid with the polynucleotide.

181. The method of claim 180, wherein the plastid is a chloroplast.

182. A transgenic host cell comprising a nucleotide sequence encoding

AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA
LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)

wherein

X_1 is T or D or E or N or H or Q or K;

X_2 is T or D or E or N or H or Q or K;

X_3 is S or D or E or N or H or Q or K;

X_4 is S or D or E or N or H or Q or K;
X_5 is S or D or E or N or H or Q or K;
X_6 is C or D or E or N or H or Q or K;
X_7 is Y or D or E or N or H or Q or K;
provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively.

183. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y.

184. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y.

185. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y.

186. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D.

187. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y.

188. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y.

189. The transgenic host cell of claim 182, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

190. The transgenic host cell of any one of claims 182 to 189, wherein the host cell is a prokaryote.

191. The transgenic host cell of claim 190, wherein the host cell is a cyanobacterium.

192. The transgenic host cell of claim 182, wherein the host cell is a plant cell.

193. The transgenic host cell of claim 192, wherein the plant cell is from a vascular plant.

194. The transgenic host cell of claim 182, wherein the plant cell is from an alga.

195. The transgenic host cell of claim 194, wherein the alga is a green alga.

196. The transgenic host cell of claim 195, wherein the green alga is a *Chlorophycean*.

197. A transgenic plastid comprising a polynucleotide encoding an acetyl CoA carboxylase comprising an amino acid sequence of:
AGEANGSPIVTGPISVNPSMSPALDPVAAAEAGKSAKAVDRSKGLWTRCDKCGTILYIKHLKEHHHICFGCNY
HLKMSSMERINHLIDAGX$_1$WRPLDEX$_2$LX$_3$PVDPLEFX$_4$DLKX$_5$YTDRIKEAQEKTGLQDGVRTGTGLLHGIPVA

LGVMDFTYMGGSMGSVVGEKLTRLIEYATQEGMPVIIVCTSGGARMQEGIFSLMQMAKISAALHVHQNX$_6$AN
LLYIAILTSPTTGGVTASFGMLGDVIIAEPQAIIGFAGRRVIEQTLQEQLPDDFQTAEYLLEHGLLDLVVPRSFLK
GALX$_7$EIIDFYRAAPYKKRGMIPFGVQHGTFLTTEEKV (SEQ ID NO: 11)

wherein

X_1 is T or D or E or N or H or Q or K;

X_2 is T or D or E or N or H or Q or K;

X_3 is S or D or E or N or H or Q or K;

X_4 is S or D or E or N or H or Q or K;

X_5 is S or D or E or N or H or Q or K;

X_6 is C or D or E or N or H or Q or K;

X_7 is Y or D or E or N or H or Q or K;

provided, however, that the combination of X_1, X_2, X_3, X_4, X_5, X_6 and X_7 is not T, T, S, S, S, C, Y, respectively.

198. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is S, X_6 is C and X_7 is Y.

199. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is D, X_6 is C and X_7 is Y.

200. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is D and X_7 is Y.

201. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is S, X_5 is S, X_6 is C and X_7 is D.

202. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is Y.

203. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is D and X_7 is Y.

204. The transgenic plastid of claim 197, wherein X_1 is T, X_2 is T, X_3 is S, X_4 is D, X_5 is D, X_6 is C and X_7 is D.

205. The transgenic plastid of any one of claims 197 to 204, wherein the plastid is a chloroplast.

206. A host cell comprising the transgenic plastid of any one of claims 197 to 205.

207. The host cell of claim 206, wherein the host cell is a prokaryote.

208. The host cell of claim 207, wherein the prokaryote is a cyanobacterium.

209. The host cell of claim 206, wherein the host cell is a plant cell.

210. The host cell of claim 209, wherein the plant cell is from a vascular plant.

211. The host cell of claim 209, wherein the plant cell is an alga.

212. The transgenic host cell of 211, wherein the alga is a green alga

213. The transgenic host cell of claim 212, wherein the green alga is a *Chlorophycean.*

214. The ACCase of claim 48, wherein the mammalian ACCase comprises the amino acid sequence of mouse (Mus Musculus: NM_133360.2 Identity: 99%); cattle (Bos Taurus: NM_174224.2. Identity: 97%); dog (Canis Lupus: XM_862501.1. Identity: 96%); chicken (Gallus gallus: NM_205505.1. Identity: 92%); or goat (Capra hircus: DQ370054.1. Identity: 98%).

215. The isolated polypeptide of claim 1, wherein the photosynthetic organism is *Chlamydomonas reinhardtii.*

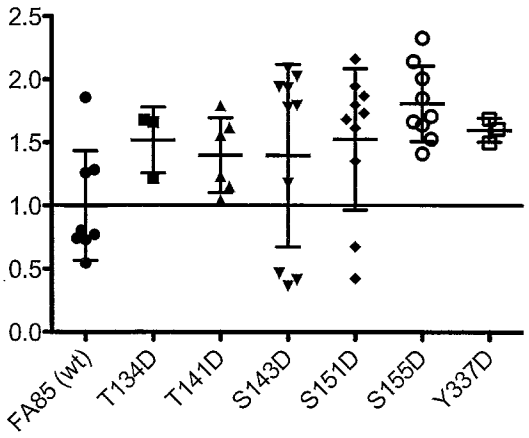


FIG. 1

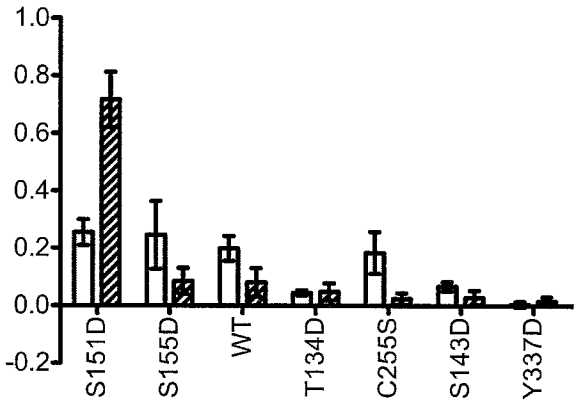


FIG. 2

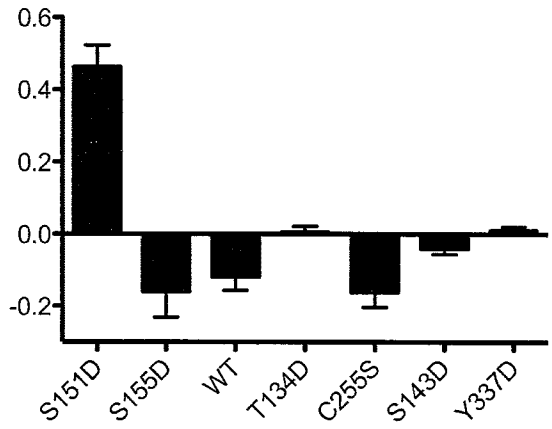


FIG. 3

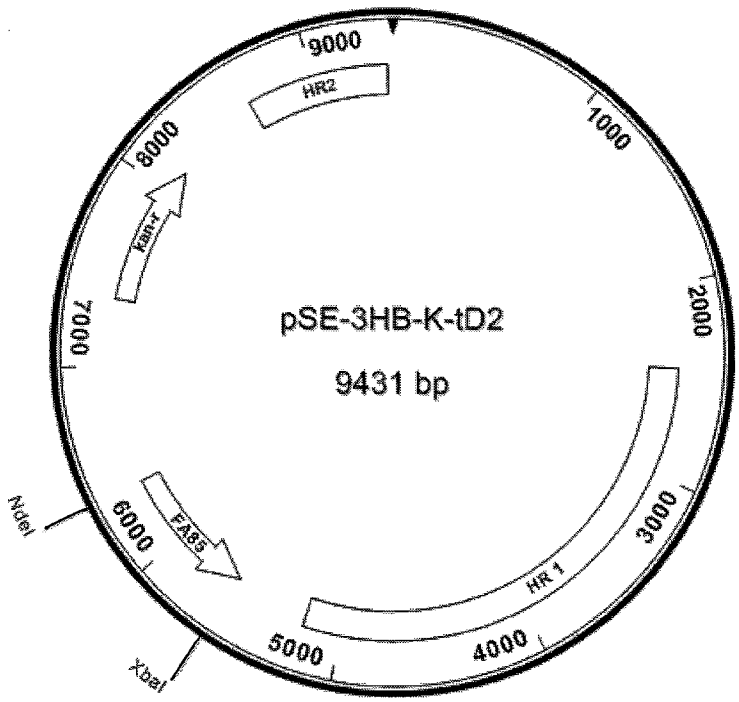

9000
HR2
1000
8000
Kan-r
2000
7000
pSE-3HB-K-tD2
9431 bp
3000
EA85
NdeI
6000
HR 1
5000
4000
XbaI

FIG. 4

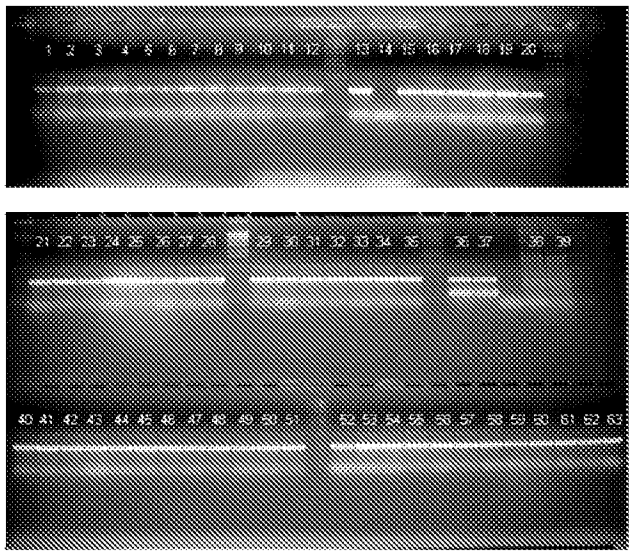


FIG. 5

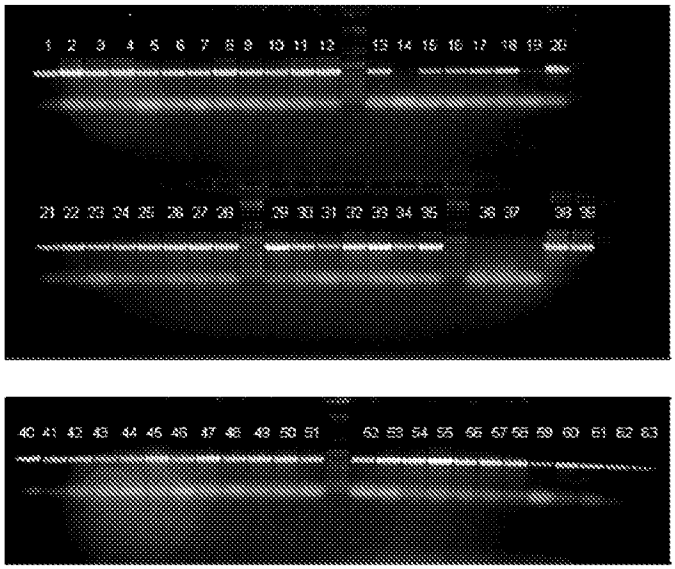


FIG. 6

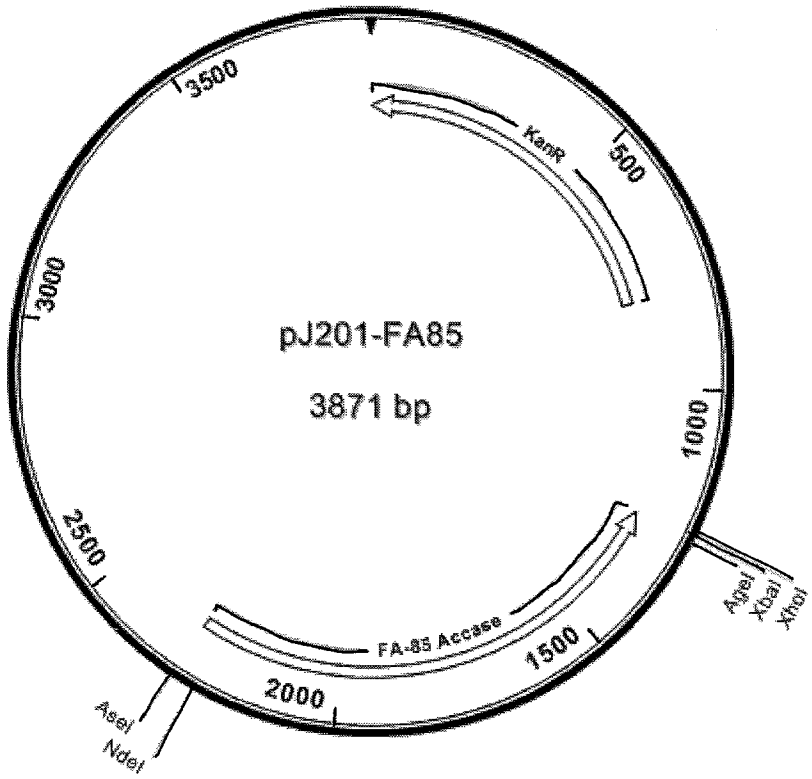

pJ201-FA85
3871 bp
KanR
FA-85 Accase
500
1000
1500
2000
2500
3000
3500
AgeI
XbaI
XhoI
AseI
NdeI

FIG. 7

FIG. 8A

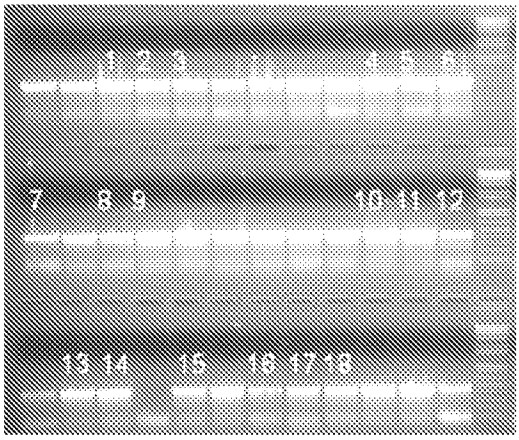
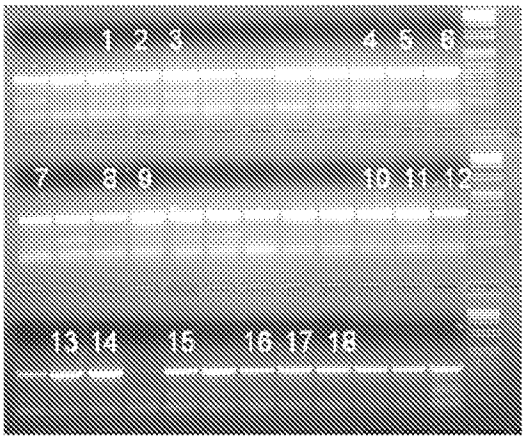

1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18

FIG. 8B


1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18

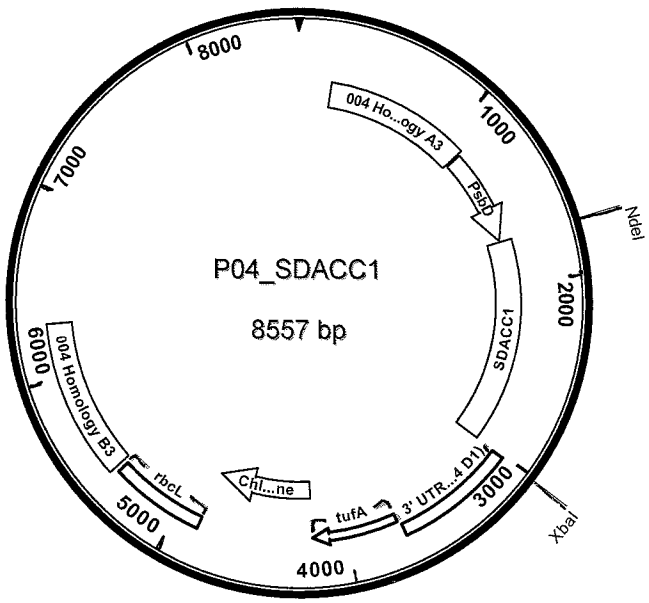

P04_SDACC1
8557 bp
1000
2000
3000
4000
5000
6000
7000
8000
004 Ho...ogy A3
PspD
SDACC1
NdeI
XbaI
3' UTR...4 D1
tufA
Chl...ne
rbcL
004 Homology B3

FIG. 9

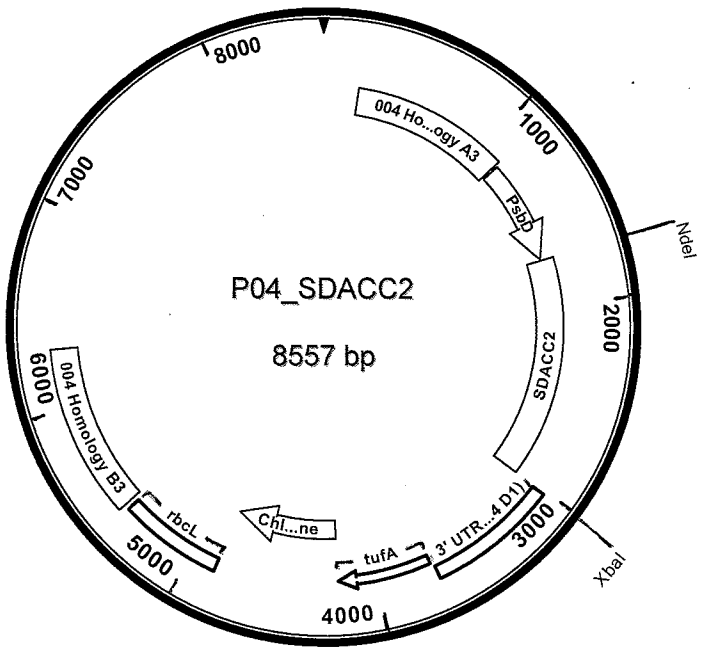

P04_SDACC2
8557 bp
1000
2000
3000
4000
5000
6000
7000
8000
004 Ho...ogy A3
PsbD
SDACC2
NdeI
XbaI
3' UTR...4 D1)
tufA
Chl...ne
rbcL
004 Homology B3

FIG. 10

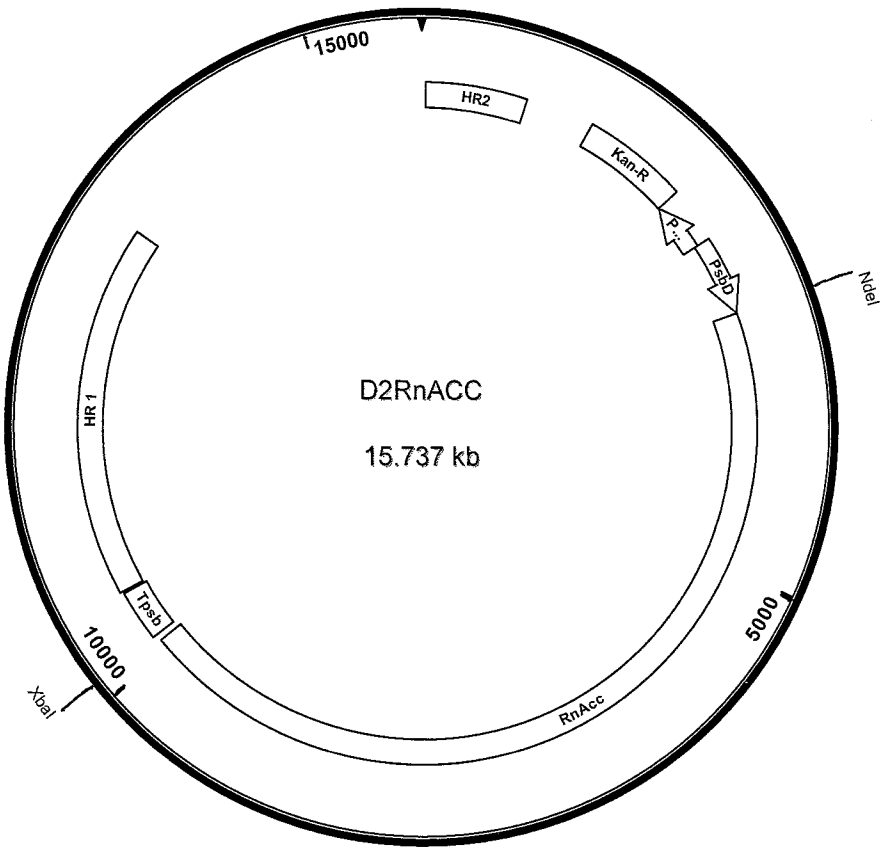

15000
HR2
Kan-R
P
PstD
NdeI
D2RnACC
15.737 kb
HR 1
Tpsb
RnAcc
10000
5000
XbaI

FIG. 11

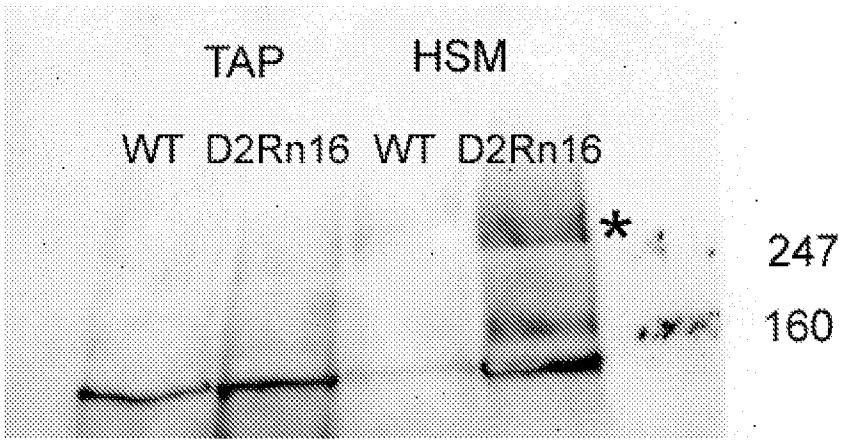


FIG. 12

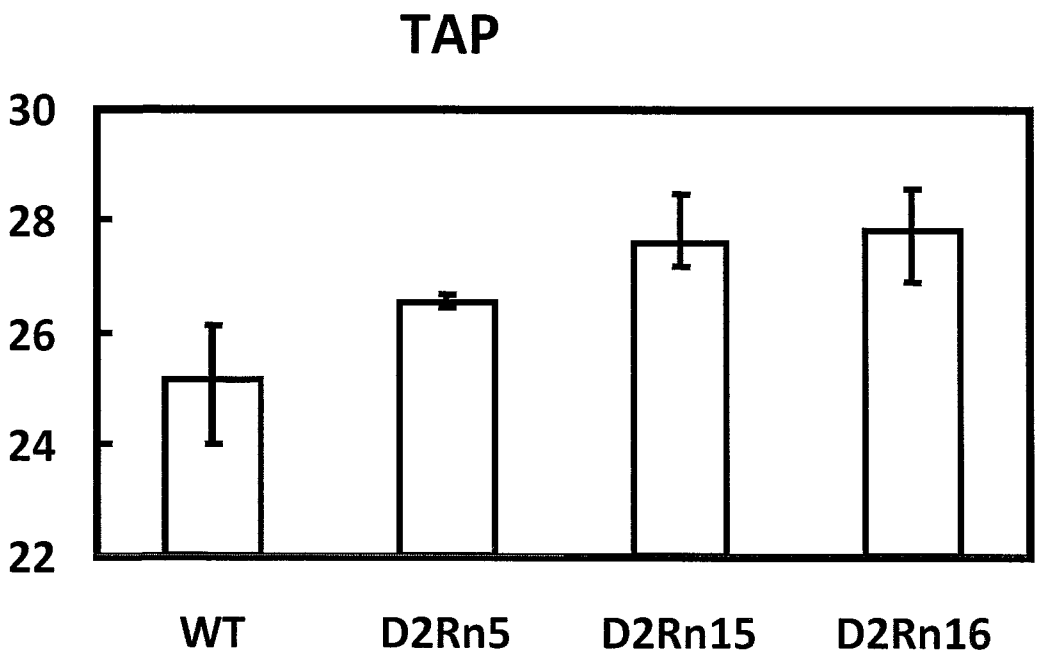


FIG. 13

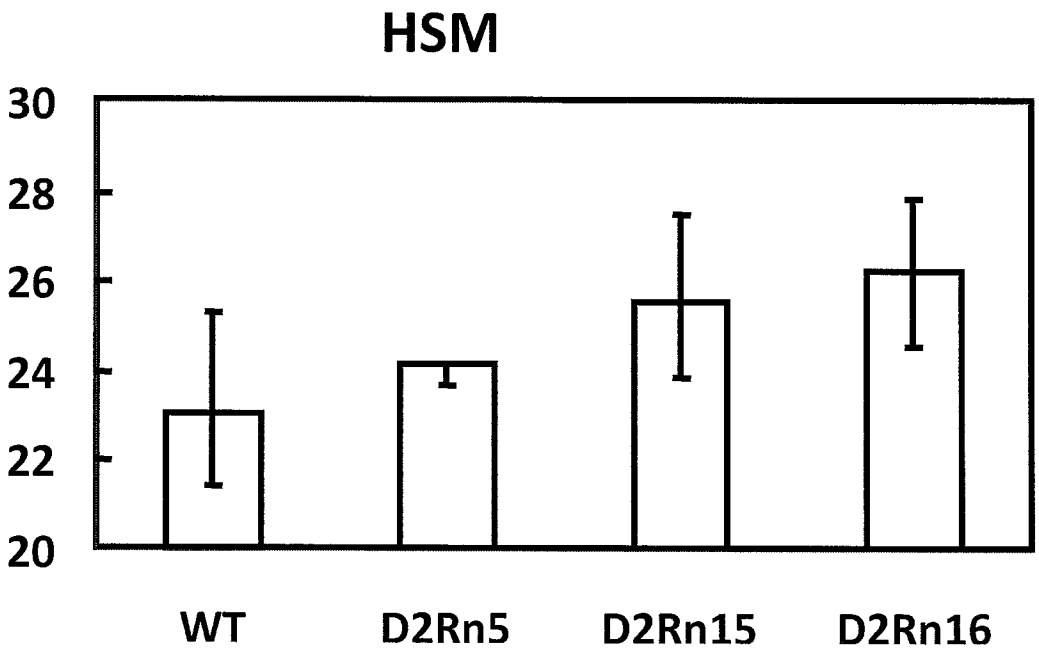
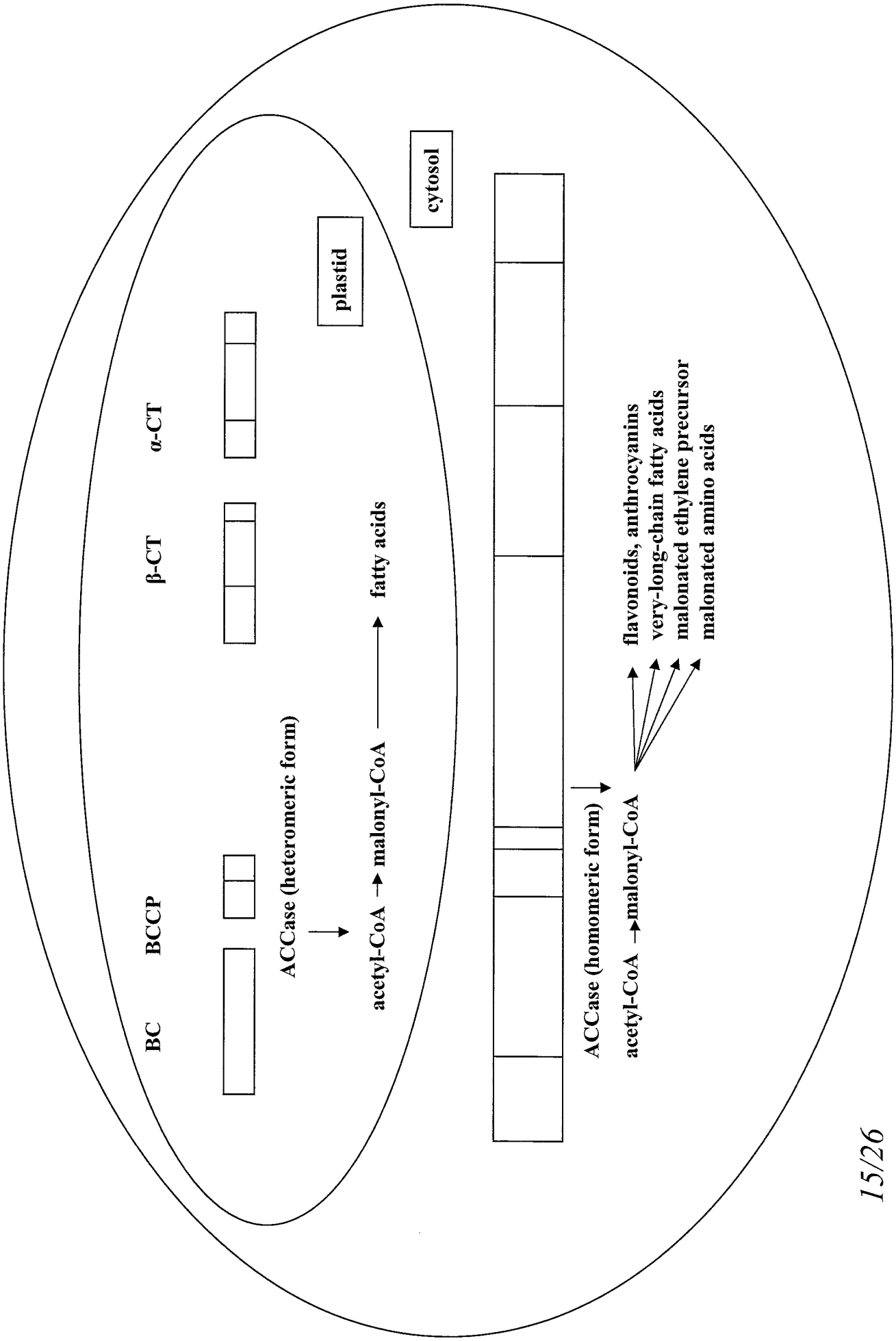


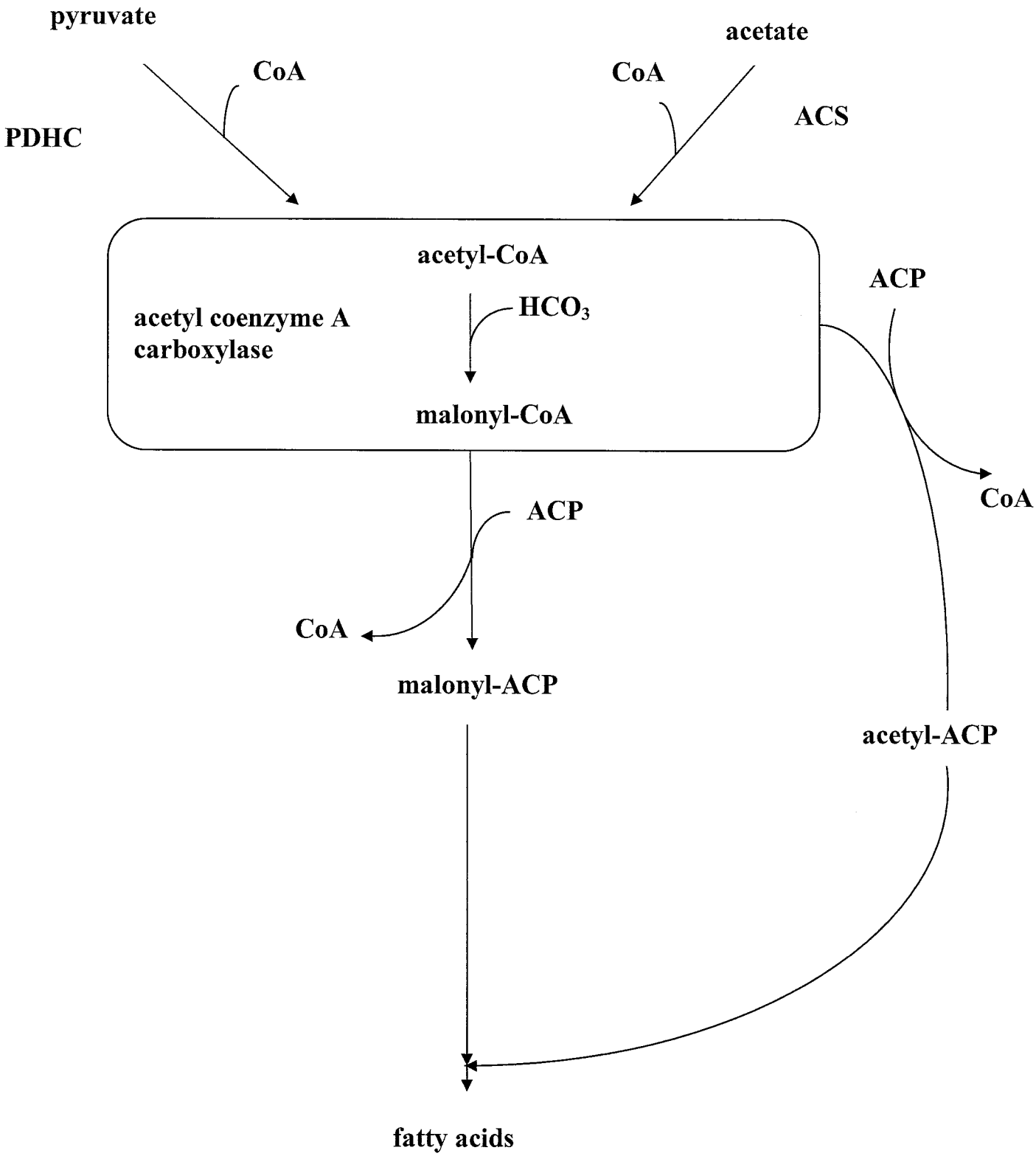
FIG. 14


BC
BCCP
β-CT
α-CT
plastid
ACCase (heteromeric form)
acetyl-CoA → malonyl-CoA → fatty acids
cytosol
ACCase (homomeric form)
acetyl-CoA → malonyl-CoA
flavonoids, anthrocyanins
very-long-chain fatty acids
malonated ethylene precursor
malonated amino acids

15/26

FIG. 15

FIG. 16


pyruvate
acetate
CoA
CoA
PDHC
ACS
acetyl-CoA
acetyl coenzyme A carboxylase
HCO3
malonyl-CoA
ACP
CoA
ACP
CoA
malonyl-ACP
acetyl-ACP
fatty acids

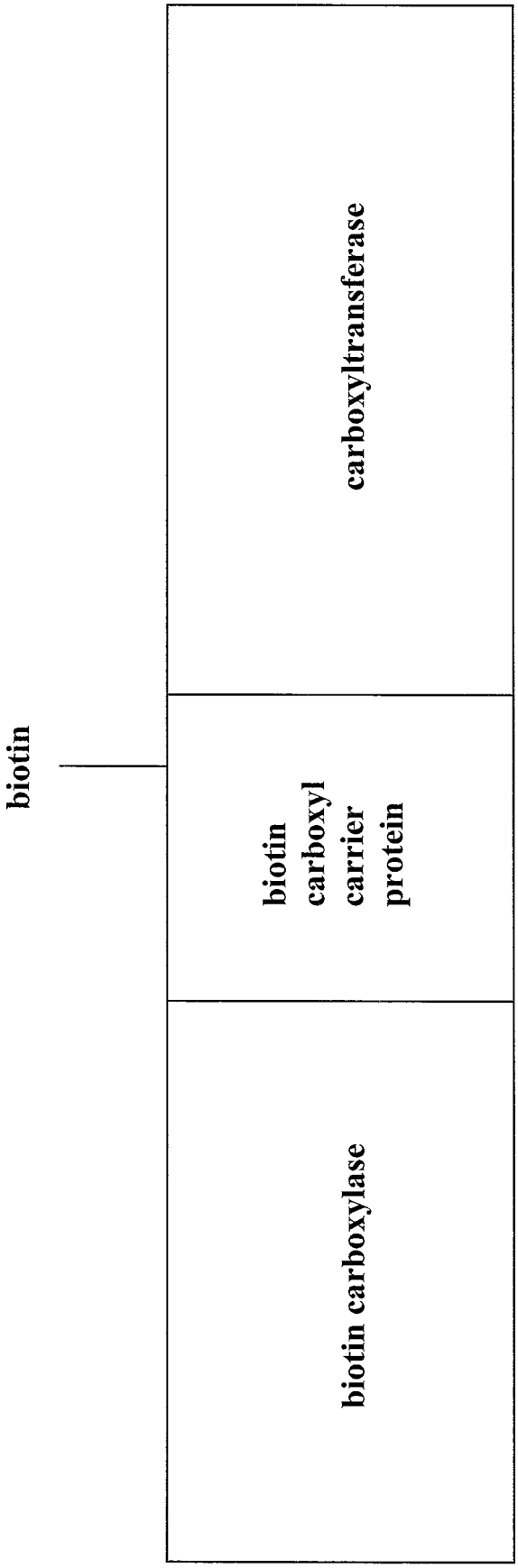

biotin
biotin carboxyl carrier protein
carboxyltransferase
biotin carboxylase

FIG. 17

ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGC
CACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCG
CTTGCAGGAACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAAC
CCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCC
ATTTCTGTGGGTGCTATGGACAAGGACTCCAAGGGCTCTTCCAAGCCTG
TTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGA
TTCTCTACATCAAGCACCTGAAGGAGCACCACCACATCTGCTTCGGCTG
CAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGAT
CGACCCAGGCTCATGGCGCCCCTTTGACGAGACGCTGTCTCCCTGCGAC
CCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGAC
AGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGCACGGG
CCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTC
ATGGGCGGCAGCATGGGCAGCGTGGTGGGGGGAGAAGCTGACGCGCCT
GATTGAGTACGCCACGCAGGAGGGGCTCACGCTGCTGGTGGTGTGCAC
CAGCGGAGGCGCGCGCATGCAGGAGGGCATCATGAGCCTGATGCAGAT
GGCCAAGATCAGCGGCGCGCTGCACGTGCACCAGAATGAGGCCAACCT
GCTGTACATCTCCATCCTGACCAGCCCCACCACAGGTGGCGTGACCGCA
AGCTTTGGCATGCTGGGGGATGTCATCATTGCTGAGCCGCAGGCCATCA
TCGGCTTTGCAGGACGGCGTGTGATCGAGCAGACGCTGCGTGAGGAGC
TGCCAGATGACTTCCAGACCGCGGAGTACCTGCTTGACAAGGGCCTGC
TCGACCTGGTGGTGCCGCGCAGCTTCCTGAAGGGCGCGCTGTTTGAGAT
CATCGACTTCTACAAGAACGCACCCTACAAGCGCCGCGGCAAGATTCC
ATTTGGCGTGCAGCGCGGTACGTACGGCCTGACCGCTGAGGAGAAGAT
GCGGCGCAGGTGGAGGGAGTGGAGCTCAGCTGGCAGCAACGGCTCGG
GCACGCCCGCGCTGGCAGCAGCAGCAGCATCAGCAGCAGTTGGGTCAG
CAGCCACTTGCGGCAGCTGCCAGCAGCAGCAGCTGGCGCTGTGGGCGG
TGCTGGCAGGCTGTGGCAGCTGTGGGCAGTGGCTGTGGTTTGCTCAGG
GGGTAGGTGCGCTTGAGCGCACAGCGGCAACAGCAGCAGTACTGAGAG
AGGGCAGCGTGCTGCTAGCAGGCGTCTGTTGTTAA

FIG. 18

ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGC
CACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCG
CTTGCAGGAACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAAC
CCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCC
ATTTCTGTGGGTGCTATGGACAAGGACTCCAAGGGCTCTTCCAAGCCTG
TTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGA
TTCTCTACATCAAGCACCTGAAGGAGCACCACCACATCTGCTTCGGCTG
CAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGAT
CGACCCAGGCTCATGGCGCCCCTTTGACGAGACGCTGTCTCCCTGCGAC
CCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGAC
AGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGCACGGG
CCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTC
ATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCT
GATTGAGTACGCCACGCAGGAGGGGCTCACGCTGCTGGTGGTGTGCAC
CAGCGGAGGCGCGCGCATGCAGGAGGGCATCATGAGCCTGATGCAGAT
GGCCAAGATCAGCGGCGCGCTGCACGTGCACCAGAATGAGGCCAACCT
GCTGTACATCTCCATCCTGACCAGCCCCACCACAGGTGGCGTGACCGCA
AGCTTTGGCATGCTGGGGGATGTCATCATTGCTGAGCCGCAGGCCATCA
TCGGCTTTGCAGGACGGCGTGTGATCGAGCAGACGCTGCGTGAGGAGC
TGCCAGATGACTTCCAGACCGCGGAGTACCTGCTTGACAAGGGCCTGC
TCGACCTGGTGGTGCCGCGCAGCTTCCTGAAGGGCGCGCTGTTTGAGAT
CATCGACTTCTACAAGAACGCACCCTACAAGCGCCGCGGCAAGATTCC
ATTTGGCGTGCAGCGCGGTACGTACGGCCTGACCGCTGAGGAGAAGAT
GCGGCGCAGGTGGAGGGAGTGGAGCTCAGTTGGCAGCATGTTGCATAG
TGTTCACTATGCAGGCCACTGGCCCTCTGGGTGTGCTGGGATGTTGCTG
GGCCAGCGCCCACTTCATATGCATTGGCATGTCAATGAAGGGTCAGGTT
GTAGCAAGACCACGTGCCAGAGCTTTAAGTATTGGTCAGCATGTGCTG
CTTGGCATGCAGTGTGCCATCGGCGAGGAACACTTCTTGAACATGAACT
TACCAAGCTGATTTCCTGGCAGTTTGATTCATGCTGTTGGCGTGCTGCC
AAAGGTATTCTGCTTAGATCTTGCAATGCTGTGTATGTATATGTGTAA

FIG. 19

ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGC
CACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCG
CTTGCAGGAACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAAC
CCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCC
ATTTCTGTGGGTGCTATGGACAAGGACTCCAAGGGCTCTTCCAAGCCTG
TTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGA
TTCTCTACATCAAGCACCTGAAGGAGCACCACCACATCTGCTTCGGCTG
CAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGAT
CGACCCAGGCTCATGGCGCCCCTTTGACGAGACGCTGTCTCCCTGCGAC
CCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGAC
AGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGCACGGG
CCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTC
ATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCT
GATTGAGTACGCCACGCAGGAGGGGCTCACGCTGCTGGTGGTGTGCAC
CAGCGGAGGCGCGCGCATGCAGGAGGGCATCATGAGCCTGATGCAGAT
GGCCAAGATCAGCGGCGCGCTGCACGTGCACCAGAATGAGGCCAACCT
GCTGTACATCTCCATCCTGACCAGCCCCACCACAGGTGGCGTGACCGCA
AGCTTTGGCATGCTGGGGGATGTCATCATTGCTGAGCCGCAGGCCATCA
TCGGCTTTGCAGGACGGCGTGTGATCGAGCAGACGCTGCGTGAGGAGC
TGCCAGATGACTTCCAGACCGCGGAGTACCTGCTTGACAAGGGCCTGC
TCGACCTGGTGGTGCCGCGCAGCTTCCTGAAGGGCGCGCTGTTTGAGAT
CATCGACTTTTACAAGAACGCACCCTACAAGCGCCGCGGCAAGATTCC
ATTTGGCGTGCAGCGCGGTACGTACGGCCTGACCGCTGAGGAGAAGAT
GCGGCGCAGGTGGAGGGAGTGGAGCTCAGCTGGCAGCAACGGCTCGG
GCACGCCCGCGCTGGCAGCAGCAGCAGCAGTGGTGGCGCCGTGCAGCA
GTGGAGGAGTTGCATGCGCACTGAGACGAGCTTGTTCAAGAGTTAGTC
GGATGGGCGGGGTGGGGAGCTTGCTACGCTGCTAG

FIG. 20

ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGC
CACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCG
CTTGCAGGAACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAAC
CCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCC
ATTTCTGTGGGTGCTATGGACAAGGACTCCAAGGGCTCTTCCAAGCCTG
TTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGA
TTCTCTACATCAAGCACCTGAAGGAGCACCACCACATCTGCTTCGGCTG
CAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGAT
CGACCCAGGCTCATGGCGCCCCTTTGACGAGACGCTGTCTCCCTGCGAC
CCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGAC
AGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGCACGGG
CCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTC
ATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCT
GATTGAGTACGCCACGCAGGAGGGGCTCACGCTGCTGGTGGTGTGCAC
CAGCGGAGGCGCGCGCATGCAGGAGGGCATCATGAGCCTGATGCAGAT
GGCCAAGATCAGCGGCGCGCTGCACGTGCACCAGAATGAGGCCAACCT
GCTGTACATCTCCATCCTGACCAGCCCCACCACAGGTGGCGTGACCGCA
AGCTTTGGCATGCTGGGGGATGTCATCATTGCTGAGCCGCAGGCCATCA
TCGGCTTTGCAGGACGGCGTGTGATCGAGCAGACGCTGCGTGAGGAGC
TGCCAGATGACTTCCAGACCGCGGAGTACCTGCTTGACAAGGGCCTGC
TCGACCTGGTGGTGCCGCGCAGCTTCCTGAAGGGCGCGCTGTTTGAGAT
CATCGACTTGTACAAGAAAGCACCCCCCAAGCGGCGGGGCAAGATTCC
ATTTGGCGTGCATAGCGGTACGTACGGCCAACCGCCGAGGAGAAGATC
CGGCGCAGGTGGAGGGAGGGGAGTTCAGCTGGCAGCAACGGGTGGGG
CACGCCCGCGCTGGCAGCAGCAGCAGGGGGGCGGTGCGGGTTTTG
GCGCCAAGCCATTCCAGGGGGTTGGTATATGTGACAGCAGCCTGTTTG
GTCACAGTCTGGATGGTGCGGCATAA

FIG. 21

ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGC
CACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCG
CTTGCAGGAACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAAC
CCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCC
ATTTCTGTGGGTGCTATGGACAAGGACTCCAAGGGCTCTTCCAAGCCTG
TTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGA
TTCTCTACATCAAGCACCTGAAGGAGCACCACCACATCTGCTTCGGCTG
CAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGAT
CGACCCAGGCTCATGGCGCCCCTTTGACGAGACGCTGTCTCCCTGCGAC
CCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGAC
AGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGCACGGG
CCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTC
ATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCT
GATTGAGTACGCCACGCAGGAGGGGCTCACGCTGCTGGTGGTGTGCAC
CAGCGGAGGCGCGCGCATGCAGGAGGGCATCATGAGCCTGATGCAGAT
GGCCAAGATCAGCGGCGCGCTGCACGTGCACCAGAATGAGGCCAACCT
GCTGTACATCTCCATCCTGACCAGCCCCACCACAGGTGGCGTGACCGCA
AGCTTTGGCATGCTGGGGGATGTCATCATTGCTGAGCCGCAGGCCATCA
TCGGCTTTGCAGGACGGCGTGTGATCGAGCAGACGCTGCGTGAGGAGC
TGCCAGATGACTTCCAGACCGCGGAGTACCTGCTTGACAAGGGCCTGC
TCGACCTGGTGGTGCCGCGCAGCTTCCTGAAGGGCGCGCTGTTTGAGAT
CATCGACTTTTACAAGAACGCACCCTGCAAGCGCCGCGGCAAGATTCC
ATTTGGCGTGCAGCGCGGTACGTACGGCCTGACCGCTGAGGAGAAGAT
GCGGCGCAGGTGGAGGGAGTGGAGCTCAGCTGGCAGCAACGGCTCGG
GCACGCCCGCGCTGGCAGCAGCAGCAGCAGAGCTGAGAGAGGGCAGC
GTGCTGCTAGCAGGCGTCTGTTGTTAA

FIG. 22

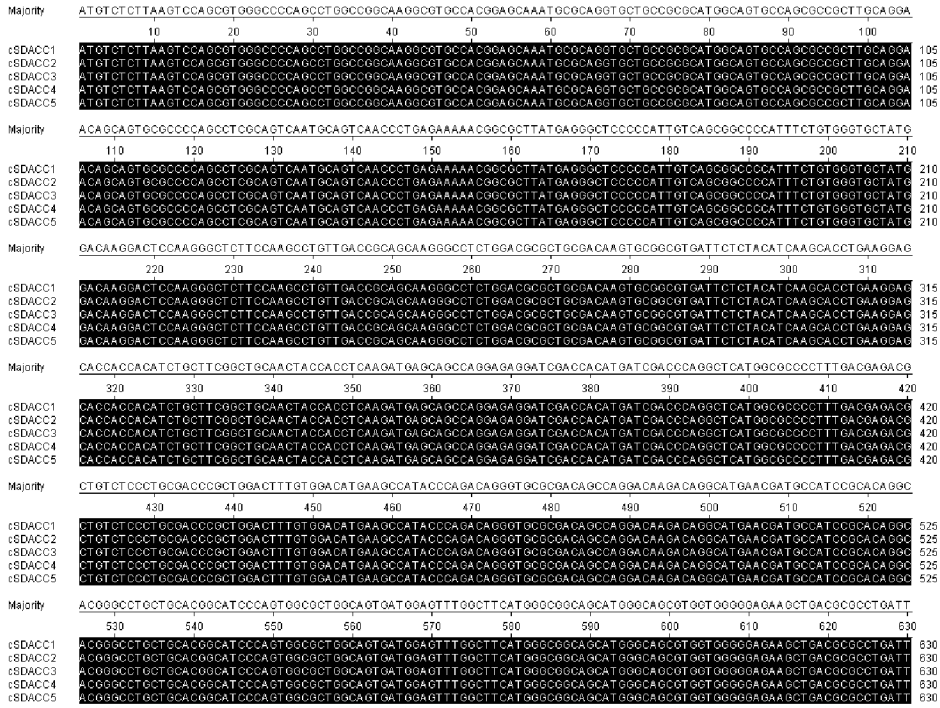

Majority ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGCCACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCGCTTGCAGGA
cSDACC1 ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGCCACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCGCTTGCAGGA 105
cSDACC2 ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGCCACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCGCTTGCAGGA 105
cSDACC3 ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGCCACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCGCTTGCAGGA 105
cSDACC4 ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGCCACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCGCTTGCAGGA 105
cSDACC5 ATGTCTCTTAAGTCCAGCGTGGGCCCCAGCCTGGCCGGCAAGGCGTGCCACGGAGCAAATGCGCAGGTGCTGCCGCGCATGGCAGTGCCAGCGCCGCTTGCAGGA 105
Majority ACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAACCCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCCATTTCTGTGGGTGCTATG
cSDACC1 ACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAACCCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCCATTTCTGTGGGTGCTATG 210
cSDACC2 ACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAACCCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCCATTTCTGTGGGTGCTATG 210
cSDACC3 ACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAACCCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCCATTTCTGTGGGTGCTATG 210
cSDACC4 ACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAACCCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCCATTTCTGTGGGTGCTATG 210
cSDACC5 ACAGCAGTGCGCCCCAGCCTCGCAGTCAATGCAGTCAACCCTGAGAAAAACGGCGCTTATGAGGGCTCCCCCATTGTCAGCGGCCCCATTTCTGTGGGTGCTATG 210
Majority GACAAGGACTCCAAGGGCTCTTCCAAGCCTGTTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGATTCTCTACATCAAGCACCTGAAGGAG
cSDACC1 GACAAGGACTCCAAGGGCTCTTCCAAGCCTGTTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGATTCTCTACATCAAGCACCTGAAGGAG 315
cSDACC2 GACAAGGACTCCAAGGGCTCTTCCAAGCCTGTTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGATTCTCTACATCAAGCACCTGAAGGAG 315
cSDACC3 GACAAGGACTCCAAGGGCTCTTCCAAGCCTGTTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGATTCTCTACATCAAGCACCTGAAGGAG 315
cSDACC4 GACAAGGACTCCAAGGGCTCTTCCAAGCCTGTTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGATTCTCTACATCAAGCACCTGAAGGAG 315
cSDACC5 GACAAGGACTCCAAGGGCTCTTCCAAGCCTGTTGACCGCAGCAAGGGCCTCTGGACGCGCTGCGACAAGTGCGGCGTGATTCTCTACATCAAGCACCTGAAGGAG 315
Majority CACCACCACATCTGCTTCGGCTGCAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGATCGACCCAGGCTCATGGCGCCCCTTTGACGAGACG
cSDACC1 CACCACCACATCTGCTTCGGCTGCAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGATCGACCCAGGCTCATGGCGCCCCTTTGACGAGACG 420
cSDACC2 CACCACCACATCTGCTTCGGCTGCAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGATCGACCCAGGCTCATGGCGCCCCTTTGACGAGACG 420
cSDACC3 CACCACCACATCTGCTTCGGGCTGCAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGATCGACCCAGGCTCATGGCGCCCCTTTGACGAGACG 420
cSDACC4 CACCACCACATCTGCTTCGGCTGCAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGATCGACCCAGGCTCATGGCGCCCCTTTGACGAGACG 420
cSDACC5 CACCACCACATCTGCTTCGGCTGCAACTACCACCTCAAGATGAGCAGCCAGGAGAGGATCGACCACATGATCGACCCAGGCTCATGGCGCCCCTTTGACGAGACG 420
Majority CTGTCTCCCTGCGACCCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGACAGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGC
cSDACC1 CTGTCTCCCTGCGACCCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGACAGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGC 525
cSDACC2 CTGTCTCCCTGCGACCCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGACAGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGC 525
cSDACC3 CTGTCTCCCTGCGACCCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGACAGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGC 525
cSDACC4 CTGTCTCCCTGCGACCCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGACAGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGC 525
cSDACC5 CTGTCTCCCTGCGACCCGCTGGACTTTGTGGACATGAAGCCATACCCAGACAGGGTGCGCGACAGCCAGGACAAGACAGGCATGAACGATGCCATCCGCACAGGC 525
Majority ACGGGCCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTCATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCTGATT
cSDACC1 ACGGGCCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTCATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCTGATT 630
cSDACC2 ACGGGCCTGCTGCACGGCATCCCAGTGGGGCTGGCAGTGATGGAGTTTGGCTTCATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCTGATT 630
cSDACC3 ACGGGCCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTCATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCTGATT 630
cSDACC4 ACGGGCCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTCATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCTGATT 630
cSDACC5 ACGGGCCTGCTGCACGGCATCCCAGTGGCGCTGGCAGTGATGGAGTTTGGCTTCATGGGCGGCAGCATGGGCAGCGTGGTGGGGGAGAAGCTGACGCGCCTGATT 630

FIG. 23

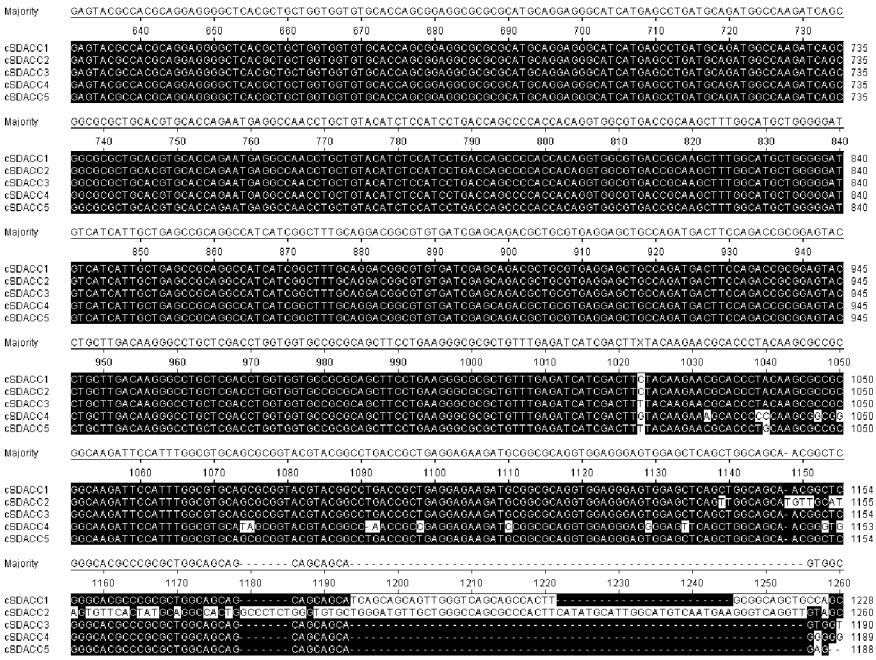


FIG. 23 (cont'd)

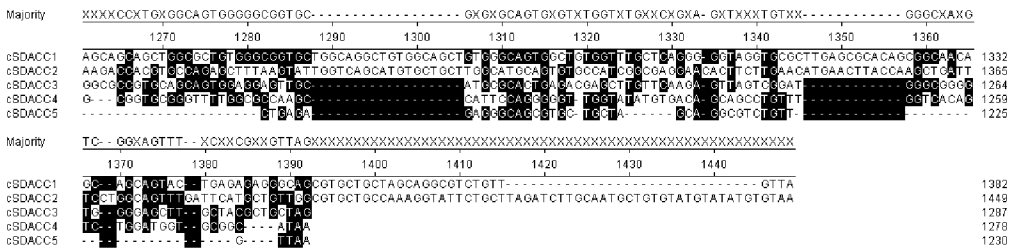


FIG. 23 (cont'd)

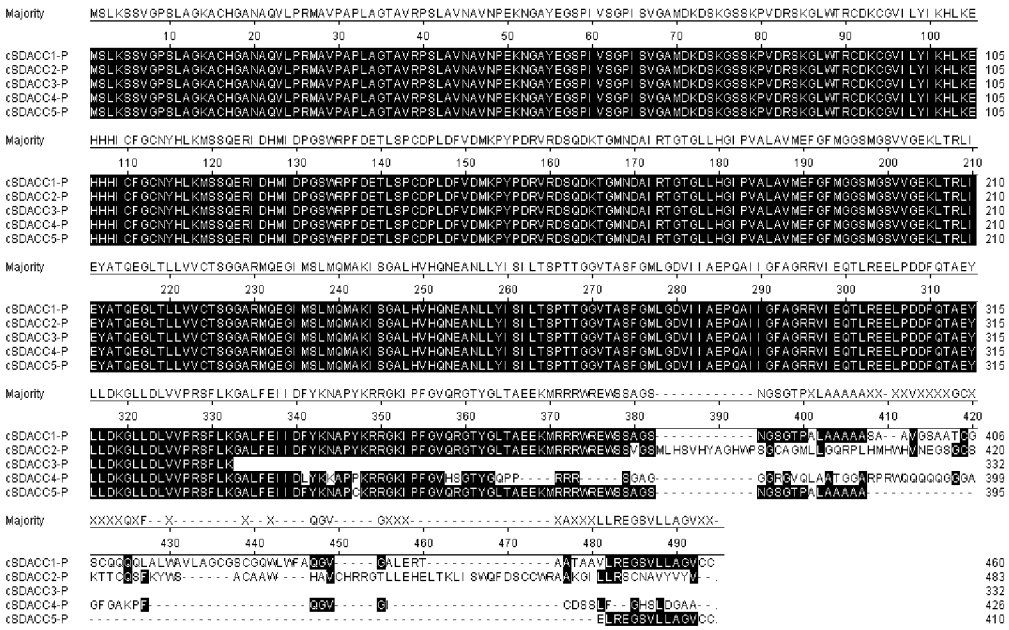


FIG. 24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/048666

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/52 (2006.01) ***C07H 21/04*** (2006.01) ***C12N 1/21*** (2006.01)
A01H 1/00 (2006.01) ***C12N 1/15*** (2006.01) ***C12N 15/82*** (2006.01)
A01H 5/00 (2006.01) ***C12N 1/19*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GenomeQuest: SEQ ID NO: 157, 114 & 115.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/087815 A2 (METANOMICS GMBH) 9 August 2007 See abstract and SEQ ID NOs: 4562, 5822, 7693, 12203, 20938 and 53747 which share 100% identity with SEQ ID NO: 157 and 115 of the present application. They also share 74.89% identity with SEQ ID NO: 114 of the present application	1, 3-11, 30 & 31
X	US 2009/0082286 A1 (HUANG, L.L. *et al.*) 26 March 2009 See abstract and SEQ ID NO: 132 which shares 92.33% identity with SEQ ID NO: 157 of the present application, 80.69% identity with SEQ ID NO: 115 of the present application, and 71.58% identity with SEQ ID NO: 114 of the present application	1, 3-11, 30 & 31
A	US 5498544 A (GENGENBACH, B. G. *et al.*) 12 March 1996 See whole document	
A	US 6222099 B1 (GENGENBACH, B.G. *et al.*) 24 April 2001 See whole document	

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
12 January 2011

Date of mailing of the international search report 14 JAN 2011

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/048666

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:
Please see Supplemental Box I

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

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

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-49, 214 & 215 (Inventions 1 & 2)

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

Supplemental Box I
(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box III

The international application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

PCT Rule 13.2 states that where a group of inventions is claimed in one and the same international application, the requirement of unity of invention referred to in Rule 13.1 shall be fulfilled only where there is a technical relationship among those inventions involving one or more of the same corresponding special technical features. Rule 13.2 states that the expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

In assessing whether there is more than one invention claimed, I have given consideration to those features which can be considered to potentially distinguish the claimed combination of features from the prior art. Where different claims have different distinguishing features they define different inventions.

The International Searching Authority has found that there are 20 different inventions as follows:

Invention 1
Claims 1, 2, 4-49, 214 & 215 (completely) and claim 3 (partially) are directed to an isolated amino acid sequence encoding a rat acetyl CoA carboxylase comprising SEQ ID NO: 157. Claims 1, 2, 4-49, 214 & 215 (completely) and claim 3 (partially) are also directed to nucleotide sequences (comprising SEQ ID NO: 115), vectors, methods, transgenic host cells and plastids comprising said sequence. Isolated amino acid sequences encoding an acetyl CoA carboxylase comprising SEQ ID NO: 157 and uses thereof are considered to define a first distinguishing feature. NOTE: we have assumed that claim 3 relates to SEQ ID NO: 115 and not SEQ ID NO: 155.

Invention 2
Claim 3 (partially) is directed to a codon optimised (for expression in the chloroplast genome of *Chlamydomonas reinhardtii*) isolated nucleotide sequence encoding an acetyl CoA carboxylase wherein the sequence comprises SEQ ID NO: 114. Isolated nucleotide sequences encoding an acetyl CoA carboxylase comprising SEQ ID NO: 114 are considered to define a second distinguishing feature.

Inventions 3-19
Claims 50-118 (partially) are directed to isolated amino acid/nucleotide sequences encoding an acetyl CoA carboxylase comprising SEQ ID NO: 15-24 & 163-169. Claims 50-118 (partially) are also directed to nucleotide sequences, vectors, methods, transgenic host cells and plastids comprising said sequences. Isolated amino acid/nucleotide sequences encoding an acetyl CoA carboxylase comprising SEQ ID NO: 15-24 & 163-169 and uses thereof are considered to define a third to nineteenth distinguishing feature, wherein each isolated amino acid/nucleotide sequence comprising one of SEQ ID NO: 15-24 & 163-169 and use thereof is considered to define a separate distinguishing feature.

Invention 20
Claims 119-213 (completely) are directed to isolated amino acid sequences encoding an acetyl CoA carboxylase comprising SEQ ID NO: 11 containing any one of the combinations defined in claim 119. Claims 119-213 are also directed to nucleotide sequences, vectors, methods, transgenic host cells and plastids comprising said sequences. Isolated amino acid sequences encoding an acetyl CoA carboxylase comprising SEQ ID NO: 11 containing any one of the combinations defined in claim 119 and uses thereof are considered to define a twentieth distinguishing feature.

The only feature common to all of the claims is that each amino acid/nucleotide sequence encodes a *mutant* acetyl CoA carboxylase or a sequence capable of transforming a photosynthetic organism. However this concepts is not novel in the light of either of the following documents:

[CONTINUED IN SUPPLEMENTAL BOX II]

Supplemental Box II
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Supplemental Box I

US 5498544 A (GENGENBACH, B. G. *et al.*) 12 March 1996

US 6222099 B1 (GENGENBACH, B.G. *et al.*) 24 April 2001

Each of these documents disclose expression cassettes encoding an acetyl CoA carboxylase, *or a functional mutant thereof*, and methods for conferring herbicide tolerance and/or altering the oil content of plants by introducing and expressing a plant acetyl CoA carboxylase gene in plant cells.

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art.

As a result of the common feature not satisfying the requirement for being a special technical feature, it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention *a posteriori*.

Accordingly, because the claims do not define inventions which share a special technical feature or single inventive concept, there is a lack of unity.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2007087815	AR	053108	AR	058785	AU	2005321630
		AU	2005339243	BR	PI0512818	BR	PI0519994
		CA	2559760	CA	2585798	CN	101421412
		CN	101675069	EP	1765857	EP	1974049
		EP	2080769	EP	2096177	EP	2199304
		MX	2007007040	WO	2006069610		
US	2009082286	US	7772444	WO	2006125000		
US	5498544	AU	41737/97	BR	9608013	CA	2217367
		EP	0820513	MX	9707681	US	4826331
		US	4911564	US	5162602	US	5290696
		US	5428001	US	6069298	US	6146867
		US	6222099	US	6268550	US	6414222
		WO	9631609	WO	9808963		

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX