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(54) **BIOCONVERSION OF SHORT-CHAIN HYDROCARBONS TO FUELS AND CHEMICALS**

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C12N 9/88	(2006.01)
C12N 9/10	(2006.01)
C12N 9/90	(2006.01)
C12P 7/40	(2006.01)
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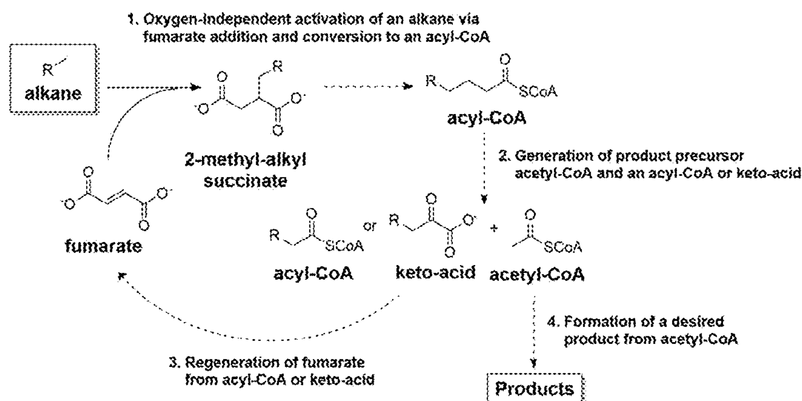
C12P 13/00	(2006.01)
C12P 13/04	(2006.01)

(52) **U.S. Cl.**

CPC ***C12P 19/32*** (2013.01); ***C12P 13/04*** (2013.01); ***C12Y 114/15003*** (2013.01); ***C12N 9/0006*** (2013.01); ***C12Y 101/01*** (2013.01); ***C12N 9/0008*** (2013.01); ***C12Y 102/01*** (2013.01); ***C12N 9/93*** (2013.01); ***C12Y 602/01*** (2013.01); ***C12N 9/88*** (2013.01); ***C12Y 401/00*** (2013.01); ***C12N 9/13*** (2013.01); ***C12Y 208/03*** (2013.01); ***C12N 9/90*** (2013.01); ***C12Y 504/99*** (2013.01); ***C12Y 401/01*** (2013.01); ***C12Y 604/01003*** (2013.01); ***C12Y 504/99001*** (2013.01); ***C12Y 504/99002*** (2013.01); ***C12Y 602/01005*** (2013.01); ***C12N 9/001*** (2013.01); ***C12Y 103/05001*** (2013.01); ***C12Y 103/99*** (2013.01); ***C12Y 402/01*** (2013.01); ***C12Y 101/03039*** (2013.01); ***C12Y 402/01002*** (2013.01); ***C12Y 401/03024*** (2013.01); ***C12Y 501/99001*** (2013.01); ***C12Y 401/01047*** (2013.01); ***C12Y 207/01031*** (2013.01); ***C12Y 504/02*** (2013.01); ***C12Y 402/01011*** (2013.01); ***C12Y 102/04001*** (2013.01); ***C12Y 203/01012*** (2013.01); ***C12Y 108/01004*** (2013.01); ***C12Y 203/01054*** (2013.01); ***C12Y 501/99002*** (2013.01); ***C12P 7/40*** (2013.01); ***C12P 7/46*** (2013.01); ***C12P 7/42*** (2013.01); ***C12P 9/00*** (2013.01); ***C12P 7/02*** (2013.01); ***C12P 7/24*** (2013.01); ***C12P 5/02*** (2013.01); ***C12P 5/026*** (2013.01); ***C12P 13/001*** (2013.01); ***C12N 9/0077*** (2013.01)

(57) **ABSTRACT**

An engineered microorganism(s) with novel pathways for the conversion of short-chain hydrocarbons to fuels and chemicals (e.g. carboxylic acids, alcohols, hydrocarbons, and their alpha-, beta-, and omega-functionalized derivatives) is described. Key to this approach is the use of hydrocarbon activation enzymes able to overcome the high stability and low reactivity of hydrocarbon compounds through the cleavage of an inert C—H bond. Oxygen-dependent or oxygen-independent activation enzymes can be exploited for this purpose, which when combined with appropriate pathways for the conversion of activated hydrocarbons to key metabolic intermediates, enables the generation of product precursors that can subsequently be converted to desired compounds through established pathways. These novel engineered microorganism(s) provide a route for the production of fuels and chemicals from short chain hydrocarbons such as methane, ethane, propane, butane, and pentane.



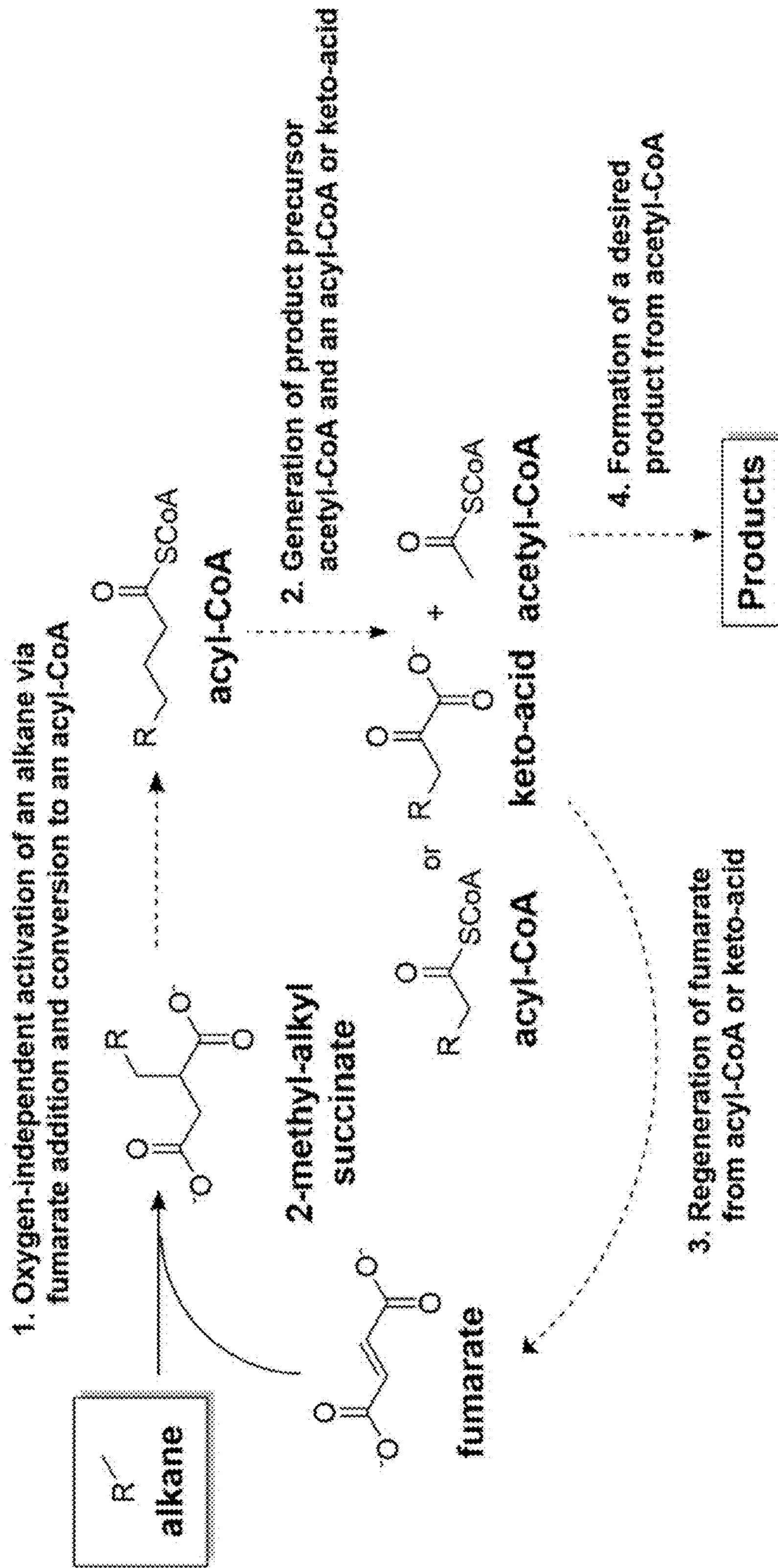


FIG. 1

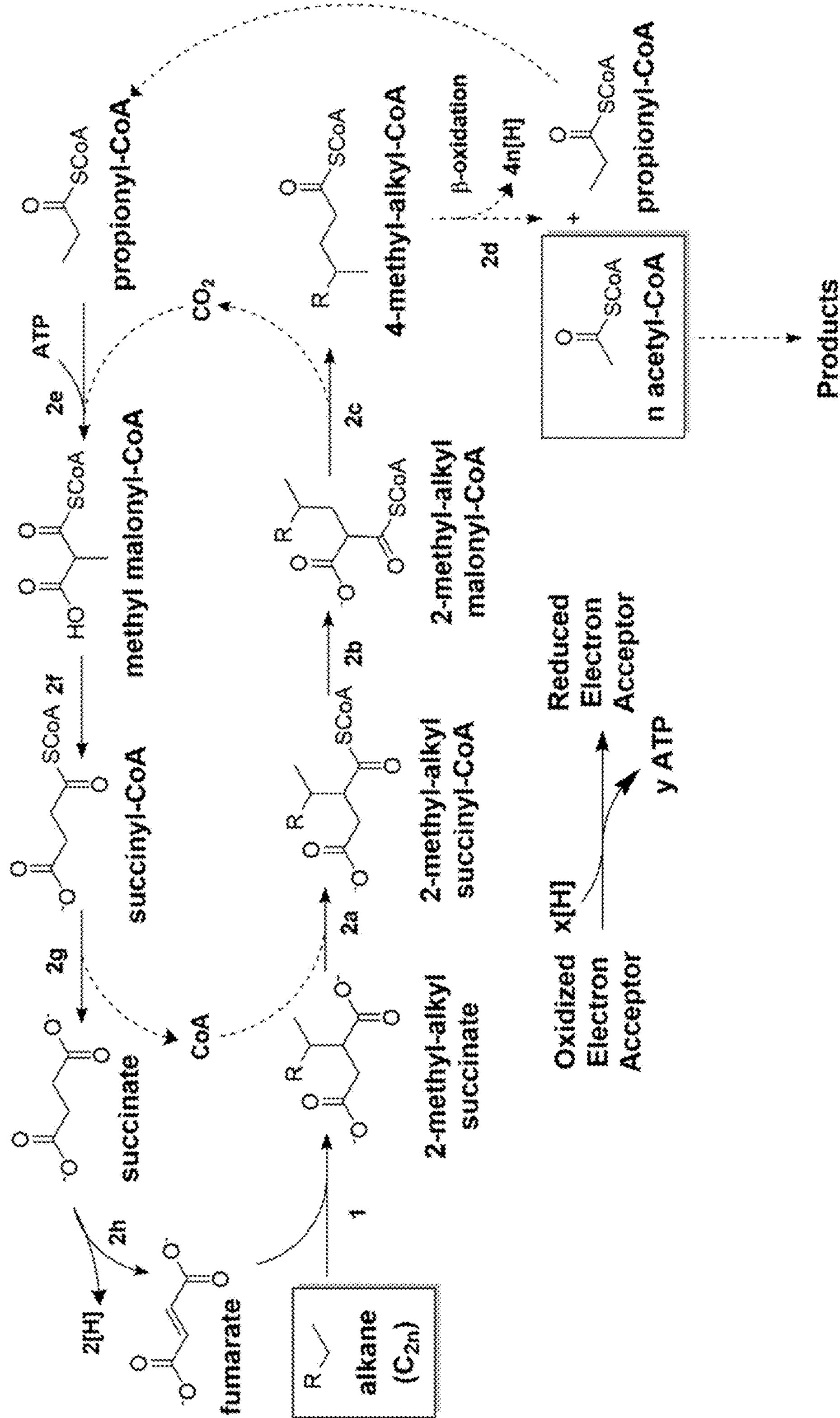


FIG. 2

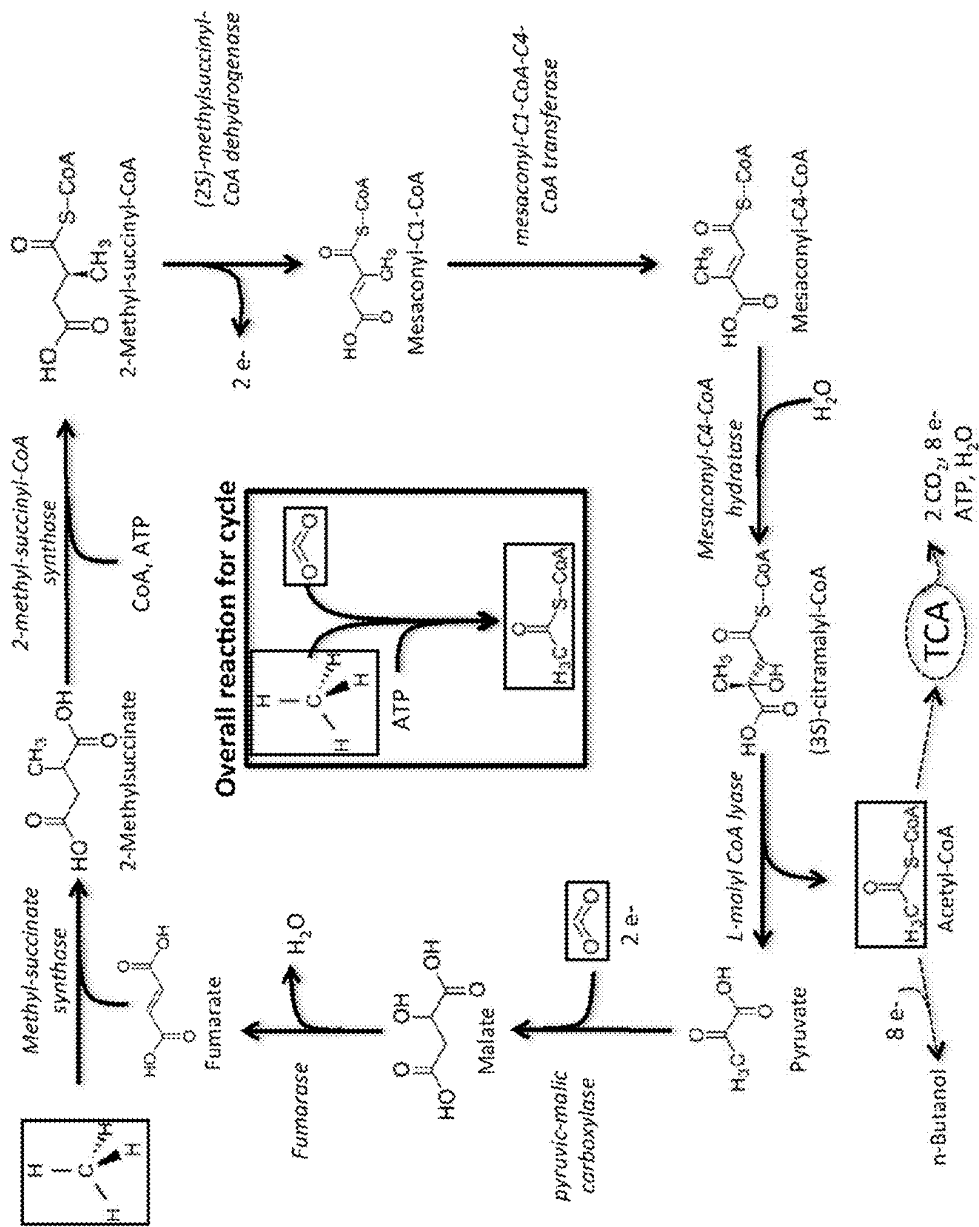


FIG. 3

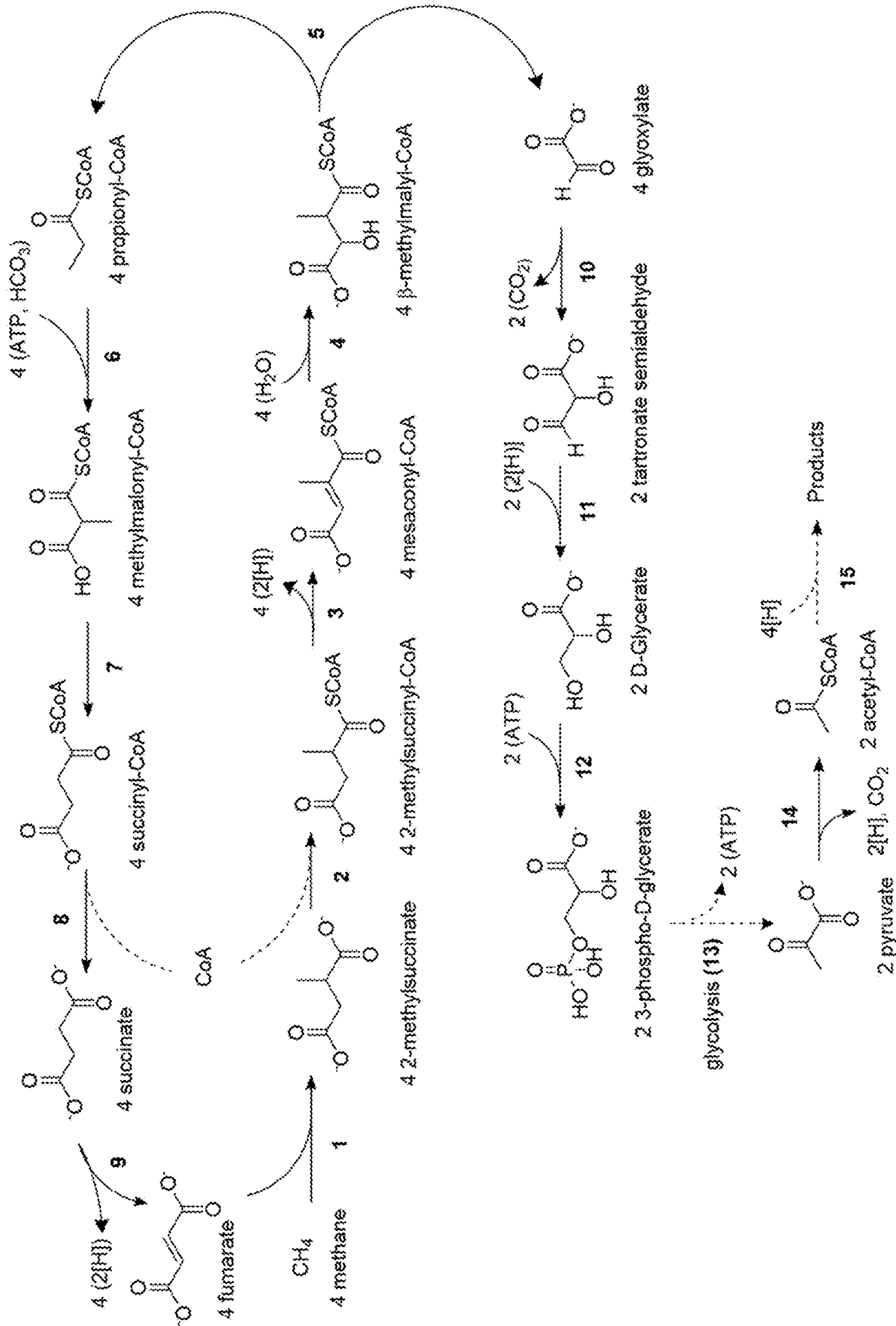


FIG. 4

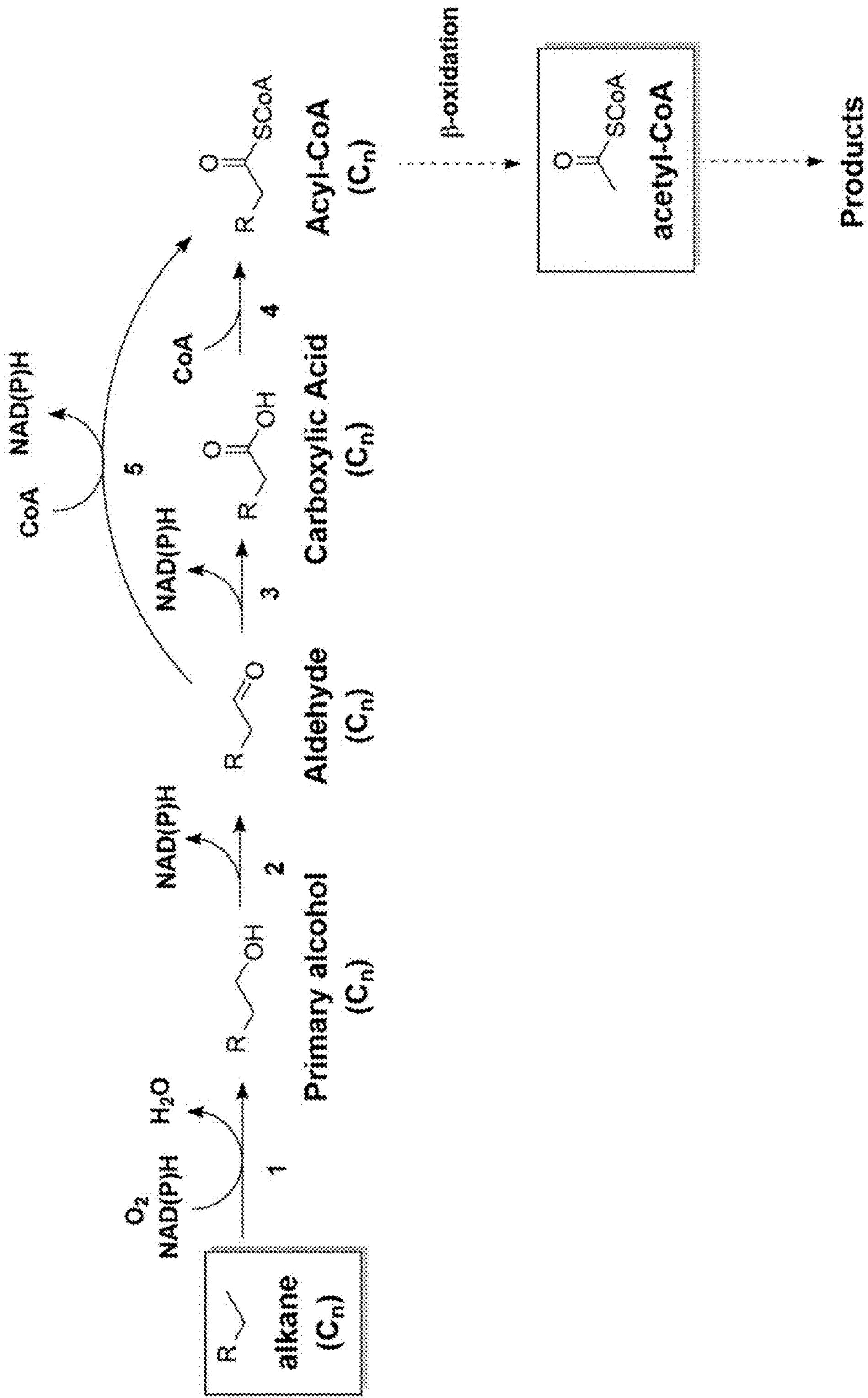


FIG. 5

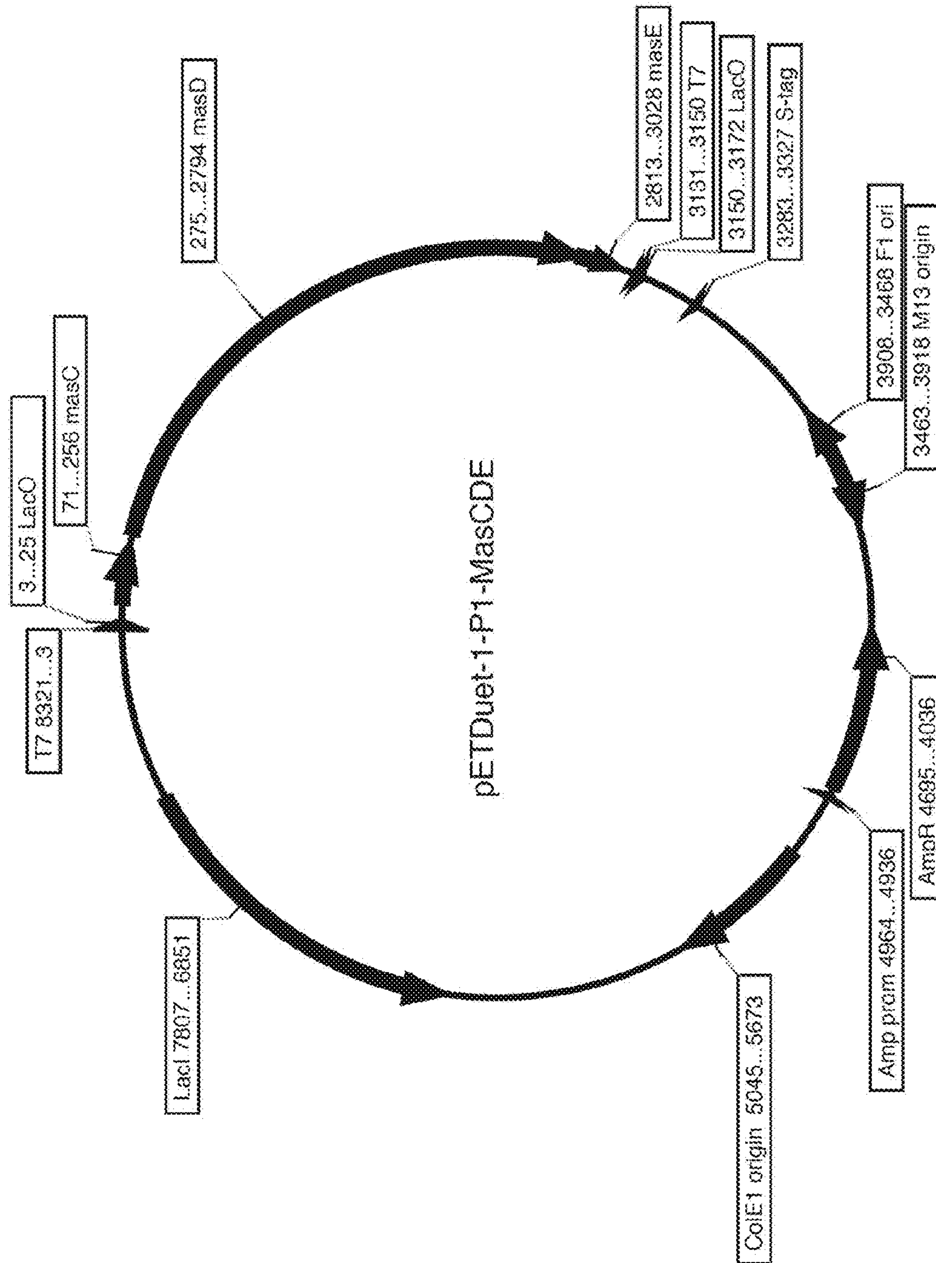


FIGURE 6A

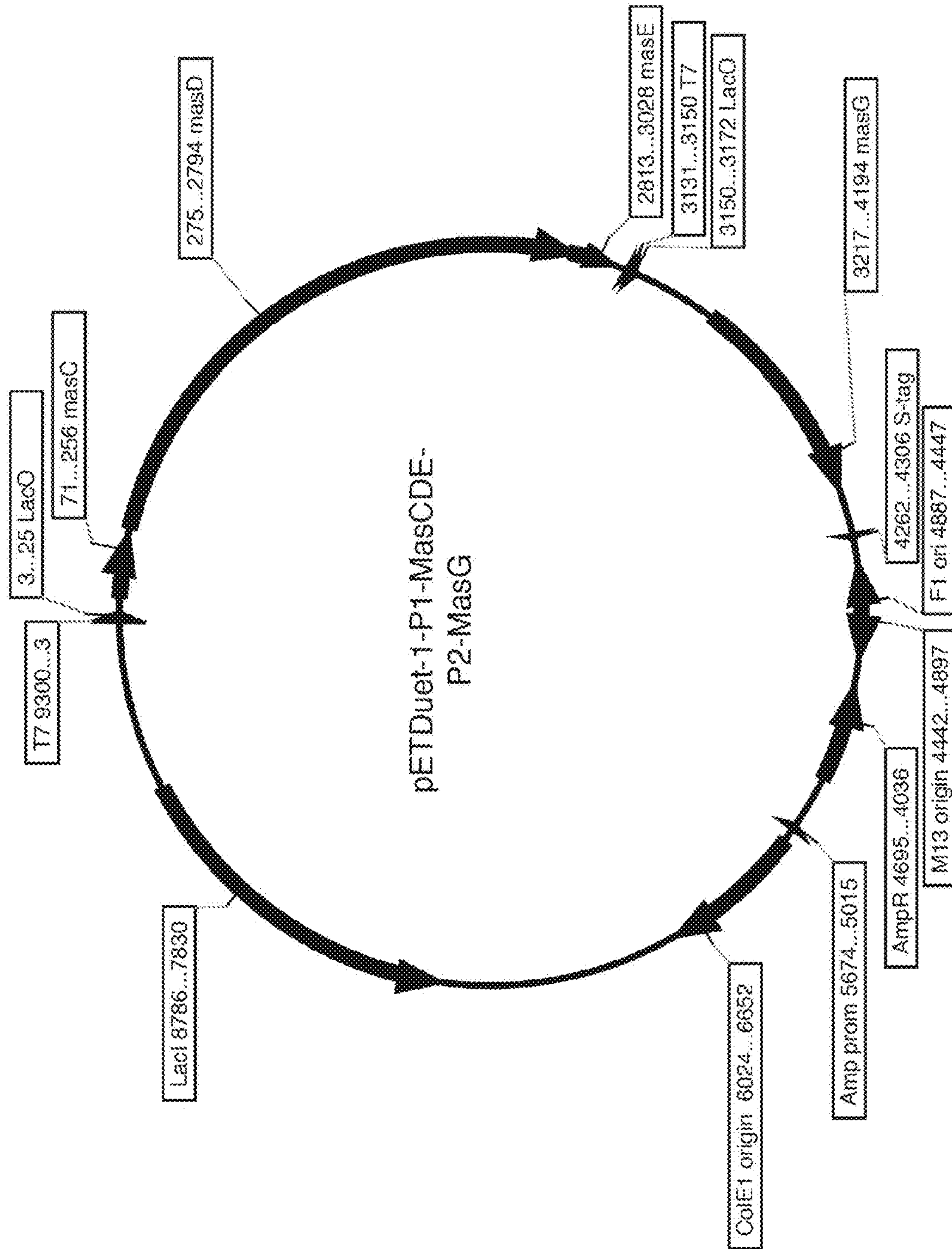


FIGURE 6B

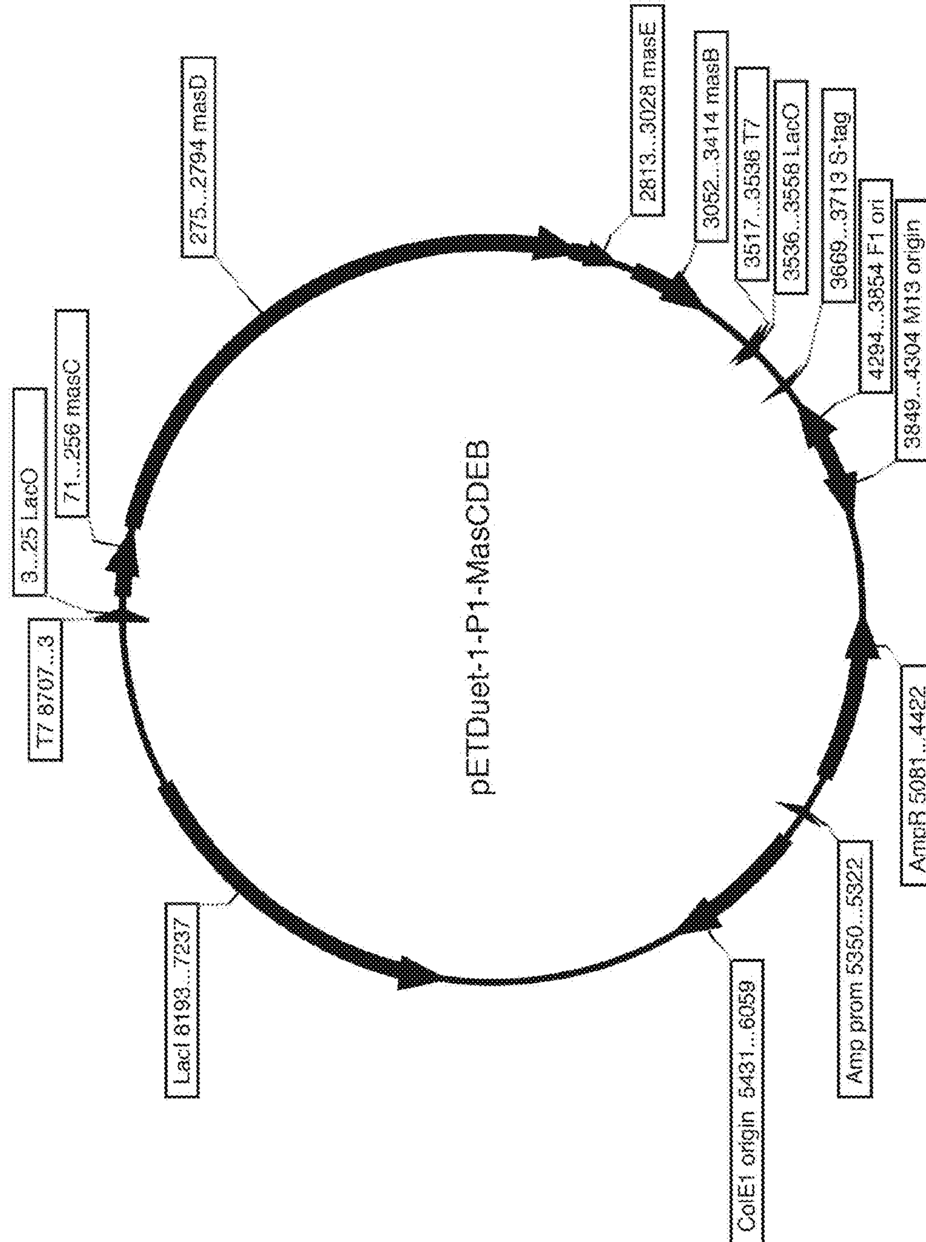


FIGURE 6C

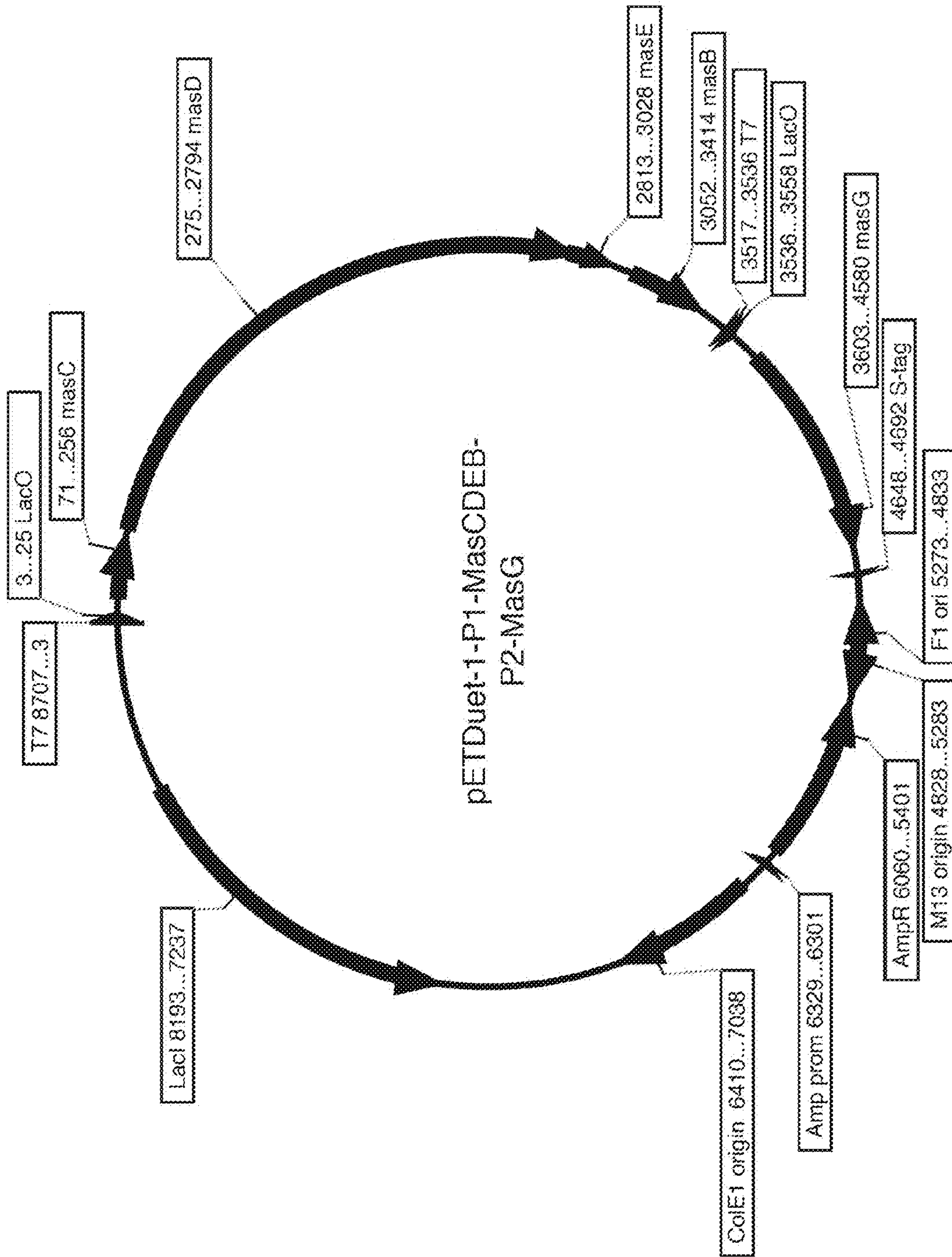


FIGURE 6D

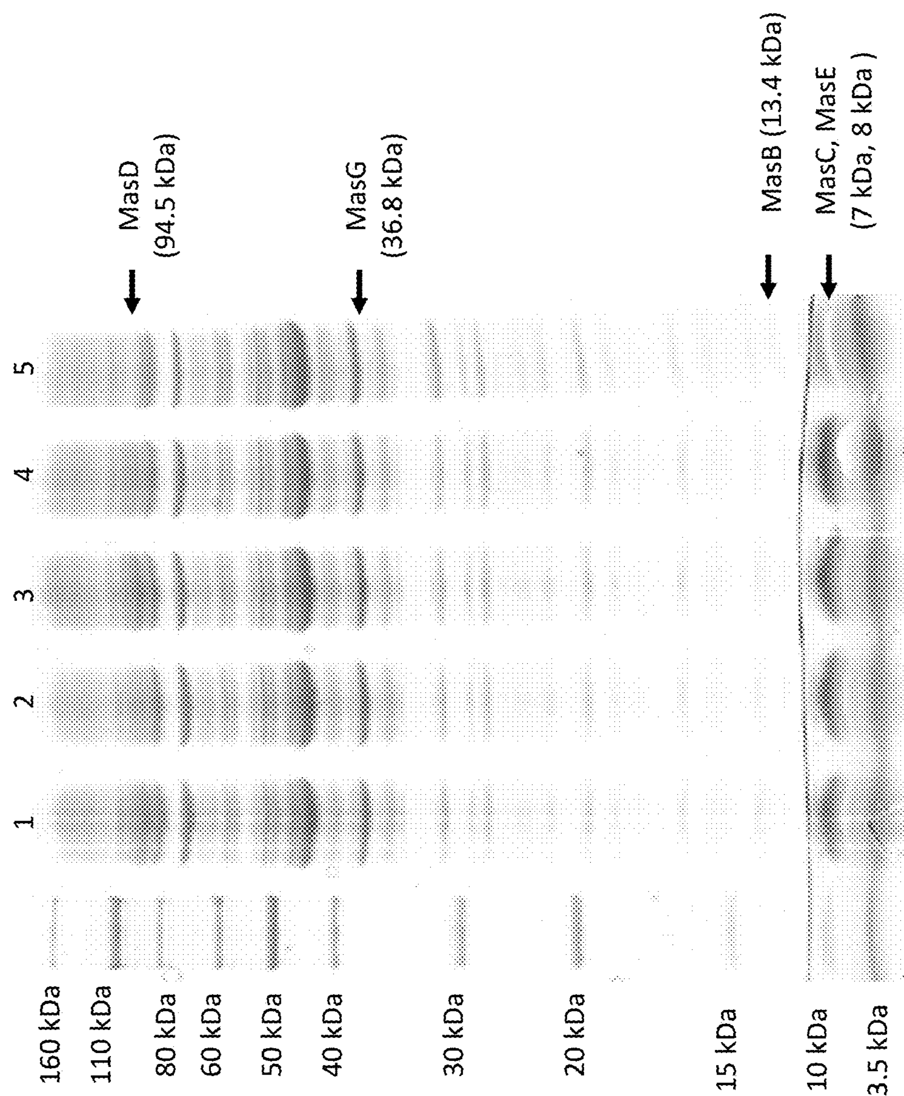


FIG. 7

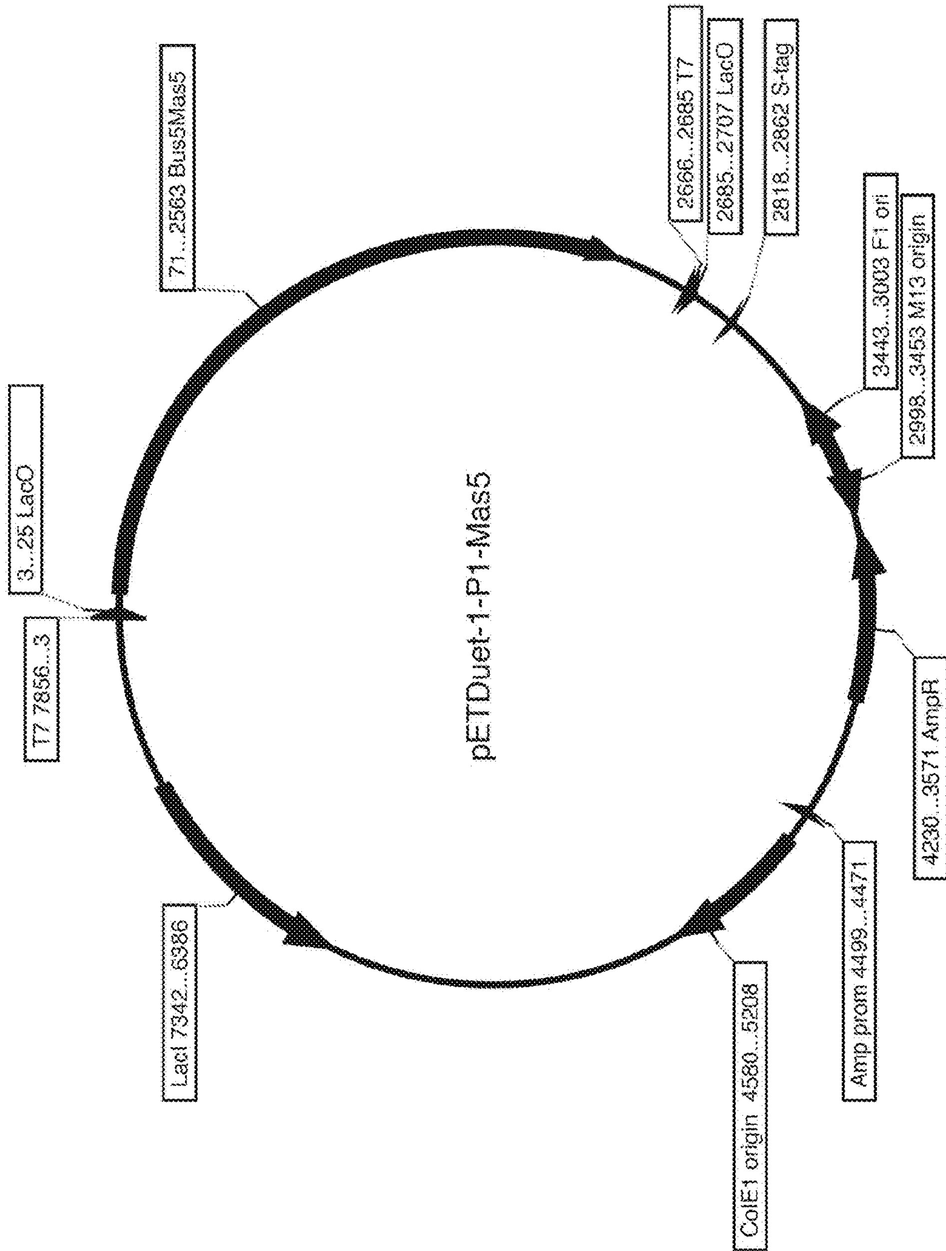


FIGURE 8A

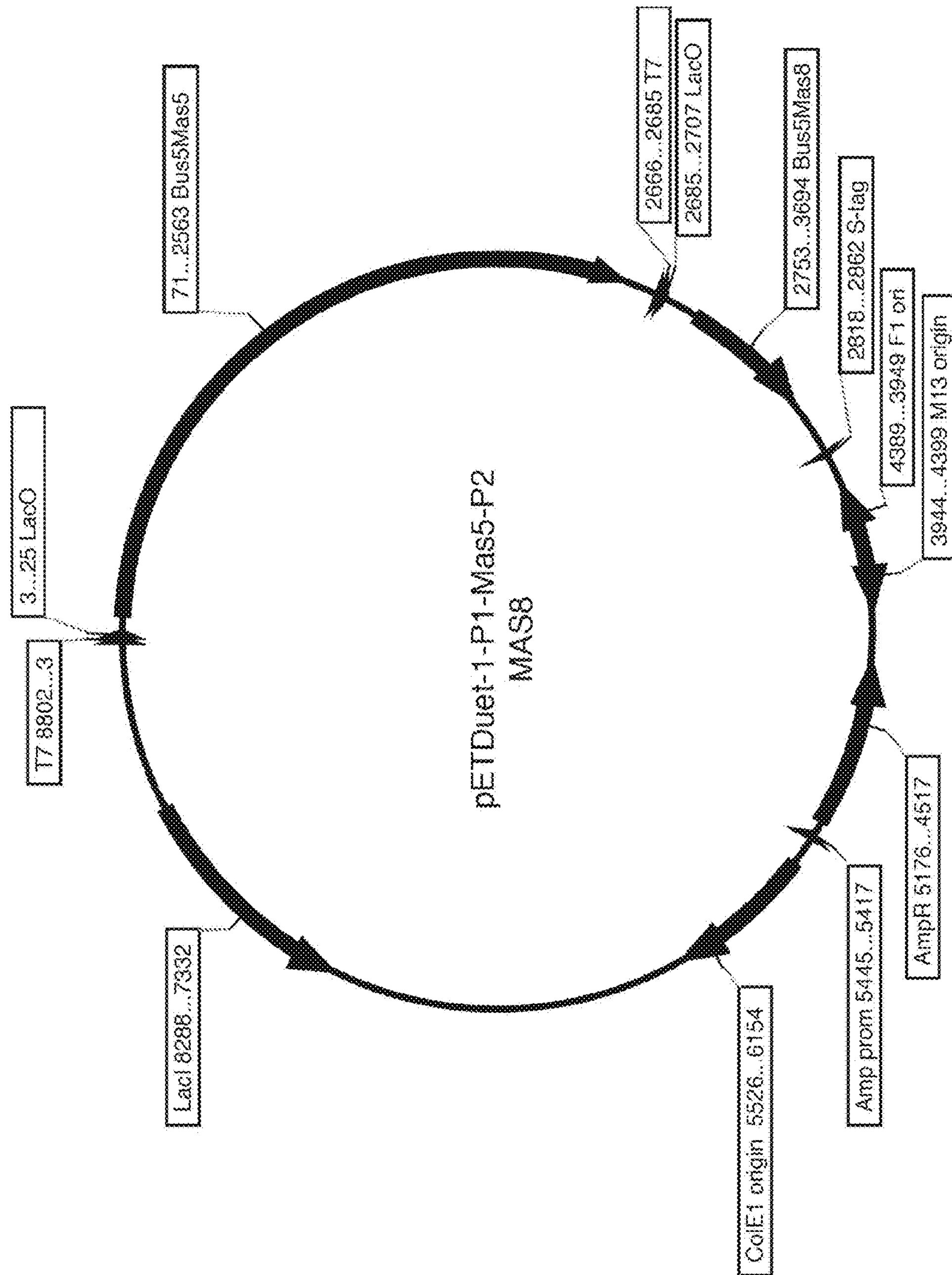


FIGURE 8B

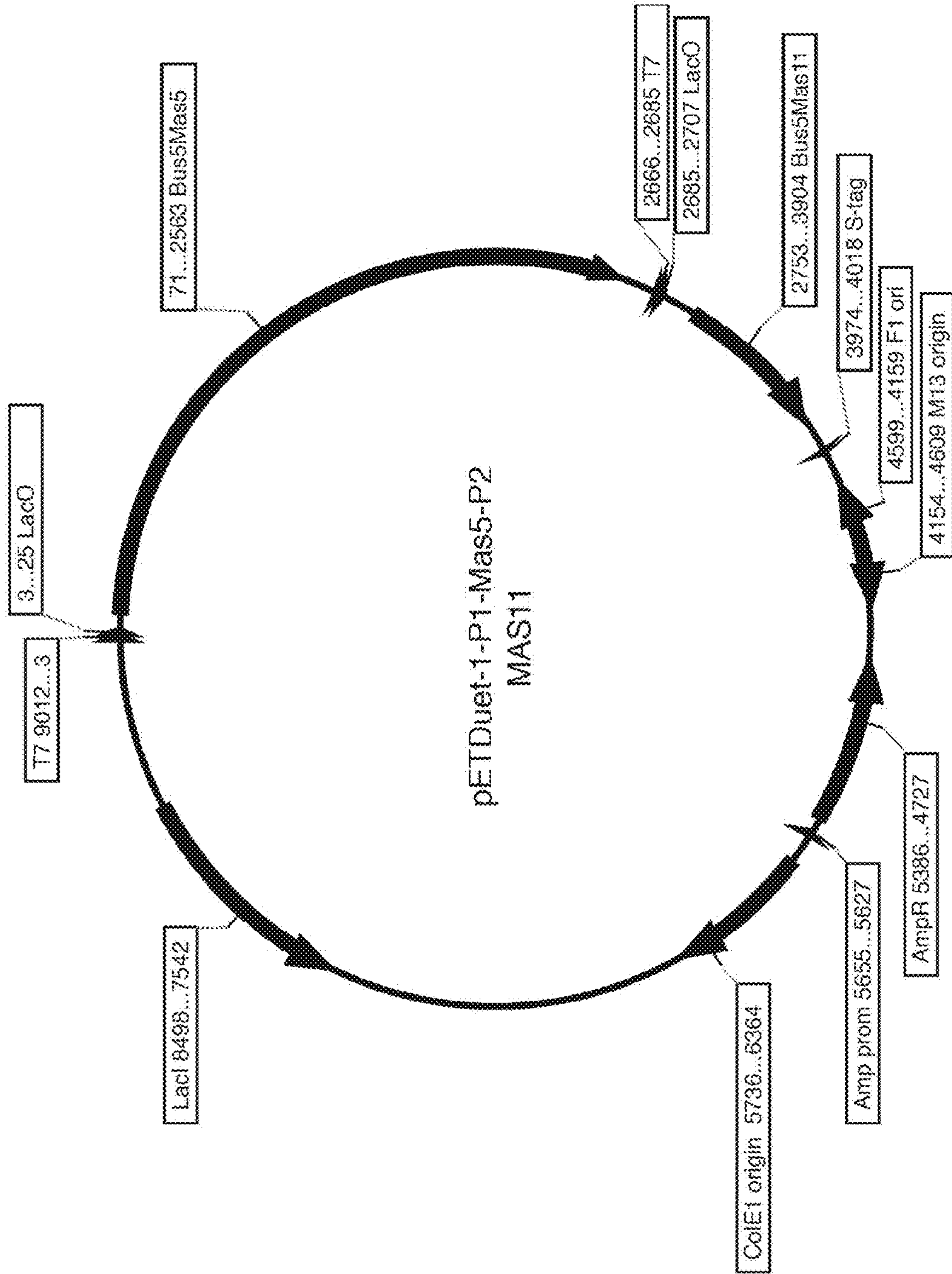


FIGURE 8C

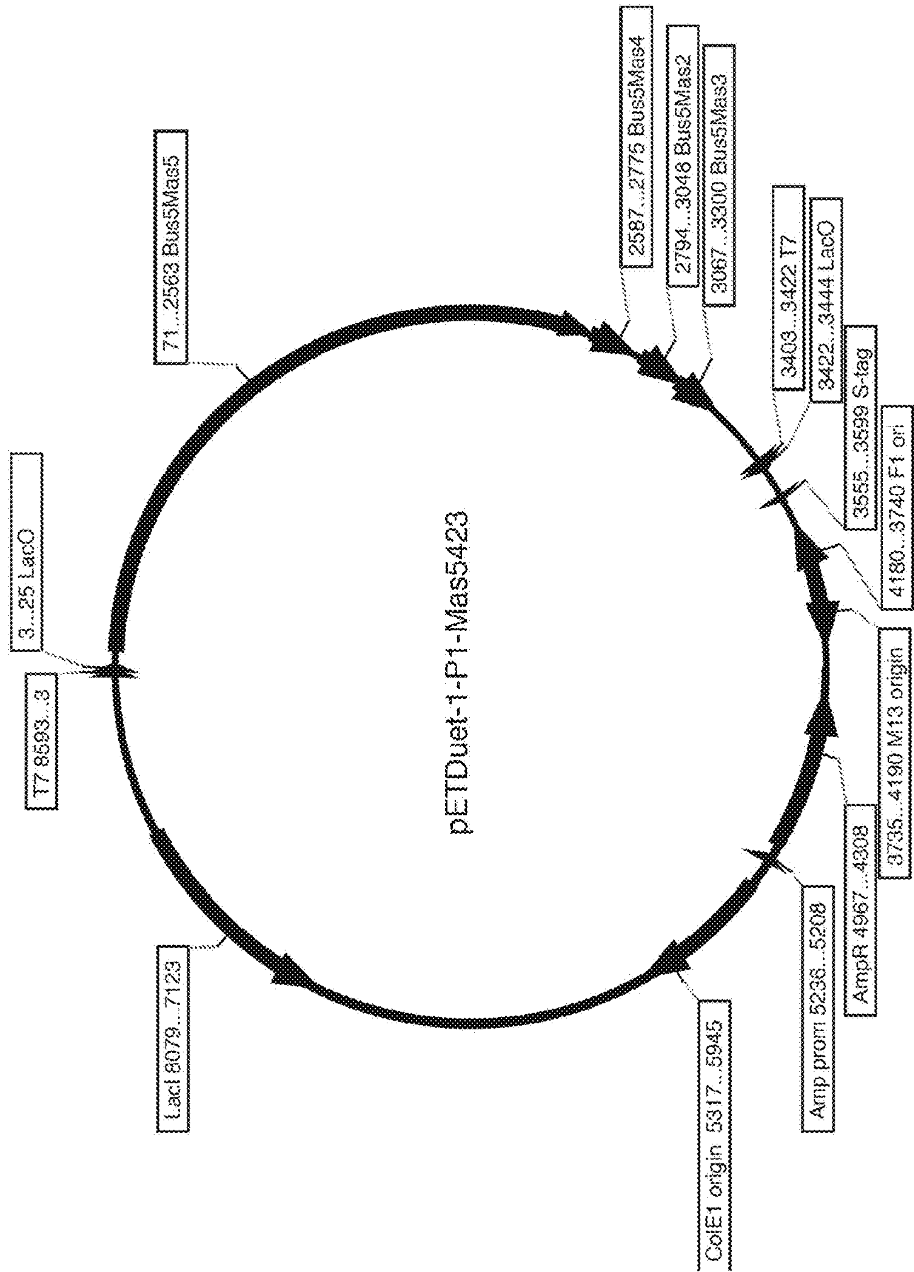


FIGURE 9A

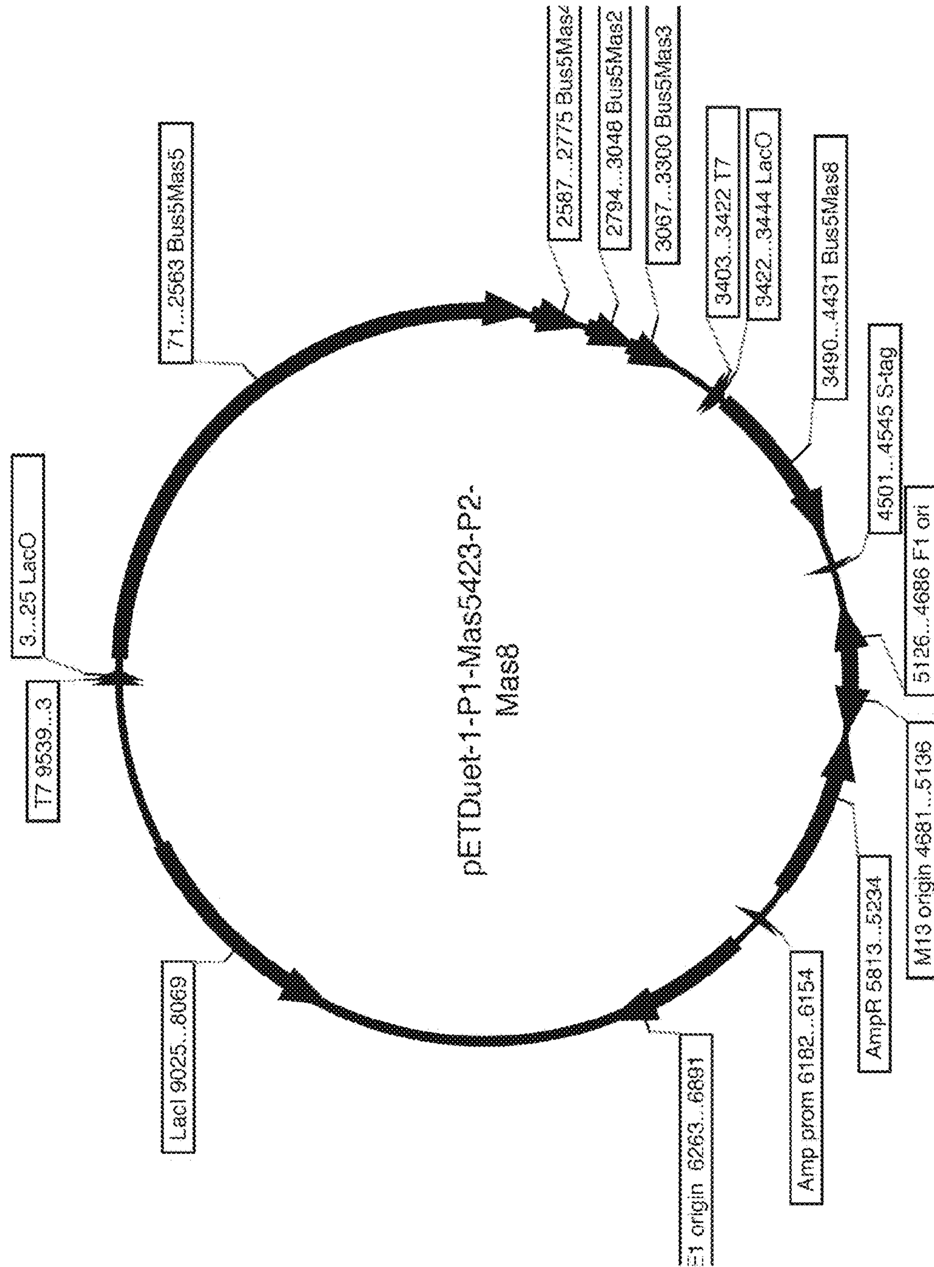


FIGURE 9B

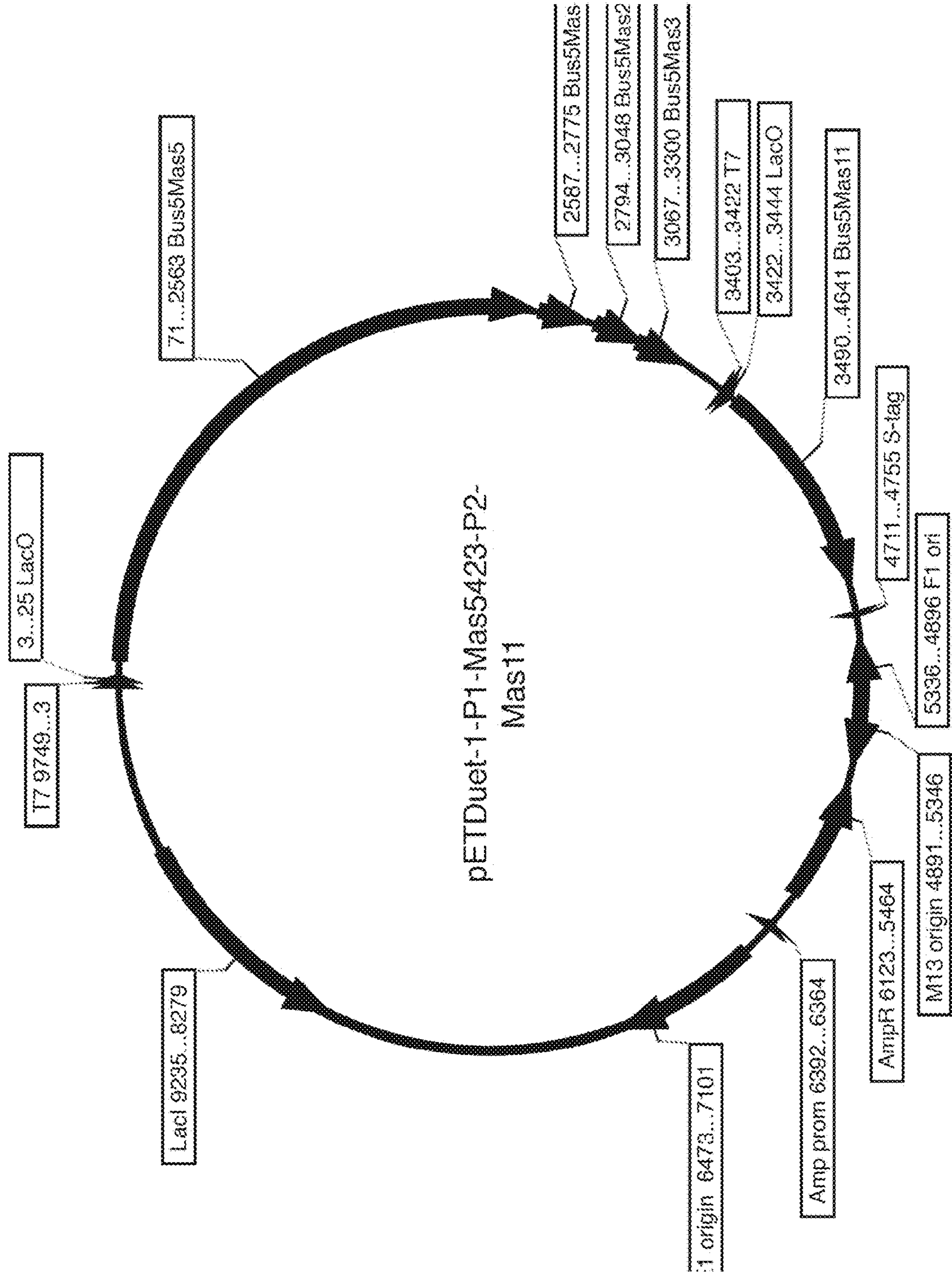


FIGURE 9C

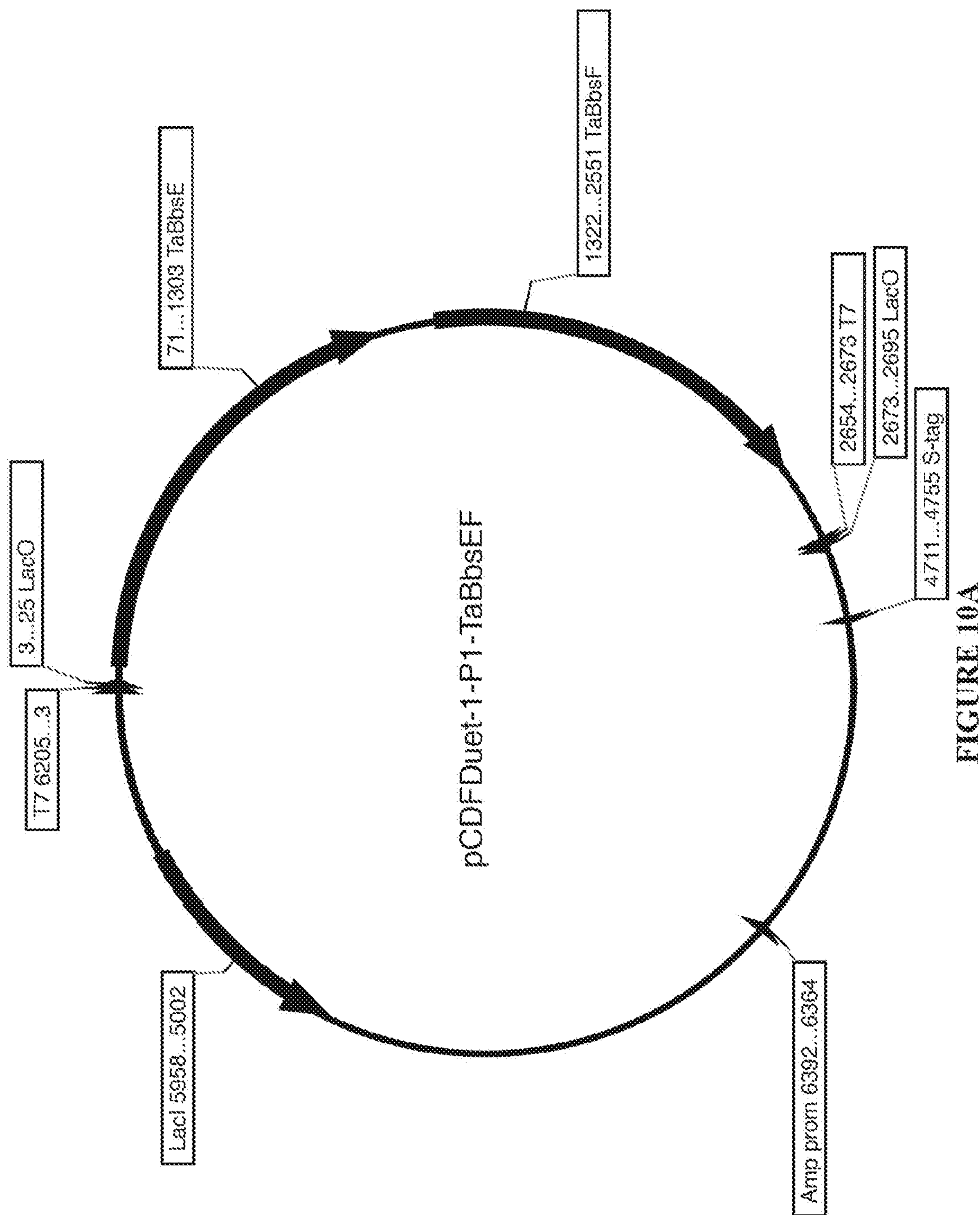


FIGURE 10A

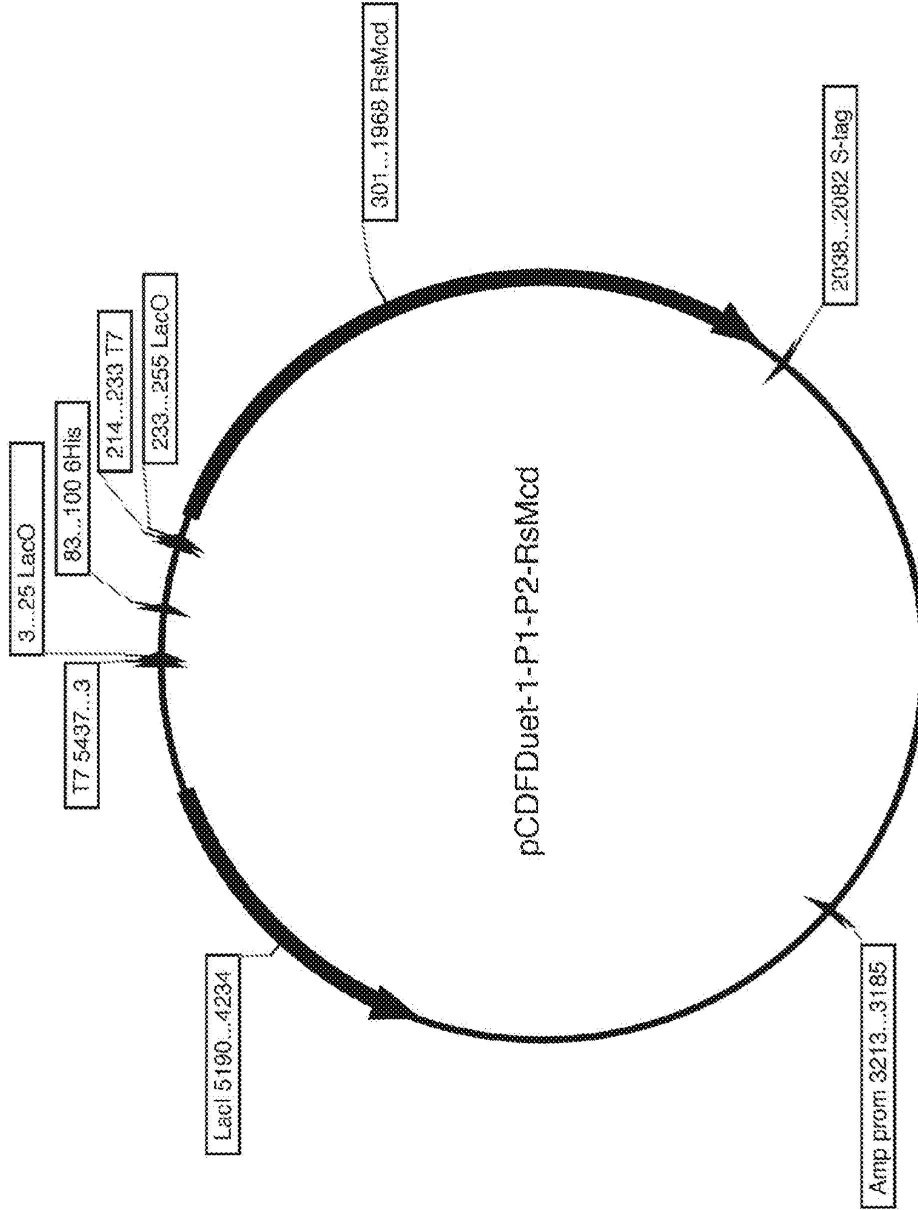


FIGURE 10B

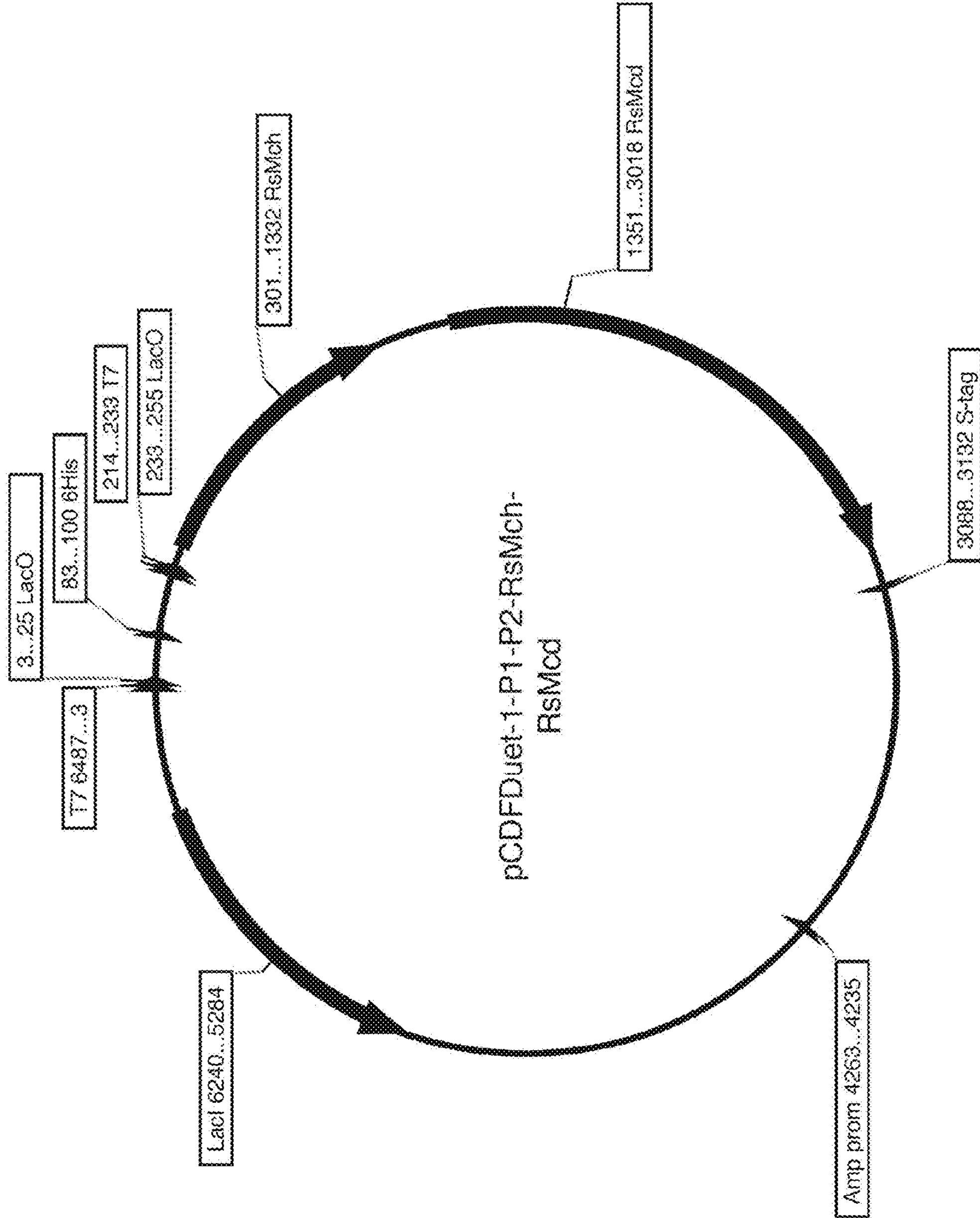


FIGURE 10C

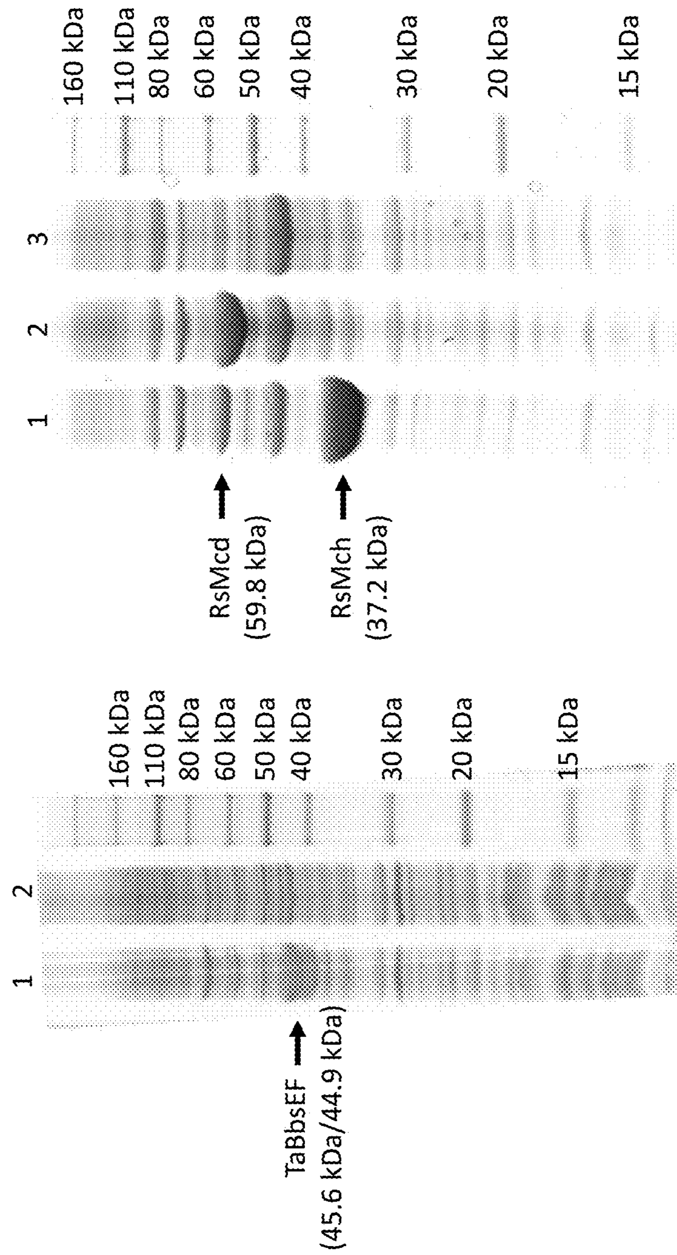


FIG. 11

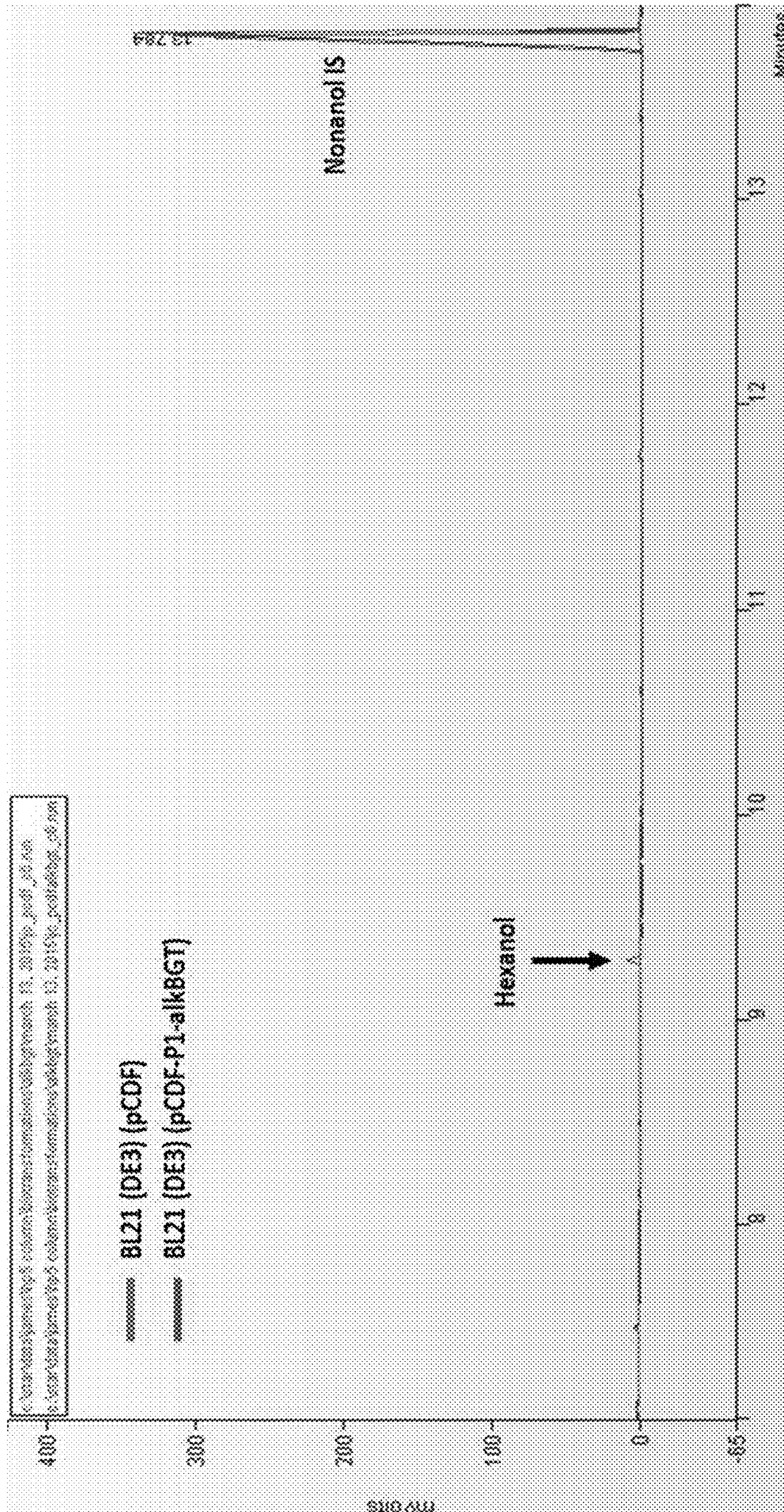


FIG. 12

Nonanol IS

Pentanol Standard
BL21(DE3) (pCDF)
BL21(DE3) (pCDF-P1-alkBGT)

Pentanol

mVolts

10
5
0
-5
-10
-11

8
9
10
11
12
13
Minutes

FIG. 13

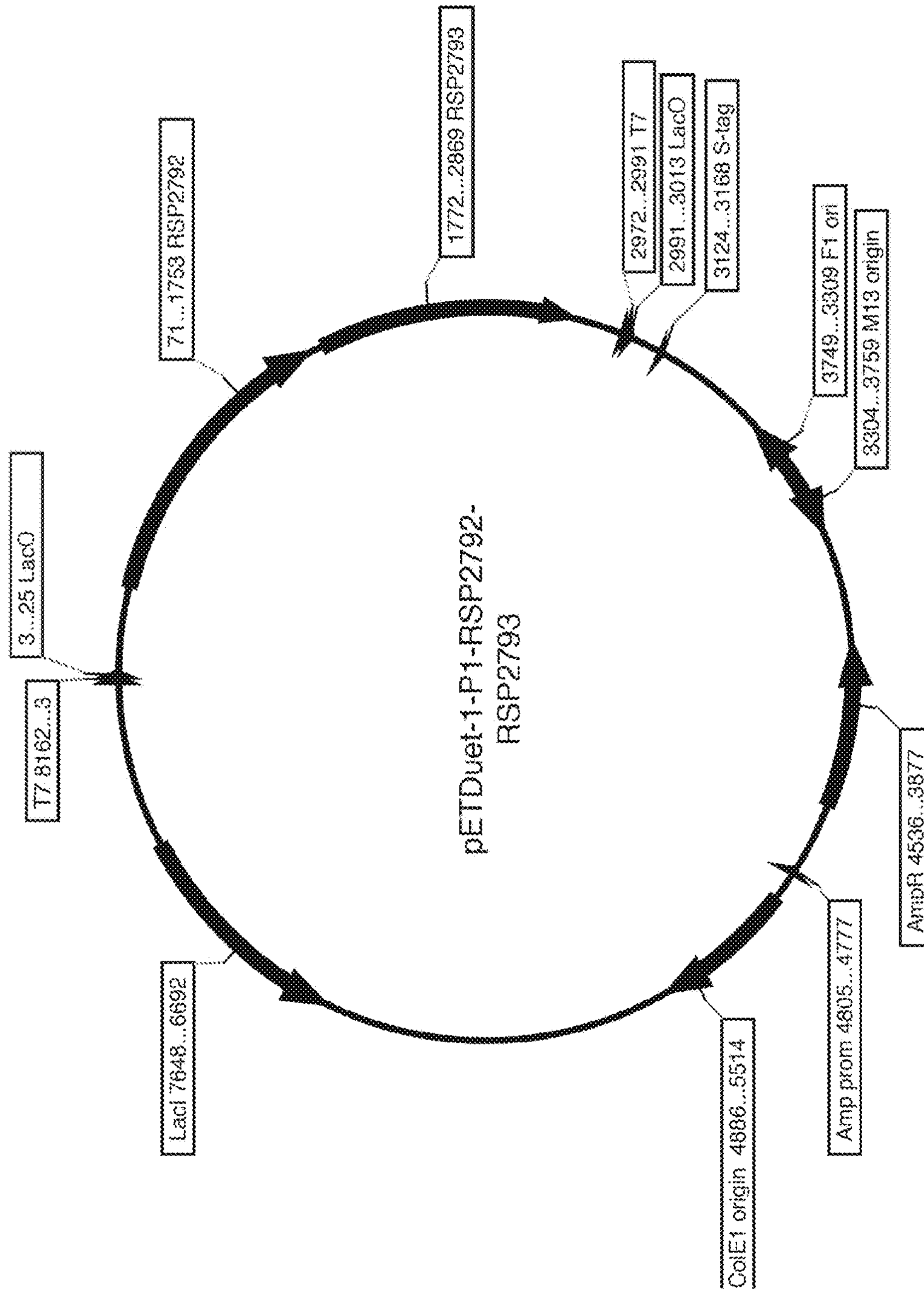


FIGURE 14A

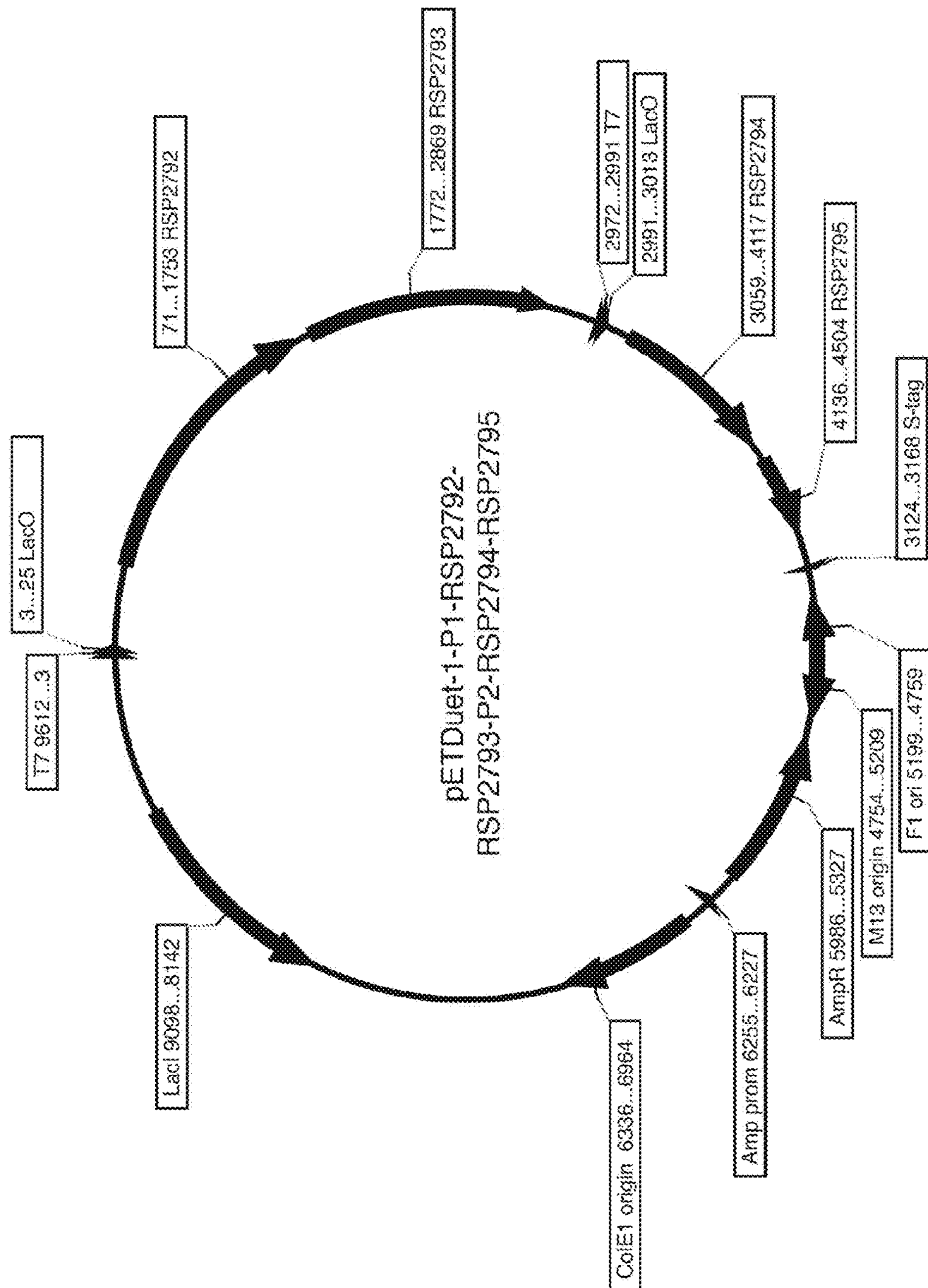


FIGURE 14B

FIGURE 15 TABLE G
A genetically engineered microorganism being a bacteria comprising one or more expression vectors or integrated sequences encoding overexpressed 1) alkane monooxygenase or alkane hydroxylase (EC 1.14.15.3), 2) alcohol dehydrogenase (EC 1.1.1.-), 3) aldehyde dehydrogenase (EC 1.2.1.-), and 4) acyl-CoA synthetase (EC 6.2.1.-); wherein said bacteria is able to convert a short chain alkane (C1-C5, preferably a C1-C4, or C1), to an acyl-CoA.
A genetically engineered microorganism being a bacteria comprising one or more expression vectors or integrated sequences encoding overexpressed 1) alkyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-malonyl-CoA mutase (EC 5.4.99.-), 4) 2-methyl-alkyl-malonyl-CoA decarboxylase (EC 4.1.1.-), 5) propionyl-CoA carboxylase (EC 6.4.1.3), 6) methylmalonyl-CoA epimerase (EC 5.1.99.1), 7) methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), and 9) succinate dehydrogenase (EC 1.3.5.1), wherein said bacteria is able to convert a short chain alkane (C1-C5, preferably a C1-C4, or C1), to an acyl-CoA through fumarate addition to said short chain alkane and subsequent regeneration of said fumarate.
A genetically engineered microorganism being a bacteria comprising one or more expression vectors or integrated sequences encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-C1-CoA-C4-CoA transferase (EC 2.8.3.-), 5) mesaconyl-C4-CoA hydratase (EC 4.2.1.153), 6) L-malyl-CoA/citramalyl-CoA lyase (EC 4.1.3.25), 7) pyruvic-malic carboxylase (EC 1.1.1.39), and 8) fumarase (EC 4.2.1.2), wherein said bacteria is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.
A genetically engineered microorganism being a bacteria comprising one or more expression vectors encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase (EC 4.2.1.148), 5) β-methylmalyl-CoA lyase (EC 4.1.3.24), 6) propionyl-CoA carboxylase (EC 6.4.1.3), 7) methylmalonyl-CoA epimerase (EC 5.1.99.1) and methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), 9) succinate dehydrogenase (EC 1.3.5.1), 10: glyoxylate carboligase (EC 4.1.1.47), 11) tartronate semialdehyde reductase (EC 1.1.1.60), 12) glycerate kinase (EC 2.7.1.31), 13:) glycolytic enzymes (phosphoglycerate mutase (EC 5.4.2.11), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40)), and 14) pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) or pyruvate formate lyase (EC 2.3.1.54), wherein said bacteria is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.
A genetically engineered microorganism being a bacteria being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) alkane monooxygenase or alkane hydroxylase (EC 1.14.15.3), 2) alcohol dehydrogenase (EC 1.1.1.-), 3) aldehyde dehydrogenase (EC 1.2.1.-), and 4) acyl-CoA synthetase (EC 6.2.1.-); wherein said bacteria is able to convert a short chain alkane (C1-C5) to an acyl-CoA.
A genetically engineered microorganism being a bacteria being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) alkyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-malonyl-CoA mutase (EC 5.4.99.-), 4) 2-methyl-alkyl-malonyl-CoA decarboxylase (EC 4.1.1.-), 5) propionyl-CoA carboxylase (EC 6.4.1.3), 6) methylmalonyl-CoA epimerase (EC 5.1.99.1), 7) methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), and 9) succinate dehydrogenase (EC 1.3.5.1), wherein said bacteria is able to converts a short chain alkane (C1-C5) to an acyl-CoA through fumarate addition to said short chain alkane and subsequent regeneration of said fumarate.
A genetically engineered microorganism being a bacteria being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-C1-CoA-C4-CoA transferase (EC 2.8.3.-), 5) mesaconyl-C4-CoA hydratase (EC 4.2.1.153), 6) L-malyl-CoA/citramalyl-CoA lyase (EC 4.1.3.25), 7) pyruvic-malic carboxylase (EC 1.1.1.39), and 8) fumarase (EC 4.2.1.2), wherein said bacteria is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.
A genetically engineered microorganism being a bacteria being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase (EC 4.2.1.148), 5) β-methylmalyl-CoA lyase (EC 4.1.3.24), 6) propionyl-CoA carboxylase (EC 6.4.1.3), 7) methylmalonyl-CoA epimerase (EC 5.1.99.1) and methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-)

FIGURE 15 TABLE G
or succinyl-CoA synthetase (EC 6.2.1.5), 9) succinate dehydrogenase (EC 1.3.5.1), 10: glyoxylate carboligase (EC 4.1.1.47), 11) tartronate semialdehyde reductase (EC 1.1.1.60), 12) glycerate kinase (EC 2.7.1.31), 13:) glycolytic enzymes (phosphoglycerate mutase (EC 5.4.2.11), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40)), and 14) pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) or pyruvate formate lyase (EC 2.3.1.54), wherein said bacteria is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.
A genetically engineered microorganism converting a short-chain (C1-C5) alkane substrate to a product, said microorganism comprising enzymes or overexpressed enzymes or genes encoding same for : a sequence of reactions for the oxygen-independent activation of a short-chain (C1-C5) alkane via fumarate addition to a 2-methyl-alkyl-succinate and subsequent conversion of said 2-methyl-alkyl-succinate to an acyl-CoA; a sequence of reactions for the generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid from said acyl-CoA; a sequence of reactions for the regeneration of fumarate through the conversion of said acyl-CoA or keto-acid to fumarate; a sequence of reactions for the formation of a desired product from said acetyl-CoA intermediate.
A genetically engineered microorganism converting a short-chain (C1-C5) alkane substrate to a product, said microorganism comprising enzymes or overexpressed enzymes or genes encoding same for: a sequence of reactions for the oxygen-dependent activation of a short-chain (C1-C5) alkane to a primary alcohol via terminal addition of a hydroxyl group and subsequent conversion of said alcohol to an acyl-CoA; a sequence of reactions for the generation of product precursor acetyl-CoA from said acyl-CoA; a sequence of reactions for the formation of a desired product from said acetyl-CoA intermediate.
Any microorganism herein described, where said expression vectors are inducible expression vectors or said integrated sequences are inducible integrated sequences.
Any microorganism herein described, wherein said pathway for the oxygen-independent activation and conversion to an acyl-CoA comprises: an overexpressed alkyl succinate synthase that catalyzes the addition of fumarate to a short-chain (C1-C5) alkane to produce a 2-methyl-alkyl-succinate; an overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or 2-methyl-alkyl-succinyl-CoA synthetase that catalyzes the conversion of said 2-methyl-alkyl-succinate to a 2-methyl-alkyl-succinyl-CoA; an overexpressed 2-methyl-alkyl-malonyl-CoA mutase that catalyzes the isomerization of said 2-methyl-alkyl-succinyl-CoA to a 2-methyl-alkyl-malonyl-CoA; an overexpressed 2-methyl-alkyl-malonyl-CoA decarboxylase that catalyzes the decarboxylation of said 2-methyl-alkyl-malonyl-CoA to an acyl-CoA;
Any microorganism herein described, wherein said pathways for the oxygen-independent activation and conversion to an acyl-CoA and generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid comprises: an overexpressed alkyl succinate synthase that catalyzes the addition of fumarate to a short-chain (C1-C5) alkane to produce a 2-methyl-alkyl-succinate; an overexpressed 2-methyl-alkyl-succinyl-CoA synthetase that catalyzes the conversion of said 2-methyl-alkyl-succinate to a 2-methyl-alkyl-succinyl-CoA; an overexpressed 2-methyl-alkyl-succinyl-CoA dehydrogenase that catalyzes the conversion of said 2-methyl-alkyl-succinyl-CoA to 2-methyl-alkyl-2-butenoyl-CoA; an overexpressed mesaconyl-C1-CoA-C4-CoA transferase that catalyzes the conversion of said 2-methyl-alkyl-2-butenoyl-CoA to 3-methyl-alkyl-2-butenoyl-CoA; an overexpressed mesaconyl-C4-CoA hydratase that catalyzes the conversion of said 3-methyl-alkyl-2-butenoyl-CoA to 3-methyl-alkyl-3-hydroxy-succinyl-CoA; an overexpressed citramalyl-CoA lyase that catalyzes the conversion of said 3-methyl-alkyl-3-hydroxy-succinyl-CoA to acetyl-CoA and a keto-acid;

FIGURE 15 TABLE G
Any microorganism herein described, wherein said pathways for the oxygen-independent activation and conversion to an acyl-CoA and generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid comprises: an overexpressed alkyl succinate synthase that catalyzes the addition of fumarate to a short-chain (C1-C5) alkane to produce a 2-methyl-alkyl-succinate; an overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or 2-methyl-alkyl-succinyl-CoA synthetase that catalyzes the conversion of said 2-methyl-alkyl-succinate to a 2-methyl-alkyl-succinyl-CoA; an overexpressed 2-methyl-alkyl-succinyl-CoA dehydrogenase that catalyzes the conversion of said 2-methyl-alkyl-succinyl-CoA to 2-methyl-alkyl-2-butenoyl-CoA; an overexpressed mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase that catalyzes the conversion of said 2-methyl-alkyl-2-butenoyl-CoA to 3-hydroxy-2-methyl-alkyl-succinyl-CoA; an overexpressed β-methylmalyl-CoA lyase that catalyzes the conversion of said 3-hydroxy-2-methyl-alkyl-succinyl-CoA to glyoxylate and an acyl-CoA; an overexpressed glyoxylate carboligase that catalyzes the conversion of said glyoxylate to tartronate semialdehyde; an overexpressed tartronate semialdehyde reductase that catalyzes the conversion of said tartronate semialdehyde to D-glycerate; an overexpressed glycerate kinase that catalyzes the conversion of said D-glycerate to 3-phospho-D-glycerate; glycolytic enzymes (phosphoglycerate mutase, enolase, pyruvate kinase) that catalyze the conversion of said 3-phospho-D-glycerate to pyruvate; a pyruvate formate lyase or pyruvate dehydrogenase that catalyze the conversion of said pyruvate to acetyl-CoA;
Any microorganism herein described, wherein said pathway for the generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid comprises: an overexpressed acyl-CoA dehydrogenase that catalyzes the conversion of said acyl-CoA to a transenoyl-CoA; an overexpressed enoyl-CoA hydratase that catalyzes the hydration of said transenoyl-CoA to a 3-hydroxyacyl-CoA; an overexpressed 3-hydroxyacyl-CoA dehydrogenase that catalyzes the oxidation of said 3-hydroxyacyl-CoA to a ß-ketoacyl-CoA; an overexpressed thiolase that catalyzes the cleavage of an acetyl-CoA from said ß-ketoacyl-CoA to produce acetyl-CoA and an acyl-CoA 2-carbons shorter than said starting acyl-CoA;
Any microorganism herein described, wherein said pathway for the regeneration of fumarate from an acyl-CoA or keto-acid comprises: an overexpressed propionyl-CoA carboxylase that catalyzes the carboxylation of propionyl-CoA to (S)-methyl-malonyl-CoA; an overexpressed methyl-malonyl-CoA epimerase that catalyzes the interconversion of said (S)-methyl-malonyl-CoA to (R)-methyl-malonyl-CoA; an overexpressed methyl-malonyl-CoA mutase that catalyzes the isomerization of said (R)-methyl-malonyl-CoA to succinyl-CoA; an overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or succinyl-CoA synthetase that catalyzes the conversion of said succinyl-CoA to succinate;
Any microorganism herein described, wherein said pathway for the regeneration of fumarate from an acyl-CoA or keto-acid comprises: an overexpressed malate dehydrogenase for the conversion of said keto-acid (pyruvate) to malate; an overexpressed fumarase for the dehydration of malate to fumarate;
Any microorganism herein described, wherein said pathway for the regeneration of fumarate from an acyl-CoA or keto-acid comprises: an overexpressed carboxylic acid omega hydroxylase that catalyzes the conversion of said keto-acid to an omega-hydroxy-2-keto-acid; an overexpressed alcohol dehydrogenase that catalyzes the conversion of said omega-hydroxyketo-acid to an omega-oxo-2-keto-acid; an overexpressed aldehyde dehydrogenase that catalyzes the conversion of said omega-oxo-keto-acid to a dicarboxylic 2-keto-acid; an overexpressed ketoreductase or malate dehydrogenase that catalyzes the conversion of said dicarboxylic 2-keto-acid to malate; an overexpressed fumarase for the dehydration of malate to fumarate.
Any microorganism herein described, wherein said pathway for the oxygen-dependent activation and conversion to an acyl-CoA comprises: an overexpressed alkane monooxygenase or alkane hydroxylase that catalyzes the terminal hydroxylation of a short-chain (C1-C5) alkane to produce a primary alcohol; an overexpressed alcohol dehydrogenase that catalyzes the oxidation of said primary alcohol to produce an aldehyde;

FIGURE 15 TABLE G
an overexpressed aldehyde dehydrogenase that catalyzes the oxidation of said aldehyde to produce a carboxylic acid and an overexpressed acyl-CoA synthetase that catalyzes the conversion of said carboxylic acid to an acyl-CoA, or an overexpressed acylating aldehyde dehydrogenase that catalyzes the conversion of said aldehyde to an acyl-CoA.
Any microorganism herein described, wherein said pathway for the generation of product precursor acetyl-CoA comprises: an overexpressed acyl-CoA dehydrogenase that catalyzes the conversion of said acyl-CoA to a transenoyl-CoA; an overexpressed enoyl-CoA hydratase that catalyzes the hydration of said transenoyl-CoA to a 3-hydroxyacyl-CoA; an overexpressed 3-hydroxyacyl-CoA dehydrogenase that catalyzes the oxidation of said 3-hydroxyacyl-CoA to a ß-ketoacyl-CoA; an overexpressed thiolase that catalyzes the cleavage of an acetyl-CoA from said ß-ketoacyl-CoA to produce acetyl-CoA and an acyl-CoA 2-carbons shorter than said starting acyl-CoA.
Any microorganism herein described, wherein said product synthesis pathway is a reverse beta oxidation (BOX-R) cycle that grows a primer by adding a 2-carbon donor thereto in each cycle, said BOX-R cycle comprising: an overexpressed thiolase that catalyzes the non-decarboxylative condensation of an acyl-CoA primer with a 2-carbon donor acetyl-CoA to produce a ß-ketoacyl-CoA; an overexpressed 3-oxoacyl-[acyl-carrier-protein] reductase or overexpressed 3-hydroxyacyl-CoA dehydrogenase that catalyzes the reduction of a ß-ketoacyl-CoA to a ß-hydroxyacyl-CoA; an overexpressed 3-hydroxyacyl-[acyl-carrier-protein] dehydratase or an overexpressed enoyl-CoA hydratase or 3-hydroxyacyl-CoA dehydratase that catalyzes the dehydration of a (3R)-ß-hydroxyacyl-CoA to a transenoyl-CoA; an overexpressed enoyl-[acyl-carrier-protein] reductase or acyl-CoA dehydrogenase or trans-enoyl-CoA reductase that catalyzes the reduction of a transenoyl-CoA to an acyl-CoA that is two carbons longer than said acyl-CoA primer; an overexpressed termination pathway that catalyzes the exit of an intermediate from said BOX-R cycle.
Any microorganism herein described, wherein said product synthesis pathway is a the fatty acid biosynthesis (FAS) pathway that grows a primer by adding a 2-carbon donor thereto in each cycle, said FAS pathway comprising: an overexpressed acetyl-CoA carboxylase that catalyzes the conversion of acetyl-CoA to malonyl-CoA; an overexpressed malonyl-CoA-[acyl-carrier-protein] ("ACP") transacylase that catalyzes the conversion of said malonyl-CoA to malonyl-ACP; an overexpressed β-ketoacyl-ACP synthase that catalyzes the decarboxylative condensation of said malonyl-ACP with an acyl-ACP primer to produce a ß-ketoacyl-ACP; an overexpressed 3-oxoacyl-ACP reductase that catalyzes the reduction of a ß-ketoacyl-ACP to a ß-hydroxyacyl-ACP; an overexpressed 3-hydroxyacyl-ACP dehydratase that catalyzes the dehydration of a (3R)-ß-hydroxyacyl-ACP to a transenoyl-ACP; an overexpressed enoyl-ACP reductase that catalyzes the reduction of a transenoyl-ACP to an acyl-ACP that is two carbons longer than said acyl-ACP primer; an overexpressed termination pathway that catalyzes the exit of an intermediate from said FAS cycle.
Any microorganism herein described, wherein said termination pathway is selected from the group consisting of i) a CoA cleaving thioesterase, ii) an acyl-CoA:acetyl-CoA transferase, and iii) a phosphotransacylase and a carboxylate kinase.
Any microorganism herein described, wherein said termination pathway is an ACP cleaving thioesterase.
Any microorganism herein described, wherein said microorganism produces a product selected from the group consisting of carboxylic acids, (3R)-β-hydroxy carboxylic acids, β-keto carboxylic acids, and α,β-unsaturated carboxylic acids.
Any microorganism herein described, wherein said termination pathway is selected from the group consisting of i) an alcohol-forming coenzyme-A thioester reductase, and ii) an aldehyde-forming CoA thioester reductase and an alcohol dehydrogenase.
Any microorganism herein described, wherein said termination pathway is selected from the group consisting of i) an alcohol-forming ACP thioester reductase, and ii) an aldehyde-forming ACP thioester reductase and an alcohol dehydrogenase.
Any microorganism herein described, wherein said microorganism produces a product selected from the group consisting of primary alcohols, 1,(3R)-β diols, β-keto primary alcohols, and α,β-unsaturated primary alcohols.
Any microorganism herein described, wherein said termination pathway consists of an aldehyde-forming CoA thioester reductase and an aldehyde decarbonylase.
Any microorganism herein described, wherein said termination pathway consists of an aldehyde-forming ACP thioester reductase and an aldehyde decarbonylase.
Any microorganism herein described, wherein said microorganism produces a product selected from the group consisting of linear alkanes, linear alkan-3-ols, linear methyl-ketones, and 1-alkenes.

FIGURE 15 TABLE G
Any microorganism herein described, wherein said termination pathway consists of an aldehyde-forming CoA thioester reductase and a transaminase.
Any microorganism herein described, wherein said termination pathway consists of an aldehyde-forming ACP thioester reductase and a transaminase.
Any microorganism herein described, wherein said microorganism produces a product selected from the group consisting of primary amines, 3-hydroxy-amines, 3-keto-amines, and α,β-unsaturated primary amines.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid omega hydroxylase and produces a product selected from the group consisting of ω-hydroxylated carboxylic acids, (3R)- β-, ω-dihydroxy carboxylic acids, β-keto, ω-hydroxy carboxylic acids, and α,β-unsaturated ω-hydroxylated carboxylic acids.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and an aldehyde dehydrogenase, and produces a product selected from the group consisting of ω-hydroxylated carboxylic acids, (3R)-β-, ω-dihydroxy carboxylic acids, β-keto, ω-hydroxy carboxylic acids, and α,β-unsaturated omega-hydroxylated carboxylic acids.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid ω hydroxylase, and produces a product selected from the group consisting of 1-,ω-diols, 1-,(3R)-β-, ω-triols, β-keto, 1-,ω-diols, and α,β-unsaturated 1-,ω-diols.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and an aldehyde dehydrogenase, and produces a product selected from the group consisting of di-carboxylic acids, (3R)-β-hydroxy di-carboxylic acids, β-keto di-carboxylic acids, and α,β-unsaturated di-carboxylic acids.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and a transaminase, and produces a product selected from the group consisting of primary alkanolamines (i.e. 1, ω-hydroxyamines), (3R)-β-hydroxy primary alkanolamines, β-keto primary alkanolamines, and α,β-unsaturated primary alkanolamines.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid ω hydroxylase, and produces a product selected from the group consisting of primary alkanolamines (i.e. 1, ω-hydroxyamines), (3R)-β-hydroxy primary alkanolamines, β-keto primary alkanolamines, and α,β-unsaturated primary alkanolamines.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and an aldehyde dehydrogenase, and produces a product selected from the group consisting of ω-amino acids, (3R)-β-hydroxy ω-amino acids, β-keto ω-amino acids, and α,β-unsaturated ω-amino acids.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid alpha hydroxylase, and produces a product selected from the group alpha-hydroxy carboxylic acids, alpha-, (3R)-β-dihydroxy carboxylic acids, α-hydroxy, β-keto carboxylic acids, and α,β-unsaturated α-hydroxy carboxylic acids.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid α hydroxylase, and produces a product selected from the group consisting of 1,2-diols, 1,2,3-triols, β-keto, 1,2-diols, and α,β-unsaturated 1,2-diols.
Any microorganism herein described, wherein said microorganism expresses a carboxylic acid α hydroxylase, and produces a product selected from the group consisting of α-hydroxylated primary amines, α-, β- dihydroxy primary amines, α-hydroxy, β-keto primary amines, and α-hydroxy, α,β-unsaturated primary amines.
Any microorganism herein described, further comprising reduced expression of fermentation enzymes leading to reduced production of lactate, acetate, ethanol and succinate.
Any microorganism herein described, wherein said overexpressed alkyl succinate synthase is encoded by *Azoarcus* sp. HxN1 *masBCDEG* (A9J4K0, A9J4K2, A9J4K4, A9J4K6, A9J4J6), *Desulfatibacillum alkenivorans assA1/assB1/assC1/assD1* (ACL03428.1, ACL03427.1, ACL03427.1, ACL03425.1), *Desulfosarcina* sp. BuS5 A39W_RS0101550/A39W_RS0101545/ A39W_RS0101540/A39W_RS0101535/A39W_RS19630/A39W_RS0101580 (WP_027352796.1, WP_027352795.1, WP_027352794.1, WP_027352793.1, WP_051374532.1, WP_027352800.1), *Desulfatibacillum alkenivorans assA2/assB2/assC2/assD2* (ACL03892.1, ACL03893.1, ACL03891.1, ACL03895.1), *Peptococcaceae* sp. *SCADC* (WP_036747468.1), *Aromatoleum* sp. OcN1 *masD* (CBK27727.1), *Desulfoglaeba alkanexedens assA* (ADJ51097.1), or homologues.
Any microorganism herein described, wherein said overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or 2-methyl-alkyl-succinyl-CoA synthetase is encoded by *Chloroflexus aurantiacus sct* (A9WGE3), *Thauera aromatica bbsEF* (Q9KJF0, Q9KJE9), *Escherichia coli sucCD* (P0A836, P0AGE9), *Desulfatibacillum alkenivorans Dalk_1737* (B8FFM9), or homologues.
Any microorganism herein described, wherein said overexpressed 2-methyl-alkyl-malonyl-CoA mutase is encoded by *Desulfatibacillum alkenivorans Dalk_0220/Dalk_0221* (ACL01930.1, ACL01929.1), or homologues.
Any microorganism herein described, wherein said overexpressed 2-methyl-alkyl-malonyl-CoA decarboxylase is encoded by *Desulfatibacillum alkenivorans Dalk_1740* (B8FFN2), or homologues.

FIGURE 15 TABLE G
Any microorganism herein described, wherein said overexpressed 2-methyl-alkyl-succinyl-CoA dehydrogenase is encoded by *Rhodobacter sphaeroides mcd* (ADC44452.1) or homologues.
Any microorganism herein described, wherein said overexpressed mesaconyl-C1-CoA-C4-CoA transferase is encoded by *Chloroflexus aurantiacus mct* (A9WC36) or homologues.
Any microorganism herein described, wherein said overexpressed mesaconyl-C4-CoA hydratase is encoded by *Chloroflexus aurantiacus meh* (A9WC41) or homologues.
Any microorganism herein described, wherein said overexpressed citramalyl-CoA lyase is encoded by *Chloroflexus aurantiacus mclA* (A9WC35) or homologues.
Any microorganism herein described, wherein said overexpressed mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase is encoded by *Chloroflexus aurantiacus mch* (A9WC34), *Rhodobacter sphaeroides mch* (Q3IZ78), or homologues.
Any microorganism herein described, wherein said overexpressed β-methylmalyl-CoA lyase is encoded by *Rhodobacter sphaeroides mcl1* (B9KLE8) or homologues.
Any microorganism herein described, wherein said overexpressed glyoxylate carboligase is encoded by *Escherichia coli gcl* (P0AEP7), or homologues.
Any microorganism herein described, wherein said overexpressed tartronate semialdehyde reductase is encoded by *Escherichia coli glxR* (P77161), or homologues.
Any microorganism herein described, wherein said overexpressed glycerate kinase is encoded by *Escherichia coli glxK* (P77364), or homologues.
Any microorganism herein described, wherein said overexpressed glycerate kinase is encoded by *Escherichia coli glxK* (P77364), or homologues.
Any microorganism herein described, wherein said phosphoglycerate mutase is encoded by *Escherichia coli gpmA* (P62707), *Escherichia coli gpmM* (P37689), or homologues.
Any microorganism herein described, wherein said enolase is encoded by *Escherichia coli eno* (P0A6P9), or homologues.
Any microorganism herein described, wherein said pyruvate kinase is encoded by *Escherichia coli pykA* (P21599), *Escherichia coli pykF* (P0AD61), or homologues.
Any microorganism herein described, wherein said pyruvate formate lyase is encoded by *Escherichia coli pflB/pflA* (P09373) or homologues.
Any microorganism herein described, wherein said pyruvate dehydrogenase is encoded by *Escherichia coli aceEF/lpd* (P0AFG8, P06959, C3TQA2) or homologues.
Any microorganism herein described, wherein said overexpressed acyl-CoA dehydrogenase is encoded by *Ascaris suum* ACDH (Q08523), *Escherichia coli fadE* (AP_000876.1), or homologues.
Any microorganism herein described, wherein said overexpressed enoyl-CoA hydratase encoded by *Pseudomonas putida fadB1x* (NP_744366.1), *Pseudomonas putida phaL* (NP_745413.1), *Alcanivorax borkumensis ech1* (YP_691868.1), *Alcanivorax borkumensis ech2* (YP_692707.1) *Alcanivorax borkumensis phaB* (YP_692246.1), *Escherichia coli fadB* (NP_418288.1), or homologues.
Any microorganism herein described, wherein said overexpressed 3-hydroxyacyl-CoA dehydrogenase is encoded by *Pseudomonas putida fadB2x* (Q88KS5), *Ascaris suum GS_18673*, *Escherichia coli fadB* (NP_418288.1), or homologues.
Any microorganism herein described, wherein said overexpressed thiolase is encoded by *Pseudomonas putida fadAx* (NP_744364.1), *Alcanivorax borkumensis fadAx* (YP_692368.1), *Escherichia coli atoB* (NP_416728.1), *Escherichia coli yqeF* (NP_417321.2), *Escherichia coli fadA* (YP_026272.1), *Escherichia coli fadI* (NP_416844.1), *Ralstonia eutropha bktB* (AAC38322.1), or homologues.
Any microorganism herein described, wherein said overexpressed propionyl-CoA carboxylase is encoded by *Chloroflexus aurantiacus Caur_2034/Caur_3435* (A9WEI4, A9WKJ2), *Rhodobacter sphaeroides pccAB* (Q3J4D9, Q3J4E3), or homologues.
Any microorganism herein described, wherein said overexpressed methyl-malonyl-CoA epimerase is encoded by *Metallosphaera sedula Msed_0639* (A4YEG2) or homologues.
Any microorganism herein described, wherein said overexpressed methyl-malonyl-CoA mutase is encoded by *Rhodobacter sphaeroides mcmA* (Q3J4D7), or homologues.
Any microorganism herein described, wherein said overexpressed succinyl-CoA synthetase is encoded by *Escherichia coli sucCD* (P0A836, P0AGE9), or homologues.
Any microorganism herein described, wherein said overexpressed malate dehydrogenase is encoded by *Escherichia coli maeA* (P26616), *Escherichia coli maeB* (P76558), or homologues.
Any microorganism herein described, wherein said overexpressed fumarase is encoded by *Escherichia coli fumA* (P0AC33), *Escherichia coli fumB* (P14407), *Escherichia coli fumC* (P05042), or homologues.

FIGURE 15 TABLE G
Any microorganism herein described, wherein said overexpressed carboxylic acid omega hydroxylase is encoded by *Pseudomonas putida alkBGT* (YP_009076004.1, Q9WWW4.1, Q9L4M8.1),*Marinobacter aquaeolei CYP153A* (ABM17701.1),*Mycobacterium marinum CYP153A16* (YP_001851443.1), *Polaromonas sp. CYP153A* (YP_548418.1), *Nicotiana tabacum CYP94A5* (AAL54887.1), *Vicia sativa CYP94A1* (AAD10204.1), *Vicia sativa CYP94A2* (AAG33645.1), *Arabidopsis thaliana CYP94B1* (BAB08810.1), *Arabidopsis thaliana CYP86A8* (CAC67445.1), *Candida tropicalis CYP52A1* (AAA63568.1, AAA34354.1, AAA34334.1), *Candida tropicalis CYP52A2* (AAA34353.2, CAA35593.1), *Homo sapiens CYP4A11* (AAQ56847.1), or homologs.
Any microorganism herein described, wherein said overexpressed alcohol dehydrogenase is encoded by *Rhodococcus ruber* SC1 *cddC* (AAL14237.1), *Acinetobacter* sp. SE19 *chnD* (AAG10028.1), *Escherichia coli betA* (NP_414845.1), *Escherichia coli dkgA* (NP_417485.4), *Escherichia coli eutG* (NP_416948.4), *Escherichia coli fucO* (NP_417279.2), *Escherichia coli ucpA* (NP_416921.4), *Escherichia coli yahK* (NP_414859.1), *Escherichia coli ybbO* (NP_415026.1), *Escherichia coli ybdH* (NP_415132.1), *Escherichia coli yiaY* (YP_026233.1), *Escherichia coli yjgB* (NP_418690.4), or homologues.
Any microorganism herein described, wherein said overexpressed aldehyde dehydrogenase is encoded by *Rhodococcus ruber* SC1 *cddD* (AAL14238.1), *Acinetobacter* sp. SE19 *chnE* (AAG10022.1), or homologues.
Any microorganism herein described, wherein said overexpressed ketoreductase/malate dehydrogenase is encoded by *Escherichia coli mdh* (P61889), or homologues.
Any microorganism herein described, wherein said overexpressed alkane monooxygenase or alkane hydroxylase is encoded by *Pseudomonas putida alkBGT* (YP_009076004.1, Q9WWW4.1, Q9L4M8.1), *Mycobacterium* sp. strain HXN-1500 CYP153A6 (Q5K1Y6), *Gordonia* sp. TY-5 *prmABCD* (AB112920.1), *Thauera butanivorans bmoXYZ/bmoC/bmoB* (Q8KQF0, Q8KQE9, Q8KQE7, Q8KQE6, Q8KQE8), *Alcanivorax borkumensis alkB1* (Q0VKZ3.1), *Alcanivorax borkumensis alkB2* (Q0VTH3.1), *Sphingopyxis macrogoltabida ahpG3* (Q5F4D3), *Methylosinus trichosporium* OB3b *mmoXYZBC/orfY* (P27353, P27354, P27355, Q53563, P27356, Q53562), *Methylococcus capsulatus Bath mmoXYZBC/orfY* (P22869, P18798, P11987, P18797, 22868, P22867), *Rhodobacter sphaeroides* RSP2792/RSP2793/RSP2794/RSP2795 (YP_352924.1, (YP_352923.1, YP_352922.1, YP_352921.1), or homologues.
Any microorganism herein described, wherein said overexpressed alcohol dehydrogenase is encoded by *Pseudomonas putida alkJ* (Q9WWW2), *Gordonia* sp. TY-5 *adh1* (AB112920.1), *Bacillus methanolicus mdh* (P31005), *Mycobacterium* sp. DSM 3803 *mdo* (C5MRT8), *Methylobacterium extorquens moxI, moxF* (P14775, P16027), *Escherichia coli betA* (NP_414845.1), *Escherichia coli dkgA* (NP_417485.4), *Escherichia coli eutG* (NP_416948.4), *Escherichia coli fucO* (NP_417279.2), *Escherichia coli ucpA* (NP_416921.4), *Escherichia coli yahK* (NP_414859.1), *Escherichia coli ybbO* (NP_415026.1), *Escherichia coli ybdH* (NP_415132.1), *Escherichia coli yiaY* (YP_026233.1), *Escherichia coli yjgB* (NP_418690.4), or homologues.
Any microorganism herein described, wherein said overexpressed aldehyde dehydrogenase is encoded by *Escherichia coli aldA* (P25553), *Escherichia coli aldB* (P37685), *Escherichia coli puuC* (P23883), *Pseudomonas putida alkH* (Q9WWW3), *Klebsiella pneumoniae KPN_01018* (A6T782), *Rhodococcus erythropolis aldhR* (Q4F895), or homologues.
Any microorganism herein described, wherein said overexpressed acyl-CoA synthetase is encoded by *Escherichia coli fadD* (P69451), *Escherichia coli fadK* (P38135), *Pseudomonas putida alkK* (Q9L4M6), or homologues.
Any microorganism herein described, wherein said overexpressed acylating aldehyde dehydrogenase is encoded by *E. coli mhpF* (NP_414885.1), *Pseudomonas* sp. CF600 *dmpF* (Q52060), or homologues.
Any microorganism herein described, wherein said overexpressed acyl-CoA dehydrogenase is encoded by *Escherichia coli fadE* (AP_000876.1), or homologues.
Any microorganism herein described, wherein said overexpressed enoyl-CoA hydratase encoded by *Escherichia coli fadB* (NP_418288.1), or homologues.
Any microorganism herein described, wherein said overexpressed 3-hydroxyacyl-CoA dehydrogenase is encoded by *Escherichia coli fadB* (NP_418288.1), or homologues.
Any microorganism herein described, wherein said overexpressed thiolase is encoded by, *Escherichia coli atoB* (NP_416728.1), *Escherichia coli yqeF* (NP_417321.2), *Escherichia coli fadA* (YP_026272.1), *Escherichia coli fadI* (NP_416844.1), or homologues.
Any microorganism herein described, wherein said overexpressed thiolase is encoded by *E. coli atoB* (NP_416728.1), *E. coli yqeF* (NP_417321.2), *E. coli fadA* (YP_026272.1), *E. coli fadI* (NP_416844.1), *Ralstonia eutropha bktB* (AAC38322.1), *Pseudomonas sp. Strain B13 catF* (AAL02407.1), *E coli paaJ* (NP_415915.1), *Pseudomonas putida pcaF* (AAA85138.1), *Rhodococcus opacus pcaF* (YP_002778248.1), *Streptomyces sp. pcaF* (AAD22035.1), *Ralstonia eutropha phaA* (AEI80291.1), *Clostridium acetobutylicum thlA* (AAC26023.1), *or Clostridium acetobutylicum thlB* (AAC26026.1), or homologues.
Any microorganism herein described, wherein said overexpressed 3-hydroxyacyl-CoA dehydrogenase or 3-oxoacyl-[acyl-carrier-protein] reductase is encoded by *E. coli fadB* (NP_418288.1), *E. coli fadJ* (NP_416843.1), *Ralstonia eutropha phaB1*

FIGURE 15 TABLE G
(YP_725942.1), *Ralstonia eutropha phaB2* (YP_726470.1), *Ralstonia eutropha phaB3* (YP_726636.1), *E. coli paaH* (P76083), *E. coli fabG* (NP_415611.1), or homologues.
Any microorganism herein described, wherein said overexpressed enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydratase, or 3-hydroxyacyl-[acyl-carrier-protein] dehydratase is encoded by *E. coli fadB* (NP_418288.1), *E. coli fadJ* (NP_416843.1), *Aeromonas caviae phaJ* (O32472.1), *Pseudomonas aeruginosa phaJ1* (BAA92740.1), *Pseudomonas aeruginosa phaJ2* (BAA92741.1), *Pseudomonas aeruginosa phaJ3* (BAC44834.1), *Pseudomonas aeruginosa phaJ4* (BAC44835.1), *E. coli paaF* (P76082), *E. coli fabA* (NP_415474.1), *E. coli fabZ* (NP_414722.1), or homologues.
Any microorganism herein described, wherein said trans-enoyl-CoA reductase or enoyl-[acyl-carrier-protein] reductase is encoded by *E. coli ydiO* (P0A9U8), *Euglena gracilis egTER* (Q5EU90.1), *Treponema denticola tdTER* (NP_971211.1), *E. coli fabI* (NP_415804.1), *Enterococcus faecalis fabK* (NP_816503.1), *Bacillus subtilis fabL* (KFK80655.1), *Vibrio cholerae fabV* (ABX38717.1), or homologues.
Any microorganism herein described, wherein said overexpressed acetyl-CoA carboxylase is encoded by *E. coli accABCD* (P0ABD5, P0ABD8, P24182, P0A9Q5), or homologues.
Any microorganism herein described, wherein said overexpressed malonyl-CoA-ACP transacylase is encoded by *E. coli fabD* (P0AAI9), or homologues.
Any microorganism herein described, wherein said overexpressed β-ketoacyl-ACP synthase is encoded by *E. coli fabB* (P0A953), *E. coli fabF* (P0AAI5), *E. coli fabH* (P0A6R0), or homologues.
Any microorganism herein described, wherein said overexpressed 3-oxoacyl-[acyl-carrier-protein] reductase is encoded by *E. coli fabG* (NP_415611.1), or homologues.
Any microorganism herein described, wherein said overexpressed 3-hydroxyacyl-[acyl-carrier-protein] dehydratase is encoded by *E. coli fabA* (NP_415474.1), *E. coli fabZ* (NP_414722.1), or homologues.
Any microorganism herein described, wherein said enoyl-[acyl-carrier-protein] reductase is encoded by *E. coli fabI* (NP_415804.1), *Enterococcus faecalis fabK* (NP_816503.1), *Bacillus subtilis fabL* (KFK80655.1), *Vibrio cholerae fabV* (ABX38717.1), or homologues.
Any microorganism herein described, wherein said overexpressed thioesterase is encoded by *E. coli tesA* (NP_415027.1), *E. coli tesB* (NP_414986.1), *E. coli yciA* (NP_415769.1), *E. coli fadM* (NP_414977.1), *E. coli ydiI* (NP_416201.1), *E. coli ybgC* (NP_415264.1), *Alcanivorax borkumensis tesB2* (YP_692749.1) *Fibrobacter succinogenes Fs2108* (YP_005822012.1), *Prevotella ruminicola Pr655* (YP_003574018.1) *Prevotella ruminicola Pr1687* (YP_003574982.1), or homologues.
Any microorganism herein described, wherein said overexpressed acyl-CoA:acetyl-CoA transferase is encoded by *E. coli atoD* (NP_416725.1), *Clostridium kluyveri cat2* (AAA92344.1), *Clostridium acetobutylicum ctfAB* (NP_149326.1, NP_149327.1) or *E. coli ydiF* (NP_416209.1),), or homologues.
Any microorganism herein described, wherein said overexpressed phosphotransacylase is encoded by *Clostridium acetobutylicum ptb* (NP_349676.1), *Enterococcus faecalis ptb* (AAD55374.1), *Salmonella enterica pduL* (AAD39011.1), or homologues.
Any microorganism herein described, wherein said overexpressed carboxylate kinase is encoded by *Clostridium acetobutylicum buk* (AAK81015.1), *Enterococcus faecalis buk* (AAD55375.1), *Salmonella enterica pduW* (AAD39021.1), or homologues.
Any microorganism herein described, wherein said overexpressed ACP-cleaving thioesterase is encoded by *E. coli tesA* (NP_415027.1), *Cuphea palustris fatB1* (AAC49179.1), *Cuphea viscosissima fatB3* (AEM72524.1), *Ulmus americana fatB1* (AAB71731.1), *Cocos nucifera fatB2* (AEM72520.1), *Elaeis guineensis PTE* (AAD42220.2), *Clostridium perfringens CPF_2954* (ABG82470.1), *Umbellularia californica fatB1* (AAA34215.1), or homologues.
Any microorganism herein described, wherein said overexpressed alcohol-forming coenzyme-A thioester reductase is encoded by *Clostridium acetobutylicum adhE2* (YP_009076789.1), *Arabidopsis thaliana At3g11980* (AEE75132.1), *Arabidopsis thaliana At3g44560* (AEE77915.1), *Arabidopsis thaliana At3g56700* (AEE79553.1), *Arabidopsis thaliana At5g22500* (AED93034.1), *Arabidopsis thaliana CER4* (AEE86278.1), *Marinobacter aquaeolei* VT8 *maqu_2220* (YP_959486.1), *Marinobacter aquaeolei* VT8 *maqu_2507* (YP_959769.1), or homologues.
Any microorganism herein described, wherein said overexpressed aldehyde-forming CoA thioester reductase is encoded by *Acinetobacter calcoaceticus acr1* (AAC45217.1), *Acinetobacter sp Strain M-1 acrM* (BAB85476.1), *Clostridium beijerinckii ald* (AAT66436.1), *E. coli eutE* (NP_416950.1), *Salmonella enterica eutE* (AAA80209.1), *E. coli mhpF* (NP_414885.1), or homologues.
Any microorganism herein described, wherein said overexpressed alcohol-forming ACP thioester reductase is encoded by *Marinobacter aquaeolei* VT8 *maqu_2220* (YP_959486.1), *Hahella chejuensis hch_05075* (ABC31758.1), *Marinobacter algicola MDG893_11561* (A6EVI7), *Bermanella marisrubri RED65_09894* (Q1N697), or homologues.
Any microorganism herein described, wherein said overexpressed aldehyde-forming ACP thioester reductase is encoded by *Nostoc punctiforme Npun_R1710* (ACC80381.1), *Synechococcus elongates Synpcc7942_1594* (Q54765), *Prochlorococcus marinus P9515_05971* (A2BVJ5), *Synechocystis* sp. PCC 6803 *sll0209* (YP_005652204.1), or homologues.

FIGURE 15 TABLE G
Any microorganism herein described, wherein said overexpressed alcohol dehydrogenase is encoded by *E. coli betA* (NP_414845.1), *E. coli dkgA* (NP_417485.4), *E. coli eutG* (NP_416948.4), *E. coli fucO* (NP_417279.2), *E. coli ucpA* (NP_416921.4), *E. coli yahK* (NP_414859.1), *E. coli ybbO* (NP_415026.1), *E. coli ybdH* (NP_415132.1), *E. coli yiaY* (YP_026233.1), *E. coli yjgB* (NP_418690.4), homologues.
Any microorganism herein described, wherein said aldehyde decarbonylase overexpressed is encoded by *Synechococcus elongatus* PCC7942 *orf1593* (Q54764.1), *Nostoc punctiforme* PCC73102 npun_R1711 (B2J1M1.1), *Prochlorococcus marinus* MIT9313 pmt1231 (Q7V6D4.1), or homologues.
Any microorganism herein described, wherein said overexpressed transaminase is encoded by *Arabidopsis thaliana At3g22200* (NP_001189947.1), *Alcaligenes denitrificans aptA* (AAP92672.1), *Bordetella bronchiseptica BB0869* (WP_015041039.1), *Bordetella parapertussis BPP0784* (WP_010927683.1), *Brucella melitensis BAWG_0478* (EEW88370.1), *Burkholderia pseudomallei BP1026B_I0669* (AFI65333.1), *Chromobacterium violaceum CV2025* (AAQ59697.1), *Oceanicola granulosus OG2516_07293* (WP_007254984.1), *Paracoccus denitrificans PD1222 Pden_3984* (ABL72050.1), *Pseudogulbenkiania ferrooxidans* ω-TA (WP_008952788.1), *Pseudomonas putida* ω -TA (P28269.1), *Ralstonia solanacearum* ω -TA (YP_002258353.1), *Rhizobium meliloti SMc01534* (NP_386510.1), and *Vibrio fluvialis* ω -TA (AEA39183.1), *Mus musculus abaT* (AAH58521.1) *E. coli gabT* (YP_490877.1), or homologues.
Any microorganism herein described, wherein said overexpressed carboxylic acid omega hydroxylase is encoded by *Pseudomonas putida alkBGT* (YP_009076004.1, Q9WWW4.1, Q9L4M8.1),*Marinobacter aquaeolei CYP153A* (ABM17701.1), *Mycobacterium marinum CYP153A16* (YP_001851443.1), *Polaromonas sp. CYP153A* (YP_548418.1), *Nicotiana tabacum CYP94A5* (AAL54887.1), *Vicia sativa CYP94A1* (AAD10204.1), *Vicia sativa CYP94A2* (AAG33645.1), *Arabidopsis thaliana CYP94B1* (BAB08810.1), *Arabidopsis thaliana CYP86A8* (CAC67445.1), *Candida tropicalis CYP52A1* (AAA63568.1, AAA34354.1, AAA34334.1), *Candida tropicalis CYP52A2* (AAA34353.2, CAA35593.1), *Homo sapiens CYP4A11* (AAQ56847.1), or homologues.
Any microorganism herein described, wherein said overexpressed alcohol oxidase is encoded by *Rhodococcus ruber* SC1 *cddC* (AAL14237.1), *Acinetobacter* sp. SE19 *chnD* (AAG10028.1), *E. coli yahK* (NP_414859.1), *E. coli yjgB* (NP_418690.4), or homologues.
Any microorganism herein described, wherein said overexpressed aldehyde dehydrogenase is encoded by *Rhodococcus ruber* SC1 *cddD* (AAL14238.1), *Acinetobacter* sp. SE19 *chnE* (AAG10022.1), or homologues.
Any microorganism herein described, wherein said overexpressed fatty acid alpha hydroxylases is encoded by *Myxococcus xanthus MXAN_0191* (YP_628473.1), *Stigmatella aurantiaca STIAU_3334* (YP_003957653.1), or homologues.
Any microorganism herein described, wherein said reduced expression of fermentation enzymes are Δ*adhE*, (Δ*pta* or Δ*ackA* or Δ*ackApta*), Δ*poxB*, Δ*ldhA*, and Δ*frdA* and less acetate, lactate, ethanol and succinate are thereby produced.
A method of a product comprising growing a genetically engineered microorganism or recombinant bacteria according to any of claims 1-114 in a culture broth containing an alkane as the sole carbon source, activating said alkane, generating precursor intermediate acetyl-CoA, producing a product from said acetyl-CoA, and isolating said product.
A method of a product comprising growing a genetically engineered microorganism or recombinant bacteria according to any of claims 1-114 in a culture broth containing an alkane as the sole carbon source and a terminal electron acceptor (such as SO_4^{2-}, NO_3^-, Fe^{3+}, O_2, Mn^{4+}), activating said alkane, generating precursor intermediate acetyl-CoA, producing a product from said acetyl-CoA, and isolating said product.

BIOCONVERSION OF SHORT-CHAIN HYDROCARBONS TO FUELS AND CHEMICALS

PRIOR RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 62/140,628, filed Mar. 31, 2015 and incorporated by reference herein in its entirety for all purposes.

FEDERALLY SPONSORED RESEARCH STATEMENT

[0002] Not applicable.

FIELD OF THE DISCLOSURE

[0003] The disclosure generally relates to the use of microorganisms with novel pathways for the conversion of short-chain hydrocarbons (e.g. methane, ethane, propane, butane, pentane) to fuels and chemicals (e.g. carboxylic acids, alcohols, hydrocarbons, and their alpha-, beta-, and omega-functionalized derivatives).

BACKGROUND OF THE DISCLOSURE

[0004] Global natural gas (CH_4) resources that are technically recoverable with new horizontal drilling and efficient extraction technologies are estimated at 7.2×10^3 trillion cubic feet (Tcf), with estimates for the US ranging between 0.65×10^3 Tcf and up to 2×10^3 Tcf. Furthermore, about 5 trillion cubic feet (Tcf) of natural gas liquids or NGLs (primarily ethane, propane and butane) are produced annually in the United States and technically recoverable reserves of this resource are estimated at over 400 Tcf based on wet natural gas estimates.

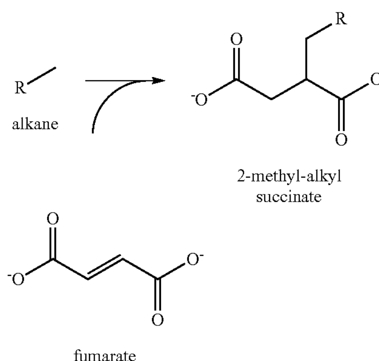
[0005] The invention re-purposes native pathways for anaerobic and aerobic activation of medium- and long-chain hydrocarbons (C_6-C_{20}) to function with short-chain substrates (C_1-C_5). Through these engineered pathways, the short-chain hydrocarbon substrates are converted to central, intracellular metabolites such as acetyl-CoA, which can in turn be converted to a variety of fuels and chemicals through other native or engineered pathways.

SUMMARY OF THE DISCLOSURE

[0006] This invention demonstrates pathways that can be utilized for the conversion of short chain hydrocarbon feedstocks to a variety of fuels and chemicals. This is accomplished through key pathway modules for hydrocarbon activation and conversion to an acyl-CoA intermediate, generation of product precursor acetyl-CoA, which in certain cases also entails the generation of an addition acyl-CoA or keto-acid, and the formation of a desired product from acetyl-CoA. Dependent on the type of hydrocarbon activation pathway utilized, the regeneration of compounds required for activation from an acyl-CoA or keto-acid intermediate are also required.

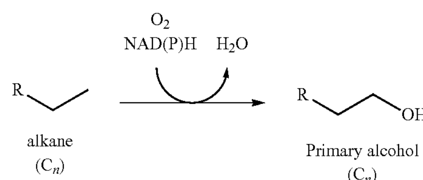
[0007] Two main approaches for short chain hydrocarbon activation are exploited: 1) the oxygen-independent activation via fumarate addition, which given the need for fumarate during oxygen-independent activation also requires pathways for the regeneration of this intermediate for continued activation cycles, and 2) the oxygen-dependent activation via the terminal addition of a hydroxyl group to the alkane.

[0008] As used herein, an "oxygen-independent activation via fumarate addition" is the addition of fumarate to an alkane leading a 2-methyl-alkyl-succinate:



[0009] The enzyme mat catalyzes this reaction is an "alkyl succinate synthase," and more than 40 such examples are exemplified herein. Alkyl succinate synthases that are specific for methane as a substrate are called "methyl succinate synthases," for ethane they are called "ethyl succinate synthases," and so on, although there are of course enzymes with broader substrate specificity.

[0010] As used herein, an "oxygen-dependent activation" is the terminal addition of a hydroxyl group to the alkane to form a primary alcohol



[0011] The enzyme that catalyzes this reaction is an "alkane monooxygenase" or "alkane hydroxylase", and as above, they may sometimes be named according the alkane substrate specificity, although there are of course enzymes in both classes with broader substrate specificity. Several examples are provided herein.

[0012] Regardless of the activation pathway utilized, the resulting activated compound is then converted into an acyl-CoA intermediate through a series of reactions. Once the given acyl-CoA intermediate is produced, various pathways dependent on the type of acyl-CoA generated can be exploited for the generation of the product precursor acetyl-CoA.

[0013] Synthesis of the desired product(s) from acetyl-CoA can be accomplished through various engineered metabolic pathways, including a reversal of the beta-oxidation cycle (BOX-R) or the fatty acid biosynthesis (FAS) pathway. Following chain elongation through either of these pathways, the selection of a given termination pathway enables the synthesis of various product families.

[0014] As used herein "termination pathway" or "termination enzyme(s)" refers to one or more enzymes (or genes encoding same) that will pull reaction intermediates out the BOX-R or FAS cycle and produce the desired end product.

[0015] By "primary termination pathway" what is meant is an intermediate from the BOX-R or FAS cycle is pulled out of the BOX-R or FAS cycle by one (which can have more than one activity) or more termination enzymes and results in i) carboxylic acids, ii) primary alcohols, iii) hydrocarbons, or iv) primary amines, from CoA intermediates as described in FIG. **1**.

[0016] By "secondary termination pathway" what is meant is that the intermediate pulled out of the BOX-R or FAS cycle by a primary termination pathway enzyme is further modified by one or more enzymes.

[0017] Many examples of termination pathways are available and the following table provides several examples:

TABLE A

Termination Pathways for Conversion of CoA Intermediates to Desired Products

Reaction	Illustration	EC Numbers	Enzyme names	Source organism and gene name	Protein Accession Numbers
Acyl-CoA → Carboxylic acid	An acyl-CoA → A carboxylic acid	3.1.2.-	Thioesterase	*E. coli* tesA	NP_415027.1
				E. coil tesB	NP_414986.1
				E. coli yciA	NP_415769.1
				E. coli fadM	NP_414977.1
				E. coli ydil	NP_416201.1
				E. coli ybgC	NP_415264.1
				Alcanivorax borkumensis tesB2	YP_692749.1
				Fibrobacter succinogenes Fs2108	YP_005822012.1
				Prevotella ruminicola Pr655	YP_003574018.1
				Prevotella ruminicola Pr1687	YP_003574982.1
		2.8.3.8	Acyl-CoA:acetyl-CoA transferase	*E. coli* atoD	NP_416725.1
				Clostridium kluyveri cat2	AAA92344.1
				Clostridium acetobutylicum ctfAB	NP_149326.1, NP_149327.1
				E. coil ydiF	NP_416209.1
		2.3.1.-; 2.7.2.1; 2.7.2.15	Phospho-transacylase + Carboxylate kinase	*Clostridium acetobutylicum* ptb	NP_349676.1
				Enterococcus faecalis ptb	AAD55374.1
				Salmonella enterica pduL	AAD39011.1
				Clostridium acetobutylicum buk	AAK81015.1
				Enterococcus faecalis buk	AAD55375.1
				Salmonella enterica pduW	AAD39021.1
Acyl-CoA → Alcohol	An acyl-CoA → An alcohol	1.2.1.84	Alcohol-forming CoA reductase	*Clostridium acetobutylicum* adhE2	YP_009076789.1
				Arabidopsis thaliana At3g11980	AEE75132.1
				Arabidopsis thaliana At3g44560	AEE77915.1
				Arabidopsis thaliana At3g56700	AEE79553.1
				Arabidopsis thaliana At5g22500	AED93034.1
				Arabidopsis thaliana CER4	AEE86278.1
				Marinobacter aquaeolei VT8 magu_2220	YP_959486.1
				Marinobacter aquaeolei VT8 maqu_2507	YP_959769.1
Acyl-CoA → Aldehyde	An acyl-CoA → An aldehyde	1.2.1.10	Aldehyde forming CoA reductase	*Acinetobacter calcoaceticus* acr1	AAC45217.1
				Acinetobacter sp Strain M-1 acrM	BAB85476.1
				Clostridium beijerinckii ald	AAT66436.1
				E. coli eut E	NP_416950.1
				Salmonella enterica eutE	AAA80209.1
				E. coli mhpF	NP_414885.1
Aldehyde → Alcohol	An aldehyde → An alcohol	1.1.1.-	Alcohol dehydro-genase	*E. coil* betA	NP_414845.1
				E. coli dkgA	NP_417485.4
				E. coli eutG	NP_416948.4
				E. Coli fucO	NP_417279.2
				E. coli ucpA	NP_416921.4
				E. coli yahK	NP_414859.1
				E. coli ybbO	NP_415026.1
				E. coli ybdH	NP_415132.1
				E. coli yiaY	YP_026233.1
				E. coli yjgB	NP_418690.4

TABLE A-continued

Termination Pathways for Conversion of CoA Intermediates to Desired Products

Reaction	Illustration	EC Numbers	Enzyme names	Source organism and gene name	Protein Accession Numbers
Aldehyde → Alkane	An aldehyde → An alkane	4.1.99.5	Aldehyde decarbonylase	*Synechococcus elongatus* PCC7942 orf1593	Q54764.1
				Nostoc punctiforme PCC73102 npun_R1711	B2J1M1.1
				Prochlorococcus marinus MIT9313 pml1231	Q7V6D4.1
Aldehyde → Amine	An aldehyde → An alkane	2.6.1.-	Transaminase	*Arabidopsis thaliana* At3g22200	NP_001189947.1
				Alcaligenes denitrificans AptA	AAP92672.1
				Bordetella bronchiseptica BB0869	WP_015041039.1
				Bordetella parapertussis BPP0784	WP_010927683.1
				Brucella melitensis BAWG_0478	EEW88370.1
				Burkholderia pseudomallei BP 1026B_I0669	AFI65333.1
				Chromobacterium violaceum CV2025	AAQ59697.1
				Oceanicola granulosus OG2516_07293	WP_007254984.1
				Paracoccus denitrificans PD1222 Pden_3984	ABL72050.1
				Pseudogulbenkiania ferrooxidans ω-TA	WP_008952788.1
				Pseudomonas putida ω-TA	P28269.1
				Ralstonia solanacearum ω-TA	YP_002258353.1
				Rhizobium meliloti SMc01534	NP_386510.1
				Vibrio fluvialis ω-TA	AEA39183.1
				Mus musculus abaT	AAH58521.1
				E. coli gabT	YP_490877.1
Carboxylic Acid → ω-hydroxyacid	Carboxylic Acid → ω-Hydroxy-Carboxylic Acid	1.14.-	Carboxylic acid omega hydroxylase	*Pseudomonas putida* alkBGT	YP_009076004.1, Q9WWW4.1, Q9L4M8.1
				Marinobacter aquaeolei CYP153A	ABM17701.1
				Mycobacterium marinum CYP153A16	YP_001851443.1
				Polaromonas sp. CYP153A	YP_548418.1
				Nicotiana tabacum CYP94A5	AAL54887.1
				Vicia sativa CYP94A1	AAD10204.1
				Vicia sativa CYP94A2	AAG33645.1
				Arabidopsis thaliana CYP94B1	BAB08810.1
				Arabidopsis thaliana CYP86A8	CAC67445.1
				Candida tropicalis CYP52A1	AAA63568.1, AAA34354.1, AAA34334.1
				Candida tropicalis CYP52A2	AAA34353.2, CAA35593.1
				Homo sapiens CYP4A11	AAQ56847.1
ω-hydroxyacid → ω-oxo-acid	ω-Hydroxy-Carboxylic Acid → ω-Oxo-Carboxylic Acid	1.1.1.-	Alcohol oxidase/ alcohol dehydro-genase	*Rhodococcus ruber* SC1 cddC	AAL14237.1
				Acinetobacter sp. SE19 chnD	AAG10028.1
				E. coli yahK	NP_414859.1
				E. coli yjgB	NP_418690.4

TABLE A-continued

Termination Pathways for Conversion of CoA Intermediates to Desired Products

Reaction	Illustration	EC Numbers	Enzyme names	Source organism and gene name	Protein Accession Numbers
ω-oxo-acid → dicarboxylic acid	ω-Oxo-Carboxylic Acid → Dicarboxylic Acid	1.2.1.-	Aldehyde dehydro-genase	*Rhodococcus ruber* SC1 cddD *Acinetobacter* sp. SE19 chnE	AAL14238.1 AAG10022.1
Carboxylic Acid → α-hydroxyacid	A carboxylic acid → An alpha-hydroxy-carboxylic acid	1.14.-	Carboxylic acid alpha hydroxylase	*Myxococcus xanthus* MXAN_0191 *Stigmatella aurantiaca* STIAU_3334	YP_628473.1 YP_003957653.1

[0018] The synthetic pathway disclosed in this invention serves as a platform for the generation of valuable chemical products from less valuable and more abundant hydrocarbon feedstocks. Methane, for example, can be converted into liquid "drop in" fuels for use in the current transportation infrastructure. The methods, materials and systems herein thus allow for various chain length hydrocarbons to be activated and assimilated into central carbon metabolism allowing for product synthesis via numerous native and synthetic pathways.

[0019] The process involves performing traditional cultures using industrial organisms (such as *E. coli, S. cerevisiae, Methylococcus capsulatus*, or *Pichia pastoris*) that convert short-chain hydrocarbons (such as methane, ethane, propane, butane, or pentane) into chemical products. These organisms are considered workhorses of modern biotechnology, and are easy to genetically engineer, and scale up for industrial production levels of desired products.

[0020] The pathways in a living system are generally made by transforming the microbe with an expression vector encoding one or more of the proteins, but the genes can also be added to the chromosome by recombineering, homologous recombination, and similar techniques. Where the needed protein is endogenous, as is the case in some instances, it may suffice as is, but it is usually overexpressed using an inducible promoter for better functionality and user-control over the level of active enzyme.

[0021] As used herein, the expressions "microorganism," "microbe," "strain" and the like may be used interchangeably and all such designations include their progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0022] As used herein, reference to a "cell" is generally understood to include a culture of such cells, as the work described herein is done in cultures having 10^{9-15} cells.

[0023] As used herein, "growing" cells used it its art accepted manner, referring to exponential growth of a culture of cells, not the few cells that may not have completed their cell cycle at stationary phase or have not yet died in the death phase or after harvesting.

[0024] As used in the claims, "homolog" means an enzyme with at least 50% identity to one of the listed sequences and also having the same general catalytic activity. While higher identity (60%, 70%, 80%) and the like may be preferred, it is typical for bacterial sequences to diverge significantly (40-60%), yet still be identifiable as homologs, while mammalian species tend to diverge less (80-90%).

[0025] Reference to proteins herein can be understood to include reference to the gene encoding such protein. Thus, a claimed "permease" protein can include the related gene encoding that permease. However, it is preferred herein to refer to the protein by standard name per ecoliwiki or HUGO since both enzymatic and gene names have varied widely, especially in the prokaryotic arts.

[0026] Once an exemplary protein is obtained, many additional examples of proteins with similar activity can be identified by BLAST search. Further, every protein record is linked to a gene record, making it easy to design overexpression vectors. Many of the needed enzymes are already available in vectors, and can often be obtained from cell depositories or from the researchers who cloned them. But, if necessary, new clones can be prepared based on available sequence information using RT-PCR techniques. Thus, it should be easily possible to obtain all of the needed enzymes for overexpression.

[0027] Another way of finding suitable enzymes/proteins for use in the invention is to consider other enzymes with the same EC number, since these numbers are assigned based on the reactions performed by a given enzyme. An enzyme that thus be obtained, e.g., from AddGene or from the author of the work describing that enzyme, and tested for functionality as described herein. In addition, many sites provide lists of proteins that all catalyze the same reaction.

[0028] Understanding the inherent degeneracy of the genetic code allows one of ordinary skill in the art to design multiple nucleotides that encode the same amino acid sequence. NCBI™ provides codon usage databases for optimizing DNA sequences for protein expression in various species. Using such databases, a gene or cDNA may be "optimized" for expression in *E. coli*, yeast, algae or other species using the codon bias for the species in which the gene will be expressed.

[0029] Initial cloning experiments have proceeded in *E. coli* for convenience since most of the required genes are already available in plasmids suitable for bacterial expression, but the addition of genes to bacteria is of nearly universal applicability. Indeed, since recombinant methods were invented in the 70's and are now so commonplace, even school children perform genetic engineering experiments using bacteria. Such species include e.g., *Bacillus, Streptomyces, Azotobacter, Trichoderma, Rhizobium, Pseudomonas, Micrococcus, Nitrobacter, Proteus, Lactobacillus, Pediococcus, Lactococcus, Salmonella, Streptococcus, Paracoccus, Methanosarcina*, and *Methylococcus*, or any of the completely sequenced bacterial species. Indeed, hundreds of bacterial genomes have been completely sequenced, and this information greatly simplifies both the generation of vectors encoding the needed genes, as well as the planning of a recombinant engineering protocol. Such species are listed along with links at http://en.wikipedia.org/wiki/List_of sequenced bacterial_genomes.

[0030] Additionally, yeast, such as *Saccharomyces*, are a common species used for microbial manufacturing, and many species can be successfully transformed. Indeed, yeast are already available that express recombinant thioesterases—one of the termination enzymes described herein—and the reverse beta oxidation pathway has also been achieved in yeast. Other species include but are not limited to *Candida, Aspergillus, Arxula adeninivorans, Candida boidinii, Hansenula polymorpha* (*Pichia angusta*), *Kluyveromyces lactis, Pichia pastoris*, and *Yarrowia lipolytica*, to name a few.

[0031] It is also possible to genetically modify many species of algae, including e.g., *Spirulina, Apergillus, Chlamydomonas, Laminaria japonica, Undaria pinnatifida, Porphyra, Eucheuma, Kappaphycus, Gracilaria, Monostroma, Enteromorpha, Arthrospira, Chlorella, Dunaliella, Aphanizomenon, Isochrysis, Pavlova, Phaeodactylum, Ulkenia, Haematococcus, Chaetoceros, Nannochloropsis, Skeletonema, Thalassiosira,* and *Laminaria japonica*, and the like. Indeed, the microalga *Pavlova lutheri* is already being used as a source of economically valuable docosahexaenoic (DHA) and eicosapentaenoic acids (EPA), and *Crypthecodinium cohnii* is the heterotrophic algal species that is currently used to produce the DHA used in many infant formulas.

[0032] Furthermore, a number of databases include vector information and/or a repository of vectors and can be used to choose vectors suitable for the chosen host species. See e.g., AddGene.org which provides both a repository and a searchable database allowing vectors to be easily located and obtained from colleagues. See also Plasmid Information Database (PlasmID) and DNASU having over 191,000 plasmids. A collection of cloning vectors of *E. coli* is also kept at the National Institute of Genetics as a resource for the biological research community. Furthermore, vectors (including particular ORFS therein) are usually available from colleagues.

[0033] The enzymes can be added to the genome or via expression vectors, as desired. Preferably, multiple enzymes are expressed in one vector or multiple enzymes can be combined into one operon by adding the needed signals between coding regions. Further improvements can be had by overexpressing one or more, or even all of the enzymes, e.g., by adding extra copies to the cell via plasmid or other vector. Initial experiments may employ expression plasmids hosting 3 or more ORFs for convenience, but it may be preferred to insert operons or individual genes into the genome for long term stability.

[0034] Still further improvements in yield can be had by reducing competing pathways, such as those pathways for making e.g., acetate, formate, ethanol, and lactate, and it is already well known in the art how to reduce or knockout these pathways. See e.g., the Rice patent portfolio by Ka-Yiu San and George Bennett (U.S. Pat. No. 7,569,380, U.S. Pat. No. 7,262,046, U.S. Pat. No. 8,962,272, U.S. Pat. No. 8,795,991) and patents by these inventors (U.S. Pat. No. 8,129,157 and U.S. Pat. No. 8,691,552) (each incorporated by reference herein in its entirety for all purposes). Many others have worked in this area as well.

[0035] In calculating "% identity" the unaligned terminal portions of the query sequence are not included in the calculation. The identity is calculated over the entire length of the reference sequence, thus short local alignments with a query sequence are not relevant (e.g., % identity=number of aligned residues in the query sequence/length of reference sequence). Alignments are performed using BLAST homology alignment as described by Tatusova T A & Madden T L (1999) FEMS Microbiol. Lett. 174:247-250, and available through the NCBI website. The default parameters were used, except the filters were turned OFF.

[0036] "Operably associated" or "operably linked", as used herein, refer to functionally coupled nucleic acid or amino acid sequences.

[0037] "Recombinant" is relating to, derived from, or containing genetically engineered material. In other words, the genetics of an organism was intentionally manipulated in some way.

[0038] "Reduced activity" is defined herein to be at least a 75% reduction in protein activity, as compared with an appropriate control species (e.g., the wild type gene in the same host species). Preferably, at least 80, 85, 90, 95% reduction in activity is attained, and in the most preferred embodiment, the activity is eliminated (100%). Proteins can be inactivated with inhibitors, by mutation, or by suppression of expression or translation, by knock-out, by adding stop codons, by frame shift mutation, and the like. All reduced activity genes or proteins are signified herein by "-".

[0039] By "null" or "knockout" what is meant is that the mutation produces undetectable active protein. A gene can be completely (100%) reduced by knockout or removal of part of all of the gene sequence. Use of a frame shift mutation, early stop codon, point mutations of critical resi-

dues, or deletions or insertions, and the like, can also completely inactivate (100%) gene product by completely preventing transcription and/or translation of active protein. All null mutants herein are signified by Δ.

[0040] "Overexpression" or "overexpressed" is defined herein to be at least 150% of protein activity as compared with an appropriate control species, or any expression in a species that lacks the activity altogether. Preferably, the activity is increased 100-500% or even ten fold. Overexpression can be achieved by mutating the protein to produce a more active form or a form that is resistant to inhibition, by removing inhibitors, or adding activators, and the like. Overexpression can also be achieved by removing repressors, adding multiple copies of the gene to the cell, or up-regulating the endogenous gene, and the like. All overexpressed genes or proteins are signified herein by "+".

[0041] In certain species it is possible to genetically engineer the endogenous protein to be overexpressed by changing the regulatory sequences or removing repressors. However, overexpressing the gene by inclusion on selectable plasmids or other vectors that exist in hundreds of copies in the cell may be preferred due to its simplicity and ease of exerting externals controls, although permanent modifications to the genome may be preferred in the long term for stability reasons.

[0042] The terms "operably associated" or "operably linked," as used herein, refer to functionally coupled nucleic acid sequences.

[0043] As used herein "recombinant" is relating to, derived from, or containing genetically engineered material. In other words, the genome was intentionally manipulated by the hand of man in some way.

[0044] The term "endogenous" or "native" means that a gene originated from the species in question, without regard to subspecies or strain, although that gene may be naturally or intentionally mutated, or placed under the control of a promoter that results in overexpression or controlled expression of said gene. Thus, genes from *Clostridia* would not be endogenous to *Escherichia*, but a plasmid expressing a gene from *E. coli* or would be considered to be endogenous to any genus of *Escherichia*, even though it may now be overexpressed.

[0045] "Expression vectors" are used in accordance with the art accepted definition of a plasmid, virus or other propagatable sequence designed for protein expression in cells. There are thousands of such vectors commercially available, and typically each has an origin of replication (ori); a multiple cloning site; a selectable marker; ribosome binding sites; a promoter and often enhancers; and the needed termination sequences. Most expression vectors are inducible, although constitutive expressions vectors also exist.

[0046] As used herein, "inducible" means that gene expression can be controlled by the hand of man, by adding e.g., a ligand to induce expression from an inducible promoter. Exemplary inducible promoters include the lac operon, inducible by IPTG, the yeast AOX1 promoter inducible with methanol, the strong LAC4 promoter inducible with lactate, and the like. Low level of constitutive protein synthesis may occur even in expression vectors with tightly controlled promoters.

[0047] As used herein, an "integrated sequence" means the sequence has been integrated into the host genome, as opposed to being maintained on an expression vector. It will still be expressable, and preferably is inducible as well.

[0048] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims or the specification means one or more than one, unless the context dictates otherwise.

[0049] The term "about" means the stated value plus or minus the margin of error of measurement or plus or minus 10% if no method of measurement is indicated.

[0050] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or if the alternatives are mutually exclusive.

[0051] The terms "comprise", "have", "include" and "contain" (and their variants) are open-ended linking verbs and allow the addition of other elements when used in a claim.

[0052] The following abbreviations are used herein:

ABBREVIATION	TERM
ACP	Acyl carrier protein
Box-R	Beta oxidation pathway in reverse.
FAS	Fatty acid biosynthesis
GC	Gas chromatograph
HPLC	High pressure liquid chromatograph
ORF	Open reading Frame

BRIEF DESCRIPTION OF THE DRAWINGS

[0053] FIG. **1**. Generalized pathway for the conversion of alkanes to desired products.

[0054] FIG. **2**. Example of oxygen-independent alkane activation cycle via fumarate addition and conversion to an acyl-CoA, generation of product precursor acetyl-CoA and propionyl-CoA, regeneration of fumarate from propionyl-CoA, and generalized product formation from acetyl-CoA. Enzymes, 1: alkyl succinate synthase, 2a: succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/2-methyl-alkyl-succinyl-CoA synthetase, 2b: 2-methyl-alkyl-malonyl-CoA mutase, 2c: 2-methyl-alkyl-malonyl-CoA decarboxylase, 2d: β-oxidation enzymes, 2e: propionyl-CoA carboxylase, 2f: methylmalonyl-CoA epimerase and methylmalonyl-CoA mutase, 2g: succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/succinyl-CoA synthetase, 2h: succinate dehydrogenase.

[0055] FIG. **3**. Example of one specific oxygen-independent alkane activation cycle via fumarate addition and conversion to an acyl-CoA, generation of product precursor acetyl-CoA and pyruvate (a keto acid), regeneration of fumarate from pyruvate, and generalized product formation from acetyl-CoA.

[0056] FIG. **4**. Example of one specific oxygen-independent alkane activation cycle via fumarate addition and conversion to an acyl-CoA, generation of product precursor acetyl-CoA and propionyl-CoA, regeneration of fumarate from propionyl-CoA, and generalized product formation from acetyl-CoA. Enzymes, 1: methyl succinate synthase, 2: succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/2-methyl-alkyl-succinyl-CoA synthetase, 3: 2-methyl-alkyl-succinyl-CoA dehydrogenase, 4: mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase, 5: β-methylmalyl-CoA lyase, 6: propionyl-CoA carboxylase, 7: methylmalonyl-CoA epimerase and methylmalonyl-CoA mutase, 8: succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/succinyl-CoA synthetase, 9: succinate dehydrogenase, 10: glyoxylate

carboligase, 11: tartronate semialdehyde reductase, 12: glycerate kinase, 13: glycolytic enzymes (phosphoglycerate mutase, enolase, pyruvate kinase), 14: pyruvate dehydrogenase or pyruvate formate lyase, 15: β-oxidation reversal or fatty acid biosynthesis pathways with desired termination pathways.

[0057] FIG. **5**. Oxygen-dependent alkane activation via terminal addition of a hydroxyl group to a primary alcohol and conversion to acyl-CoA, generation of precursor acetyl-CoA, and generalized product synthesis from acetyl-CoA. Enzymes, 1: alkane monooxygenase or alkane hydroxylase, 2: alcohol dehydrogenase, 3: aldehyde dehydrogenase, 4: acyl-CoA synthetase, 5: acylating aldehyde dehydrogenase.

[0058] FIG. **6**A-D. Vectors expressing the components of the oxygen-independent activation enzyme alkyl-succinate synthase from *Azoarcus* sp. HxN1. MasB (A9J4K0), MasC (A9J4K2), MasD (A9J4K4), MasE (A9J4K6), and MasG (A9J4J6) have been proposed as required components for enzyme function.

[0059] FIG. **7**. Expression of components of the alkyl-succinate synthase from *Azoarcus* sp. HxN1, part of the oxygen-independent alkane activation/utilization pathway. Soluble cell extract of *E. coli* cells expressing MasB (A9J4K0), MasC (A9J4K2), MasD (A9J4K4), MasE (A9J4K6), and MasG (A9J4J6). Lane 1: pETDuet-1-P1-MasCDEB-P2-MasG; Lane 2: pETDuet-1-P1-MasCDE-P2-MasG; Lane 3: pETDuet-1-P1-MasCDEB; Lane 4: pETDuet-1-P1-MasCDE; Lane 5: pETDuet-1.

[0060] FIG. **8**A-C. Vectors expressing the components of the oxygen-independent activation enzyme alkyl-succinate synthase from *Desulfosarcina* sp. BuS5. Proteins identified through similarity to *Azoarcus* sp. HxN1 MasBCDEG as well as physical location in *Desulfosarcina* sp. BuS5 genome. Mas5: WP_027352796.1; Mas8: WP_051374532.1; Mas11: WP_027352800.1.

[0061] FIG. **9**A-C. Vectors expressing the components of the oxygen-independent activation enzyme alkyl-succinate synthase from *Desulfosarcina* sp. BuS5. Proteins identified through similarity to *Azoarcus* sp. HxN1 MasBCDEG as well as physical location in *Desulfosarcina* sp. BuS5 genome. Mas5: WP_027352796.1; Mas4: WP_027352795.1; Mas3: WP_027352794.1; Mas2: WP_027352793.1; Mas8: WP_051374532.1; Mas11: WP_027352800.1.

[0062] FIG. **10**A-C. Vectors expressing enzymes for the conversion of fumarate activated hydrocarbon intermediates to product precursors. TaBbsEF: *T. aromatica* succinyl-CoA:(R)-benzylsuccinate CoA-transferase (Q9KJF0, Q9KJE9); RsMcd: *R. sphaeroides* (2S)-methylsuccinyl-CoA dehydrogenase (ADC44452.1); RsMch: *R. sphaeroides* mesaconyl-coenzyme A hydratase (Q3IZ78).

[0063] FIG. **11**. Expression of enzymes involved in the oxygen-independent alkane utilization pathway. Left: Whole cell extract of *E. coli* cells expressing *T. aromatica* succinyl-CoA:(R)-benzylsuccinate CoA-transferase TaBbsEF (Q9KJF0, Q9KJE9), Lane 1: pCDFDuet-1-P1-TaBbsEF; Lane 2: pCDFDuet-1. Right: Soluble cell extract of *E. coli* cells expressing *R. sphaeroides* (2S)-methylsuccinyl-CoA dehydrogenase RsMcd (ADC44452.1) and *R. sphaeroides* mesaconyl-coenzyme A hydratase RsMch (Q3IZ78), Lane 1: pCDFDuet-1-P1-P2-RsMch-RsMcd; Lane 2: pCDFDuet-1-P1-P2-RsMch; Lane 3: pCDFDuet-1. RsMcd: *R. sphaeroides* (2S)-methylsuccinyl-CoA dehydrogenase (ADC44452.1); RsMch: *R. sphaeroides* mesaconyl-coenzyme A hydratase (Q3IZ78).

[0064] FIG. **12**. Oxygen-dependent alkane activation of hexane with *P. putida* alkane hydroxylase AlkBGT. GC-FID chromatogram for products of whole cell biotransformation of BL21(DE3) cells with indicated vectors shown. 1 mL of cell suspension ($OD_{550\ nm}$=12) incubated with 250 μL hexane at 30° C. for 2 hours. Hexanol only observed only in cells expressing AlkBGT.

[0065] FIG. **13**. Oxygen-dependent alkane activation of pentane with *P. putida* alkane hydroxylase AlkBGT. GC-FID chromatogram for products of whole cell biotransformation of BL21(DE3) cells with indicated vectors shown. 1 mL of cell suspension ($OD_{550\ nm}$=12) incubated with 250 μL pentane at 30° C. for 2 hours. Pentanol only observed only in cells expressing AlkBGT.

[0066] FIG. **14**A-B. Vectors expressing the putative alkane monooxygenase from *Rhodobacter sphaeroides*. RSP2792: monooxygenase alpha subunit (YP_352924.1); RSP2793: monooxygenase reductase component (YP_352923.1); RSP2794: monooxygenase beta subunit (YP_352922.1); RSP2795: regulatory protein of multicomponent monooxygenase (YP_352921.1).

[0067] FIG. **15**. TABLE G: list of some possible embodiments.

DETAILED DESCRIPTION

[0068] The activation of the short chain hydrocarbon substrates, such as methane of LNG components, requires the high stability and low reactivity of hydrocarbon compounds to be overcome through the cleavage of an inert C—H bond. Two main biological approaches can be exploited here for this purpose, 1) the oxygen-independent activation through the addition of fumarate to form a 2-methyl-alkyl-succinyl-CoA, or 2) the oxygen-dependent activation through the terminal addition of a hydroxyl group to the alkane to form a primary alcohol.

[0069] The first approach leverages a native pathway for anaerobic hydrocarbon activation used by certain microorganisms for the degradation of C_6-C_{20} alkanes (Callaghan, 2013). In this pathway, an alkyl-succinate synthase first forms a branched methyl-alkyl-succinate adduct from the addition of fumarate to the alkane, with further metabolism and fumarate regeneration steps resulting in the conversion of hydrocarbon substrates to central, intracellular metabolites (FIG. **2**).

[0070] In order to meet ATP requirements of this cycle, native hydrocarbon degrading microorganisms utilize the electrons generated during substrate oxidation for anaerobic respiration with nitrate, metal ions, or sulfate, thereby making hydrocarbon degradation thermodynamically favorable (Mbadinga et al., 2011). An example of this type of process is found in strain HxN1, which can grow anaerobically on alkanes such as hexane when coupled to denitrification, with the key genes required for initial alkane activation proposed (Grundmann et al., 2008). Reports also have suggested similar activation with shorter chain hydrocarbons in bacterial consortiums, although the enzymes responsible have not been identified (Duncan et al., 2009; Kniemeyer et al., 2007).

[0071] Furthermore, several potential variations of this pathway exist in which various biological reactions can be utilized to convert the 2-methyl-alkyl-succinate adduct formed via fumarate addition into central metabolic intermediates (FIG. **3** and FIG. **4**). Regardless of the pathway selected, the end result of the oxygen-independent hydro-

carbon activation pathway is the formation of an acyl-CoA molecule that can be used to generate the product precursor acetyl-CoA. While in certain cases acetyl-CoA is directly formed as a result of initial acyl-CoA formation (FIG. **3** and FIG. **4**), other initial acyl-CoA intermediates require further oxidation for the generation of acetyl-CoA (FIG. **2**). In all cases, the end result is the generation of acetyl-CoA to serve as a product precursor, as well as another acyl-CoA of keto-acid compound that can be used for fumarate regeneration.

[0072] Considering the use of fumarate in the alkane activation cycle, this oxygen-independent activation approach also requires pathways for the regeneration of this compound from an acyl-CoA or keto-acid. Details on the pathways leading to the regeneration of fumarate from an acyl-CoA, such as propionyl-CoA, or a keto-acid, such as pyruvate, are shown in FIG. **2**, FIG. **3**, and FIG. **4**. This regeneration enables the continued operation of the hydrocarbon activation cycle via fumarate addition.

[0073] In contrast to hydrocarbon activation via fumarate addition, an alternative pathway for the cleavage of an inert C—H bond is the use of an oxygen-dependent activation mechanism in which an alkane hydroxylase/monooxygenase adds a terminal alcohol group to the hydrocarbon, which is then further oxidized (FIG. **5**). The end result of the oxygen-dependent activation pathway is an acyl-CoA intermediate than can be used for the generation of the product precursor acetyl-CoA. Given the direct use of oxygen in this pathway for activation, no additional pathways are required to regenerate an activation compounds as in the above oxygen-independent approach.

[0074] Once the product precursor acetyl-CoA has been formed through the above described approaches, the desired products can then be synthesized through various native or engineered metabolic pathways. These include both the reversal of the β-oxidation cycle and the fatty acid biosynthesis pathway, which both proceed from acetyl-CoA as the starting compound. While the type of condensation reaction for chain elongation varies between the 2 pathways (reversal of the β-oxidation cycle utilizes a non-decarboxylative condensation while the fatty acid biosynthesis pathway employs a decarboxylative condensation), both proceed through a series of condensation, reduction, dehydration, and reduction reactions that result in an intermediate 2 carbons longer than the starting unit. As such, each of these pathways can be exploited for the synthesis of a wide range of products through the selection of termination pathways that convert cycle intermediates to products such as carboxylic acids and alcohols among others.

[0075] Thus, either of these product synthesis pathways, combined with the key pathway modules for hydrocarbon activation and conversion to an acyl-CoA intermediate, generation of product precursor acetyl-CoA, and generation of an addition acyl-CoA or keto-acid for fumarate when required, and the formation of a desired product from acetyl-CoA enables an engineered microorganism capable of the conversion of short-chain hydrocarbons (e.g. methane, ethane, propane, butane, pentane) to fuels and chemicals (e.g. carboxylic acids, alcohols, hydrocarbons, and their alpha-, beta-, and omega-functionalized derivatives).

[0076] The following description provides additional details, any one of which can be subject to patenting in combination with any other. The specification in its entirety is to be treated as providing a variety of details that can be used interchangeably with other details, as the specification would be of inordinate length if one were to list every possible combination of genes/vectors/enzymes/hosts that can be made to convert short chain hydrocarbons to desired fuels and chemicals of interest. Some possible combinations are listed in Table G, however, (FIG. **15**).

[0077] Enzymes of interest can be expressed from vectors such as pETDuet-1 or pCDFDuet-1 (MERCK, Germany), which makes use of the DE3 expression system. Genes can be codon optimized according to the codon usage frequencies of the host organism and synthesized by a commercial vendor or in-house. However, thousands of expression vectors and hosts are available, and this is a matter of convenience.

[0078] The genes can be amplified by PCR using primers designed with 15-22 base pairs of homology for the appropriate vector cut site. For enzymes that will not require a 6×-histadine tag fusion for purification, pCDFDuet-1 can be linearized with NcoI and EcoRI. Enzymes that will be purified by Ni-NTA column will make use of the 6×-HIS tag in pCDFDuet-1. The vector can be linearized using only EcoRI in this case.

[0079] The PCR product can be inserted into the vector using e.g., the In-Fusion HD EcoDry Cloning System and the vector transformed by heat shock into competent *E. coli* cells. Transformants can be selected on solid media containing the appropriate antibiotic. Plasmid DNA can be isolated using any suitable method, including QIAprep Spin Miniprep Kit (QIAGEN, Limburg), and the construct confirmed by PCR and sequencing. Confirmed constructs can be transformed by e.g., electroporation into a host strain such as *E. coli* for expression, but other host species can be used with suitable expression vectors and possible codon optimization for that host species.

[0080] Expression of the desired enzymes from the constructed strain can be conducted in liquid culture, e.g., shaking flasks, bioreactors, chemostats, fermentation tanks and the like. Gene expression is typically induced by the addition of a suitable inducer, when the culture reaches an $OD_{550\ nm}$ of approximately 0.5-0.8. Induced cells can be grown for about 4-8 hours, at which point the cells can be pelleted and saved to −20° C. Expression of the desired protein can be confirmed by running samples on SDS-PAGE.

[0081] The expressed enzyme can be directly assayed in crude cell lysates, simply by breaking the cells by chemical, enzymatic, heat or mechanical means. Depending on the expression level and activity of the enzyme, however, purification may be required to be able to measure enzyme activity over background levels. Purified enzymes can also allow for the in vitro assembly of the pathway, allowing for its controlled characterization.

[0082] N- or C-terminal HIS-tagged proteins can be purified using e.g., a Ni-NTA Spin Kit (Qiagen, Venlo, Limburg) following the manufacturer's protocol, or other methods could be used. The HIS-tag system was chosen for convenience only, and other tags are available for purification uses. Further, the proteins in the final assembled pathway need not be tagged if they are for in vivo use. Tagging was convenient, however, for the enzyme characterization work performed hereunder.

[0083] Reaction conditions for enzyme assays can vary greatly with the type of enzyme to be tested. In general, however, enzyme assays follow a similar general protocol.

Purified enzyme or crude lysate is added to suitable reaction buffer. Reaction buffers typically contain salts, necessary enzyme cofactors, and are at the proper pH. Buffer compositions often change depending on the enzyme or reaction type. The reaction is initiated by the addition of substrate, and some aspect of the reaction related either to the consumption of a substrate or the production of a product is monitored.

[0084] Choice of the appropriate monitoring method depends on the compound to be measured. Spectrophotometric assays are convenient because they allow for the real time determination of enzyme activity by measuring the concentration dependent absorbance of a compound at a certain wavelength. There are not always compounds with a measureable absorbance at convenient wavelengths in the reaction, unfortunately. In these situations, other methods of chemical analysis may be necessary to determine the concentration of the involved compounds.

[0085] As an example, cultures for enzymatic assays were conducted in 125 mL Erlenmeyer flasks containing 25 mL LB media inoculated at 3% from an overnight culture. *E. coli* strains containing constructs expressing genes of interest were grown under appropriate conditions until an optical density of ~0.5 was reached, at which point inducer(s) were added and the cells incubated for an additional 4 hrs. Cell harvesting and preparation of crude cell extracts for enzyme assays was conducted as described elsewhere (Dellomonaco et al., 2011). Enzymatic reactions were then monitored on either a Synergy HT plate reader (BioTek Instruments, Inc., Winooski, Vt.) or a Biomate 5 Spectrophotometer (Thermo Scientific, Waltham, Mass.) according to established protocols.

[0086] Furthermore, whole cell biotransformations represent another method to determine enzyme/pathway functionality. As an example, biotransformations for alkane activation were conducted using *E. coli* BL21(DE3) with appropriate vectors. 750 μL of an overnight LB culture with appropriate antibiotics was used to inoculate 25 mL LB media with 10 μM $FeSO_4$ in a 125 mL Pyrex Erlenmeyer flask. Flasks were incubated at 37° C. and 200 rpm in an NBS Benchtop Incubator Shaker until an optical density of ~0.5 was reached, at which point appropriate inducers were added. Cultures were grown for 4 hrs post-induction and then centrifuged (8000 rpm, 22° C., 5 min) and re-suspended to an optical density ~12 in 100 mM KP_i buffer (pH 7.0) with 1% glycerol (Koch et al., 2009). 250 μL alkane was then added to a 2 mL cell suspension in a 5 mL glass vial and incubated with rotation (60 rpm) at 30° C. for 2 hrs. Following the biotransformations, products (alcohols) were extracted and analyzed as previously described (Kim et al., 2015).

[0087] Gas chromatography (GC) is convenient for the quantification of volatile substances, of which fatty acids and alcohols are of particular relevance. Internal standards, typically one or more molecules of similar type not involved in the reaction, are added to the reaction mixture, and the reaction mixture is extracted with an organic solvent, such as hexane. Fatty acid samples, for example, can be dried under a stream of nitrogen and converted to their trimethylsilyl derivatives using N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine in a 1:1 ratio. After 30 minutes incubation, the samples are once again dried and resuspended in hexane to be applied to the gas chromatograph (GC). Aldehyde samples do not need to be derivatized. Samples can be run e.g., on a Varian CP-3800 gas chromatograph (VARIAN ASSOCIATES, Palo Alto, Calif.) equipped with a flame ionization detector and HP-5 capillary column (AGILENT TECH., CA).

[0088] Once pathways have been fully studied in vitro, they can be constructed in vivo with greater confidence. The strain construction for the in vivo pathway operation should allow for the well-defined, controlled expression of the enzymes of the pathway. As before, *E. coli* or yeast will be a host of choice for the in vivo pathway, but other hosts could be used. The Duet system, for example, allows for the simultaneous expression of up to eight proteins by induction with IPTG in *E. coli*, and initial experiments will use this host.

[0089] Pathway enzymes can also be inserted into the host chromosome, allowing for the maintenance of the pathway without requiring antibiotics to ensure the continued upkeep of plasmids. A large number of genes that can be placed on the chromosome, as chromosomal expression does not require separate origins of replication as is the case with plasmid expression.

[0090] DNA constructs for chromosomal integration usually include an antibiotic resistance marker with flanking FRT sites for removal, as described by Datsenko and Wanner, a well characterized promoter, a ribosome binding site, the gene of interest, and a transcriptional terminator. The overall product is a linear DNA fragment with 50 base pairs of homology for the target site on the chromosome flanking each side of the construct.

[0091] However, the Flp-FRT recombination method is only one system for adding genes to a chromosome, and other systems are available, such as the RecBCD pathway, the RecF pathway, RecA recombinase, non-homologous end joining (NHEJ), Cre-Lox recombination, TYR recombinases and integrases, SER resolvases/invertases, SER integrases, PhiC31 Integrase, and the like. Chromosomal modifications in *E. coli* can also achieved by the method of recombineering, as originally described by Datsenko and Wanner.

[0092] In a recombineering method, for example, the cells are prepared for electroporation following standard techniques, and the cells transformed with linear DNA that contains flanking 50 base pair targeting homology for the desired modification site. For seamless integration of a DNA construct, a two-step approach can be taken using a cassette that contains both positive and negative selection markers, such as the combination of cat and sacB. In the first round of recombineering, the cat-sacB cassette with targeting homology for the desired modification site is introduced to the cells. The cat gene provides resistance to chloramphenicol, which allows for positive recombinants to be selected for on solid media containing chloramphenicol.

[0093] A positive isolate can be subjected to a second round of recombineering introducing the desired DNA construct with targeting homology for sites that correspond to the removal of the cat-sacB cassette. The sacB gene encodes for an enzyme that provides sensitivity to sucrose. Thus, growth on media containing sucrose allows for the selection of recombinants in which the cat-sacB construct was removed. P1 phage lysates can be made from isolates confirmed by PCR and sequencing. The lysates can be used to transduce the modification into desired strains, as described previously.

[0094] Engineered strains expressing the designed pathway can be cultured under the following or similar conditions. Overnight cultures started from a single colony can be used to inoculate flasks containing appropriate media. Cultures are grown for a set period of time, and the culture media analyzed. The conditions will be highly dependent on the specifications of the actual pathway and what exactly is to be tested. For example, the ability for the pathway to be used for hydrocarbon utilization can be tested by the use of short-chain alkanes as a substrate in MOPS minimal media, as described by Neidhardt, supplemented with appropriate antibiotics, and inducers.

[0095] Analysis of culture media after fermentation provides insight into the performance of the engineered pathway. Quantification of hydrocarbons and longer chain fatty acid and alcohol products can be analyzed by GC. Other metabolites, such as short chain organic acids and alcohols can be analyzed by high pressure liquid chromatograph (HPLC). Once the pathway is fully functional, the cultures can be grown in chemostat, providing continuous uninterrupted production of product if desired.

[0096] Various-omics techniques, such as microarray or 2D-PAGE can give information about gene expression or protein expression, respectively. Genome scale modeling allows for the identification of additional modifications to the host strain that might lead to improved performance. Deletion of competing pathways, for example, might increase carbon flux through the engineered pathway for product production.

[0097] Standard molecular biology techniques were used for gene cloning, plasmid isolation, and *E. coli* transformation. Native *E. coli* genes were amplified from *E. coli* MG1655 genomic DNA using primers to append 15 bp of homology on each end of the gene insert for recombination into the vector backbone. Genes from other organisms were codon optimized and synthesized by either GeneArt (LIFE TECH., CA or GENSCRIPT, NJ). Plasmids were linearized by the appropriate restriction enzymes and recombined with the gene inserts using the In-Fusion HD Eco-Dry Cloning system (CLONTECH LAB. CA,). The mixture was subsequently transformed into Stellar competent cells (CLONTECH LAB.).

[0098] Transformants that grew on solid media (LB+Agar) supplemented with the appropriate antibiotic were isolated and screened for the gene insert by PCR. Plasmid was isolated from the verified transformants and the sequence of the gene insert was further confirmed by DNA sequencing (LONE STAR LABS, TX). Plasmids (also referred to as vectors) in each case contain at least one promoter, a ribosome binding site for each gene, the gene(s) of interest, at least one terminator, an origin of replication, and an antibiotic resistance marker. Exemplary plasmids are shown in FIGS. **6**A-D, **8**A-C, **9**A-C, **10**A-C and **14**.

[0099] Genes that encode the enzymes of the engineered pathway were cloned and expressed as described above. The crude protein extracts or purified enzymes were assessed for their ability to catalyze the proposed reactions. Tables B-F below describe the characterization of enzymes for required pathway steps depicted in FIG. **2**, FIG. **3**, FIG. **4**, and FIG. **5**. In addition to the experimental data provided, several literature examples are listed to provide additional evidence to the ability of known enzymes to possess the required enzymatic activity of several pathway steps. Thus, a great variety of enzymes are exemplified herein.

[0100] TABLE B describes the characterization of enzymes involved in hydrocarbon activation/utilization and conversion to acyl-CoA intermediates through the fumarate addition pathway as depicted in FIG. **2**.

TABLE B

Characterization of enzymes involved in the pathway for bioconversion of hydrocarbons via fumarate addition as depicted in FIG. 2

Enzyme Number	Enzyme class	Enzyme	Substrate	Measured specific activity (μmol/mg protein/min)	Reference
1	alkylsuccinate synthase			See Table C for details	
2a	succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/2-methyl-alkyl-succinyl-CoA synthetase	*T. aromatica* BSCT	Methylsuccinate and succinyl-CoA	Methylsuccinyl-CoA formation observed	Leutwein and Heider (2001)
2b	2-methyl-alkyl-malonyl-CoA mutase	*R. Sphaeroides* Ecm	ethylmalonyl-CoA	0.05, K_m: 60 μM (methylsuccinyl-CoA product)	Erb et al (2008)
2c	2-methyl-alkyl-malonyl-CoA decarboxylase	*M. musculus* ECHDC1	ethylmalonyl-CoA	8800, K_m: 0.96 μM	Linster et al (2011)
			methylmalonyl-CoA	K_m: 3.1 μM	Linster et al (2011)
2e	propionyl-CoA carboxylase	*M. sedula* Pcc	propionyl-CoA	3.3, K_m: 70 μM (75° C.)	Hugler et al (2003)
		S. coelicolor Pcc	propionyl-CoA	0.2, K_m: 76 μM	Arabolaza et al (2010)
2f	methylmalonyl-CoA epimerase	*P. horikoshii* Mce	(S)-2-methylmalonyl-CoA	162, K_m: 79 μM	Bobik and Rasche (2004)
	methylmalonyl-CoA mutase	*P. freudenreichii* subsp. *shermanii* Mcm	(R)-2-methylmalonyl-CoA	26, K_m: 124 μM	Chowdhury et al (1999); Padovani et al (2006)
2g	succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/succinyl-CoA synthetase	*E. coli* SucCD	Succinate (reversible reaction)	18.6, K_m: 141 μM	Nolte et al (2014)

TABLE B-continued

Characterization of enzymes involved in the pathway for bioconversion of hydrocarbons via fumarate addition as depicted in FIG. 2					
Enzyme Number	Enzyme class	Enzyme	Substrate	Measured specific activity (μmol/mg protein/min)	Reference
2h	succinate dehydrogenase	*E. coli* SdhCDAB	succinate	K_m: 2.5 μM	Maklashina et al (2001)
		2d: β-oxidation enzymes			
—	Acyl-CoA dehydrogenase	*E. coli* FadE	butyryl-CoA	0.008 ± 0.001	This work
—	Enoyl-CoA hydratase	*E. coli* FadB	crotonyl-CoA	0.051 ± 0.004	This work
—	3-hydroxyacyl-CoA dehydrogenase	*E. coli* FadB	3-hydroxybutyryl-CoA	0.185 ± 0.001	This work
—	3-ketoacyl-CoA thiolase	*E. coli* AtoB	acetoacetyl-CoA	17.1 ± 1.2	This work
		E. coli FadA	acetoacetyl-CoA	0.013 ± 0.002	This work
		R. eutropha BktB	acetoacetyl-CoA	27.0 ± 1.1	This work
		S. collinus FadA	acetoacetyl-CoA	115.6 ± 1.0	This work
		P. putida FadAx	acetoacetyl-CoA	30.9 ± 0.3	This work

[0101] Additional details on alkylsuccinate synthase enzymes, required for the activation of hydrocarbons via fumarate addition are provided in TABLE C:

TABLE C

Observed alkane activation via fumarate addition (alkylsuccinate synthases)				
Hydrocarbon Substrate	Organism(s)	Product	Literature Evidence	Reference
Methane	bacterial consortium (Alaskan North Slope)	methylsuccinate	Product detected (2.08 μM) in oil field sample	Duncan et al. (2009)
	bacterial consortium	methylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Ethane	bacterial consortium (Alaskan North Slope)	ethylsuccinate	Product detected (1.77 μM) in oil field sample	Duncan et al. (2009)
	bacterial consortium	ethylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Propane	bacterial consortium (Alaskan North Slope)	methylethylsuccinate	Product detected (2.18 μM) in oil field sample	Duncan et al. (2009)
	Desulfosarcina sp. BuS5	methylethylsuccinate/n-propylsuccinate	Terminal and sub-terminal product formation from bacterial cultures	Kniemeyer et al. (2007)
	bacterial consortium	methylethylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Butane	bacterial consortiums (Alaskan North Slope)	methylpropylsuccinate	Product detected (0.76 μM) in oil field sample	Duncan et al. (2009)
	Desulfosarcina sp. BuS5	methylpropylsuccinate	Terminal and sub-terminal product formation from bacterial cultures	Kniemeyer et al. (2007)
	bacterial consortium	methylpropylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Pentane	*Azoarcus* sp. HxN1	Methylbutylsuccinate	Product formation from crude enzyme assay (~5% relative to hexane)	Webner (2012)
	bacterial consortium	methylbutylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Hexane	*Azoarcus* sp. HxN1	methylpentylsuccinate	Product formation from crude enzyme assay (55 μM in crude extract assays)	Webner (2012)
	bacterial consortium	methylpentylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Heptane	*Azoarcus* sp. HxN1	methylhexylsuccinate	Product formation from crude enzyme assay (~120% relative to hexane)	Webner (2012)
	bacterial consortium	methylhexylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)
Octane	*Azoarcus* sp. HxN1	methylheptylsuccinate	Product formation from crude enzyme assay (~50% relative to hexane)	Webner (2012)

TABLE C-continued

Observed alkane activation via fumarate addition (alkylsuccinate synthases)				
Hydrocarbon Substrate	Organism(s)	Product	Literature Evidence	Reference
	bacterial consortium	methylheptylsuccinate	Product detected in production field sample from oil reservoir	Bian et al (2015)

[0102] Included in these alkylsuccinate synthase enzymes are those from *Azoarcus* sp. HxN1 (Grundmann et al., 2008) and *Desulfosarcina* sp. BuS5 (Kniemeyer et al., 2007) for which the catalytic subunit, and associated subunits for the case of *Azoarcus* sp. HxN1, have been proposed. Genes encoding the proposed subunits of the *Azoarcus* sp. HxN1 alkylsuccinate synthase have been cloned into required expression vectors as shown in FIG. **6**. Evaluation of the expression of alkylsuccinate synthase subunits in *E. coli* is shown in FIG. **7**.

[0103] Furthermore, several candidate alkylsuccinate synthase subunits and associated proteins have been identified through a BLAST search of *Azoarcus* sp. HxN1 alkyl succinate synthase subunits against the recently sequenced *Desulfosarcina* sp. BuS5 genome, a strain that degrades propane and butane via fumarate addition. This search identified several enzymes within close physical proximity to the large catalytic MasD subunit (WP_027352796.1), including 2 enzymes with high similarity to the *Azoarcus* sp. HxN1 MasE subunit (WP_027352794.1 and WP_027352793.1), a protein with similarity to alkylsuccinate synthase gamma subunits from *Desulfatibacillum alkenivorans* AK-01 and *Smithella* sp. SCADC (WP_027352795.1), and enzymes with similarity to the *Azoarcus* sp. HxN1 MasG activating enzyme (WP_051374532.1) and other radical SAM enzymes (WP_027352800.1). In all, 13 proteins appear to be encoded from an operon within the *Desulfosarcina* sp. BuS5 genome spanning the locus from 66239-79342 in the associated genome sequence NZ_AXAM01000003.1. Genes encoding potential subunits of the alkylsuccinate synthase (WP_027352793.1, WP_027352794.1, WP_ 027352795.1, and WP_027352796.1) and activation enzyme (WP_051374532.1 and WP_027352800.1) have been cloned into required expression vectors as shown in FIG. **8** and FIG. **9**.

[0104] This type of homology search can be further exploited to identify other potential alkylsuccinate synthase enzymes/operons through a BLAST search of these enzymes against other hydrocarbon degrading species. For example, a search of the *Desulfosarcina* sp. BuS5 MasD subunit (WP_027352796.1) was used to identify potential MasD subunits from species such as *Peptococcaceae bacterium* SCADC1_2_3 (WP_036734374.1), *Desulfoglaeba alkanexedens* ALDC (ADJ51097.1), and *Peptococcaceae bacterium* BRH_c4a (KJS01634.1), among others.

[0105] Furthermore, additional enzymes required for conversion of fumarate activated hydrocarbon intermediates to product precursors have been investigated. Enzymes such as *T. aromatica* succinyl-CoA:(R)-benzylsuccinate CoA-transferase (Q9KJF0, Q9KJE9), *R. sphaeroides* (2S)-methylsuccinyl-CoA dehydrogenase (ADC44452.1), and *R. sphaeroides* mesaconyl-coenzyme A hydratase (Q31Z78) enable the conversion of the fumarate activated hydrocarbons to product precursors, which can then be converted to desired products. Genes encoding the above proteins have been cloned into required expression vectors (FIG. **10**A-C) and their expression in *E. coli* evaluated (FIG. **11**).

[0106] The above described enzymes for hydrocarbon activation/utilization and conversion to acyl-CoA intermediates through the fumarate addition pathway provide a route for the bioconversion of short-chain hydrocarbons of varying chain length. For the specific case of methane activation/utilization, the pathways depicted in FIG. **3** and FIG. **4** can also be exploited for the conversion of fumarate activated methane into product precursors as well as for the regeneration of fumarate. TABLE D and TABLE E describe the characterization of enzymes involved in these versions of the bioconversion pathway:

TABLE D

Characterization of enzymes involved in the pathway for bioconversion of hydrocarbons via fumarate addition as depicted in FIG. 3				
Enzyme name	Enzyme	Substrate	Measured specific activity (μmol/mg protein/min)	Reference
alkylsuccinate synthase			See Table B for details	
succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/2-methyl-alkyl-succinyl-CoA synthetase	*T. aromatica* BSCT	Methylsuccinate and succinyl-CoA	Methylsuccinyl-CoA formation observed	Leutwein and Heider (2001)
2-methyl-alkyl-succinyl-CoA dehydrogenase	*R. sphaeroides* Mcd	(2S)-methylsuccinyl-CoA	mesaconyl-CoA formation observed	Erb et al (2009)
mesaconyl-C1-CoA-C4 transferase	*C. aurantiacus* Mct	mesaconyl-C1-CoA	mesaconyl-C4-CoA formation observed	Zarzycki et al (2009)
mesaconyl-C4-CoA hydratase	*C. aurantiacus* Meh	mesaconyl-C4-CoA	(S)-citramalyl-CoA formation observed	Zarzycki et al (2009)
L-malyl-CoA-citramalyl-CoA lyase	*C. aurantiacus* Mmc	(S)-citramalyl-CoA	Acetyl-CoA and pyruvate formation observed	Zarzycki et al (2009)

TABLE D-continued

Characterization of enzymes involved in the pathway for bioconversion of hydrocarbons via fumarate addition as depicted in FIG. 3

Enzyme name	Enzyme	Substrate	Measured specific activity (μmol/mg protein/min)	Reference
pyruvic-malic carboxylase	*E. coli* MaeA	pryuvate	1.3, K_m: 16 mM	Stols and Donnelly (1997)
fumarase	*E. coli* FumA	L-malate	706, K_m: 700 mM	van Vugt-Lussenburg et al (2013)

TABLE E

Characterization of enzymes involved in the pathway for bioconversion of hydrocarbons via fumarate addition as depicted in FIG. 4

Enzyme Number	Enzyme class	Enzyme	Substrate	Measured specific activity (μmol/mg protein/min)	Reference
1	alkylsuccinate synthase	See Table C for details			
2	succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/2-methyl-alkyl-succinyl-CoA synthetase	*T. aromatica* BSCT	Methylsuccinate and succinyl-CoA	Methylsuccinyl-CoA formation observed	Leutwein and Heider (2001)
3	2-methyl-alkyl-succinyl-CoA dehydrogenase	*R. sphaeroides* Mcd	(2S)-methylsuccinyl-CoA	mesaconyl-CoA formation observed	Erb et al (2009)
4	mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase	*R. sphaeroides* Mch	β-methylmalyl-CoA (reverse reaction)	1400, mesaconyl-CoA formation observed	Zarzycki et al (2008)
5	β-methylmalyl-CoA lyase	*R. sphaeroides* Mcl1	β-methylmalyl-CoA	26, K_m:10 μM	Erb et al (2010)
6	propionyl-CoA carboxylase	*M. sedula* Pcc	propionyl-CoA	3.3, K_m:70 μM (75° C.)	Hugler et al (2003)
		S. coelicolor Pcc	propionyl-CoA	0.2, K_m:76 μM	Arabolaza et al (2010)
7	methylmalonyl-CoA epimerase	*P. horikoshii* Mce	(S)-2-methylmalonyl-CoA	162, K_m:79 μM	Bobik and Rasche (2004)
	methylmalonyl-CoA mutase	*P. freudenreichii* subsp. *shermanii* Mcm	(R)-2-methylmalonyl-CoA	26, K_m:124 μM	Chowdhury et al (1999); Padovani et al (2006)
8	succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase/succinyl-CoA synthetase	*E. coli* SucCD	Succinate (reversible reaction)	18.6, K_m:141 μM	Nolte et al (2014)
9	succinate dehydrogenase	*E. coli* SdhCDAB	succinate	K_m:2.5 μM	Maklashina et al (2001)
10	glyoxylate carboligase	*E. coli* Gcl	glyoxylate	17.5, K_m:900 μM	Kaplun et al (2008)
11	tartronate semialdehyde reductase	*E. coli* GarR	tartronate emialdehyde	K_m:280 μM	Njau et al (2000)
12	glycerate kinase	*E. coli* GlxK	D-glycerate	K_m:70 μM	Ornston and Ornston (1969)

[0107] Together, these enzymatic components described above provide a full route for the conversion of short chain hydrocarbons to acyl-CoA (e.g. acetyl-CoA) product precursors, which can subsequently be converted into fuels and chemicals (e.g. carboxylic acids, alcohols, hydrocarbons, and their alpha-, beta-, and omega-functionalized derivatives) through numerous product synthesis pathways, such as beta-oxidation reversal (BOX-R) or fatty acid biosynthesis. BOX-R is not described in great detail herein, since it has been described in the inventor's prior patents and publications.

[0108] The use of an oxygen-dependent activation mechanism in which an alkane hydroxylase adds a terminal alcohol group to the hydrocarbon can also be exploited for the bioconversion of short chain hydrocarbons to fuels and chemicals as depicted in FIG. **5**. Characterization of required enzymes for hydrocarbon activation and conversion to product precursors is shown in TABLE F:

TABLE F

Characterization of enzymes involved in the pathway for bioconversion of hydrocarbons via hydroxylation as depicted in FIG. 5

Enzyme Number	Enzyme class	Enzyme	Substrate	Measured specific activity (μmol/mg protein/min)	Reference
1	alkane monooxygenase or alkane hydroxylase	*M. capsulatus* (Bath) sMMO	Methane	$K_{m,methane}$: 3 μM	Green and Dalton (1986)
		M. capsulatus (Bath) pMMO		0.139 ± 0.005	Sirajuddin et al. (2014)
		Thauera butanivorans sBMO		$K_{m,methane}$: 1100 μM	Cooley et al. (2009)
		Thauera butanivorans sBMO	Ethane	$K_{m,ethane}$: 2.2 μM	Cooley et al. (2009)
		P. putida AlkBFG	Propane	Propanol formation observed	Koch et al. (2009)
		Mycobacterium sp. strain HXN-1500 CYP153A6		Propanol formation observed	Koch et al. (2009)
		Thauera butanivorans sBMO		$K_{m,propane}$: 0.94 μM	Cooley et al. (2009)
		P. putida AlkBFG	Butane	Butanol formation observed	Koch et al. (2009)
		Mycobacterium sp. strain HXN-1500 CYP153A6		Butanol formation observed	Koch et al. (2009)
		Thauera butanivorans sBMO		$K_{m,butane}$: 0.24 μM	Cooley et al. (2009)
		P. putida AlkBGT	Pentane	Pentanol formation observed, see FIG. 13	This work
		P. putida AlkBGT	Hexane	Hexanol formation observed, see FIG. 12	This work
2	alcohol dehydrogenase	*E. coli* FucO	butryaldehyde	5.08 ± 0.08	This work
3	Coenzyme A-acylating aldehyde dehydrogenase	*L. Reuteri* PduP	propionaldehyde	25 ± 0.2, K_m: 28 mM	Sabet-Azad et al. (2013)
4	acyl-CoA synthetase	*E. coli* Acs	acetate	K_m: 200 μM	Brown et al. (1977)
		P. Aeruginosa butyryl-CoA synthetase	butyrate	0.255, K_m: 10 μM	Shimizu et al. (1981)
5	acylating aldehyde dehydrogenase	*E. coli* AdhE	butyryl-CoA	0.073 ± 0.00	This work
		E. coli MhpF	butyryl-CoA	0.009 ± 0.003	This work
		β-oxidation enzymes			
—	Acyl-CoA dehydrogenase	*E. coli* FadE	butyryl-CoA	0.008 ± 0.001	This work
—	Enoyl-CoA hydratase	*E. coli* FadB	crotonyl-CoA	0.051 ± 0.004	This work
—	3-hydroxyacyl-CoA dehydrogenase	*E. coli* FadB	3-hydroxybutyryl-CoA	0.185 ± 0.001	This work
—	3-ketoacyl-CoA thiolase	*E. coli* AtoB	acetoacetyl-CoA	17.1 ± 1.2	This work
		E. coli FadA	acetoacetyl-CoA	0.013 ± 0.002	This work
		R. eutropha BktB	acetoacetyl-CoA	27.0 ± 1.1	This work
		S. collinus FadA	acetoacetyl-CoA	115.6 ± 1.0	This work
		P. putida FadAx	acetoacetyl-CoA	30.9 ± 0.3	This work

[0109] In addition to the demonstrated activation of hydrocarbon such as pentane and hexane to the associated alcohols, the enzymes required for the conversion of these alcohols to product precursors have also been characterized. The combination of these enzymatic components provide a full route for the conversion of short chain hydrocarbons to acyl-CoA (e.g. acetyl-CoA) product precursors, which can subsequently be converted into fuels and chemicals (e.g. carboxylic acids, alcohols, hydrocarbons, and their alpha-, beta-, and omega-functionalized derivatives) through numerous product synthesis pathways, such as beta-oxidation reversal or fatty acid biosynthesis.

[0110] The enzymes described in TABLE F represent a number of examples of enzymes with required pathway activity, however this list is not representative of all available enzymes. Many additional enzymes have been shown to possess the required activities in literature and as such, can easily be integrated with the pathway framework. Furthermore, sequence similarity can also be used for the identification of additional enzymes. As an example, components of putative alkane monooxygenase system in *Rhodobacter sphaeroides* were identified through a BLAST using the soluble methane monoxygenase (sMMO) system of *Methylococcus capsulatus* (Bath). Required hydroxylase alpha (YP_352924.1) and reductase (YP_352923.1) subunits were identified, which are part of a 4 gene operon also including a hydroxylase beta subunit (YP_352922.1) and a regulatory protein (YP_352921.1). Genes encoding potential subunits this putative alkane monooxygenase have been cloned into required expression vectors as shown in FIG. **14**A-B.

[0111] By exploiting either of these hydrocarbon activation described above, various fuels and chemicals (e.g. carboxylic acids, alcohols, hydrocarbons, and their alpha-, beta-, and omega-functionalized derivatives) can be synthe-

sized from short-chain hydrocarbons through the metabolic pathways described and demonstrated herein.

[0112] Each of the following is incorporated by reference herein in its entirety for all purposes:

[0113] U.S. 61/440,192, filed Feb. 7, 2011, WO2013036812, 0520130316413 Reverse beta oxidation pathway by Clomburg et al.

[0114] U.S. 61/531,911, filed Sep. 7, 2011, WO2013036812 U520140273110 Functionalized carboxylic acids and alcohols by reverse fatty acid oxidation by Gonzalez & Clomburg

[0115] 61/932,057, filed Jan. 27, 2014, WO2015112988, Type II fatty acid synthesis enzymes in reverse beta-oxidation by Gonzalez & Clomburg

[0116] All accession numbers (generally in brackets after a gene or protein) are expressly incorporated by reference for all purposes herein. Inclusion of the information at each accession entry, would render the patent of inordinate length, and thus, incorporation of all sequences (and other information found therein) by reference is preferred. A person of ordinary skill in the art will recognize the accession numbers and be able to access them from a variety of databases.

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We claim:

1. A genetically engineered microorganism with one or more expression vectors or integrated sequences encoding overexpressed 1) alkane monooxygenase or alkane hydroxylase (EC 1.14.15.3), 2) alcohol dehydrogenase (EC 1.1.1.-), 3) aldehyde dehydrogenase (EC 1.2.1.-), and 4) acyl-CoA synthetase (EC 6.2.1.-); wherein said microorganism is able to convert a short chain alkane (C1-C5) to an acyl-CoA.

2. A genetically engineered microorganism with one or more expression vectors or integrated sequences encoding overexpressed 1) alkyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-malonyl-CoA mutase (EC 5.4.99.-), 4) 2-methyl-alkyl-malonyl-CoA decarboxylase (EC 4.1.1.-), 5) propionyl-CoA carboxylase (EC 6.4.1.3), 6) methylmalonyl-CoA epimerase (EC 5.1.99.1), 7) methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), and 9) succinate dehydrogenase (EC 1.3.5.1), wherein said microorganism is able to convert a short chain alkane (C1-C5) to an acyl-CoA through fumarate addition to said short chain alkane and subsequent regeneration of said fumarate.

3. A genetically engineered microorganism with one or more expression vectors or integrated sequences encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-C1-CoA-C4-CoA transferase (EC 2.8.3.-), 5) mesaconyl-C4-CoA hydratase (EC 4.2.1.153), 6) L-malyl-CoA/citramalyl-CoA lyase (EC 4.1.3.25), 7) pyruvic-malic carboxylase (EC 1.1.1.39), and 8) fumarase (EC 4.2.1.2), wherein said microorganism is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.

4. A genetically engineered microorganism with one or more expression vectors encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase (EC 4.2.1.148), 5) β-methylmalyl-CoA lyase (EC 4.1 3.24), 6) propionyl-CoA carboxylase (EC 6.4.1.3), 7) methylmalonyl-CoA epimerase (EC 5.1.99.1) and methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), 9) succinate dehydrogenase (EC 1.3.5.1), 10: glyoxylate carboligase (EC 4.1.1.47), 11) tartronate semialdehyde reductase (EC 1.1.1.60), 12) glycerate kinase (EC 2.7.1.31), 13:) glycolytic enzymes (phosphoglycerate mutase (EC 5.4.2.11), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40)), and 14) pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) or pyruvate formate lyase (EC 2.3.1.54), wherein said microorganism is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.

5. A genetically engineered microorganism being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) alkane monooxygenase or alkane hydroxylase (EC 1.14.15.3), 2) alcohol dehydrogenase (EC 1.1.1.-), 3) aldehyde dehydrogenase (EC 1.2.1.-), and 4) acyl-CoA synthetase (EC 6.2.1.-); wherein said microorganism is able to convert a short chain alkane (C1-C5) to an acyl-CoA.

6. A genetically engineered microorganism being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) alkyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-malonyl-CoA mutase (EC 5.4.99.-), 4) 2-methyl-alkyl-malonyl-CoA decarboxylase (EC 4.1.1.-), 5) propionyl-CoA carboxylase (EC 6.4.1.3), 6) methylmalonyl-CoA epimerase (EC 5.1.99.1), 7) methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), and 9) succinate dehydrogenase (EC 1.3.5.1), wherein said microorganism is able to converts a short chain alkane (C1-C5) to an acyl-CoA through fumarate addition to said short chain alkane and subsequent regeneration of said fumarate.

7. A genetically engineered microorganism being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-C1-CoA-C4-CoA transferase (EC 2.8.3.), 5) mesaconyl-C4-CoA hydratase (EC 4.2.1.153), 6) L-malyl-CoA/citramalyl-CoA lyase (EC 4.1.3.25), 7) pyruvic-malic carboxylase (EC 1.1.1.39), and 8) fumarase (EC 4.2.1.2), wherein said microorganism is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.

8. A genetically engineered microorganism being *E. coli* and comprising one or more expression vectors or integrated sequences encoding overexpressed 1) methyl succinate synthase (EC 4.1.-), 2) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or 2-methyl-alkyl-succinyl-CoA synthetase (EC 6.2.1.-), 3) 2-methyl-alkyl-succinyl-CoA dehydrogenase (EC 1.3.99.-), 4) mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase (EC 4.2.1.148), 5) β-methylmalyl-CoA lyase (EC 4.1.3.24), 6) propionyl-CoA carboxylase (EC 6.4.1.3), 7) methylmalonyl-CoA epimerase (EC 5.1.99.1) and methylmalonyl-CoA mutase (EC 5.1.99.2), 8) succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase (EC 2.8.3.-) or succinyl-CoA synthetase (EC 6.2.1.5), 9) succinate dehydrogenase (EC 1.3.5.1), 10: glyoxylate carboligase (EC 4.1.1.47), 11) tartronate semialdehyde reductase (EC 1.1.1.60), 12) glycerate kinase (EC 2.7.1.31), 13:) glycolytic enzymes (phosphoglycerate mutase (EC 5.4.2.11), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40)), and 14) pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) or pyruvate formate lyase (EC 2.3.1.54), wherein said microorganism is able to convert methane to an acyl-coA through fumarate addition to said methane and subsequent regeneration of said fumarate.

9. The genetically engineered microorganism of claims **1-8** wherein said expression vectors are inducible expression vectors or said integrated sequences are inducible integrated sequences.

10. A genetically engineered microorganism converting a short-chain (C1-C5) alkane substrate to a product, said microorganism comprising enzymes or overexpressed enzymes catalyzing:

a) a sequence of reactions a pathway for the oxygen-independent activation of a short-chain (C1-C5) alkane via fumarate addition to a 2-methyl-alkyl-succinate and subsequent conversion of said 2-methyl-alkyl-succinate to an acyl-CoA;

b) a sequence of reactions of a pathway for the generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid from said acyl-CoA;

c) a sequence of reactions of a pathway for the regeneration of fumarate from said acyl-CoA or keto-acid to fumarate through conversion;

d) a sequence of reactions of a pathway for the formation of a desired product from said acetyl-CoA intermediate.

11. A genetically engineered microorganism converting a short-chain (C1-C5) alkane substrate to a product, said microorganism comprising enzymes or overexpressed enzymes catalyzing:

a) a sequence of reactions of a pathway for the oxygen-dependent activation of a short-chain (C1-C5) alkane to a primary alcohol via terminal addition of a hydroxyl group and subsequent conversion of said alcohol to an acyl-CoA;

b) a sequence of reactions for the generation of product precursor acetyl-CoA from said acyl-CoA; and

c) a sequence of reactions of a pathway for the formation of a desired product from said acetyl-CoA intermediate.

12. The microorganism of claim **10**, wherein said pathway for the oxygen-independent activation and conversion to an acyl-CoA comprises:

a) an overexpressed alkyl succinate synthase that catalyzes the addition of fumarate to a short-chain (C1-C5) alkane to produce a 2-methyl-alkyl-succinate;

b) an overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or 2-methyl-alkyl-succinyl-CoA synthetase that catalyzes the conversion of said 2-methyl-alkyl-succinate to a 2-methyl-alkyl-succinyl-CoA;

c) an overexpressed 2-methyl-alkyl-malonyl-CoA mutase that catalyzes the isomerization of said 2-methyl-alkyl-succinyl-CoA to a 2-methyl-alkyl-malonyl-CoA; and

d) an overexpressed 2-methyl-alkyl-malonyl-CoA decarboxylase that catalyzes the decarboxylation of said 2-methyl-alkyl-malonyl-CoA to an acyl-CoA.

13. The microorganism of claim **10**, wherein said pathways for the oxygen-independent activation and conversion to an acyl-CoA and generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid comprises:

a) an overexpressed alkyl succinate synthase that catalyzes the addition of fumarate to a short-chain (C1-C5) alkane to produce a 2-methyl-alkyl-succinate;

b) an overexpressed 2-methyl-alkyl-succinyl-CoA synthetase that catalyzes the conversion of said 2-methyl-alkyl-succinate to a 2-methyl-alkyl-succinyl-CoA;

c) an overexpressed 2-methyl-alkyl-succinyl-CoA dehydrogenase that catalyzes the conversion of said 2-methyl-alkyl-succinyl-CoA to 2-methyl-alkyl-2-butenoyl-CoA;

d) an overexpressed mesaconyl-C1-CoA-C4-CoA transferase that catalyzes the conversion of said 2-methyl-alkyl-2-butenoyl-CoA to 3-methyl-alkyl-2-butenoyl-CoA;

e) an overexpressed mesaconyl-C4-CoA hydratase that catalyzes the conversion of said 3-methyl-alkyl-2-butenoyl-CoA to 3-methyl-alkyl-3-hydroxy-succinyl-CoA; and

f) an overexpressed citramalyl-CoA lyase that catalyzes the conversion of said 3-methyl-alkyl-3-hydroxy-succinyl-CoA to acetyl-CoA and a keto-acid.

14. The microorganism of claim **10**, wherein said pathways for the oxygen-independent activation and conversion to an acyl-CoA and generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid comprises:

a) an overexpressed alkyl succinate synthase that catalyzes the addition of fumarate to a short-chain (C1-C5) alkane to produce a 2-methyl-alkyl-succinate;

b) an overexpressed succinyl-CoA2-methyl-alkyl-succinyl-CoA transferase or 2-methyl-alkyl-succinyl-CoA synthetase that catalyzes the conversion of said 2-methyl-alkyl-succinate to a 2-methyl-alkyl-succinyl-CoA;

c) an overexpressed 2-methyl-alkyl-succinyl-CoA dehydrogenase that catalyzes the conversion of said 2-methyl-alkyl-succinyl-CoA to 2-methyl-alkyl-2-butenoyl-CoA;

d) an overexpressed mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase that catalyzes the conversion of said 2-methyl-alkyl-2-butenoyl-CoA to 3-hydroxy-2-methyl-alkyl-succinyl-CoA;

e) an overexpressed 3-methylmalyl-CoA lyase that catalyzes the conversion of said 3-hydroxy-2-methyl-alkyl-succinyl-CoA to glyoxylate and an acyl-CoA;

f) an overexpressed glyoxylate carboligase that catalyzes the conversion of said glyoxylate to tartronate semialdehyde;

g) an overexpressed tartronate semialdehyde reductase that catalyzes the conversion of said tartronate semialdehyde to D-glycerate;
h) an overexpressed glycerate kinase that catalyzes the conversion of said D-glycerate to 3-phospho-D-glycerate;
i) glycolytic enzymes (phosphoglycerate mutase, enolase, pyruvate kinase) that catalyze the conversion of said 3-phospho-D-glycerate to pyruvate; and
j) a pyruvate formate lyase or pyruvate dehydrogenase that catalyze the conversion of said pyruvate to acetyl-CoA.

15. The microorganism of claim **12**, wherein said pathway for the generation of product precursor acetyl-CoA and an acyl-CoA or keto-acid comprises:
a) an overexpressed acyl-CoA dehydrogenase that catalyzes the conversion of said acyl-CoA to a transenoyl-CoA;
b) an overexpressed enoyl-CoA hydratase that catalyzes the hydration of said transenoyl-CoA to a 3-hydroxyacyl-CoA;
c) an overexpressed 3-hydroxyacyl-CoA dehydrogenase that catalyzes the oxidation of said 3-hydroxyacyl-CoA to a β-ketoacyl-CoA; and
d) an overexpressed thiolase that catalyzes the cleavage of an acetyl-CoA from said β-ketoacyl-CoA to produce acetyl-CoA and an acyl-CoA 2-carbons shorter than said starting acyl-CoA.

16. The microorganism of claim **12** or **14**, wherein said pathway for the regeneration of fumarate from an acyl-CoA or keto-acid comprises:
a) an overexpressed propionyl-CoA carboxylase that catalyzes the carboxylation of propionyl-CoA to (S)-methyl-malonyl-CoA;
b) an overexpressed methyl-malonyl-CoA epimerase that catalyzes the interconversion of said (S)-methyl-malonyl-CoA to (R)-methyl-malonyl-CoA;
c) an overexpressed methyl-malonyl-CoA mutase that catalyzes the isomerization of said (R)-methyl-malonyl-CoA to succinyl-CoA; and
d) an overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or succinyl-CoA synthetase that catalyzes the conversion of said succinyl-CoA to succinate.

17. The microorganism of claim **13**, wherein said pathway for the regeneration of fumarate from an acyl-CoA or keto-acid comprises:
a) an overexpressed malate dehydrogenase for the conversion of said keto-acid (pyruvate) to malate; and
b) an overexpressed fumarase for the dehydration of malate to fumarate.

18. The microorganism of claim **13**, wherein said pathway for the regeneration of fumarate from an acyl-CoA or keto-acid comprises:
a) an overexpressed carboxylic acid omega hydroxylase that catalyzes the conversion of said keto-acid to an omega-hydroxy-2-keto-acid;
b) an overexpressed alcohol dehydrogenase that catalyzes the conversion of said omega-hydroxyketo-acid to an omega-oxo-2-keto-acid;
c) an overexpressed aldehyde dehydrogenase that catalyzes the conversion of said omega-oxo-keto-acid to a dicarboxylic 2-keto-acid;
d) an overexpressed ketoreductase or malate dehydrogenase that catalyzes the conversion of said dicarboxylic 2-keto-acid to malate; and
e) an overexpressed fumarase for the dehydration of malate to fumarate.

19. The microorganism of claim **11**, wherein said pathway for the oxygen-dependent activation and conversion to an acyl-CoA comprises:
a) an overexpressed alkane monooxygenase or alkane hydroxylase that catalyzes the terminal hydroxylation of a short-chain (C1-C5) alkane to produce a primary alcohol;
b) an overexpressed alcohol dehydrogenase that catalyzes the oxidation of said primary alcohol to produce an aldehyde;
c) an overexpressed aldehyde dehydrogenase that catalyzes the oxidation of said aldehyde to produce a carboxylic acid and an overexpressed acyl-CoA synthetase that catalyzes the conversion of said carboxylic acid to an acyl-CoA, or an overexpressed acylating aldehyde dehydrogenase that catalyzes the conversion of said aldehyde to an acyl-CoA.

20. The microorganism of claim **12**, wherein said pathway for the generation of product precursor acetyl-CoA comprises:
a) an overexpressed acyl-CoA dehydrogenase that catalyzes the conversion of said acyl-CoA to a transenoyl-CoA;
b) an overexpressed enoyl-CoA hydratase that catalyzes the hydration of said transenoyl-CoA to a 3-hydroxyacyl-CoA;
c) an overexpressed 3-hydroxyacyl-CoA dehydrogenase that catalyzes the oxidation of said 3-hydroxyacyl-CoA to a β-ketoacyl-CoA;
d) an overexpressed thiolase that catalyzes the cleavage of an acetyl-CoA from said β-ketoacyl-CoA to produce acetyl-CoA and an acyl-CoA 2-carbons shorter than said starting acyl-CoA.

21. The microorganism of claim **10** or **11**, wherein said pathway for the formation of a desired product from said acetyl-CoA intermediate is a reverse beta oxidation (BOX-R) cycle that grows a primer by adding a 2-carbon donor thereto in each cycle, said BOX-R cycle comprising:
a) an overexpressed thiolase that catalyzes the non-decarboxylative condensation of an acyl-CoA primer with a 2-carbon donor acetyl-CoA to produce a β-ketoacyl-CoA;
b) an overexpressed 3-oxoacyl-[acyl-carrier-protein] reductase or overexpressed 3-hydroxyacyl-CoA dehydrogenase that catalyzes the reduction of a β-ketoacyl-CoA to a β-hydroxyacyl-CoA;
c) an overexpressed 3-hydroxyacyl-[acyl-carrier-protein] dehydratase or an overexpressed enoyl-CoA hydratase or 3-hydroxyacyl-CoA dehydratase that catalyzes the dehydration of a (3R)-β-hydroxyacyl-CoA to a transenoyl-CoA;
d) an overexpressed enoyl-[acyl-carrier-protein] reductase or acyl-CoA dehydrogenase or trans-enoyl-CoA reductase that catalyzes the reduction of a transenoyl-CoA to an acyl-CoA that is two carbons longer than said acyl-CoA primer; and
e) an overexpressed termination pathway that catalyzes the exit of an intermediate from said BOX-R cycle.

22. The microorganism of claim **10** or **11**, wherein said pathway for the formation of a desired product from said acetyl-CoA intermediate is a the fatty acid biosynthesis (FAS) pathway that grows a primer by adding a 2-carbon donor thereto in each cycle, said FAS pathway comprising:

a) an overexpressed acetyl-CoA carboxylase that catalyzes the conversion of acetyl-CoA to malonyl-CoA;

b) an overexpressed malonyl-CoA-[acyl-carrier-protein] ("ACP") transacylase that catalyzes the conversion of said malonyl-CoA to malonyl-ACP;

c) an overexpressed 3-ketoacyl-ACP synthase that catalyzes the decarboxylative condensation of said malonyl-ACP with an acyl-ACP primer to produce a β-ketoacyl-ACP;

d) an overexpressed 3-oxoacyl-ACP reductase that catalyzes the reduction of a β-ketoacyl-ACP to a β-hydroxyacyl-ACP;

e) an overexpressed 3-hydroxyacyl-ACP dehydratase that catalyzes the dehydration of a (3R)-β-hydroxyacyl-ACP to a transenoyl-ACP;

f) an overexpressed enoyl-ACP reductase that catalyzes the reduction of a transenoyl-ACP to an acyl-ACP that is two carbons longer than said acyl-ACP primer; and

g) an overexpressed termination pathway that catalyzes the exit of an intermediate from said FAS cycle.

23. The microorganism of claim **21**, wherein said termination pathway is selected from the group consisting of i) a CoA cleaving thioesterase, ii) an acyl-CoA:acetyl-CoA transferase, and iii) a phosphotransacylase and a carboxylate kinase.

24. The microorganism of claim **22**, wherein said termination pathway is an ACP cleaving thioesterase.

25. The microorganism of claim **23** or **24**, wherein said microorganism produces a product selected from the group consisting of carboxylic acids, (3R)-β-hydroxy carboxylic acids, β-keto carboxylic acids, and α,β-unsaturated carboxylic acids.

26. The microorganism of claim **21**, wherein said termination pathway is selected from the group consisting of i) an alcohol-forming coenzyme-A thioester reductase, and ii) an aldehyde-forming CoA thioester reductase and an alcohol dehydrogenase.

27. The microorganism of claim **21**, wherein said termination pathway is selected from the group consisting of i) an alcohol-forming ACP thioester reductase, and ii) an aldehyde-forming ACP thioester reductase and an alcohol dehydrogenase.

28. The microorganism of claim **26** or **27**, wherein said microorganism produces a product selected from the group consisting of primary alcohols, 1,(3R)-β diols, β-keto primary alcohols, and α,β-unsaturated primary alcohols.

29. The microorganism of claim **21**, wherein said termination pathway consists of an aldehyde-forming CoA thioester reductase and an aldehyde decarbonylase.

30. The microorganism of claim **22**, wherein said termination pathway consists of an aldehyde-forming ACP thioester reductase and an aldehyde decarbonylase.

31. The microorganism of claim **29** or **30**, wherein said microorganism produces a product selected from the group consisting of linear alkanes, linear alkan-2-ols, linear methyl-ketones, and 1-alkenes.

32. The microorganism of claim **21**, wherein said termination pathway consists of an aldehyde-forming CoA thioester reductase and a transaminase.

33. The microorganism of claim **22**, wherein said termination pathway consists of an aldehyde-forming ACP thioester reductase and a transaminase.

34. The microorganism of claim **32** or **33**, wherein said microorganism produces a product selected from the group consisting of primary amines, 3-hydroxy-amines, 3-keto-amines, and α,β-unsaturated primary amines.

35. The microorganism of claim **25**, wherein said microorganism expresses a carboxylic acid omega hydroxylase and produces a product selected from the group consisting of w-hydroxylated carboxylic acids, (3R)-β-, ω-dihydroxy carboxylic acids, β-keto, co-hydroxy carboxylic acids, and α,β-unsaturated ω-hydroxylated carboxylic acids.

36. The microorganism of claim **28**, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and an aldehyde dehydrogenase, and produces a product selected from the group consisting of ω-hydroxylated carboxylic acids, (3R)-β-, ω-dihydroxy carboxylic acids, β-keto, ω-hydroxy carboxylic acids, and α,β-unsaturated omega-hydroxylated carboxylic acids.

37. The microorganism of claim **28**, wherein said microorganism expresses a carboxylic acid ω hydroxylase, and produces a product selected from the group consisting of 1-,ω-diols, 1-,(3R)-β-, ω-triols, β-keto, 1-,ω-diols, and α,β-unsaturated 1-,ω-diols.

38. The microorganism of claim **25**, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and an aldehyde dehydrogenase, and produces a product selected from the group consisting of di-carboxylic acids, (3R)-β-hydroxy di-carboxylic acids, β-keto di-carboxylic acids, and α,β-unsaturated di-carboxylic acids.

39. The microorganism of claim **28**, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and a transaminase, and produces a product selected from the group consisting of primary alkanolamines (i.e. 1, ω-hydroxyamines), (3R)-β-hydroxy primary alkanolamines, β-keto primary alkanolamines, and α,β-unsaturated primary alkanolamines.

40. The microorganism of claim **34**, wherein said microorganism expresses a carboxylic acid ω hydroxylase, and produces a product selected from the group consisting of primary alkanolamines (i.e. 1, ω-hydroxyamines), (3R)-β-hydroxy primary alkanolamines, β-keto primary alkanolamines, and α,β-unsaturated primary alkanolamines.

41. The microorganism of claim **34**, wherein said microorganism expresses a carboxylic acid ω hydroxylase, an alcohol oxidase, and an aldehyde dehydrogenase, and produces a product selected from the group consisting of ω-amino acids, (3R)-β-hydroxy ω-amino acids, 3-keto ω-amino acids, and α,β-unsaturated ω-amino acids.

42. The microorganism of claim **25**, wherein said microorganism expresses a carboxylic acid alpha hydroxylase, and produces a product selected from the group alpha-hydroxy carboxylic acids, alpha-, (3R)-β-dihydroxy carboxylic acids, α-hydroxy, β-keto carboxylic acids, and α,β-unsaturated α-hydroxy carboxylic acids.

43. The microorganism of claim **28**, wherein said microorganism expresses a carboxylic acid α hydroxylase, and produces a product selected from the group consisting of 1,2-diols, 1,2,3-triols, β-keto, 1,2-diols, and α,β-unsaturated 1,2-diols.

44. The microorganism of claim **34**, wherein said microorganism expresses a carboxylic acid α hydroxylase, and

produces a product selected from the group consisting of α-hydroxylated primary amines, α-, β-dihydroxy primary amines, α-hydroxy, β-keto primary amines, and α-hydroxy, α,β-unsaturated primary amines.

45. The microorganisms of claim **10** or **11**, further comprising reduced expression of fermentation enzymes leading to reduced production of lactate, acetate, ethanol and succinate.

46. The microorganism of claim **12**, **13**, or **14**, wherein said overexpressed alkyl succinate synthase is encoded by *Azoarcus* sp. HxN1 masBCDEG (A9J4K0, A9J4K2, A9J4K4, A9J4K6, A9J4J6), *Desulfatibacillum alkenivorans* assA1/FassB1/assC1/assD1 (ACL03428.1, ACL03427.1, ACL03427.1, ACL03425.1), *Desulfosarcina* sp. BuS5 A39W_RS0101550/A39W_RS0101545/A39W RS0101540/A39W_RS0101535/A39W_RS19630/A39W_RS0101580 (WP_027352796.1, WP_027352795.1, WP_027352794.1, WP_027352793.1, WP_051374532.1, WP_027352800.1), *Desulfatibacillum alkenivorans* assA2/assB2/assC2/assD2 (ACL03892.1, ACL03893.1, ACL03891.1, ACL03895.1), *Peptococcaceae* sp. SCADC (WP_036747468.1), *Aromatoleum* sp. OcN1 masD (CBK27727.1), *Desulfoglaeba alkanexedens* assA (ADJ51097.1), or homologues thereof.

47. The microorganism of claim **12** or **14**, wherein said overexpressed succinyl-CoA:2-methyl-alkyl-succinyl-CoA transferase or 2-methyl-alkyl-succinyl-CoA synthetase is encoded by *Chloroflexus aurantiacus* sct (A9WGE3), *Thauera aromatica* bbsEF (Q9KJF0, Q9KJE9), *Escherichia coli* sucCD (P0A836, P0AGE9), *Desulfatibacillum alkenivorans* Dalk_1737 (B8FFM9), or homologues thereof.

48. The microorganism of claim **12**, wherein said overexpressed 2-methyl-alkyl-malonyl-CoA mutase is encoded by *Desulfatibacillum alkenivorans* Dalk_0220/Dalk_0221 (ACL01930.1, ACL01929.1), or homologues.

49. The microorganism of claim **12**, wherein said overexpressed 2-methyl-alkyl-malonyl-CoA decarboxylase is encoded by *Desulfatibacillum alkenivorans* Dalk_1740 (B8FFN2), or homologues thereof.

50. The microorganism of claim **13** or **14**, wherein said overexpressed 2-methyl-alkyl-succinyl-CoA dehydrogenase is encoded by *Rhodobacter sphaeroides* mcd (ADC44452.1) or homologues thereof.

51. The microorganism of claim **13**, wherein said overexpressed mesaconyl-C1-CoA-C4-CoA transferase is encoded by *Chloroflexus aurantiacus* mct (A9WC36) or homologues thereof.

52. The microorganism of claim **13**, wherein said overexpressed mesaconyl-C4-CoA hydratase is encoded by *Chloroflexus aurantiacus* meh (A9WC41) or homologues thereof.

53. The microorganism of claim **13**, wherein said overexpressed citramalyl-CoA lyase is encoded by *Chloroflexus aurantiacus* mclA (A9WC35) or homologues thereof.

54. The microorganism of claim **14**, wherein said overexpressed mesaconyl-CoA hydratase/β-methylmalyl-CoA dehydratase is encoded by *Chloroflexus aurantiacus* mch (A9WC34), *Rhodobacter sphaeroides* mch (Q31Z78), or homologues thereof.

55. The microorganism of claim **14**, wherein said overexpressed β-methylmalyl-CoA lyase is encoded by *Rhodobacter sphaeroides* mclI (B9KLE8) or homologues thereof.

56. The microorganism of claim **14**, wherein said overexpressed glyoxylate carboligase is encoded by *Escherichia coli* gcl (P0AEP7), or homologues thereof.

57. The microorganism of claim **14**, wherein said overexpressed tartronate semialdehyde reductase is encoded by *Escherichia coli* glxR (P77161), or homologues thereof.

58. The microorganism of claim **14**, wherein said overexpressed glycerate kinase is encoded by *Escherichia coli* glxK (P77364), or homologues thereof.

59. The microorganism of claim **14**, wherein said overexpressed glycerate kinase is encoded by *Escherichia coli* glxK (P77364), or homologues thereof.

60. The microorganism of claim **14**, wherein said phosphoglycerate mutase is encoded by *Escherichia coli* gpmA (P62707), *Escherichia coli* gpmM (P37689), or homologues thereof.

61. The microorganism of claim **14**, wherein said enolase is encoded by *Escherichia coli* eno (P0A6P9), or homologues thereof.

62. The microorganism of claim **14**, wherein said pyruvate kinase is encoded by *Escherichia coli* pykA (P21599), *Escherichia coli* pykF (POAD61), or homologues thereof.

63. The microorganism of claim **14**, wherein said pyruvate formate lyase is encoded by *Escherichia coli* pfiB/pflA (P09373) or homologues thereof.

64. The microorganism of claim **14**, wherein said pyruvate dehydrogenase is encoded by *Escherichia coli* aceEF/lpd (P0AFG8, P06959, C3TQA2) or homologues.

65. The microorganism of claim **14**, wherein said overexpressed acyl-CoA dehydrogenase is encoded by *Ascaris suum* ACDH (Q08523), *Escherichia coli* fadE (AP 000876.1), or homologues thereof.

66. The microorganism of claim **15**, wherein said overexpressed enoyl-CoA hydratase encoded by *Pseudomonas putida* fadBlx (NP 744366.1), *Pseudomonas putida* phaL (NP_745413.1), *Alcanivorax borkumensis* ech1 (YP_691868.1), *Alcanivorax borkumensis* ech2 (YP_692707.1) *Alcanivorax borkumensis* phaB (YP_692246.1), *Escherichia coli* fadB (NP_418288.1), or homologues thereof.

67. The microorganism of claim **15**, wherein said overexpressed 3-hydroxyacyl-CoA dehydrogenase is encoded by *Pseudomonas putida* fadB2x (Q88KS5), *Ascaris suum* GS 18673, *Escherichia coli* fadB (NP_418288.1), or homologues thereof.

68. The microorganism of claim **15**, wherein said overexpressed thiolase is encoded by *Pseudomonas putida* fadAx (NP_744364.1), *Alcanivorax borkumensis* fadAx (YP_692368.1), *Escherichia coli* atoB (NP_416728.1), *Escherichia coli* yqeF (NP_417321.2), *Escherichia coli* fadA (YP_026272.1), *Escherichia coli* fadI (NP_416844.1), *Ralstonia eutropha* bklB (AAC38322.1), or homologues thereof.

69. The microorganism of claim **16**, wherein said overexpressed propionyl-CoA carboxylase is encoded by *Chloroflexus aurantiacus* Caur_2034/Caur_3435 (A9WEI4, A9WKJ2), *Rhodobacter sphaeroides* pccAB (Q3J4D9, Q3J4E3), or homologues thereof.

70. The microorganism of claim **16**, wherein said overexpressed methyl-malonyl-CoA epimerase is encoded by *Metallosphaera sedula* Msed_0639 (A4YEG2) or homologues thereof.

71. The microorganism of claim **16**, wherein said overexpressed methyl-malonyl-CoA mutase is encoded by *Rhodobacter sphaeroides* mcmA (Q3J4D7), or homologues thereof.

72. The microorganism of claim **16**, wherein said overexpressed succinyl-CoA synthetase is encoded by *Escherichia coli* sucCD (P0A836, P0AGE9), or homologues thereof.

73. The microorganism of claim **17**, wherein said overexpressed malate dehydrogenase is encoded by *Escherichia coli* maeA (P26616), *Escherichia coli* maeB (P76558), or homologues thereof.

74. The microorganism of claim **17** or **18**, wherein said overexpressed fumarase is encoded by *Escherichia coli* fumA (POAC33), *Escherichia coli* fumB (P14407), *Escherichia coli* fumC (P05042), or homologues thereof.

75. The microorganism of claim **18**, wherein said overexpressed carboxylic acid omega hydroxylase is encoded by *Pseudomonas putida* alkBGT (YP_009076004.1, Q9WWW4.1, Q9L4M8.1), *Marinobacter aquaeolei* CYP153A (ABM17701.1), *Mycobacterium marinum* CYP153A16 (YP_001851443.1), *Polaromonas* sp. CYP153A (YP_548418.1), *Nicotiana tabacum* CYP94A5 (AAL54887.1), *Vicia sativa* CYP94A1 (AAD10204.1), *Vicia sativa* CYP94A2 (AAG33645.1), *Arabidopsis thaliana* CYP94B1 (BAB08810.1), *Arabidopsis thaliana* CYP86A8 (CAC67445.1), *Candida tropicalis* CYP52A1 (AAA63568.1, AAA34354.1, AAA34334.1), *Candida tropicalis* CYP_52A2 (AAA34353.2, CAA35593.1), *Homo sapiens* CYP4A11 (AAQ56847.1), or homologs thereof.

76. The microorganism of claim **18**, wherein said overexpressed alcohol dehydrogenase is encoded by *Rhodococcus ruber* SC1 cddC (AAL14237.1), *Acinetobacter* sp. SE19 chnD (AAG10028.1), *Escherichia coli* betA (NP_414845.1), *Escherichia coli* dkgA (NP_417485.4), *Escherichia coli* eutG (NP_416948.4), *Escherichia coli* fucO (NP_417279.2), *Escherichia coli* ucpA (NP_416921.4), *Escherichia coli* yahK (NP_414859.1), *Escherichia coli* ybbO (NP_415026.1), *Escherichia coli* ybdH (NP_415132.1), *Escherichia coli* yiaY (YP_026233.1), *Escherichia coli* yjgB (NP_418690.4), or homologues thereof.

77. The microorganism of claim **18**, wherein said overexpressed aldehyde dehydrogenase is encoded by *Rhodococcus ruber* SC1 cddD (AAL14238.1), *Acinetobacter* sp. SE19 chnE (AAG10022.1), or homologues thereof.

78. The microorganism of claim **18**, wherein said overexpressed ketoreductase/malate dehydrogenase is encoded by *Escherichia coli* mdh (P61889), or homologues thereof.

79. The microorganism of claim **19**, wherein said overexpressed alkane monooxygenase or alkane hydroxylase is encoded by *Pseudomonas putida* alkBGT (YP_009076004.1, Q9WWW4.1, Q9L4M8.1), *Mycobacterium* sp. strain HXN-1500 CYP153A6 (Q5K1Y6), *Gordonia* sp. TY-5 prmABCD (AB112920.1), *Thauera butanivorans* bmoXYZ/bmoC/bmoB (Q8KQF0, Q8KQE9, Q8KQE7, Q8KQE6, Q8KQE8), *Alcanivorax borkumensis* alkB1 (Q0VKZ3.1), *Alcanivorax borkumensis* alkB2 (QOVTH3.1), *Sphingopyxis macrogoltabida* ahpG3 (Q5F4D3), *Methylosinus trichosporium* OB3b mmoXYZBC/orfY (P27353, P27354, P27355, Q53563, P27356, Q53562), *Methylococcus capsulatus* Bath mmoXYZBC/orfY (P22869, P18798, P11987, P18797, 22868, P22867), *Rhodobacter sphaeroides* RSP2792/RSP2793/RSP2794/RSP2795 (YP_352924.1, (YP_352923.1, YP_352922.1, YP_352921.1), or homologues thereof.

80. The microorganism of claim **19**, wherein said overexpressed alcohol dehydrogenase is encoded by *Pseudomonas putida* (Q9WWW2), *Gordonia* sp. TY-5 adhI (AB112920.1), *Bacillus methanolicus* mdh (P31005), *Mycobacterium* sp. DSM 3803 mdo (C5MRT8), *Methylobacterium extorquens* moxI, moxF (P14775, P16027), *Escherichia coli* betA (NP_414845.1), *Escherichia coli* dkgA (NP_417485.4), *Escherichia coli* eutG (NP_416948.4), *Escherichia coli* fucO (NP_417279.2), *Escherichia coli* ucpA (NP_416921.4), *Escherichia coli* yahK (NP_414859.1), *Escherichia coli* ybbO (NP_415026.1), *Escherichia coli* ybdH (NP_415132.1), *Escherichia coli* yiaY (YP_026233.1), *Escherichia coli* yjgB (NP_418690.4), or homologues thereof.

81. The microorganism of claim **19**, wherein said overexpressed aldehyde dehydrogenase is encoded by *Escherichia coli* aldA (P25553), *Escherichia coli* aldB (P37685), *Escherichia coli* punC (P23883), *Pseudomonas putida* alkH (Q9WWW3), *Klebsiella pneumoniae* KPN_01018 (A6T782), *Rhodococcus erythropolis* aldhR (Q4F895), or homologues thereof.

82. The microorganism of claim **19**, wherein said overexpressed acyl-CoA synthetase is encoded by *Escherichia coli* fadD (P69451), *Escherichia coli* fadK (P38135), *Pseudomonas putida* alkK (Q9L4M6), or homologues thereof.

83. The microorganism of claim **19**, wherein said overexpressed acylating aldehyde dehydrogenase is encoded by *E. coli* mhpF (NP_414885.1), *Pseudomonas* sp. CF600 drnpF (Q52060), or homologues thereof.

84. The microorganism of claim **20**, wherein said overexpressed acyl-CoA dehydrogenase is encoded by *Escherichia coli* fadE (AP_000876.1), or homologues thereof.

85. The microorganism of claim **10**, wherein said overexpressed enoyl-CoA hydratase encoded by *Escherichia coli* fadB (NP_418288.1), or homologues thereof.

86. The microorganism of claim **10**, wherein said overexpressed 3-hydroxyacyl-CoA dehydrogenase is encoded by *Escherichia coli* fadB (NP_418288.1), or homologues thereof.

87. The microorganism of claim **10**, wherein said overexpressed thiolase is encoded by, *Escherichia coli* atoB (NP_416728.1), *Escherichia coli* yqeF (NP_417321.2), *Escherichia coli* fadA (YP_026272.1), *Escherichia coli* fadI (NP_416844.1), or homologues thereof.

88. The microorganism of claim **21**, wherein said overexpressed thiolase is encoded by *E. coli* atoB (NP_416728.1), *E. coli* yqeF (NP_417321.2), *E. coli* fadA (YP_026272.1), *E. coli* fadI (NP_416844.1), *Ralstonia eutropha* bktB (AAC38322.1), *Pseudomonas* sp. Strain B13 catF (AAL02407.1), *E. coli* paaJ (NP_415915.1), *Pseudomonas putida* pcaF (AAA85138.1), *Rhodococcus opacus* pcaF (YP_002778248.1), *Streptomyces* sp. pcaF (AAD22035.1), *Ralstonia eutropha* phaA (AEI80291.1), *Clostridium acetobutylicum* thIA (AAC26023.1), or *Clostridium acetobutylicum* thIB (AAC26026.1), or homologues thereof.

89. The microorganism of claim **21**, wherein said overexpressed 3-hydroxyacyl-CoA dehydrogenase or 3-oxoacyl-[acyl-carrier-protein] reductase is encoded by *E. coli* fadB (NP_418288.1), *E. coli* fadJ (NP_416843.1), *Ralstonia eutropha* phaB1 (YP_725942.1), *Ralstonia eutropha* phaB2

(YP_726470.1), *Ralstonia eutropha* phaB3 (YP_726636.1), *E. coli* paaH (P76083), *E. coli* fabG (NP_415611.1), or homologues thereof.

90. The microorganism of claim **21**, wherein said overexpressed enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydratase, or 3-hydroxyacyl-[acyl-carrier-protein] dehydratase is encoded by *E. coli* fadB (NP_418288.1), *E. coli* fadJ (NP_416843.1), *Aeromonas caviae* phaJ (032472.1), *Pseudomonas aeruginosa* phaJ1 (BAA92740.1), *Pseudomonas aeruginosa* phaJ2 (BAA92741.1), *Pseudomonas aeruginosa* phaJ3 (BAC44834.1), *Pseudomonas aeruginosa* phaJ4 (BAC44835.1), *E. coli* paaF (P76082), *E. coli* fabA (NP_415474.1), *E. coli* fabZ (NP_414722.1), or homologues thereof.

91. The microorganism of claim **21**, wherein said trans-enoyl-CoA reductase or enoyl-[acyl-carrier-protein] reductase is encoded by *E. coli* ydiO (P0A9U8), *Euglena gracilis* egTER (Q5EU90.1), *Treponema denticola* tdTER (NP_971211.1), *E. coli* fabI (NP_415804.1), *Enterococcus faecalis* fabK (NP_816503.1), *Bacillus subtilis* fabL (KFK80655.1), *Vibrio cholerae* fabV (ABX38717.1), or homologues thereof.

92. The microorganism of claim **22**, wherein said overexpressed acetyl-CoA carboxylase is encoded by *E. coli* accABCD (P0ABD5, P0ABD8, P24182, P0A9Q5), or homologues thereof.

93. The microorganism of claim **22**, wherein said overexpressed malonyl-CoA-ACP transacylase is encoded by *E. coli* fabD (P0AAI9), or homologues thereof.

94. The microoroganism of claim **22**, wherein said overexpressed β-ketoacyl-ACP synthase is encoded by *E. coli* fabB (P0A953), *E. coli* fabF (P0AAI5), *E. coli* fabH (P0A6R0), or homologues thereof.

95. The microorganism of claim **22**, wherein said overexpressed 3-oxoacyl-[acyl-carrier-protein] reductase is encoded by *E. coli* fabG (NP_415611.1), or homologues thereof.

96. The microorganism of claim **22**, wherein said overexpressed 3-hydroxyacyl-[acyl-carrier-protein] dehydratase is encoded by *E. coli* fabA (NP_415474.1), *E. coli* fabZ (NP_414722.1), or homologues thereof.

97. The microorganism of claim **22**, wherein said enoyl-[acyl-carrier-protein] reductase is encoded by *E. coli* fabI (NP_415804.1), *Enterococcus faecalis* fabK (NP_816503.1), *Bacillus subtilis* fabL (KFK80655.1), *Vibrio cholerae* fabV (ABX38717.1), or homologues thereof.

98. The microorganism of claim **23**, wherein said overexpressed thioesterase is encoded by *E. coli* tesA (NP_415027.1), *E. coli* tesB (NP_414986.1), *E. coli* yciA (NP_415769.1), *E. coli* (NP_414977.1), *E. coli* ydiI (NP_416201.1), *E. coli* ybgC (NP_415264.1), *Alcanivorax borkumensis* tesB2 (YP_692749.1) *Fibrobacter succinogenes* Fs2108 (YP_005822012.1), *Prevotella ruminicola* Pr655 (YP_003574018.1) *Prevotella ruminicola* Pr1687 (YP_003574982.1), or homologues thereof.

99. The microorganism of claim **23**, wherein said overexpressed acyl-CoA:acetyl-CoA transferase is encoded by *E. coli* atoD (NP_416725.1), *Clostridium kluyveri* call (AAA92344.1), *Clostridium acetobutylicum* ctfAB (NP_149326.1, NP_149327.1) or *E. coli* ydiF (NP_416209.1), or homologues thereof.

100. The microorganism of claim **23**, wherein said overexpressed phosphotransacylase is encoded by *Clostridium acetobutylicum* ptb (NP_349676.1), *Enterococcus faecalis* ptb (AAD55374.1), *Salmonella enterica* pduL (AAD39011.1), or homologues thereof.

101. The microorganism of claim **23**, wherein said overexpressed carboxylate kinase is encoded by *Clostridium acetobutylicum* buk (AAK81015.1), *Enterococcus faecalis* buk (AAD55375.1), *Salmonella enterica* pduW (AAD39021.1), or homologues thereof.

102. The microorganism of claim **24**, wherein said overexpressed ACP-cleaving thioesterase is encoded by *E. coli* tesA (NP_415027.1), *Cuphea palustris* fatB1 (AAC49179.1), *Cuphea viscosissima* fatB3 (AEM72524.1), *Ulmus americana* fatB1 (AAB71731.1), *Cocos nucifera* fatB2 (AEM72520.1), *Elaeis guineensis* PTE (AAD42220.2), *Clostridium perfringens* CPF 2954 (ABG82470.1), *Umbellularia californica* fatB1 (AAA34215.1), or homologues thereof.

103. The microorganism of claim **26**, wherein said overexpressed alcohol-forming coenzyme-A thioester reductase is encoded by *Clostridium acetobutylicum* adhE2 (YP_009076789.1), *Arabidopsis thaliana* At3g11980 (AEE75132.1), *Arabidopsis thaliana* At3g44560 (AEE77915.1), *Arabidopsis thaliana* At3g56700 (AEE79553.1), *Arabidopsis thaliana* At5g22500 (AED93034.1), *Arabidopsis thaliana* CER4 (AEE86278.1), *Marinobacter aquaeolei* VT8 maqu_2220 (VP_959486.1), *Marinobacter aquaeolei* VT8 maqu_2507 (YP_959769.1), or homologues thereof.

104. The microorganism of claim **26** or **29**, wherein said overexpressed aldehyde-forming CoA thioester reductase is encoded by *Acinetobacter calcoaceticus* acr1 (AAC45217.1), *Acinetobacter* sp Strain M-1 acrM (BAB85476.1), *Clostridium beijerinckii* ald (AAT66436.1), *E. coli* eutE (NP_416950.1), *Salmonella enterica* eutE (AAA80209.1), *E. coli* mhpF (NP_414885.1), or homologues thereof.

105. The microorganism of claim **27**, wherein said overexpressed alcohol-forming ACP thioester reductase is encoded by *Marinobacter aquaeolei* VT8 maqu_2220 (VP_959486.1), *Hahella chejuensis* hch_05075 (ABC31758.1), *Marinobacter algicola* MDG893_11561 (A6EVI7), *Bermanella marisrubri* RED65_09894 (Q1N697), or homologues thereof.

106. The microorganism of claim **27** or **30**, wherein said overexpressed aldehyde-forming ACP thioester reductase is encoded by *Nostoc punctiforme* Npun_R1710 (ACC80381.1), *Synechococcus elongates* Synpcc7942_1594 (Q54765), *Prochlorococcus marinus* P9515_05971 (A2BVJ5), *Synechocystis* sp. PCC 6803 sll0209 (YP_005652204.1), or homologues thereof.

107. The microorganism of claim **26** or **27**, wherein said overexpressed alcohol dehydrogenase is encoded by *E. coli* betA (NP_414845.1), *E. coli* dkgA (NP_417485.4), *E. coli* eutG (NP_416948.4), *E. coli* fucO (NP_417279.2), *E. coli* ucpA (NP_416921.4), *E. coli* yahK (NP_414859.1), *E. coli* ybbO (NP_415026.1), *E. coli* ybdH (NP_415132.1), *E. coli* yiaY (YP_026233.1), *E. coli* yjgB (NP_418690.4), or homologues thereof.

108. The microorganism of claim **29** or **30**, wherein said overexpressed aldehyde decarbonylase is encoded by *Synechococcus elongates* PCC7942 orf1593 (Q54764.1), *Nostoc punctiforme* PCC73102 npun_R1711 (B2J1M1.1), *Prochlorococcus marinus* MIT9313 pmt1231 (Q7V6D4.1), or homologues thereof.

109. The microorganism of claim **32**, **33**, or **39**, wherein said overexpressed transaminase is encoded by *Arabidopsis thaliana* At3g22200 (NP_001189947.1), *Alcaligenes denitrificans* aptA (AAP_92672.1), *Bordetella bronchiseptica* BB0869 (WP_015041039.1), *Bordetella parapertussis* BPP0784 (WP_010927683.1), *Brucella melitensis* BAWG 0478 (EEW88370.1), *Burkholderia pseudomallei* BP1026B_I0669 (AFI65333.1), *Chromobacterium violaceum* CV2025 (AAQ59697.1), *Oceanicola granulosus* OG2516_07293 (WP_007254984.1), *Paracoccus denitrificans* PD1222 Pden 3984 (ABL72050.1), *Pseudogulbenkiania ferrooxidans* ω-TA (WP_008952788.1), *Pseudomonas putida* ω-TA (P28269.1), *Ralstonia solanacearum* ω-TA (YP_002258353.1), *Rhizobium meliloti* SMc01534 (NP_386510.1), and *Vibrio fluvialis* ω-TA (AEA39183.1), *Mus musculus* abaT (AAH58521.1) *E. coli* gabT (YP_490877.1), or homologues thereof.

110. The microorganism of claims **35**-**41**, wherein said overexpressed carboxylic acid omega hydroxylase is encoded by *Pseudomonas putida* alkBGT (YP_009076004.1, Q9WWW4.1, Q9L4M8.1), *Marinobacter aquaeolei* CYP_153A (ABM17701.1), *Mycobacterium marinum* CYP_153A16 (YP_001851443.1), *Polaromonas* sp. CYP_153A (YP_548418.1), *Nicotiana tabacum* CYP94A5 (AAL54887.1), *Vicia sativa* CYP94A1 (AAD10204.1), *Vicia sativa* CYP94A2 (AAG33645.1), *Arabidopsis thaliana* CYP94B1 (BAB08810.1), *Arabidopsis thaliana* CYP86A8 (CAC67445.1), *Candida tropicalis* CYP52A1 (AAA63568.1, AAA34354.1, AAA34334.1), *Candida tropicalis* CYP52A2 (AAA34353.2, CAA35593.1), *Homo sapiens* CYP4A11 (AAQ56847.1), or homologues thereof.

111. The microorganism of claim **36**, **38**, **39**, or **41**, wherein said overexpressed alcohol oxidase is encoded by *Rhodococcus ruber* SC1 cddC (AAL14237.1), *Acinetobacter* sp. SE19 chnD (AAG10028.1), *E. coli* yahK (NP_414859.1), *E. coli* yjgB (NP_418690.4), or homologues thereof.

112. The microorganism of claim **36**, **38**, or **41**, wherein said overexpressed aldehyde dehydrogenase is encoded by *Rhodococcus ruber* SC1 cddD (AAL14238.1), *Acinetobacter* sp. SE19 chnE (AAG10022.1), or homologues thereof.

113. The microorganism of claim **42**, **43**, or **44**, wherein said overexpressed fatty acid alpha hydroxylases is encoded by *Myxococcus xanthus* MXAN_0191 (YP_628473.1), *Stigmatella aurantiaca* STIAU_3334 (YP_003957653.1), or homologues thereof.

114. The microorganism of claim **45**, wherein said reduced expression of fermentation enzymes are ΔadhE, (Δpta or ΔackA or ΔackApta), ΔpoxB, ΔldhA, and ΔfrdA and less acetate, lactate, ethanol and succinate are thereby produced.

115. A method of a product comprising growing a genetically engineered microorganism or recombinant bacteria according to any of claims **1**-**114** in a culture broth containing an alkane as the sole carbon source, activating said alkane, generating precursor intermediate acetyl-CoA, producing a product from said acetyl-CoA, and isolating said product.

116. A method of a product comprising growing a genetically engineered microorganism or recombinant bacteria according to any of claims **1**-**114** in a culture broth containing an alkane as the sole carbon source and a terminal electron acceptor (such as SO_4^{2-}, NO_3^-, Fe^{3+}, O_2, Mn^{4+}), activating said alkane, generating precursor intermediate acetyl-CoA, producing a product from said acetyl-CoA, and isolating said product.

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