(12) STANDARD PATENT (11) Application No. **AU 2014227811 C1**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Use of phosphoketolase and phosphotransacetylase for production of acetyl-coenzyme a derived compounds

(51) International Patent Classification(s)
C12P 19/00 (2006.01) ***C12P 7/00*** (2006.01)
C12N 9/10 (2006.01) ***C12P 15/00*** (2006.01)
C12N 9/16 (2006.01) ***C12P 17/00*** (2006.01)
C12N 9/88 (2006.01) ***C12P 23/00*** (2006.01)
C12P 5/00 (2006.01) ***C12P 33/00*** (2006.01)

(21) Application No: **2014227811** (22) Date of Filing: **2014.03.14**

(87) WIPO No: **WO14/144135**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/800,356	**2013.03.15**	**US**

(43) Publication Date: **2014.09.18**
(44) Accepted Journal Date: **2018.03.22**
(44) Amended Journal Date: **2018.09.27**

(71) Applicant(s)
Amyris, Inc.;Total Marketing Services

(72) Inventor(s)
Hawkins, Kristy Michelle;Mahatdejkul-Meadows, Tina Tipawan;Meadows, Adam Leon;Pickens, Lauren Barbara;Tai, Anna;Tsong, Annie Ening

(74) Agent / Attorney
Phillips Ormonde Fitzpatrick, PO Box 323, Collins Street West, VIC, 8007, AU

(56) Related Art
US 2012276587 A1
EP 2546336 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/144135 A3

(51) International Patent Classification:
C12P 19/00 (2006.01) *C12P 15/00* (2006.01)
C12P 23/00 (2006.01) *C12P 17/00* (2006.01)
C12P 33/00 (2006.01) *C12N 9/10* (2006.01)
C12P 5/00 (2006.01) *C12N 9/16* (2006.01)
C12P 7/00 (2006.01) *C12N 9/88* (2006.01)

(21) International Application Number:
PCT/US2014/028421

(22) International Filing Date:
14 March 2014 (14.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/800,356 15 March 2013 (15.03.2013) US

(71) Applicants: **AMYRIS, INC.** [US/US]; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US). **TOTAL MARKETING SERVICES** [FR/FR]; 24, Cours Michelet, F-92800 Puteaux (FR).

(72) Inventors: **HAWKINS, Kristy Michelle**; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US). **MAHATDEJKUL-MEADOWS, Tina Tipawan**; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US). **MEADOWS, Adam Leon**; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US). **PICKENS, Lauren Barbara**; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US). **TAI, Anna**; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US). **TSONG, Annie Ening**; 5885 Hollis Street, Suite 100, Emeryville, California 94608 (US).

(74) Agents: **PATHAK, Rahul** et al.; Squire Patton Boggs (US) LLP, 275 Battery Street, Suite 2600, San Francisco, California 94111 (US).

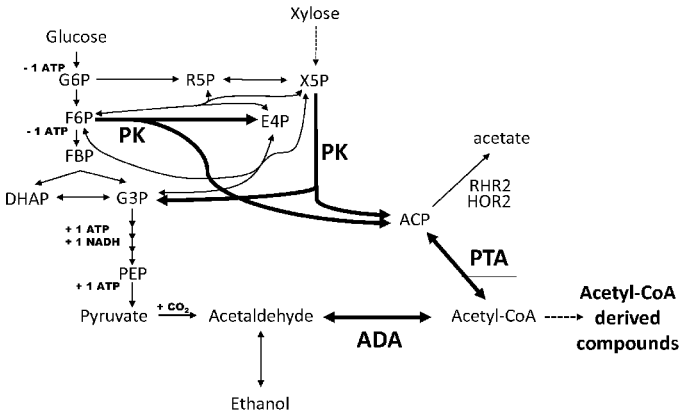
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP,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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: USE OF PHOSPHOKETOLASE AND PHOSPHOTRANSACETYLASE FOR PRODUCTION OF ACETYL-COENZYME A DERIVED COMPOUNDS

Figure 1



(57) Abstract: Provided herein are compositions and methods for improved production of acetyl-CoA and acetyl-CoA derived compounds in a host cell. In some embodiments, the host cell is genetically modified to comprise a heterologous nucleotide sequence encoding a phosphoketolase (PK), and a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the host cell further comprises a heterologous nucleotide sequence encoding a phosphotransacetylase (PTA). In some embodiments, the enzyme that converts acetyl phosphate to acetate is a glycerol-1-phosphatase. In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2. The compositions and methods described herein provide an efficient route for the heterologous production of acetyl-CoA-derived compounds, including but not limited to, isoprenoids, polyketides, and fatty acids.

WO 2014/144135 A3

Declarations under Rule 4.17:

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

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

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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

(88) Date of publication of the international search report:
23 October 2014

USE OF PHOSPHOKETOLASE AND PHOSPHOTRANSACETYLASE FOR PRODUCTION OF ACETYL-COENZYME A DERIVED COMPOUNDS

[0001] This application claims benefit of priority of U.S. Provisional Application No. 61/800,356, filed on March 15, 2013, the contents of which are hereby incorporated by reference in their entirety.

1. FIELD OF THE INVENTION

[0002] The present disclosure relates to compositions and methods for producing acetyl-CoA derived compounds in engineered host cells.

2. BACKGROUND

[0003] Acetyl coenzyme A (acetyl-CoA) is a key intermediate in the synthesis of essential biological compounds, including polyketides, fatty acids, isoprenoids, phenolics, alkaloids, vitamins, and amino acids. Among the metabolites derived from acetyl-CoA are primary and secondary metabolites, including compounds of industrial utility. In yeast, acetyl-CoA is biosynthesized from pyruvate metabolism (**FIG. 1**). However, in this biosynthetic pathway, CO_2 is lost via the reactions catalyzed by pyruvate carboxylase and/or pyruvate dehydrogenase. In an industrial fermentation setting, one benefit of providing an alternative to pyruvate metabolism and lower glycolysis is that less CO_2 is produced in the decarboxylation of pyruvate, and thus more carbon can be captured in the end product, thereby increasing the maximum theoretical yield. A second benefit is that less NADH is produced, and therefore significantly less oxygen is needed to reoxidize it. This can be accomplished by expressing phosphoketolase (PK; EC 4.1.2.9) in conjunction with phosphoacetyltransferase (PTA; EC 2.3.1.8).

[0004] PK and PTA catalyze the reactions to convert fructose-6-phosphate (F6P) or xylulose-5-phosphate (X5P) to acetyl-CoA. As shown in **FIG. 1**, PK draws from the pentose phosphate intermediate xyulose 5-phosphate, or from the upper glycolysis intermediate D-fructose 6-phosphate (F6P). PK splits X5P into glyceraldehyde 3-phosphate (G3P) and acetyl phosphate, or F6P into erythrose 4-phosphate (E4P) and acetyl phosphate. PTA then converts the acetyl phosphate into acetyl-CoA. G3P can re-enter lower glycolysis, and E4P can re-enter the pentose phosphate pathway or glycolysis by cycling through the non-oxidative pentose phosphate pathway network of transaldolases and transketolases.

[0005] The applicants have previously described the improved efficiency of heterologous isoprenoid production that can be gained with the introduction of PK and PTA enzymes. *See* U.S. Application No. 13/673,819 (now U.S. Patent No. 8,415,136), filed on

November 9, 2012, the contents of which are hereby incorporated by reference in their entirety. In particular, when cytosolic acetyl-CoA is synthesized from glucose using only the chemical reactions which occur in the native yeast metabolic network, the maximum possible stoichiometric yield for conversion of glucose to the isoprenoid farnesene via the mevalonate pathway is 23.6 wt%. By including the reactions catalyzed by acetaldehyde dehydrogenase, acetylating (ADA; EC 1.2.1.10) and NADH-using HMG-CoA reductase into the metabolic network for mevalonate production, the maximum theoretical stoichiometric yield is improved to 25.2 wt%. With the further introduction of PK and PTA, the reaction network, at optimality, is able to reach 29.8 wt% mass yield or greater, a significant increase in maximum theoretical yield.

[0006] Sondregger *et al.* have also described the benefits of PK and PTA with respect to ethanol production in a xylose-utilizing yeast strain. *See* Sondregger *et al.*, *Applied and Environmental Microbiology* 70(5):2892-2897 (2004), the contents of which are hereby incorporated by reference in their entirety. The heterologous phosphoketolase pathway (PK, PTA, and ADA) was introduced in *S. cerevisiae* to address low ethanol yields that result from overexpression of NAD(P)H-dependent xylose reductase and NAD^+-dependent xylitol dehydrogenase from *Pichia stipitis*. The different cofactor preferences in the two oxidoreductase reactions caused an anaerobic redox balancing problem that manifested in the extensive accumulation of the reduced reaction intermediate xylitol, and thus, low ethanol yields. Redox metabolism was balanced by introducing the phosphoketolase pathway, which lead to the net reoxidation of one NADH per xylose converted to ethanol, and an improvement in ethanol yield by 25%. However, overexpression of PK also leads to an increase in acetate accumulation and a reduction in fermentation rate. Although some acetate accumulation could be reduced by combining the phosphoketolase pathway with a mutation of ALD6, which converts acetaldehyde to acetate, the flux through the recombinant phosphoketolase pathway was about 30% of the optimum flux that would be required to completely eliminate xylitol and glycerol accumulation. The authors suggested that higher activities of phosphotransacetylase and/or acetaldehyde dehydrogenase may be necessary to prevent phosphoketolase pathway-based acetate formation.

[0007] Thus, while the introduction of a heterologous PK pathway can lead to substantial improvements in the yields of acetyl-CoA derived compounds, further improvements in the implementation of this pathway appear to be required to achieve optimal

carbon flux through PK and PTA. The compositions and methods provided herein address this need and provide related advantages as well.

3. SUMMARY OF THE INVENTION

[0008] Provided herein are compositions and methods for the improved utilization of phosphoketolase (PK) and phosphotransacetylase (PTA) for the production of industrially useful compounds. These compositions and methods are based on the surprising discovery that phosphoketolase pathway-based acetate accumulation results from the enzyme-catalyzed hydrolysis of acetyl phosphate, the product of PK catalysis. Hydrolysis of acetyl phosphate is an undesirable side-reaction that can negatively impact production, via depletion of carbon, of any type of product derived from acetyl-CoA, including isoprenoids, polyketides, and fatty acids. By functionally disrupting native enzymes in the host cell that catalyze acetyl phosphate hydrolysis, acetate accumulation is reduced and carbon flux through the PK/PTA pathway towards acetyl-CoA production is increased.

[0009] The compositions and methods provided herein are further based on the unexpected discovery of native enzymes in yeast that catalyze the hydrolysis of acetyl phosphate to acetate, namely GPP1/RHR2, and its closely related homolog GPP2/HOR2. Both of these enzymes have only been previously characterized as having glycerol-1-phosphatase (EC 3.1.3.21; alternately referred to as "glycerol-3-phosphatase") activity, and thus, the promiscuous acetyl-phosphatase activity of these enzymes is unexpected. In cells heterologously expressing PK and PTA, deletion of one or both of the genes encoding RHR2 and HOR2 leads to a reduction in acetate accumulation, with deletion of the gene encoding RHR2 alone leading to a substantial reduction in acetate levels. Moreover, deletion of the *RHR2* gene in cells engineered to comprise PK, PTA and a mevalonate pathway resulted in a substantial increase in the production of farnesene, an acetyl-CoA derived isoprenoid.

[0010] Thus, provided herein are genetically modified host cells and methods of their use for the production of industrially useful compounds. In one aspect, provided herein is a genetically modified host cell comprising: a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9); and a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC 2.3.1.8).

[0011] In another aspect, provided herein is a genetically modified host cell comprising: a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC

2.3.1.8); and a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9).

[0012] In some embodiments, the enzyme that converts acetyl phosphate to acetate is a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is selected from the group consisting of GPP1/RHR2, GPP2HOR2, and homologues and variants thereof. In some embodiments, the genetically modified host cell comprises a functional disruption of GPP1/RHR2. In some embodiments, the genetically modified host cell comprises a functional disruption of GPP2/HOR2. In some embodiments, the genetically modified host cell comprises a functional disruption of both GPP1/RHR2 and GPP2/HOR2.

[0013] In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding an acylating acetylaldehyde dehydrogenase (ADA; EC 1.2.1.10). In some embodiments, the genetically modified host cell further comprises a functional disruption of one or more enzymes of the native pyruvate dehydrogenase (PDH) - bypass. In some embodiments, the one or more enzymes of the PDH-bypass are selected from acetyl-CoA synthetase 1 (ACS1), acetyl-CoA synthetase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6).

[0014] In some embodiments, the genetically modified host cell is capable of producing a heterologous acetyl-CoA derived compound. In some embodiments, the heterologous acetyl-CoA derived compound is selected from the group consisting of an isoprenoid, a polyketide, and a fatty acid. In particular embodiments, the genetically modified host cell is capable of producing an isoprenoid.

[0015] In some embodiments, the genetically modified host cell comprises one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate. In some embodiments, the one or more enzymes of the MEV pathway comprise an NADH-using HMG-CoA reductase. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that converts HMG-CoA to mevalonate. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate. In some embodiments, the one or

more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate. In some embodiments, the one or more enzymes of the MEV pathway are selected from HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase and mevalonate pyrophosphate decarboxylase. In some embodiments, the host cell comprises a plurality of heterologous nucleic acids encoding all of the enzymes of the MEV pathway. In some embodiments, the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of a single transcriptional regulator. In some embodiments, the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of multiple heterologous transcriptional regulators. In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can convert isopentenyl pyrophosphate (IPP) into dimethylallyl pyrophosphate (DMAPP). In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can condense IPP and/or DMAPP molecules to form a polyprenyl compound. In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound.

[0016] In some embodiments, the enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound is selected from the group consisting of carene synthase, geraniol synthase, linalool synthase, limonene synthase, myrcene synthase, ocimene synthase, α-pinene synthase, β-pinene synthase, γ-terpinene synthase, terpinolene synthase, amorphadiene synthase, α-farnesene synthase, β-farnesene synthase, farnesol synthase, nerolidol synthase, patchouliol synthase, nootkatone synthase, and abietadiene synthase.

[0017] In some embodiments, the isoprenoid is selected from the group consisting of a hemiterpene, monoterpene, diterpene, triterpene, tetraterpene, sesquiterpene, and polyterpene. In some embodiments, the isoprenoid is a sesquiterpene. In some embodiments, the isoprenoid is a C_5-C_{20} isoprenoid. In some embodiments, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, isoprene, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpinolene, and valencene.

[0018] In another aspect, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate; a heterologous nucleic acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0019] In another aspect, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate; a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase, acetylating (ADA); a heterologous nucleic acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0020] In another aspect, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate; a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase, acetylating (ADA); a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthetase 1 (ACS1), acetyl-CoA synthetase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6); a heterologous nucleic acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0021] In another aspect, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate, wherein the one or more enzymes comprise a NADH-using HMG-CoA

reductase; a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase, acetylating (ADA); a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthetase 1 (ACS1), acetyl-CoA synthetase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6); a heterologous nucleic acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0022] In another aspect, provided herein is genetically modified host cell capable of producing an isoprenoid, the cell comprising: one or more heterologous nucleic acids encoding a plurality of enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate, wherein the plurality of enzymes comprise an acetyl-CoA:malonyl-CoA acyltransferase; a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase, acetylating (ADA); a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthetase 1 (ACS1), acetyl-CoA synthetase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6); a heterologous nucleic acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0023] In another aspect, provided herein is a genetically modified host cell capable of producing an polyketide, the cell comprising: one or more heterologous nucleic acids encoding one or more enzymes of polyketide biosynthetic pathway; a heterologous nucleic acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0024] In another aspect, provided herein is a genetically modified host cell capable of producing a fatty acid, the cell comprising: one or more heterologous nucleic acids encoding one or more enzymes of fatty acid biosynthetic pathway; a heterologous nucleic

acid encoding a phosphoketolase (PK); a heterologous nucleic acid encoding a phosphotransacetylase (PTA); and a functional disruption of a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2, or a homologue or variant thereof. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2, or a homologue or variant thereof.

[0025] In some embodiments, the genetically modified host cell provided herein is selected from the group consisting of a bacterial cell, a fungal cell, an algal cell, an insect cell, and a plant cell. In some embodiments, the cell is a yeast cell. In some embodiments, the yeast is *Saccharomyces cerevisiae*.

[0026] In some embodiments, the genetically modified host cell produces an increased amount of an acetyl-CoA derived compound (*e.g.,* an isoprenoid, polyketide, or fatty acid) compared to a yeast cell not comprising a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate.

[0027] In another aspect, provided herein are methods for producing a heterologous acetyl-CoA derived compound, the method comprising: culturing a population of genetically modified host cells, capable of producing a heterologous acetyl-CoA derived compound as described herein, in a medium with a carbon source under conditions suitable for making said heterologous acetyl-CoA derived compound; and recovering said heterologous acetyl-CoA derived compound from the medium. In some embodiments, heterologous acetyl-CoA derived compound is selected from the group consisting of an isoprenoid, a polyketide, and a fatty acid.

[0028] In another aspect, provided herein is a method for increasing the production of acetyl-CoA or an acetyl-CoA derived compound in a host cell, the method comprising: expressing in the host cell a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9); and functionally disrupting an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the method further comprises expressing in the host cell a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC 2.3.1.8).

[0029] In another aspect, provided herein is a method for increasing the production of acetyl-CoA in a host cell, the method comprising: expressing in the host cell a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC 2.3.1.8); and functionally disrupting an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the method further comprises expressing in the host cell a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9).

[0030] In some embodiments, the enzyme that converts acetyl phosphate to acetate is a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the glycerol-1-phosphatase is selected from GPP1/RHR2, GPP2/HOR2, and homologues and variants thereof. In some embodiments, GPP1/RHR2, or a homologue or variant thereof, is functionally disrupted. In some embodiments, GPP2/HOR2, or a homologue or variant thereof, is functionally disrupted. In some embodiments, both GPP1/RHR2 and GPP2/HOR2, or both a homologue or variant of GPP1/RHR2 and a homologue or variant of GPP2/HOR2, are functionally disrupted. In some embodiments, the host cell is selected from the group consisting of a bacterial cell, a fungal cell, an algal cell, an insect cell, and a plant cell. In some embodiments, the host cell is a yeast cell. In some embodiments, the yeast is *Saccharomyces cerevisiae*. In some embodiments, the host cell produces an increased amount of acetyl-CoA or an acetyl-CoA derived compound compared to a yeast cell not comprising a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate.

[0030a] In one aspect, provided herein is a genetically modified yeast host cell comprising: (a) a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9); (b) a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC 2.3.1.8); and (c) a functional disruption of an endogenous glycerol-1-phosphatase enzyme (EC 3.1.3.21) that converts acetyl phosphate to acetate, wherein conversion from acetyl phosphate to acetate is functionally disrupted.

[0030b] In another aspect, provided herein is a method for increasing the production of acetyl-CoA or an isoprenoid in a yeast host cell, the method comprising: (a) expressing in the yeast host cell a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9) and a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC2.3.1.8); and (b) functionally disrupting an endogenous glycerol-1-phosphatase enzyme (EC 3.1.3.21) that converts acetyl phosphate to acetate, wherein conversion from acetyl phosphate to acetate is functionally disrupted.

4. BRIEF DESCRIPTION OF THE FIGURES

[0031] **FIG. 1** provides a schematic representation of the pathways involved in the conversion of sugar (glucose and xylose) to acetyl-CoA, and acetyl-CoA derived compounds, in a yeast host cell. The bold arrows indicate the recombinant phosphoketolase pathway. Acetyl phosphate is an intermediate of the phosphoketolase (PK) / phosphotransacetyklase (PTA) pathway to acetyl-CoA, and is hydrolyzed to acetate by RHR2 and HOR2.

2014227811 28 Feb 2018

Abbreviations: G6P, glucose-6-phosphate; R5P, ribulose-5-phosphate; X5P, xyulose-5-phosphate; F6P, fructose-6-phosphate; E4P, eryhtrose-4-phosphate; FBP, fructose-1,6-biphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde-3-phosphate; PEP, phosphoenolpyruvate; ADA, acetaldehyde dehydrogenase, acetylating; ACP, acetyl phosphate.

[0032] **FIG. 2** provides representative enzymes of the mevalonate pathway for isoprenoid production. Abbreviations: AcCoA, acetyl-CoA; AcAcCoA, acetoacetyl-CoA; HMGCoA, 3-hydroxy-3-methylglutaryl-CoA; Mev5P, mevalonate-5-phosphate; Mev5DP, mevalonate-5-diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl pyrophosphate; Erg10, acetyl-CoA thiolase; ACC1, acetyl-CoA carboxylase; AACS, acetoacetyl-CoA synthase; Erg13, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGr, 3-hydroxy-3-methylglutaryl-CoA reductase; Erg12, mevalonate kinase; Erg8, phosphomevalonate kinase; Erg19, mevalonate pyrophosphate decarboxylase.

[0033] **FIGS. 3A-3B** provides the sugar consumption (**A**) and acetate production (**B**) of wild-type (strain Y967, left) and recombinant yeast cells (middle, right) comprising: a heterologous acetaldehyde dehydrogenase acylating (Dz.eutE) and deletion of the native PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ) (strain Y12869; middle); and further comprising a heterologous phosphoketolase (Lm.PK) and phosphotransacetylase (Ck.PTA) (strain Y12746; right).

[0034] **FIGS. 3C-3D** provides the sugar consumption (**C**) and acetate production (**D**) of recombinant yeast cells comprising: a heterologous acetaldehyde dehydrogenase acylating (Dz.eutE) and deletion of the native PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ) (strain Y12869; left); and further comprising a heterologous phosphoketolase (Lm.PK) (strain Y19390; middle) or phosphotransacetylase (Ck.PTA) (strain Y19391; right).

[0035] **FIG. 4** provides a demonstration of acetyl phosphate hydrolysis in cell free extracts (CFE) of wild-type *S. cerevisiae* strain Y967 over a 120 minute timecourse. Shown are CFE only (left); CFE plus 30 mM sodium fluoride, a broad spectrum phosphatase inhibitor (middle); and CFE that has been heat inactivated (right).

[0036] **FIG. 5** provides results of anion exchange chromatography on Y967 cell free extracts. Protein was eluted with a 0-100% gradient of buffer B (20 mM Tris-Cl pH 7, 1M NaCl, 10% glycerol) over 30 column volumes at a flow rate of 0.5 mL/minute, and 1 mL fractions were collected, analyzed by protein gel electrophoresis (**FIG. 5B),** and assayed for acetyl phosphatase activity (**FIG. 5A**). ACP, acetyl phosphate.

[0037] **FIG. 6A** provides results of anion exchange chromatography on fraction #10 of Y967. The most active fraction from this purification, # 14, was analyzed by mass spectrometry to determine the identity of the proteins in the fraction (**FIG. 6B**). RHR2 was identified as a phosphatase in the active fraction.

[0038] **FIG. 7** provides results of acetyl phosphatase activity assays on CFEs of a wild-type yeast strain (Y968) or recombinant yeast strains comprising a deletion of *RHR2*, *HOR2* or both *RHR2* and *HOR2*.

[0039] **FIGS. 8A-8C** provides acetate levels (**A**), glycerol levels (**B**) and optical densities (**C**) of recombinant yeast strain populations. Strain Y12746.ms63909.ms64472 comprises a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), and heterologously expresses acetaldehyde dehydrogenase aceylating (Dz.eutE), phosphoketolase (Lm.PK), phosphotransacetylase (Ck.PTA), and genes in the farnesene production pathway. Strain

Y12746.ms63909.ms64472 rhr2^ is isogenic to strain Y12746.ms63909.ms64472 but further comprises a deletion of RHR2 (rhr2^).

[0040] **FIGS. 8D-8E** provides acetate levels (**D**) and optical densities (**E**) of recombinant yeast strain populations. Strain Y12745 comprises a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), and heterologously expresses acetaldehyde dehydrogenase aceylating (Dz.eutE), phosphoketolase (Lm.PK), and phosphotransacetylase (Ck.PTA). Strain Y12746 rhr2^ is isogenic to strain Y12746 but further comprises a deletion of RHR2 (rhr2^).

[0041] **FIG. 9** provides relative farnesene levels (top) and relative optical densities (bottom) of recombinant yeast strain populations wherein the *RHR2* gene is intact (RHR2+) or deleted (rhr2$^-$). Y968 (right panel) is a wild-type yeast strain. Y12869.ms63907.ms64472 ("Y12869"; 2nd from right panel) comprises a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), and heterologously expresses acetaldehyde dehydrogenase aceylating (Dz.eutE) and genes in the farnesene production pathway, but does not express phosphoketolase or phosphotransacetylase. Y12746.ms63907.ms64472 ("Y12746"; 2nd from left panel) comprises a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), and heterologously expresses acetaldehyde dehydrogenase aceylating (Dz.eutE) and genes in the farnesene production pathway, and uses phosphoketolase and phosphotransacetylase as a pathway to produce cytosolic acetyl-CoA, which is used for synthesis of farnesene. Y12745.ms63907.ms64472 ("Y12745"; left panel) comprises a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), and genes in the farnesene production pathway, and uses phosphoketolase and phosphotransacetylase as a pathway to produce cytosolic acetyl-CoA, which is used for synthesis of farnesene.

5. DETAILED DESCRIPTION OF THE EMBODIMENTS

5.1 Terminology

[0042] As used herein, the term "heterologous" refers to what is not normally found in nature. The term "heterologous nucleotide sequence" refers to a nucleotide sequence not normally found in a given cell in nature. As such, a heterologous nucleotide sequence may be: (a) foreign to its host cell (*i.e.,* is "exogenous" to the cell); (b) naturally found in the host cell (*i.e.,* "endogenous") but present at an unnatural quantity in the cell (*i.e.,* greater or lesser quantity than naturally found in the host cell); or (c) be naturally found in the host cell but positioned outside of its natural locus. The term "heterologous enzyme" refers to an enzyme that is not normally found in a given cell in nature. The term encompasses an enzyme that is: (a) exogenous to a given cell (*i.e.*, encoded by a nucleotide sequence that is not naturally

present in the host cell or not naturally present in a given context in the host cell); and (b) naturally found in the host cell (*e.g.*, the enzyme is encoded by a nucleotide sequence that is endogenous to the cell) but that is produced in an unnatural amount (*e.g.*, greater or lesser than that naturally found) in the host cell.

[0043] On the other hand, the term "native" or "endogenous" as used herein with reference to molecules, and in particular enzymes and nucleic acids, indicates molecules that are expressed in the organism in which they originated or are found in nature, independently of the level of expression that can be lower, equal, or higher than the level of expression of the molecule in the native microorganism. It is understood that expression of native enzymes or polynucleotides may be modified in recombinant microorganisms.

[0044] As used herein, to "functionally disrupt" or a "functional disruption" *e.g.*, of a target gene, for example, one or more genes of the PDH-bypass, means that the target gene is altered in such a way as to decrease in the host cell the activity of the protein encoded by the target gene. In some embodiments the functional disruption of a target gene results in a reduction by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the expression level of the target gene compared to its expression when not functionally disrupted. Similarly, to "functionally disrupt" or a "functional disruption" *e.g.*, of a target protein, for example, a protein having acetyl phosphatase activity, means that the target protein is altered in such a way as to decrease in the host cell the activity of the protein. In some embodiments the functional disruption of a target protein results in a reduction by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the activity or expression level of the target protein compared to its activity or expression when not functionally disrupted. In some embodiments, the activity of the target protein encoded by the target gene is eliminated in the host cell. In other embodiments, the activity of the target protein encoded by the target gene is decreased in the host cell. Functional disruption of the target gene may be achieved by deleting all or a part of the gene so that gene expression is eliminated or reduced, or so that the activity of the gene product is eliminated or reduced. Functional disruption of the target gene may also be achieved by mutating a regulatory element of the gene, *e.g.,* the promoter of the gene so that expression is eliminated or reduced, or by mutating the coding sequence of the gene so that the activity of the gene product is eliminated or reduced. In some embodiments, functional disruption of the target gene results in the removal of the complete open reading frame of the target gene.

[0045] As used herein, the term "parent cell" refers to a cell that has an identical genetic background as a genetically modified host cell disclosed herein except that it does not comprise one or more particular genetic modifications engineered into the modified host cell, for example, one or more modifications selected from the group consisting of: heterologous expression of an ADA, heterologous expression of an NADH-using HMG-CoA reductase, heterologous expression of an AACS, heterologous expression of a phosphoketolase, heterologous expression of a phosphotransacetylase, and heterologous expression of one or more enzymes of the mevalonate pathway.

[0046] As used herein, the term "production" generally refers to an amount of an isoprenoid produced by a genetically modified host cell provided herein. In some embodiments, production is expressed as a yield of isoprenoid by the host cell. In other embodiments, production is expressed as a productivity of the host cell in producing the isoprenoid.

[0047] As used herein, the term "productivity" refers to production of an isoprenoid by a host cell, expressed as the amount of isoprenoid produced (by weight) per amount of fermentation broth in which the host cell is cultured (by volume) over time (per hour).

[0048] As used herein, the term "yield" refers to production of an isoprenoid by a host cell, expressed as the amount of isoprenoid produced per amount of carbon source consumed by the host cell, by weight.

[0049] As used herein, the phrase "acetyl-CoA derived compound" refers to a compound which uses acetyl-CoA as a substrate in its biosynthesis. Exemplary acetyl-CoA derived compounds include, but are not limited to, isoprenoids, polyketides, fatty acids, and alcohols. In some embodiments, an acetyl-CoA derived compound is ethanol, for example, bioethanol produced from pentose substrates, as described in U.S. Patent No. 7,253,001, the contents of which are hereby incorporated by reference in their entirety.

[0050] As used herein, the term "variant" refers to a polypeptide differing from a specifically recited "reference" polypeptide (*e.g.,* a wild-type sequence) by amino acid insertions, deletions, mutations, and substitutions, but retains an activity that is substantially similar to the reference polypeptide. In some embodiments, the variant is created by recombinant DNA techniques, such as mutagenesis. In some embodiments, a variant polypeptide differs from its reference polypeptide by the substitution of one basic residue for another (*i.e.* Arg for Lys), the substitution of one hydrophobic residue for another (*i.e.* Leu for Ile), or the substitution of one aromatic residue for another (*i.e.* Phe for Tyr), *etc.* In some

embodiments, variants include analogs wherein conservative substitutions resulting in a substantial structural analogy of the reference sequence are obtained. Examples of such conservative substitutions, without limitation, include glutamic acid for aspartic acid and vice-versa; glutamine for asparagine and vice-versa; serine for threonine and vice-versa; lysine for arginine and vice-versa; or any of isoleucine, valine or leucine for each other.

5.2 Host Cells

[0051] Host cells useful compositions and methods provided herein include archae, prokaryotic, or eukaryotic cells.

[0052] Suitable prokaryotic hosts include, but are not limited, to any of a variety of gram-positive, gram-negative, or gram-variable bacteria. Examples include, but are not limited to, cells belonging to the genera: *Agrobacterium*, *Alicyclobacillus*, *Anabaena*, *Anacystis*, *Arthrobacter*, *Azobacter*, *Bacillus*, *Brevibacterium*, *Chromatium*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Erwinia*, *Escherichia*, *Lactobacillus*, *Lactococcus*, *Mesorhizobium*, *Methylobacterium*, *Microbacterium*, *Phormidium*, *Pseudomonas*, *Rhodobacter*, *Rhodopseudomonas*, *Rhodospirillum*, *Rhodococcus*, *Salmonella*, *Scenedesmun*, *Serratia*, *Shigella*, *Staphlococcus*, *Strepromyces*, *Synnecoccus*, and *Zymomonas*. Examples of prokaryotic strains include, but are not limited to: *Bacillus subtilis*, *Bacillus amyloliquefacines*, *Brevibacterium ammoniagenes*, *Brevibacterium immariophilum*, *Clostridium beigerinckii*, *Enterobacter sakazakii*, *Escherichia coli*, *Lactococcus lactis*, *Mesorhizobium loti*, *Pseudomonas aeruginosa*, *Pseudomonas mevalonii*, *Pseudomonas pudica*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rhodospirillum rubrum*, *Salmonella enterica*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Staphylococcus aureus*. In a particular embodiment, the host cell is an *Escherichia coli* cell.

[0053] Suitable archae hosts include, but are not limited to, cells belonging to the genera: *Aeropyrum*, *Archaeglobus*, *Halobacterium*, *Methanococcus*, *Methanobacterium*, *Pyrococcus*, *Sulfolobus*, and *Thermoplasma*. Examples of archae strains include, but are not limited to: *Archaeoglobus fulgidus*, *Halobacterium sp.*, *Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*, *Thermoplasma acidophilum*, *Thermoplasma volcanium*, *Pyrococcus horikoshii*, *Pyrococcus abyssi*, and *Aeropyrum pernix*.

[0054] Suitable eukaryotic hosts include, but are not limited to, fungal cells, algal cells, insect cells, and plant cells. In some embodiments, yeasts useful in the present methods include yeasts that have been deposited with microorganism depositories (*e.g.* IFO, ATCC,

etc.) and belong to the genera *Aciculoconidium, Ambrosiozyma, Arthroascus, Arxiozyma, Ashbya, Babjevia, Bensingtonia, Botryoascus, Botryozyma, Brettanomyces, Bullera, Bulleromyces, Candida, Citeromyces, Clavispora, Cryptococcus, Cystofilobasidium, Debaryomyces, Dekkara, Dipodascopsis, Dipodascus, Eeniella, Endomycopsella, Eremascus, Eremothecium, Erythrobasidium, Fellomyces, Filobasidium, Galactomyces, Geotrichum, Guilliermondella, Hanseniaspora, Hansenula, Hasegawaea, Holtermannia, Hormoascus, Hyphopichia, Issatchenkia, Kloeckera, Kloeckeraspora, Kluyveromyces, Kondoa, Kuraishia, Kurtzmanomyces, Leucosporidium, Lipomyces, Lodderomyces, Malassezia, Metschnikowia, Mrakia, Myxozyma, Nadsonia, Nakazawaea, Nematospora, Ogataea, Oosporidium, Pachysolen, Phachytichospora, Phaffia, Pichia, Rhodosporidium, Rhodotorula, Saccharomyces, Saccharomycodes, Saccharomycopsis, Saitoella, Sakaguchia, Saturnospora, Schizoblastosporion, Schizosaccharomyces, Schwanniomyces, Sporidiobolus, Sporobolomyces, Sporopachydermia, Stephanoascus, Sterigmatomyces, Sterigmatosporidium, Symbiotaphrina, Sympodiomyces, Sympodiomycopsis, Torulaspora, Trichosporiella, Trichosporon, Trigonopsis, Tsuchiyaea, Udeniomyces, Waltomyces, Wickerhamia, Wickerhamiella, Williopsis, Yamadazyma, Yarrowia, Zygoascus, Zygosaccharomyces, Zygowilliopsis*, and *Zygozyma*, among others.

[0055] In some embodiments, the host microbe is *Saccharomyces cerevisiae, Pichia pastoris, Schizosaccharomyces pombe, Dekkera bruxellensis, Kluyveromyces lactis* (previously called *Saccharomyces lactis*), *Kluveromyces marxianus, Arxula adeninivorans*, or *Hansenula polymorpha (*now known as *Pichia angusta)*. In some embodiments, the host microbe is a strain of the genus *Candida*, such as *Candida lipolytica, Candida guilliermondii, Candida krusei, Candida pseudotropicalis*, or *Candida utilis*.

[0056] In a particular embodiment, the host microbe is *Saccharomyces cerevisiae*. In some embodiments, the host is a strain of *Saccharomyces cerevisiae* selected from the group consisting of Baker's yeast, CBS 7959, CBS 7960, CBS 7961, CBS 7962, CBS 7963, CBS 7964, IZ-1904, TA, BG-1, CR-1, SA-1, M-26, Y-904, PE-2, PE-5, VR-1, BR-1, BR-2, ME-2, VR-2, MA-3, MA-4, CAT-1, CB-1, NR-1, BT-1, and AL-1. In some embodiments, the host microbe is a strain of *Saccharomyces cerevisiae* selected from the group consisting of PE-2, CAT-1, VR-1, BG-1, CR-1, and SA-1. In a particular embodiment, the strain of *Saccharomyces cerevisiae* is PE-2. In another particular embodiment, the strain of *Saccharomyces cerevisiae* is CAT-1. In another particular embodiment, the strain of *Saccharomyces cerevisiae* is BG-1.

[0057] In some embodiments, the host microbe is a microbe that is suitable for industrial fermentation. In particular embodiments, the microbe is conditioned to subsist under high solvent concentration, high temperature, expanded substrate utilization, nutrient limitation, osmotic stress due to sugar and salts, acidity, sulfite and bacterial contamination, or combinations thereof, which are recognized stress conditions of the industrial fermentation environment.

5.3 The Phosphoketolase (PK) / Phosphotransacetylase (PTA) Pathway to Acetyl-CoA

[0058] In some embodiments, the phosphoketolase pathway is activated in the genetically modified host cells provided herein by engineering the cells to express polynucleotides and/or polypeptides encoding phosphoketolase and, optionally, phosphotransacetylase. Thus, in some embodiments, the genetically modified host cells provided herein comprise a heterologous polynucleotide encoding a polypeptide having phosphoketolase activity. In other embodiments, particularly where acetyl phosphate can be supplied as a metabolic intermediate independent of phosphoketolase activity, the genetically modified host cells provided herein comprise a heterologous polynucleotide encoding a polypeptide having phosphotransacetylase activity. In other embodiments, the genetically modified host cells provided herein comprise both a heterologous polynucleotide encoding a polypeptide having phosphoketolase activity and a heterologous polynucleotide encoding a polypeptide having phosphotransacetylase activity.

5.3.1 Phosphoketolase (PK)

[0059] Phosphoketolase (EC 4.1.2.9) catalyzes the conversion of xylulose 5-phosphate into glyceraldehyde 3-phosphate and acetyl phosphate; and/or the conversion of fructose-6-phosphate into erythrose-4-phosphate and acetyl phosphate. Phosphoketolase activity has been identified in several yeast strains growing with xylose as the sole carbon source but not in yeast strains grown with glucose (Evans and Ratledge, *Arch. Microbiol.* 139: 48-52; 1984). Inhibitors of phosphoketolase include, but are not limited to, erythrose 4-phosphate and glyceraldehyde 3-phosphate.

[0060] Numerous examples of polynucleotides, genes and polypeptides encoding phosphoketolase activity are known in the art and can be used in the genetically modified host cell provided herein. In some embodiments, such a polynucleotide, gene and/or polypeptide is the xylulose 5-phosphateketolase (XpkA) of *Lactobacillus pentosus* MD363 (Posthuma *et al.*, *Appl. Environ. Microbiol.* 68: 831-7; 2002). XpkA is the central enzyme of

the phosphoketolase pathway (PKP) in lactic acid bacteria, and exhibits a specific activity of 4.455 μmol/min/mg (Posthuma *et al.*, *Appl. Environ. Microbiol.* 68: 831-7; 2002). In other embodiments, such a polynucleotide, gene and/or polypeptide is the phosphoketolase of *Leuconostoc mesenteroides* (Lee *et al.*, *Biotechnol Lett.* 27(12);853-858 (2005)), which exhibits a specific activity of 9.9 μmol/min/mg and is stable at pH above 4.5 (Goldberg *et al.*, *Methods Enzymol.* 9: 515-520; 1966). This phosphoketolase exhibits a Km of 4.7 mM for D-xylulose 5- phosphate and a Km of 29 mM for fructose 6-phosphate (Goldberg et al., Methods Enzymol. 9: 515-520; 1966). Representative phosphoketolase nucleotide sequences of *Leuconostoc mesenteroides* includes accession number AY804190, and SEQ ID NO: 1 as provided herein. Representative phosphoketolase protein sequences of *Leuconostoc mesenteroides* include accession numbers YP_819405, AAV66077.1, and SEQ ID NO: 2 as provided herein. In other embodiments, such a polynucleotide, gene and/or polypeptide is the D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase gene xfp from *B. lactis*, as described, for example, in a pentose-metabolizing *S. cerevisiae* strain by Sonderegger *et al.* (*Appl. Environ. Microbiol.* 70: 2892-7; 2004).

[0061] Other useful phosphoketolases include, but are not limited to, those from *Bifidobacterium dentium* ATCC 27678 (ABIX02000002.1:2350400..2352877; EDT46356.1); *Bifidobacterium animalis* (NC_017834.1:1127580..1130057; YP_006280131.1); and *Bifidobacterium pseudolongum* (AY518216.1:988..3465; AAR98788.1); *Aspergillus nidulans FGSC A4* (CBF76492.1); *Bifidobacterium longum* (AAR98787.1); *Bifidobacterium bifidum NCIMB 41171* (ZP 03646196.1); *Bifidobacterium animalis subsp. lactis HN019* (ZP 02962870.1); *Lactobacillus plantarum WCFS1* (NP_786060.1); *Lactobacillus brevis subsp. gravesensis ATCC 27305* (ZP_03940142.1); *Lactobacillus reuteri 100-23* (ZP_03073172.1); and *Leuconostoc mesenteroides subsp. mesenteroides ATCC 8293* (YP_818922.1).

[0062] Other useful phosphoketolases include those described in International Publication No. WO 2011/15985, the contents of which are hereby incorporated by reference in their entirety. These phosphoketolases include: (YP_001601863.1; *Gluconacetobacter diazotrophicus Pal 5*), (YP_001093221.1; *Shewanella loihica PV-4*), (YP_926792.1; *Shewanella amazonensis SB2B*), (YP_735093.1; *Shewanella sp. MR-4*), (YP_001049439.1; *Shewanella baltica OS155*), (ZP_02157884.1; *Shewanella benthica KT99*), (YP_001472925.1; *Shewanella sediminis HAW-EB3*), (YP_001759669.1; *Shewanella woodyi ATCC 51908*), (YP_001673352.1; *Shewanella halifaxensis HA W-EB4*), (YP_563733.1;

Shewanella denitrificans OS217), (ZP_05111697.1; *Legionella drancourtii LLAP 12*), (EEQ84307.1; *Ajellomyces dermatitidis ER-3*), (XP_002626734.1; *Ajellomyces dermatitidis SLH14081*), (XP_001539009.1; *Ajellomyces capsulatus NAm1*), (EEH04133.1; *Ajellomyces capsulatus G186AR*), (EEH20258.1; *Paracoccidioides brasiliensis Pb03*), (EEH44652.1; *Paracoccidioides brasiliensis Pb 18*), (XP_002582752.1; *Uncinocarpus reesii 1704*), (EER26377.1; *Coccidioides posadasii C735 delta SOWgp*), (EEQ28085.1; *Microsporum canis CBS 113480*), (XP_001819785.1; *Aspergillus oryzae RIB40*), (XP_001399780.1; *Aspergillus niger*), (XP_001263382.1; *Neosartorya fischeri NRRL 181*), (XP_001271080.1; *Aspergillus clavatus NRRL 1*), (XP_001213784.1; *Aspergillus terreus NIH2624*), (CBF76492.1; *Aspergillus nidulans FGSCA4*), (XP_002561913.1; *Penicillium chrysogenum Wisconsin 54-1255*), (XP_002480391.1; *Talaromyces stipitatus ATCC 10500*), (XP_002144014.1; *Penicillium stipitatus ATCC 10500*), (XP_002144014.1; *Penicillium mameffei ATCC 18224*), (XP_754543.1; *Aspergillus fumigatus Af293*), (XP_001556635.1; *Botryotinia fuckeliana B05.1 0*), (XP_001592549.1; *Sclerotinia sclerotiorum 1980*), (XP_386729.1; *Gibberella zeae PH-1*), (EEU47171.1; *Nectria haematococca mp VI 77-13-4),* (EEY16637.1; *Verticillium alboatrum VaMs.l 02*), (XP_956649.1; *Neurospora crassa OR74A*), (XP_364271.2; *Magnaporthe grisea 70-15*), (XP_001904585.1; *Podospora anserine*), (XP_001836159.1; *Coprinopsis cinerea okayama7#130*), (NP_595963.1; *Schizosaccharomyc es pombe*), (XP_002173441.1; *Schizosaccharomyc es japonicus yFS275*), (XP_570860.1; *Cryptococcus neoformans var. neoformans JEC21*), (XP_759561.1; *Ustilago maydis 521*), (ZP_05027078.1; *Microcoleus chthonoplastes PCC 7420*), (YP_003101114.1; *Actinosynnema mirum DSM 43827*), (ZP_03568244.1; *Atopobium rimae ATCC 49626*), (YP_003180237.1; *Atopobium parvulum DSM 20469*), (ZP_03946928.1; *Atopobium vaginae DSM 15829*), (ZP_03296299.1; *Collinsella stercoris DSM 13279*), (AAR98787.1; *Bifidobacterium longum*), (ZP_03618909.1; *Bifidobacterium breve DSM 20213*), (ZP_03646196.1; *Bifidobacterium bifidum NCIMB 41171*), (ZP_04448101.1; *Bifidobacterium angulatum DSM 20098*), (ZP_03324204.1; *Bifidobacterium catenulatum DSM 16992*), (AAR98790.1; *Bifidobacterium sp. CFAR 172*), (AAR98789.1; *Bifidobacterium pullorum*), (ZP_03937610.1; *Gardnerella vaginalis ATCC 14019*), (ZP_05965201.1; *Bifidobacterium gallicum DSM 20093*), (ZP_02962870.1; *Bifidobacterium animalis subsp. lactis HNO19*), (AAR98788.1; *Bifidobacterium pseudolongum subsp. Globosum*), (ZP_03946518.1; *Atopobium vaginae DSM 15829*), (YP_001511171.1; *Frankia sp. EANlpec*), (YP_713678.1; *Frankia alni ACN14a*), (YP_002778395.1; *Rhodococcus*

opacus B4), (YP_701466.1; *Rhodococcus jostii RHA1*), (ZP_04383880.1; *Rhodococcus erythropolis SK121*), (YP 947598.1; *Arthrobacter aurescens TC 1*), (CAD48946.1; *Propionibacterium freudenreichii subsp. Shermanii*), (NP_791495.1*; Pseudomonas syringae pv. Tomato str. DC3000*), (YP_003125992.1; *Chitinophaga pinensis DSM 2588*), (ABX56639.1; *Verrucomicrobiae bacterium V4*), (YP_002371883.1; *Cyanothece sp. PCC 8801*), (YP_001806596.1; *Cyanothece sp. ATCC 51142*), (ZP_01730652.1; *Cyanothece sp. CCY0110*), (CAQ48286.1; *Planktothrix rubescens NIVA-CYA 98*), (ZP_03276298.1; *Arthrospira maxima CS-328*), (ZP_03157277.1*; Cyanothece sp. PCC 7822*), (YP_002379031.1; *Cyanothece sp. PCC 7424*), (YP_001658501.1; *Microcystis aeruginosa NIES-843*), (ZP_01621774.1; *Lyngbya sp. PCC 8106*), (NP_485524.1; *Nostoc sp. PCC 7120*), (ZP_05036350.1; *Synechococcus sp. PCC 7335*), (YP_001514813.1; *Acaryoch1oris marina MBIC 11 017*), (ZP_05039537.1; *Synechococcus sp. PCC 7335*), (ZP_02886235.1; *Burkho1deria graminis C4 DIM*), (ZP_03264503.1; *Burkholderia sp. H160*), (ZP_01085819.1; *Synechococcus sp. WH 5701*), (ZP_05045603.1; *Cyanobium sp. PCC 7001*), (ZP_01123645.1; *Synechococcus sp. WH 7805*), (YP_001223932.1; *Synechococcus sp. WH 7803*), (ZP_01079038.1; *Synechococcus sp. RS9917*), (YP_001889002.1; *Burkho1deria phytofirmans PsJN*), (YP_553967.1; *Burkholderia xenovorans LB400*), (ZP_02881709.1; *Burkholderia graminis C4DIM*), (ZP_03270532.1; *Burkholderia sp. H160*), (YP_001861620.1; *Burkholderia phymatum STM815*), (YP_002755285.1; *Acidobacterium capsulatum ATCC 51196*), (EDZ38884.1; Leptospi*rillum sp. Group II '5-way CO'*), (EES53204.1; *Leptospirillum ferrodiazotrophum*), (YP_172723.1; *Synechococcus elongatus PCC 6301*), (NP_681976.1; *Thermosynechococcus elongatus BP-1*), (YP_114037.1; *Methylococcus capsulatus str. Bath*), (YP_002482577.1; *Cyanothece sp. PCC 7425*), (NP_442996.1; *Synechocystis sp. PCC 6803*), (YP_002482735.1; *Cyanothece sp. PCC 7425*), (ZP_04774866.1; *Allochromatium vinosum DSM 180*), (ZP_01453148.1; *Mariprofundus ferrooxydans PV-l*), (ZP_04830548.1; *Gallionella ferruginea ES-2*), (XP_001273863.1; *Aspergillus clavatus NRRL 1*), (XP_001258643.1; *Neosartorya fischeri NRRL 181*), (XP_001727680.1; *Aspergillus oryzae RIB40*), (XP_001396306.1; *Aspergillus niger*), (XP_001216075.1; *Aspergillus terreus NIH2624*), (XP_002567130.1; *Penicillium chrysogenum Wisconsin 54-1255*), (XP_002143851.1*; Penicillium marneffei ATCC 18224*), (XP_002480216.1; T*alaromyces stipitatus ATCC 10500*), (XP_001559949.1; *Botryotinia fuckeliana B05.10*), (XP_001593100.1; *Sclerotinia sclerotiorum 1980*), (XP_001932192.1; *Pyrenophora triticirepentis Pt-lC-BFP*), (XP_001793729.1; *Phaeosphaeria nodorum SN 15*),

(XP_567776.1; *Cryptococcus neoforrnans var. neoforrnans JEC21*), (XP_386504.1; *Oibberella zeae PH-1*), (EEU46265.1; *Nectria haematococca mp VI 77-13-4*), (AC024516.1; *Metarhizium anisop1iae*), (XP_959985.1; *Neurospora crassa OR74A*), (XP_001904686.1; *Podospora anserine*), (YP_002220141.1; *Acidithiobacillus ferrooxidans ATCC 53993*), (YP_001220128.1; *Acidiphilium cryptum JF -5*), (YP_001471202.1; *Thermotoga lettingae TMO*), (YP_002352287.1; *Dictyoglomus turgidum DSM 6724*), (YP_571790.1; *Nitrobacter hamburgensis X14*), (ZP_01092401.1; *Blastopirellula marina DSM 3645*), (YP_001340809.1; *Marinomonas sp. MWYLI*), (NP_866384.1; *Rhodopirellula baltica SH 1*), (ZP_05108502.1; *Legionella drancourtii LLAP 12*), (ZP_04995817.1; *Streptomyces sp. Mg1*), (ZP_04023055.1; *Lactobacillus reuteri SD2112*), (ZP_03960060.1; *Lactobacillus vaginalis ATCC 49540*), (ZP_03073172.1; *Lactobacillus reuteri 100-23*), (ZP_05553031.1; *Lactobacillus coleohominis 101-4-CHN*), (ZP_05863347.1; *Lactobacillus fermentum 28-3-CHN*), (ZP_04021289.1; *Lactobacillus acidophilus ATCC 4796*), (ZP_03995194.1; *Lactobacillus crispatus lV-VOl*), (ZP_04010922.1; *Lactobacillus ultunensis DSM 16047*), (ZP_05549961.1; *Lactobacillus crispatus 125-2-CRN*), (ZP_03951361.1; *Lactobacillus gasseri lV-V03*), (ZP_05744515.1; *Lactobacillus iners DSM 13335*), (YP_618635.1; *Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842*), (ZP_03955917.1; *Lactobacillus jensenii lV-VI6*), (ZP_03942415.1; *Lactobacillus buchneri ATCC 11577*), (ZP_01544800.1; *Oenococcus oeni ATCC BAA-1163*), (NP_786060.1; *Lactobacillus plantarum WCFSI*), (Q937F6; *XPKA_LACPE*), (YP_394903.1; *Lactobacillus sakei subsp. sakei 23K*), (YP_803891.1; *Pediococcus pentosaceus ATCC 25745*), (BAI40727.1; *Lactobacillus rhamnosus GG*), (ZP_03940142.1; *Lactobacillus brevis subsp. Gravesensis ATCC 27305*), (ZP_04009273.1; *Lactobacillus salivarius ATCC 11741*), (ZP_03958643.1; *Lactobacillus ruminis ATCC 25644*), (ZP_04431433.1; *Bacillus coagulans 36D1*), (ZP_04601906.1; *Kingella oralis ATCC 51147*), (ZP_05736927.1; *Granulicatella adiacens ATCC 49175*), (YP_001449631.1; *Streptococcus gordonii str. Challis substr. CHI*), (NP_736274.1; *Streptococcus agalactiae NEM316*), (ZP_04442854.1; *Listeria grayi DSM 20601*), (ZP_05646360.1; *Enterococcus casseliflavus EC30*), (ZP_05650322.1; *Enterococcus gallinarum EG2*), (ZP_05675307.1; *Enterococcus faecium Com12*), (BAH69929.1; *Mycoplasma fermentans PG 18*), (YP_002000006.1; *Mycoplasma arthritidis 15 8L3-1*), (YP_001256266.1; *Mycoplasma agalactiae PG2*), (YP_001988835.1; *Lactobacillus casei BL23*), (NP_786753.1; *Lactobacillus plantarum WCFS 1*), (ZP_04009976.1; *Lactobacillus salivarius ATCC 11741*), (YP_818922.1; *Leuconostoc mesenteroides subsp. Mesenteroides*

ATCC 8293), (YP_794669.1; *Lactobacillus brevis ATCC 367*), (ZP_04782553.1; *Weissella paramesenteroides ATCC 33313*), (YP_001727454.1; *Leuconostoc citreum KM20*), (YP_819405.1; *Leuconostoc mesenteroides subsp. mesenteroides ATCC 8293*), (ABX75772.1; *Lactococcus lactis subsp. Lactis*), (YP_811314.1; *Oenococcus oeni PSU-1*), (ZP_02951191.1; *Clostridium butyricum 5521*), (ZP_05390294.1; *Clostridium carboxidivorans P7*), (NP_347971.1; *Clostridium acetobutylicum ATCC 824*), (ZP_03800296.1; *Coprococcus comes ATCC 27758*), (ZP_04857624.1; *Ruminococcus sp. 5_1_39B FAA*), (ZP_04743029.2; *Roseburia intestinalis L 1-82*), (ZP_02038271.1; *Bacteroides capillosus ATCC 29799*), (XP_002180542.1; *Phaeodactylum tricomutum CCAP 1055/1*), (YP_568630.1; *Rhodopseudomonas palustris BisB5*), (YP_487462.1; *Rhodopseudomonas palustris HaA2*), (NP_947019.1; *Rhodopseudomonas palustris CGA009*), (YP_533660.1; *Rhodopseudomonas palustris BisB18*), (YP_973512.1; *Polaromonas naphthalenivorans CJ2*), (ZP_01464191.1; *Stigmatella aurantiaca DW4/3-1*), (YP_001267778.1; *Pseudomonas putida F1*), (YP_829644.1; *Arthrobacter sp. FB24*), (YP_002486392.1; *Arthrobacter chlorophenolicus A6*), (ZP_05816651.1; *Sanguibacter keddieii DSM 10542*), (YP_002883053.1; *Beutenbergia cavemae DSM 12333*), (YP_003161540.1; *Jonesia denitrificans DSM 20603*), (ZP_03911482.1; *Xylanimonas cellulosilytica DSM 15894*), (CAJ57850.1; *Cellulomonas flavigena*), (YP_001134605.1; *Mycobacterium gilvum PYR-GCK*), (YP 953877.1; *Mycobacterium vanbaalenii PYR-1*), (YP_003155611.1; *Brachybacterium faecium DSM 4810*), (YP_003148127.1; *Kytococcus sedentarius DSM 20547*), (YP_001221168.1; *Clavibacter michiganensis subsp. michiganensis NCPPB 382*), (YP_001158426.1; *Salinispora tropica CNB-440*), (YP_001536420.1; *Salinispora arenicola CNS-205*), (ZP_04608302.1; *Micromonospora sp. ATCC 39149*), (YP_887914.1; *Mycobacterium smegmatis str. MC2 155*), (YP_639956.1; *Mycobacterium sp. MCS*), (ZP_04749157.1; *Mycobacterium kansasii ATCC 12478*), (YP_001851039.1; *Mycobacterium marinumM*), (NP_960507.1; *Mycobacterium avium subsp. paratuberculosis K-10*), (ZP_05224330.1; *Mycobacterium intracellulare ATCC 13950*), (YP_001703240.1; *Mycobacterium abscessus*), (ZP_00995133.1; *Janibacter sp. HTCC2649*), (YP_291026.1; *Thermobifida fusca YX*), (ZP_04031845.1; *Thermomonospora curvata DSM 43183*), (ZP_04475514.1; *Streptosporangium roseum DSM 43021*), (ZP_04335641.1; *Nocardiopsis dassonvillei subsp. dassonvillei DSM 43111*), (ZP_04482201.1; *Stackebrandtia nassauensis DSM 44728*), (YP_003099712.1; *Actinosynnema mirum DSM 43827*), (NP_733508.1; *Streptomyces coelicolor A3(2)*),

(CAJ88379.1; *Streptomyces ambofaciens ATCC 23877*), (ZP_05536883.1; *Streptomyces griseoflavus Tu4000*), (ZP_05020421.1; *Streptomyces sviceus ATCC 29083*), (CBG67625.1; *Streptomyces scabiei 87.22*), (NP_822448.1; *Streptomyces avermitilis MA-4680*), (ZP_04689547.1; *Streptomyces ghanaensis ATCC 14672*), (ZP_05530021.1; *Streptomyces viridochromogenes DSM 40736*), (ZP_05512501.1; *Streptomyces hygroscopicus ATCC 53653*), (ZP_05800927.1; *Streptomyces flayogriseus ATCC 33331*), (YP_001828275.1; *Streptomyces griseus subsp. griseus NBRC 13350*), (ZP_04705493.1; *Streptomyces albus JI074*), (ZP_04996963.1; *Streptomyces sp. Mgl*), (ZP_05485309.1; *Streptomyces sp. SPB78*), (ZP_03860882.1; *Kribbella flayida DSM 17836*), (YP_117539.1; *Nocardia farcinica IFM 10152*), (YP_001505556.1; *Frankia sp. EANlpec*), (YP_482627.1; *Frankia sp. CcI3*), (YP_003116893.1; *Catenulispora acidiphila DSM 44928*), (YP_872280.1; *Acidothermus lolyticus lIB*), (YP_924807.1; *Nocardioides sp. JS614*), (YP_001104157.1; *Saccharopolyspora erythraea NRRL 2338*), (YP_002282673.1; *Rhizobium leguminosarum by. trifolii WSM2304*), (YP_002977256.1; *Rhizobium leguminosarum by. trifolii WSM1325*), (YP_001979796.1; *Rhizobium etli CIAT 652*), (YP_470926.1; *Rhizobium etli CFN 42*), (YP_002540633.1; *Agrobacterium radiobacter K84*), (ZP_05182366.1; *Brucella sp. 83/13*), (ZP_04683384.1; *Ochrobactrum intermedium LMG 3301*), (YP_001373254.1; *Ochrobactrum anthropi ATCC 49188*), (YP_001204109.1; *Bradyrhizobium sp. ORS278*), (YP_001238418.l; *Bradyrhizobium sp. BTAil*), (NP_769158.1; *Bradyrhizobium japonicum USDA 110*), (YP_577164.1; *Nitrobacter hamburgensis X14*), (YP_002961612.1; *Methylobacterium extorquens AM 1*), (YP_674792.1; *Mesorhizobium sp. BNCI*), (ZP_05813617.1; *Mesorhizobium opportunistum WSM2075*), (YP_318559.1; *Nitrobacter winogradskyi Nb-255*), (YP_001755280.1; *Methylobacterium radiotolerans JCM 2831*), (YP_001753119.l; *Methylobacterium radiotolerans JCM 2831*), (YP_003066011.1; *Methylobacterium extorquens DM4*), (YP_002964777.1; *Methylobacterium extorquens AM 1*), (YP_002501292.1; *Methy1obacterium nodu1ans ORS 2060*), (YP_002495265.1; *Methy1obacterium nodu1ans ORS 2060*), (YP_001770387.1; *Methylobacterium sp.4-46*), (YP_002944712.1; *Variovorax paradoxus S110*), (ZP_01156757.1; *Oceanicola granulosus HTCC2516*), (ZP_01628787.1; *Nodu1aria spumlgena CCY9414*), (YP_001865546.1; *Nostoc punctiforme PCC 73102*), (YP_321015.1; *Anabaena variabi1is ATCC 29413*), (ZP_03769140.1; *Nostoc azollae' 0708*), (NP_923943.1; *Gloeobacter vio1aceus PCC 7421*), (YP_477385.1; *Synechococcussp. JA-2-3B'a(2-13)*), (YP_001328659.1; *Sinorhizobium medicae WSM419*), (YP_765670.1; *Rhizobium leguminosarum bv. viciae 3841*),

(*NP_384212.2; Sinorhizobium meliloti 1021*), (ZP_02928455.1; *Verrucomicrobium spinosum DSM 4136*), (YP_001637539.1; *Methylobacterium extorquens Pal*), (ZP_01045825.1; *Nitrobacter sp. Nb-311A*), (ZP_02736602.1; *Gemmata obscuriglobus UQM 2246*), (YP_003157871.1; *Desulfomicrobium baculatum DSM 4028*), (ZP_03631304.1*; bacterium Ellin514*), (ZP_04577558.1; *Oxalobacter formigenes HOxBLS*), (ZP_04579712.1; *Oxalobacter formigenes OXCC13*), (YP_826169.1; *Solibacter usitatus Ellin6076*), (YP_002018753.1; *Pelodictyon phaeoclathratiforme BU-1*), (YP_002016285.1; *Prosthecochloris aestuarii DSM 271*), (YP_001943369.1; *Chlorobium limicola DSM 245*), (NP_662409.1; *Chlorobium tepidum TLS*), (ZP_01386179.1; *Chlorobium ferrooxidans DSM 13031*), (YP_375422.1; *Chlorobium luteolum DSM 273*), (YP_285277.1; *Dechloromonas aromatica RCB*), (YP_314589.1; *Thiobacillus denitrificans ATCC 25259*), (YP_545002.1; *Methylobacillus flagellatus KT*), (NP_842139.1; *Nitrosomonas europaea ATCC 19718*), (YP_748274.1; *Nitrosomonas eutropha C91*), (YP_411688.1; *Nitrosospira multiformis ATCC 25196*), (YP_344700.1; *Nitrosococcus oceani ATCC 19707*), (YP_007004.1; *Candidatus Protochlamydia amoebophila UWE25*), (NP_435833.1; *Sinorhizobium meliloti 1021*), (ZP_04421874.1; *Sulfurospirillum deleyianum DSM 6946*), (NP_107054.1; *Mesorhizobium loti MAFF303099*), (YP_002289797.1; *Oligotropha carboxidovorans OM5*), (YP_001833312.1; *Beijerinckia indica subsp. indica ATCC 9039*).

[0063] Phosphoketolases also useful in the compositions and methods provided herein include those molecules which are said to be "derivatives" of any of the phosphoketolases described herein. Such a "derivative" has the following characteristics: (1) it shares substantial homology with any of the phosphoketolases described herein; and (2) is capable of catalyzing the conversion of X5P into glyceraldehyde 3-phosphate (G3P) and acetyl phosphate; or F6P into erythrose 4-phosphate (E4P) and acetyl phosphate. A derivative of a phosphoketolase is said to share "substantial homology" with the phosphoketolase if the amino acid sequences of the derivative is at least 80%, and more preferably at least 90%, and most preferably at least 95%, the same as that of the phosphoketolase.

5.3.2 Phosphotransacetylase (PTA)

[0064] In some embodiments, the genetically modified host cell provided herein comprises a heterologous nucleotide sequence encoding a phosphotransacetylase. Phosphotransacetylase (EC 2.3.1.8) converts acetyl phosphate into acetyl-CoA.

[0065] Numerous examples of polynucleotides, genes and polypeptides encoding phosphotransacetylase activity are known in the art and can be used in the genetically modified host cell provided herein. In some embodiments, such a polynucleotide, gene and/or polypeptide is the phosphotransacetylase from *Clostridium kluyveri*. Representative phosphotransacetylase nucleotide sequences of *Clostridium kluyveri* includes accession number NC_009706.1:1428554..1429555, and SEQ ID NO: 3 as provided herein. Representative phosphotransacetylase protein sequences of *Clostridium kluyveri* include accession number YP_001394780 and SEQ ID NO: 4 as provided herein. Other useful phosphotransacetylases include, but are not limited to, those from *Lactobacillus reuteri* (NC_010609.1:460303..461277; YP_001841389.10); *Bacillus subtilis* (NC_014479.1:3671865..3672836; YP_003868063.1); *Methanosarcina thermophile* (L23147.1:207..1208; AAA72041.1); *Lactobacillus sanfranciscensis* (BAB19267.1); *Lactobacillus plantarum WCFS1* (NP_784550.1); *Lactobacillus fermentum ATCC 14931* (ZP 03944466.1); *Bacillus subtilis subsp. subtilis str. 168* (NP_391646.1); *Methanosarcina thermophila* (AAA72041.1); *Clostridium thermocellum DSM 4150* (ZP 03152606.1); *Clostridium acetobutylicum ATCC 824* (NP_348368.1); *Clostridium kluyveri DSM 555* (YP 001394780.1); *Veillonella parvula DSM 2008* (ZP 03855267.1); and *Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150* (YP_ 149725.1).

[0066] Other useful phosphotransacetylases include those described in International Publication No. WO 2011/15985, the contents of which are hereby incorporated by reference in their entirety. These phosphotransacetylases include: (ZP_05427766.1; *Eubacterium saphenum ATCC 49989*), (ZP_03627696.1; *bacterium Ellin514*),), (ZP_03131770.1; *Chthonio bacter flavus Ellin428*), (YP_001878031.1; *Akkermansia muciniphila TCCBAA-835*), (ZP_04562924.1; *Citrobacter sp.30_2*), (YP_001451936.1; *Citrobacter koseri ATCC BAA-895*), (YP_149725.1; *Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150*), (YP_001569496.1; *Salmonella enterica subsp. anzonae serovar 62:z4,z23:--*), (NP_416953.1; *Escherichia coli str. K-12 substr. MG1655*), (YP_002920654.1; *Klebsiella pneumomae NTUH-K2044*), (ZP_04637797.1; *Yersinia intermedia ATCC 29909*), (ZP_01222604.1; *Photobacterium profundum 3TCK*), (ZP_02156855.1; *Shewanella benthica KT99*), (YP_958508.1; *Marinobacter aquaeolei VT8*), (YP_066771.1; *Desulfotalea psychrophila LSv54*), (YP_002780531.1; *Rhodococcus opacus B4*), (YP_703506.1; *Rhodococcus jostii RHA1*), (ZP_05479963.1; *Streptomyces sp. AA4*), (YP_002761398.1; *Gemmatimonas aurantiaca T-27*), (ZP_04670189.1; *Clostridiales bacterium 1_7_47FAA*),

(ZP_05493958.1; *Clostridium papyrosolvens DSM 2782*), (YP_003143506.1; *Slackia heliotrinireducens DSM 20476*), (ZP_05090822.1; *Ruegeria sp. R11*), (ZP_01748021.1; *Sagittula stellata E-37*), (NP_604069.1; *Fusobacterium nucleatum subsp. nucleatum ATCC 25586*), (ZP_05814734.1; *Fusobacterium sp. 3_1_33*), (ZP_06026613.1; *Fusobacterium periodonticum ATCC 33693*), (ZP_05617632.1; Fusobacterium sp. 3_1_5R), (ZP_05628030.1; *Fusobacterium sp. D12*), (ZP_04860946.1; *Fusobacteriumvanum ATCC 27725*), (ZP_04567444.1; *Fusobacterium mortiferum ATCC 9817*), (YP_001489437.1; *Arcobacter butzleri RM4018*), (YP_003163236.1; *Leptotrichia buccalis C-1013-b*), (ZP_05902420.1; *Leptotrichia hofstadii F0254*), (ZP_06011308.1; *Leptotrichia goodfellowii F0264*), (ZP_04479548.1; *Streptobacillus moniliformis DSM 12112*), (ZP_03855267.1; *Veillonella parvula DSM 2008*), (ZP_03928523.1; *Acidaminococcus sp. D21*), (NP_970659.1; *Treponema denticola ATCC 35405*), (ZP_05621510.1; *Treponema vincentii ATCC 35580*), (NP_218534.1; *Treponema pallidum subsp. pallidum str. Nichols*), (ZP_04047318.1; *Brachyspira murdochii DSM 12563*), (YP_002720478.1; *Brachyspira hyodysenteriae WAl*), (YP_001740706.1; *Candidatus Cloacamonas acidaminovorans*), (EER05013.1; *Perkinsus mannus ATCC 50983*), (YP_945582.1; *Borrelia turicatae 91E135*), (YP_001884013.1; *Borrelia hermsii DAH*), (YP_002222233.1; *Borrelia duttonii Ly*), (ZP_03675306.1; *Borrelia spielmanii A14S*), (ZP_03435394.1; *Borrelia afzelii ACA-l*), (ZP_03540018.1; *Borrelia garinii Far04*), (ZP_03672928.1; *Borrelia valaisiana VS116*), (NP_212723.1; *Borrelia burgdorferi B31*), (YP_001956287.1; *uncultured Termite group 1 bacterium phylotype Rs-D17*), (NP_975268.1; *Mycoplasma mycoides subsp. mycoides SC str. PGl*), (YP_424216.1; *Mycoplasma capricolum subsp. capricolum ATCC 27343*), (YP_053283.1; *Mesoplasma florum Ll*), (CAK99540.1; *Spiroplasma citri*), (NP_072966.1; *Mycoplasma genitalium G37*), (NP_110116.1; *Mycoplasma pneumomae M129*), (NP_853403.1; *Mycoplasma gallisepticum R*), (NP_757889.1; *Mycoplasma penetrans HF-2*), (YP_116016.1; *Mycoplasma hyopneumoniea 232*), (YP_002960607.1; *Mycoplasma conjunctivae*), (YP_001256282.1; *Mycoplasma agalactiae PG2*), (BAH69503.1; *Mycoplasma fermentans PG18*), (YP_278771.1; *Mycoplasma synoviae 53*), (NP_326068.1; *Mycoplasma pulmonis UAB CTIP*), (YP_015865.1; *Mycoplasma mobile 163K*), (YP_001256630.1; *Mycoplasma agalactiae PG2*), (YP_802685.1; *Buchnera aphidicola str. Cc (Cinara cedri)*), (YP_001885432.1; *Clostridium botulinum B str. Eklund 17B*), (YP_001308302.1; *Clostridium beijerinckii NClMB 8052*), (ZP_05131280.1; *Clostridium sp. 7_2_43FAA*), (ZP_02948604.1; *Clostridium butyricum 5521*), (NP_562641.1; *Clostridium*

perfringens str. 13), (ZP_05391232.1; *Clostridium carboxidivorans P7*), (YP_001394780.1; *Clostridium kluyveri DSM 555*), (ZP_02995419.1; *Clostridium sporogenes ATCC 15579*), (NP_781870.1; *Clostridium tetani E88*), (ZP_04862192.1; *Clostridium botulinum D str. 1873*), (YP_878298.1; *Clostridium novyi NT*), (ZP_04804960.1; *Clostridium cellulovorans 743B*), (NP_348368.1; *Clostridium acetobutylicum ATCC 824*), (ACA51668.1; *Thermoanaero bacterium saccharolyticum*), (ZP_05336886.1; *Thermoanaero bacterium thermosaccharolyticum SM 571*), (NP_623097.1; *Thermoanaero bacter tengcongensis MB4*), (YP_001663354.1; *Thermoanaero bacter sp. X514*), (YP_002508771.1; *Halothermoth rix orenii H 168*), (YP_003190679.1; *Desulfotomaculum acetoxidans DSM 771*), (YP_001917776.1; *Natranaerobius thermophiles JWINM-WN-LF*), (YP_360288.1; *Carboxydothermus hydrogenoformans Z-2901*), (EY83551.1; *Bacteroides sp.2_1_33B*), (ZP_02033408.1; *Parabacteroides merdae ATCC 43184*), (NP_905297.1; *Porphyromonas gingivalis W83*), (ZP_04056000.1; *Porphyromonas uenonis 60-3*), (ZP_04389884.1; *Porphyromonas endodontalis ATCC 35406*), (ZP_02068815.1; *Bacteroides uniformis ATCC 8492*), (ZP_03460749.1; *Bacteroides eggerthii DSM 20697*), (ZP_03676944.1; *Bacteroides cellulosilyticus DSM 14838*), (YP_097761.1; *Bacteroides fragilis YCH46*), (ZP_04545825.1; *Bacteroides sp. D1*), (ZP_03643544.1; *Bacteroides coprophilus DSM 18228*), (ZP_03207078.1; *Bacteroides plebeius DSM 17135*), (YP_001297855.1; *Bacteroides vulgatus ATCC 8482*), (ZP_05736702.1; *Prevotella tannerae ATCC 51259*), (ZP_06007587.1; *Prevotella bergensis DSM 17361*), (ZP_05858935.1; *Prevotella veroralis F0319*), (ZP_05916997.1; *Prevotella sp. oral taxon 472 str. F0295*), (YP_002308782.1; *Candidatus Azo bacteroides pseudotrichon ymphae genomovar. CFP2*), (YP_753459.1; *Syntrophomonas wolfei subsp. wolfei str. Goettingen*), (ZP_01771389.1; *Collinsella aerofaciens ATCC 25986*), (ZP_03296849.1; *Collinsella stercoris DSM 13279*), (ZP_04445308.1; *Collinsella ntestinalis DSM 13280*), (ZP_03567515.1; *Atopobium rimaeATCC 49626*), (YP_003179667.1; *Atopobium parvulum DSM 20469*), (ZP_03946133.1; *Atopobium vaginae DSM 15829*), (ZP_03990654.1; *Oribacterium sinus F0268*), (ZP_04450849.1; *Abiotrophia defective ATCC 49176*), (ZP_05797601.1; *Oribacterium sp. oral taxon 078 str. F0262*), (ZP_03730247.1; *Clostridium sp. M62/1*), (ZP_04856252.1; *Ruminococcus sp. 5_1_39BFAA*), (ZP_01966332.1; *Ruminococcus obeum ATCC 29174*), (ZP_05345616.1; *Bryantella formatexigens DSM 14469*), (ZP_03780829.1; *Blautia hydrogenotro phica DSM 10507*), (ZP_03289360.1; *Clostridium nexile DSM 1787*), (ZP_02042092.1; *Ruminococcus gnavus ATCC 29149*), (ZP_03168112.1; *Ruminococcus*

lactaris ATCC 29176), (ZP_01968837.1; *Ruminococcus torques ATCC 27756*), (ZP_02430426.1; *Clostridium scindens ATCC 35704*), (ZP_03779744.1; *Clostridium hylemonae DSM 15053*), (ZP_02234595.1; *Dorea formicigenerans ATCC 27755*), (ZP_01994673.1; *Dorea longicatena DSM 13814*), (YP_001558442.1; *Clostridium phytofermentans ISDg*), (ZP_04667085.1; *Clostridiales bacterium 1_7_47FAA*), (ZP_02085391.1; *Clostridium bolteae ATCC BAA-613*), (ZP_05790853.1; *Butyrivibrio crossotus DSM 2876*), (ZP_02026034.1; *Eubacterium ventriosum ATCC 27560*), (YP_002930513.1; *Eubacterium eligens ATCC 27750*), (ZP_04808213.1; *Helicobacter pullorum MIT 98-5489*), (ZP_03656120.1; *Helicobacter Canadensis MIT 98-5491*), (ZP_04583217.1; *Helicobacter winghamensis ATCCBAA-430*), (NP_860840.1; *Helicobacter hepaticus ATCC 51449*), (ZP_03657896.1; *Helicobacter cinaedi CCUG 18818*), (ZP_02417779.1; *Anaerostipes caccae DSM 14662*), (ZP_02437622.1; *Clostridium sp. SS211*), (ZP_02205430.1; *Coprococcus eutactus ATCC 27759*), (ZP_02692616.1; *Epulopiscium sp. 'N.t. morphotype B'*), (YP_003182082.1; *Eggerthella lenta DSM 2243*), (YP_003151027.1; *Cryptobacterium curtum DSM 15641*), (YP_003143601.1; *Slackia heliotrinireducens DSM 20476*), (ZP_05498135.1; *Clostridium papyrosolvens DSM 2782*), (ZP_03152606.1; *Clostridium thermocellum JW20*), (YP_001180817.1; *Caldicellulosiruptor saccharolyticus DSM 8903*), (AAA72041.1; *Methanosarcina thermophile*), (NP_618482.1; *Methanosarcina acetivorans C2A*), (YP_305342.1; *Methanosarcina barkeri str. Fusaro*), (ZP_02142278.1; *Roseobacter litoralis Och 149*), (YP_681184.1; *Roseobacter denitrificans OCh 114*), (YP_001533168.1; *Dinoroseo bacter shibae DFL 12*), (ZP_05124935.1; *Rhodobacteraceae bacterium KLH11*), (ZP_05786337.1; *Silicibacter lacuscaerulensis ITI-1157*), (YP_001313586.1; *Sinorhizobium medicae WSM419*), (NP_437512.1; *Sinorhizobium meliloti 1021*), (ZP_04682129.1; *Ochrobactrum intermedium LMG 3301*), (YP_001372036.1; *Ochrobactrum anthropic ATCC 49188*), (YP_001888115.1; *Burkholderia phytofirmans PsJN*), (YP_554613.1; *Burkholderia xenovorans LB400*), (YP_001862297.1; *Burkholderia phymatum STM815*), (YP_297974.1; *Ralstonia eutropha JMP134*), (YP_002008219.1; *Cupriavidus taiwanensis*), (YP_001584488.1; *Burkholderia multivorans multivorans*), (YP_002233797.1; *Burkholderia cenocepacia J2315*), (ZP_01220235.1; *Photobacterium profundum 3TCK*), (ZP_03698361.1; *Lutiella nitroferrum 2002*), (ZP_01811515.1; *Vibrionales bacterium SWAT-3*), (ZP_00988349.1; *Vibrio splendidus 12B01*), (ZP_01866234.1; *Vibrio shilonii AK1*), (ZP_05885163.1; *Vibrio coralliilyticus ATCCBAA-450*), (AAS78789.1; *Paracoccus denitrificans*), (YP_345196.1; *Rhodobacter*

sphaeroides 2.4.1), (AAN08490.1; *Castellaniella defragrans*), (ZP_00961345.1; *Roseovarius nubinhibens ISM*), (YP_168755.1; *Ruegeria pomeroyi DSS-3*), (ZP_01901193.1; *Roseobacter sp. AzwK-3b*), (ZP_01752570.1; *Roseobacter sp. SK209-2-6*), (ZP_02140073.1; *Roseobacter litoralis Och 149*), (YP_510789.1; *Jannaschia sp. CCS1*), (ZP_05073153.1; *Rhodobacteral es bacterium HTCC2083*), (YP_822367.1; *Candidatus Solibacter usitatus Ellin6076*), (ZP_01313101.1; *Desulfuromon as acetoxidans DSM 684*), (YP_357950.1; *Pelobacter carbinolicus DSM 2380*), (YP_002537084.1; *Geobacter sp. FRC-32*), (YP_001232124.1; *Geobacter uraniireducens Rf4*), (NP_953751.1; *Geobacter sulfurreducens PCA*), (YP_384000.1; *Geobacter metallireducens GS-15*), (YP_900968.1; *Pelobacter propionicus DSM 2379*), (YP_001951452.1; *Geobacter lovleyi SZ*), (ZP_05311922.1; *Geobacter sp. M18*), (YP_003021758.1; *Geobacter sp. M21*), (YP_358255.1; *Pelobacter carbinolicus DSM 2380*), (ZP_03906856.1; *Denitrovibrio acetiphilus DSM 12809*), (YP_001997093.1; *Chloroherpeton thalassium ATCC 35110*), (ZP_01924858.1; *Victivallis vadensis ATCCBAA-548*), (ZP_03439825.1; *Helicobacter pylori 98-10*), (YP_003057614.1; *Helicobacter pylori B38*), (YP_001910417.1; *Helicobacter pylori Shi470*), (NP_223559.1; *Helicobacter pylori J99*), (YP_665033.1; *Helicobacter acinonychis str. Sheeba*), (ZP_01810337.1; *Campylobacter jejuni subsp. jejuni CG8486*), (ZP_00366840.1; *Campylobacter coli RM2228*), (ZP_00370527.1; *Campylobacter upsaliensis RM3195*), (YP_002575219.1; *Campylobacter lari RM2100*), (YP_001406718.1; *Campylobacter hominis ATCCBAA- 381*), (ZP_05624820.1; *Campylobacter gracilis RM3268*), (YP_891988.1; *Campylobacter fetus subsp. fetus 82-40*), (YP_001466901.1; *Campylobacter concisus 13826*), (YP_001408221.1; *Campylobacter curvus 525.92*), (ZP_05363348.1; *Campylobacter showae RM3277*), (ZP_03742933.1; *Bifidobacterium pseudocatenulatum DSM 20438*), (ZP_02918887.1; *Bifidobacterium dentium ATCC 27678*), (ZP_02028883.1; *Bifidobacterium adolescentis L2-32*), (ZP_04448100.1; *Bifidobacterium angulatum DSM 20098*), (ZP_03618886.1; *Bifidobacterium breve DSM 20213*), (ZP_03976084.1; *Bifidobacterium longum subsp. infantis ATCC 55813*), (YP_002323183.1; *Bifidobacterium longum subsp. infantis ATCC 15697*), (ZP_03646187.1; *Bifidobacterium bifidum NCIMB 41171*), (ZP_03937611.1; *Gardnerella vaginalis ATCC 14019*), (ZP_02962869.1; *Bifidobacterium animalis subsp. lactis HN019*), (ZP_05965185.1; *Bifidobacterium gallicum DSM 20093*), (ZP_02043408.1; *Actinomyces odontolyticus ATCC 17982*), (ZP_03925176.1; *Actinomyces coleocanis DSM 15436*), (NP_601948.1; *Corynebacterium glutamicum ATCC 13032*), (NP_739201.1; *Corynebacteriurn efficiens YS-*

314), (NP_940379.1; *Corynebacterium diphtheria NCTC 13129*), (ZP_04835255.1; *Corynebacteriurn glucuronolyticum ATCC 51867*), (ZP_05708623.1; *Corynebacteriurn genitalium ATCC 33030*), (ZP_03977910.1; *Corynebacterium lipophiloflavum DSM 44291*), (ZP_03932064.1; *Corynebacterium accolens ATCC 49725*), (ZP_05366890.1; *Corynebacterium tuberculostearicum SK141*), (YP_002835817.1; *Corynebacterium aunmucosum ATCC 700975*), (YP_250020.1; *Corynebacterium jeikeium K411*), (YP_001801132.1; *Corynebacterium urealyticum DSM 7109*), (YP_002906954.1; *Corynebacterium kroppenstedtii DSM 44385*), (ZP_03393297.1; *Corynebacterium amycolatum SK46*), (ZP_03718987.1; *Neisseria flavescens NRL30031/H 210*), (ZP_05318956.1; *Neisseria sicca ATCC 29256*), (YP_001598731.1; *Neisseria meningitides 053442*), (ZP_04602977.1; *Kingella oralis ATCC 51147*), (YP_426466.1; *Rhodospirillum rubrum ATCC 11170*), (NP_871183.1; *Wigglesworthia glossinidia endosymbiont of Glossina brevipalpis*), (NP_777793.1; *Buchnera aphidicola str. Bp (Baizongia pistaciae)*), (YP_003249406.1; *Fibrobacter succmogenes subsp. succmogenes S85*), (ZP_03535302.1; *Mycobacterium tuberculosis T17*), (ZP_04056438.1; *Capnocytophaga gingivalis ATCC 33624*), (YP_003108500.1; *Candidatus Sulcia muelleri SMDSEM*), (P77844; *Corynebacterium glutamicum*), (ZP_03994160.1; *Mobiluncus mulieris ATCC 35243*), (ZP_03922640.1; *Mobiluncus curtisii ATCC 43063*), (ZP_03716209.1; *Eubacterium hallii DSM 3353*), (ZP_03718143.1; *Eubacterium hallii DSM 3353*), (ZP_05614434.1; *Faecalibacteri um prausnitzii A2-165*), (ZP_02034852.1; *Bacteroides capillosus ATCC 29799*), (ZP_03753543.1; *Roseburia inulinivorans DSM 16841*), (ZP_04745275.2; *Roseburia intestinalis Ll-82*), (YP_002937332.1; *Eubacterium rectale ATCC 33656*), (ZP_02074244.1; *Clostridium sp. L2-50*), (ZP_04455374.1; *Shuttleworthia satelles DSM 14600*), (ZP_03488480.1; *Eubacterium biforme DSM 3989*), (ZP_02078327.1; *Eubacterium dolichum DSM 3991*), (ZP_02077559.1; *Eubacterium dolichum DSM 3991*), (ZP_03305532.1; *Anaerococcus hydrogenalis DSM 7454*), (ZP_05473291.1; *Anaerococcus vaginalis ATCC 51170*), (ZP_03931050.1; *Anaerococcus tetradius ATCC 35098*), (YP_003153463.1; *Anaerococcus prevotii DSM 20548*), (ZP_03916048.1; *Anaerococcus lactolyticus ATCC 51172*), (NP_607213.1; *Streptococcus pyogenes MGAS8232*), (AAK34003.1; *Streptococcus pyogenes M1GAS*), (YP_002562185.1; *Streptococcus uberis 01401*), (YP_002744451.1; *Streptococcus equi subsp. Zooepidemicus*), (BAH88016.1; *Streptococcus mutans NN2025*), (ZP_02920305.1; *Streptococcus infantarius subsp. infantarius ATCCBAA-102*), (YP_329798.1; *Streptococcus agalactiae A909*),

(ZP_04061789.1; *Streptococcus salivarius SK126*), (YP_139881.1; *Streptococcus thermophiles LMG 18311*), (ZP_04525024.1; *Streptococcus pneumomae CCRI 1974*), (ZP_06060573.1; *Streptococcus sp. 2_1_36FAA*), (YP_001198423.1; *Streptococcus suis 05ZYH33*), (NP_964739.1; *Lactobacillus johnsonii NCC 533*), (YP_193610.1; *Lactobacillus acidophilus NCFM*), (ZP_04011019.1; *Lactobacillus ultunensis DSM 16047*), (ZP_03995297.1; *Lactobacillus crispatus JV- VOl*), (ZP_05752753.1; *Lactobacillus helveticus DSM 20075*), (ZP_03956024.1; *Lactobacillus jensenii JV-V16*), (ZP_04645187.1; *Lactobacillus jensenii 269-3*), (YP_618719.1; *Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842*), (ZP_05744366.1; *Lactobacillus iners DSM 13335*), (NP_391646.1; *Bacillus subtilis subsp. subtilis str. 168*), (YP_001423045.1; *Bacillus amyloliquefaciens FZB42*), (YP_081073.1; *Bacillus licheniformis ATCC 14580*), (ZP_03055101.1; *Bacillus pumilus ATCC 7061*), (YP_002317098.1; *Anoxybacillus flavithermus WKI*), (YP_002951270.1; *Geobacillus sp. WCH70*), (YP_001127443.1; *Geobacillus thermodenitrificans NG80-2*), (YP_149268.1; *Geobacillus kaustophilus HTA426*), (ZP_01861251.1; *Bacillus sp. SG-l*), (ZP_03228176.1; *Bacillus coahuilensis m4-4*), (ZP_01173945.1; *Bacillus sp. NRRLB-14911*), (NP_693944.1; *Oceanobacillus iheyensis HTE831*), (ZP_04314753.1; *Bacillus cereus BGSC 6E1*), (YP_014727.1; *Listeria monocytogen es str. 4b F2365*), (ZP_04443757.1; *Listeria grayi DSM 20601*), (NP_244690.1; *Bacillus halodurans C-125*), (YP_177402.1; *Bacillus clausii KSM-K16*), (YP_002885816.1; *Exiguo bacteriumsp. AT1b*), (YP_001812721.1; *Exiguo bacterium sibiricum 255-15*), (ZP_02169346.1; *Bacillus selenitireducens MLS10*), (ZP_04818386.1; *Staphylococcus epidermidis M23864:W1*), (ZP_03612973.1; *Staphylococcus capitis SK14*), (ZP_04677798.1; *Staphylococcus wameri L37603*), (NP_763914.1; *Staphylococcus epidermidis ATCC 12228*), (ZP_05685678.1; *Staphylococcus aureus A9635*), (YP_254319.1; *Staphylococcus haemolyticus JCSC1435*), (ZP_04059818.1; *Staphylococcus hominis SKl19*), (ABR57177.1; *Staphylococcus xylosus*), (YP_302214.1; *Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305*), (YP_002633340.1; *Staphylococcus camosus subsp. camosus TM300*), (YP_002561236.1; *Macrococcus caseolyticus JCSC5402*), (ZP_03944466.1; *Lactobacillus fermentum ATCC 14931*), (ZP_05553502.1; *Lactobacillus coleohominis 101-4-CHN*), (ZP_03959629.1; *Lactobacillus vaginalis ATCC 49540*), (YP_001271004.1; *Lactobacillus reuteri DSM 20016*), (ZP_05745668.1; *Lactobacillus antri DSM 16041*), (YP_818931.1; *Leuconostoc mesenteroides subsp. mesenteroides ATCC 8293*), (YP_001727831.1; *Leuconostoc citreum KM20*), (ZP_04782044.1; *Weissella paramesentero ides ATCC 33313*), (ZP_01544468.1;

Oenococcus oeniATCC BAA-1163), (ZP_05737294.1; *Granulicatella adiacens ATCC 49175*), (ZP_05851915.1; *Granulicatella elegans ATCC 700633*), (ZP_02183965.1; *Camobacterium sp.AT7*), (ZP_05649755.1; *Enterococcus gallinarum EG2*), (ZP_03947918.1; *Enterococcus faecalis TX0104*), (ZP_03982224.1; *Enterococcus faecium TX1330*), (YP_395954.1; *Lactobacillus sakei subsp. sakei 23K*), (ZP_04449762.1; *Catonella morbi ATCC 51271*), (YP_001032100.1; *Lactococcus lactis subsp. cremons MG1363*), (YP_806234.1; *Lactobacillus casei ATCC 334*), (NP_784550.1; *Lactobacillus plantarum WCFS1*), (YP_794848.1; *Lactobacillus brevis ATCC 367*), (ZP_03954831.1; *Lactobacillus hilgardii ATCC 8290*), (BABI9267.1; *Lactobacillus sanfranciscensis*), (ZP_03958288.1; *Lactobacillus ruminis ATCC 25644*), (YP_536042.1; *Lactobacillus salivarius UCC118*), (ZP_05747635.1; *Erysipelothrix rhusiopathiae ATCC 19414*), (YP_803875.1; *Pediococcus pentosaceus ATCC 25745*), (ZP_02093784.1; *Parvimonas micraATCC 33270*), (YP_001692923.1; *Finegoldia magnaATCC 29328*), (ZP_04431499.1; *Bacillus coagulans 36Dl*), (ZP_04775813.1; *Gemella haemolysans ATCC 10379*), (YP_001360609.1; *Kineococcus radiotolerans SRS30216*), (ZP_01115869.1; *Reinekea blandensis MED297*), (YP_003074238.1; *Teredinibac turnterrae T7901*), (YP_958411.1; *Marinobacter quaeolei VT8*), (YP_435580.1; *Hahella chejuensis KCTC 2396*), (YP_001189125.1; *Pseudomonas mendocina ymp*), (YP_792443.1; *Pseudomonas aerugmosa UCBPP-PA14*), (NP_791001.1; *Pseudomonas synngae pv. tomato str. DC3000*), (YP_258069.1; *Pseudomonas fluorescens Pf-5*), (YP_606637.1; *Pseudomonas entomophila L48*), (YP_002800579.1; *Azotobacter vinelandii DJ*), (YP_001171663.1; *Pseudomonas stutzeri A1501*), (NP_840385.1; *Nitrosomonas europaea ATCC 19718*), (YP_002801221.1; *Azotobacter vinelandii DJ*), (YP_002787111.1; *Deinococcus deserti VCDl15*), (YP_603523.1; *Deinococcus geothermalis DSM 11300*), (NP_293799.1; *Deinococcus radiodurans R1*), (YP_521550.1; *Rhodoferax ferrireducens T118*), (YP_530962.1; *Rhodopseudo monas palustris BisB18*), (YP_531882.1; *Rhodopseudo monas palustris BisA53*), (ZP_02367347.1; *Burkholderia oklahomensis C6786*), (YP_428079.1; *Rhodospirillum rubrum ATCC 11170*), (YP_530535.1; *Rhodopseudo monas palustris BisB18*), (NP_901200.1; *Chromobacterium violaceum ATCC 12472*), (ZP_03698345.1; *Lutiella nitroferrum 2002*), (YP_001279250.1; *Psychrobacter sp. PRwf-1*), (YP_579484.1; *Psychrobacter cryohalolentis K5*), (ZP_05618978.1; *Enhydrobacter aerosaccus SK60*), (ZP_05362319.1; *Acinetobacter radioresistens SK82*), (YP_045288.1; *Acinetobacter sp. ADP1*), (ZP_05823314.1; *Acinetobacter sp. RUH2624*), (ZP_03824416.1; *Acinetobacter sp. ATCC 27244*), (YP_001380280.1; *Anaeromyxobacter sp.*

Fw109-5), (YP_466103.1; *Anaeromyxobacter dehalogenans 2CP-C*), (YP_088190.1; *Mannheimia succiniciproducens MBEL55E*), (YP_001344949.1; *Actinobacillus succmogenes 130Z*), (YP_003007411.1; *Aggregatibacter aphrophilus NJ8700*), (ZP_01788798.1; *Haemophilus influenzae 3655*), (YP_719012.1; *Haemophilus somnus 129PT*), (NP_245642.1; *Pasteurella multocida subsp. multocida str. Pm70*), (ZP_05920444.1; *Pasteurella dagmatis ATCC 43325*), (ZP_00133992.2; *Actinobacillus pleuropneumoniae serovar 1 str. 4074*), (ZP_04753547.1; *Actinobacillus minor NM305*), (NP_873873.1; *Haemophilus ducreyi 35000HP*), (ZP_04978908.1; *Mannheimia haemolytica PHL213*), (YP_002475022.1; *Haemophilus parasuis SH0165*), (ZP_05730581.1; *Pantoea sp. At-9b*), (YP_001907133.1; *Erwinia tasmaniensis Et1/99*), (YP_455287.1; *Sodalis glossinidius str. 'morsitans'*), (ZP_05723922.1; *Dickeya dadantii Ech586*), (YP_003258889.1; *Pectobacterium wasabiae WPP163*), (YP_002988159.1; *Dickeya dadantii Ech703*), (NP_668938.1; *Yersinia pestis KIM 10*), (YP_001479543.1; *Serratia proteamaculans 568*), (YP_002934098.1; *Edwardsiella ictaluri 93-146*), (YP_002151502.1; *Proteus mirabilis HI4320*), (NP_930328.1; *Photorhabdus luminescens subsp. laumondii TTO1*), (YP_002920553.1; *Klebsiella pneumomae NTUH-K2044*), (YP_001177557.1; *Enterobacter sp.638*), (YP_003211286.1; *Cronobacter turicensis*), (BAA04663.1; *Escherichia coli*), (YP_002924403.1; *Candidatus Hamiltonella defensa 5AT (Acyrthosiphon pisum)*), (ZP_03827735.1; *Pectobacterium carotovorum subsp. brasiliensis PBR1692*), (ZP_01159282.1; *Photobacterium sp. SKA34*), (YP_130973.1; *Photobacterium profundum SS9*), (ZP_06052481.1; *Grimontia hollisae CIP 101886*), (ZP_05877035.1; *Vibrio fumissii CIP 102972*), (ZP_05881960.1; *Vibrio metschnikoyii CIP 69.14*), (ZP_05881960.1; *Vibrio metschnikoyii CIP 69.14*), (ZP_02196748.1; *Vibrio sp. AND4*), (NP_934927.1; *Vibrio vulnificus YJ016*), (ZP_01866446.1; *Vibrio shilonii AKI*), (YP_002416612.1; *Vibrio splendidus LGP32*), (YP_002263486.1; *Aliiyibrio salmonicida LFI1238*), (ZP_04415114.1; *Vibrio cholerae by. albensis VL426*), (YP_001143125.1; *Aeromonas salmonicida subsp. salmonicida A449*), (YP_002892091.1; *Tolumonas auensis DSM 9187*), (ZP_01215350.1; *Psychromonas sp. CNPT3*), (YP_944598.1; *Psychromonas ingrahamii 37*), (YP_001473443.1; *Shewanella sediminis HAW-EB3*), (YP_001761257.1; *Shewanella woodyi ATCC 51908*), (YP_001094519.1; *Shewanella loihica PV -4*), (YP_001674811.1; *Shewanella halifaxensis HAW-EB4*), (YP_869191.1; *Shewanella sp. ANA-3*), (YP_927371.1; *Shewanella amazonensis SB2B*), (YP_751160.1; *Shewanella frigidimarina NCIMB 400*), (YP_563413.1; *Shewanella denitrificans OS217*), (YP_001475272.1; *Shewanella sediminis HAW-EB3*),

(YP_001674949.1; *Shewanella halifaxensis HAW-EB4*), (ZP_04716660.1; *Alteromonas macleodii ATCC 27126*), (YP_662160.1; *Pseudoalteromonas atlantica T6c*), (ZP_01612225.1; *Alteromonadales bacterium TW-7*), (ZP_01134640.1; *Pseudoalteromonas tunicate D2*), (YP_269873.1; *Colwellia psychrerythrae a 34H*), (YP_001341167.1; *Marinomonas sp. MWYL1*), (ZP_01077352.1; *Marinomonas sp. MED121*), (YP_001209362.1; *Dichelobacter nodosus VCS1703A*), (ZP_05705193.1; *Cardiobacterium hominis ATCC 15826*), (EEY62817.1; *Phytophthora infestans T30-4*), (EEY62816.1; *Phytophthora infestans T30-4*), (XP 001694504.1; *Chlamydomonas reinhardtii*), (XP 001753120.1; *Physcomitrella patens subsp. Patens*), (YP_001804510.1; *Cyanothece sp. ATCC 51142*), (ZP_01729220.1; *Cyanothece sp. CCY0110*), (YP_003138337.1; *Cyanothece sp. PCC 8802*), (YP_002380034.1; *Cyanothece sp. PCC 7424*), (YP_001661110.1; *Microcystis aerugmosa NIES-843*), (YP_002485151.1; *Cyanothece sp. PCC 7425*), (NP_441027.1; *Synechocystis sp. PCC 6803*), (ZP_01061171.1; *Leeuwenhoeki ella blandensis MED217*), (YP_001195862.1; *Flavobacterium johnsoniae UW101*), (YP_003194927.1; *Robiginitalea biformata HTCC2501*), (ZP_01107792.1; *Flavobacteriales bacterium HTCC2170*), (ZP_01051731.1; *Polaribacter sp. MED152*), (ZP_01119204.1; *Polaribacter irgensii 23-P*), (ZP_03390929.1; *Capnocytophaga sputigena ATCC 33612*), (YP_003141977.1; *Capnocytophaga ochracea DSM 7271*), (YP_012240.1; *Desulfovibrio vulgaris str. Hildenborough*), (YP_002436276.1; *Desulfovibrio vulgaris str. 'Miyazaki F'*), (YP_389730.1; *Desulfovibrio desulfuricans subsp. desulfuricans str. G20*), (YP_002992165.1; *Desulfovibrio salexigens DSM 2638*), (YP_003197901.1; *Desulfohalobium retbaense DSM 5692*), (YP_003157577.1; *Desulfomicrobium baculatum DSM 4028*), (ZP_03737911.1; *Desulfonatronospira thiodismutans AS03-1*), (YP_002990332.1; *Desulfovibrio salexigens DSM 2638*), (ZP_03312237.1; *Desulfovibrio piger ATCC 29098*), (YP_002478890.1; *Desulfovibrio desulfuricans subsp. desulfuricans str. ATCC 27774*), (YP_064294.1; *Desulfotalea psychrophila LSv54*), (YP_594656.1; *Lawsonia intracellularis PHE/MN1-00*), (ZP_01621820.1; *Lyngbya sp. PCC 8106*), (ZP_03272899.1; *Arthrospira maxima CS-328*), (YP_845596.1; *Syntrophobacter fumaroxidans MPOB*), (ZP_04773932.1; *Allochromatium vinosum DSM 180*), (NP_869002.1; *Rhodopirellula baltica SH 1*), (YP_392571.1; *Sulfurimonas denitrificans DSM 1251*), (ZP_05071717.1; *Campylobacterales bacterium GD 1*), (ZP_04421899.1; *Sulfurospirillum deleyianum DSM 6946*), (YP_001359295.1; *Sulfurovum sp. NBC37-1*), (YP_951544.1; *Mycobacterium vanbaalenii PYR-1*), (YP_001131488.1; *Mycobacterium gilvum PYR-GCK*), (YP_637714.1;

Mycobacterium sp. MCS), (YP_885188.1; *Mycobacterium smegmatis str. MC2 155*), (YP_001704953.1; *Mycobacterium abscessus*), (ZP_04747529.1; *Mycobacterium kansasii ATCC 12478*), (YP_001849024.1; *Mycobacterium marinum M*), (NP_214922.1; *Mycobacterium tuberculosis H37Rv*), (NP_962819.1; *Mycobacterium avium subsp. paratuberculosis K-10*), (ZP_05223872.1; *Mycobacterium intracellulare ATCC 13950*), (YP_002764919.1; *Rhodococcus erythropolis PR4*), (YP_702162.1; *Rhodococcus jostii RHAI*), (YP_121562.1; *Nocardia farcinica IFM 10152*), (ZP_04025361.1; *Tsukamurella paurometabola DSM 20162*), (YP_003275431.1; *Gordonia bronchialis DSM 43247*), (YP_003160610.1; *Jonesia denitrificans DSM 20603*), (ZP_05816650.1; *Sanguibacter keddieii DSM 10542*), (ZP_04368027.1; *Cellulomonas flavigena DSM 20109*), (YP_002883054.1; *Beutenbergia cavemae DSM 12333*), (ZP_03911481.1; *Xylanimonas cellulosilytica DSM 15894*), (YP_924143.1; *Nocardioides sp.1S614*), (ZP_03864789.1; *Kribbella flavida DSM 17836*), (ZP_01131057.1; *marine actinobacterium PHSC20C1*), (YP_001708941.1; *Clavibacter michiganensis subsp. Sepedonicus*), (YP_061462.1; *Leifsonia xyli subsp. xyli str. CTCB07*), (YP_748183.1; *Nitrosomonas eutropha C91*), (YP_003116892.1; *Catenulispora acidiphila DSM 44928*), (YP_003199983.1; *Nakamurella muitipartita DSM 44233*), (YP_003154321.1; *Brachybacterium faecium DSM 4810*), (ZP_03927492.1; *Actinomyces urogenitalis DSM 15434*), (YP_003148931.1; *Kytococcus sedentarius DSM 20547*), (ZP_05803950.1; *Streptomyces flavogriseus ATCC 33331*), (YP_001823623.1; *Streptomyces griseus subsp. griseus NBRC 13350*), (ZP_05002693.1; *Streptomyces clavuligerus ATCC 27064*), (ZP_05015493.1; *Streptomyces sviceus ATCC 29083*), (ZP_05538660.1; *Streptomyces griseoflavus Tu4000*), (ZP_04685789.1; *Streptomyces ghanaensis ATCC 14672*), (ZP_05534308.1; *Streptomyces viridochromogenes DSM 40736*), (ZP_05523554.1; *Streptomyces lividans TK24*), (NP_823999.1; *Streptomyces avermitilis MA-4680*), (CBG69921.1; *Streptomyces scabiei 87.22*), (ZP_04704905.1; *Streptomyces albus 11074*), (ZP_04997745.1; *Streptomyces sp. Mgl*), (ZP_05509147.1; *Streptomyces sp. C*), (ZP_05514718.1; *Streptomyces hygroscopicus ATCC 53653*), (ZP_04994290.1; *Streptomyces sp. SPB74*), (ZP_04474082.1; *Streptosporangium roseum DSM 43021*), (YP_001160501.1; *Salinispora tropica CNB-440*), (YP_001538853.1; *Salinispora arenicola CNS-205*), (ZP_04605575.1; *Micromonospora sp. ATCC 39149*), (YP_832716.1; *Arthrobacter sp. FB24*), (ABR13603.1; *Arthrobacter oxydans*), (YP_002956296.1; *Micrococcus luteus NCTC 2665*), (ZP_05367249.1; *Rothia mucilaginosa ATCC 25296*), (YP_001854004.1; *Kocuria rhizophila DC2201*), (ZP_04984463.1;

Francisella tularensis subsp. holarctica FSC022), (YP_001677422.1; *Francisella philomiragia subsp. philomiragia ATCC 25017*), (YP_588827.1; *Baumannia cicadellinicola str. Hc (Homalodisca oagulata)*), (NP_240007.1; *Buchnera aphidicola str. APS (Acyrthosiphonpisum)*), (ZP_05057494.1; *Verrucomicrobiae bacterium DG1235*), (ZP_02930252.1; *Verrucomicrobium spinosum DSM 4136*), (ZP_01452386.1; *Mariprofundus ferrooxydans PV-l*), and (ZP_01307392.1; *Bermanella marisrubri*).

[0067] Phosphotransacetylases also useful in the compositions and methods provided herein include those molecules which are said to be "derivatives" of any of the phosphotransacetylases described herein. Such a "derivative" has the following characteristics: (1) it shares substantial homology with any of the phosphotransacetylases described herein; and (2) is capable of catalyzing the conversion of acetyl phosphate into acetyl-CoA. A derivative of a phosphotransacetylase is said to share "substantial homology" with the phosphotransacetylase if the amino acid sequences of the derivative is at least 80%, and more preferably at least 90%, and most preferably at least 95%, the same as that of the phosphotransacetylase.

5.4 Functional Disruption of Acetyl Phosphatase Activity

[0068] In some embodiments, the genetically modified host cell provided herein comprises a functional disruption in an enzyme that converts acetyl phosphate to acetate. In some embodiments, the enzyme is native to the host cell.

[0069] In some embodiments, the enzyme that converts acetyl phosphate to acetate is a glycerol-1-phosphatase (EC 3.1.3.21). In some embodiments, the enzyme having glycerol-1-phosphatase activity is RHR2 (GPP1/RHR2; systematic name: YIL053W), or a homolog or variant thereof. GPP1/RHR2 is a constitutively expressed glycerol-1-phosphatase involved in glycerol biosynthesis, and is induced in response to both anaerobic and osmotic stress. *See, e.g.,* Norbeck *et al.*, *J Biol Chem* 271(23): 13875-13881 (1996); Norbeck *et al.*, *J Biol Chem* 272(9): 13875-13881 (1996); Pahlman *et al.*, *J Biol Chem* 276(5): 3555-3563 (2001); Nevoigt and Stahl, FEMS Microbiol Rev 21(3):231-41 (1997); Byrne and Wolf, *Genome Res* 15(10):1456-61; and Hirayama *et al., Mol Gen Genet* 249(2):127-38, the contents of each of which are hereby incorporated by reference in their entireties. The sequence of the *GPP1/RHR2* gene of *S. cerevisiae* has been previously described. *See, e.g.,* Norbeck *et al.*, *J Biol Chem* 271(23): 13875-13881 (1996); and Pahlman *et al.*, *J Biol Chem* 276(5): 3555-3563 (2001). Gpp1/Rhr2 has been previously described as catalyzing the following reaction:

[0070] glycerol-1-phosphate + H2O ⇔ glycerol + phosphate.

[0071] Representative *GPP1/RHR2* nucleotide sequences of *Saccharomyces cerevisiae* include accession number NM_001179403.1, and SEQ ID NO:5 as provided herein. Representative Gpp1/Rhr2 protein sequences of *Saccharomyces cerevisiae* include accession number NP_012211, and SEQ ID NO:6 as provided herein.

[0072] A closely related homolog of GPP1/RHR2 which also catalyzes the hydrolysis of acetyl phosphate to acetate is HOR2 (GPP2/HOR2; systematic name: YER062C). Gpp2/Hor2 has also been previously described as a glycerol-1-phosphatase capable of catalyzing the following reaction: glycerol-1-phosphate + H2O⇔ glycerol + phosphate. Accordingly, functional disruption of GPP2/HOR2 also finds use in the compositions and methods provided herein. The sequence of the *GPP2/HOR2* gene of *S. cerevisiae* has been previously described. *See, e.g.,* Norbeck *et al., J. of Biological Chemistry* 271(23): 13875-13881 (1996); and Pahlman *et al., J. of Biological Chemistry* 276(5): 3555-3563 (2001). Representative *GPP2/HOR2* nucleotide sequences of *Saccharomyces cerevisiae* include accession number NM_001178953.3, and SEQ ID NO:7 as provided herein. Representative Gpp1/Rhr2 protein sequences of *Saccharomyces cerevisiae* include accession number NP_010984, and SEQ ID NO:8 as provided herein.

[0073] As would be understood in the art, naturally occurring homologs of GPP1/RHR2 and/or GPP2/HOR2 in yeast other than *S. cerevisiae* can similarly be inactivated using the methods described herein. Moreover, a polynucleotide, gene and/or polypeptide encoding acetyl-phosphatase activity (*e.g.,* RHR2 and/or HOR2) can be used to identify other polynucleotide, gene and/or polypeptide sequences or to identify homologs having acetyl-phosphatase activity in other host cells. Such sequences can be identified, for example, in the literature and/or in bioinformatics databases well known to the skilled person. For example, the identification of sequences encoding acetyl-phosphatase activity in other cell types using bioinformatics can be accomplished through BLAST (as described above) searching of publicly available databases with known DNA and polypeptide sequences encoding acetyl-phosphatase and/or glycerol-1-phosphatase activity, such as those provided herein. Identities can be based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

[0074] In some embodiments, the activity or expression of an endogenous enzyme that converts acetyl phosphate to acetate (*e.g.,* RHR2 or HOR2) is reduced by at least about 50%. In another embodiment, the activity or expression of an endogenous enzyme that

converts acetyl phosphate to acetate is reduced by at least about 60%, by at least about 65%, by at least about 70%, by at least about 75%, by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95%, or by at least about 99% as compared to a recombinant microorganism not comprising a reduction or deletion of the activity or expression of an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the endogenous enzyme that converts acetyl phosphate to acetate is RHR2, or homologues thereof. In some embodiments, the endogenous enzyme that converts acetyl phosphate to acetate is HOR2, or homologues thereof.

[0075] As is understood by those skilled in the art, there are several mechanisms available for reducing or disrupting the activity of a protein that converts acetyl phosphate to acetate, such as a glycerol-1-phosphatase (*e.g.,* RHR2 and/or HOR2), including, but not limited to, the use of a regulated promoter, use of a weak constitutive promoter, disruption of one of the two copies of the gene encoding the protein in a diploid yeast, disruption of both copies of the gene in a diploid yeast, expression of an anti-sense nucleic acid, expression of an siRNA, over expression of a negative regulator of the endogenous promoter, alteration of the activity of an endogenous or heterologous gene, use of a heterologous gene with lower specific activity, the like or combinations thereof.

[0076] In some embodiments, the genetically modified host cell comprises a mutation in at least one gene encoding acetyl-phosphatase activity (*e.g.,* RHR2, HOR2 or a homolog or variant thereof), resulting in a reduction of activity of a polypeptide encoded by said gene. In another embodiment, the genetically modified host cell comprises a partial deletion of a gene encoding acetyl-phosphatase activity (*e.g.,* RHR2, HOR2 or a homolog or variant thereof), resulting in a reduction of activity of a polypeptide encoded by the gene. In another embodiment, the genetically modified host cell comprises a complete deletion of a gene encoding acetyl-phosphatase activity (*e.g.,* RHR2, HOR2 or a homolog or variant thereof), resulting in a reduction of activity of a polypeptide encoded by the gene. In yet another embodiment, the genetically modified host cell comprises a modification of the regulatory region associated with the gene encoding acetyl-phosphatase activity (*e.g.,* RHR2, HOR2 or a homolog or variant thereof), resulting in a reduction of expression of a polypeptide encoded by said gene. In yet another embodiment, the genetically modified host cell comprises a modification of the transcriptional regulator resulting in a reduction of transcription of a gene encoding acetyl-phosphatase activity (*e.g.,* RHR2, HOR2 or a homolog or variant thereof).

[0077] In some embodiments, disruption of one or more genes encoding a protein capable of catalyzing the conversion of acetyl phosphate to acetate is achieved by using a "disruption construct" that is capable of specifically disrupting such a gene (*e.g., RHR2* or *HOR2*) upon introduction of the construct into the microbial cell, thereby rendering the disrupted gene non-functional. In some embodiments, disruption of the target gene prevents the expression of a functional protein. In some embodiments, disruption of the target gene results in expression of a non-functional protein from the disrupted gene. In some embodiments, disruption of a gene encoding a protein capable of converting acetyl phosphate to acetate is achieved by integration of a "disrupting sequence" within the target gene locus by homologous recombination. In such embodiments, the disruption construct comprises a disrupting sequence flanked by a pair of nucleotide sequences that are homologous to a pair of nucleotide sequences of the target gene locus (homologous sequences). Upon replacement of the targeted portion of the target gene by the disruption construct, the disrupting sequence prevents the expression of a functional protein, or causes expression of a non-functional protein, from the target gene.

[0078] Disruption constructs capable of disrupting a gene may be constructed using standard molecular biology techniques well known in the art. *See, e.g.,* Sambrook *et al.*, 2001, *Molecular Cloning -- A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, and Ausubel *et al., eds.*, Current Edition, *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, NY. Parameters of disruption constructs that may be varied in the practice of the present methods include, but are not limited to, the lengths of the homologous sequences; the nucleotide sequence of the homologous sequences; the length of the disrupting sequence; the nucleotide sequence of the disrupting sequence; and the nucleotide sequence of the target gene. In some embodiments, an effective range for the length of each homologous sequence is 50 to 5,000 base pairs. In particular embodiments, the length of each homologous sequence is about 500 base pairs. For a discussion of the length of homology required for gene targeting, *see* Hasty *et al., Mol Cell Biol* 11:5586-91 (1991). In some embodiments, the homologous sequences comprise coding sequences of the target gene. In other embodiments, the homologous sequences comprise upstream or downstream sequences of the target gene. Is some embodiments, one homologous sequence comprises a nucleotide sequence that is homologous to a nucleotide sequence located 5' of the coding sequence of the target gene, and the other homologous sequence comprises a nucleotide sequence that is homologous to a

nucleotide sequence located 3' of the coding sequence of the target gene. In some embodiments, the disrupting sequence comprises a nucleotide sequence encoding a selectable marker that enables selection of microbial cells comprising the disrupting sequence. Thus, in such embodiments, the disruption construct has a dual function, *i.e.,* to functionally disrupt the target gene and to provide a selectable marker for the identification of cells in which the target gene is functionally disrupted. In some embodiments, a termination codon is positioned in-frame with and downstream of the nucleotide sequence encoding the selectable marker to prevent translational read-through that might yield a fusion protein having some degree of activity of the wild type protein encoded by the target gene. In some embodiments, the length of the disrupting sequence is one base pair. Insertion of a single base pair can suffice to disrupt a target gene because insertion of the single base pair in a coding sequence could constitute a frame shift mutation that could prevent expression of a functional protein. In some embodiments, the sequence of the disruption sequence differs from the nucleotide sequence of the target gene located between the homologous sequences by a single base pair. Upon replacement of the nucleotide sequence within the target gene with the disrupting sequence, the single base pair substitution that is introduced could result in a single amino acid substitution at a critical site in the protein and the expression of a non-functional protein. It should be recognized, however, that disruptions effected using very short disrupting sequences are susceptible to reversion to the wild type sequence through spontaneous mutation, thus leading to restoration of acetyl-phosphatase function to the host strain. Accordingly, in particular embodiments, the disrupting sequences are longer than one to a few base pairs. At the other extreme, a disrupting sequence of excessive length is unlikely to confer any advantage over a disrupting sequence of moderate length, and might diminish efficiency of transfection or targeting. Excessive length in this context is many times longer than the distance between the chosen homologous sequences in the target gene. Thus, in certain embodiments, the length for the disrupting sequence can be from 2 to 2,000 base pairs. In other embodiments, the length for the disrupting sequence is a length approximately equivalent to the distance between the regions of the target gene locus that match the homologous sequences in the disruption construct.

[0079] In some embodiments, the disruption construct is a linear DNA molecule. In other embodiments, the disruption construct is a circular DNA molecule. In some embodiments, the circular disruption construct comprises a pair of homologous sequences separated by a disrupting sequence, as described above. In some embodiments, the circular

disruption construct comprises a single homologous sequence. Such circular disruption constructs, upon integration at the target gene locus, would become linearized, with a portion of the homologous sequence positioned at each end and the remaining segments of the disruption construct inserting into and disrupting the target gene without replacing any of the target gene nucleotide sequence. In particular embodiments, the single homologous sequence of a circular disruption construct is homologous to a sequence located within the coding sequence of the target gene.

[0080] Disruption constructs can be introduced into a microbial cell by any method known to one of skill in the art without limitation. Such methods include, but are not limited to, direct uptake of the molecule by a cell from solution, or facilitated uptake through lipofection using, *e.g.,* liposomes or immunoliposomes; particle-mediated transfection; *etc.* See, *e.g.,* U.S. Patent No. 5,272,065; Goeddel et al., eds, 1990, Methods in Enzymology, vol. 185, Academic Press, Inc., CA; Krieger, 1990, Gene Transfer and Expression -- A Laboratory Manual, Stockton Press, NY; Sambrook et al., 1989, Molecular Cloning -- A Laboratory Manual, Cold Spring Harbor Laboratory, NY; and Ausubel et al., eds., Current Edition, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY. Particular methods for transforming yeast cells are well known in the art. *See* Hinnen *et al.,* Proc. Natl. Acad. Sci. USA 75:1292-3 (1978); Cregg *et al.,* Mol. Cell. Biol. 5:3376-3385 (1985). Exemplary techniques include, but are not limited to, spheroplasting, electroporation, PEG 1000 mediated transformation, and lithium acetate or lithium chloride mediated transformation.

5.5 Additional Modifications to Improve Acetyl-CoA Production

5.5.1 ADA

[0081] In some embodiments, the genetically modified host cells provided herein further comprise one or more heterologous nucleotide sequences encoding acylating acetaldehyde dehydrogenase (alternately referred to as "acetylaldehyde dehydrogenase, acetylating," "acetylaldehyde dehydrogenase, acylating," or ADA (EC 1.2.1.10)).

[0082] Proteins capable of catalyzing this reaction that are useful for the compositions and methods provided herein include the following four types of proteins:

[0083] (1) Bifunctional proteins that catalyze the reversible conversion of acetyl-CoA to acetaldehyde, and the subsequent reversible conversion of acetaldehyde to ethanol. An example of this type of protein is the AdhE protein in *E. coli* (Gen Bank No: NP_415757). AdhE appears to be the evolutionary product of a gene fusion. The NH_2-terminal region of

the AdhE protein is highly homologous to aldehyde:NAD^+ oxidoreductases, whereas the COOH-terminal region is homologous to a family of Fe^{2+}-dependent ethanol:NAD^+ oxidoreductases (Membrillo-Hernandez *et al.*, (2000) *J. Biol. Chem.* 275: 33869-33875). The *E. coli* AdhE is subject to metal-catalyzed oxidation and therefore oxygen-sensitive (Tamarit *et al.* (1998) *J. Biol. Chem.* 273:3027-32).

[0084] (2) Proteins that catalyze the reversible conversion of acetyl-CoA to acetaldehyde in strictly or facultative anaerobic microbes but do not possess alcohol dehydrogenase activity. An example of this type of protein has been reported in *Clostridium kluyveri* (Smith *et al.* (1980) *Arch. Biochem. Biophys.* 203: 663-675). An ADA has been annotated in the genome of *Clostridium kluyveri* DSM 555 (accession no: EDK33116). A homologous protein AcdH is identified in the genome of *Lactobacillus plantarum* (accession no: NP_784141). Another example of this type of protein is the *ald* gene product in *Clostridium beijerinckii* NRRL B593 (Toth *et al.* (1999) *Appl. Environ. Microbiol.* 65: 4973-4980, accession no: AAD31841).

[0085] (3) Proteins that are involved in ethanolamine catabolism. Ethanolamine can be utilized both as carbon and nitrogen source by many enterobacteria (Stojiljkovic *et al.* (1995) *J. Bacteriol.* 177: 1357-1366). Ethanolamine is first converted by ethanolamine ammonia lyase to ammonia and acetaldehyde, subsequently, acetaldehyde is converted by ADA to acetyl-CoA. An example of this type of ADA is the EutE protein in *Salmonella typhimurium* (Stojiljkovic *et al.* (1995) *J. Bacteriol.* 177: 1357-1366, accession no: AAL21357; *see* also U18560.1). *E. coli* is also able to utilize ethanolamine (Scarlett *et al.* (1976) *J. Gen. Microbiol.* 95:173-176) and has an EutE protein (accession no: AAG57564; *see* also EU897722.1) which is homologous to the EutE protein in *S. typhimurium*.

[0086] (4) Proteins that are part of a bifunctional aldolase-dehydrogenase complex involved in 4-hydroxy-2-ketovalerate catabolism. Such bifunctional enzymes catalyze the final two steps of the meta-cleavage pathway for catechol, an intermediate in many bacterial species in the degradation of phenols, toluates, naphthalene, biphenyls and other aromatic compounds (Powlowski and Shingler (1994) *Biodegradation* 5, 219-236). 4-Hydroxy-2-ketovalerate is first converted by 4-hydroxy-2-ketovalerate aldolase to pyruvate and acetaldehyde, subsequently acetaldehyde is converted by ADA to acetyl-CoA. An example of this type of ADA is the DmpF protein in *Pseudomonas sp* CF600 (accession no: CAA43226) (Shingler *et al.* (1992) *J. Bacteriol.* 174:71 1-24). *E. coli* has a homologous

MphF protein (Ferrandez *et al.* (1997) J. Bacteriol. 179: 2573-2581 , accession no: NP_414885) to the DmpF protein in *Pseudomonas sp.* CF600.

[0087] In some embodiments, an ADA (or nucleic acid sequence encoding such activity) useful for the compositions and methods described herein is selected from the group consisting of *Escherichia coli* adhE, *Entamoeba histolytica* adh2, *Staphylococcus aureus* adhE, *Piromyces sp.E2* adhE, *Clostridium kluyveri* (EDK33116), *Lactobacillus plantarum* acdH, and *Pseudomonas putida* (YP 001268189), as described in International Publication No. WO 2009/013159, the contents of which are incorporated by reference in their entirety. In some embodiments, the ADA is selected from the group consisting of *Clostridium botulinum* eutE (FR745875.1), *Desulfotalea psychrophila* eutE (CR522870.1), *Acinetobacter sp. HBS-2* eutE (ABQ44511.2), *Caldithrix abyssi* eutE (ZP_09549576), and *Halorubrum lacusprofundi* ATCC 49239 (YP_002565337.1).

[0088] In particular embodiments, the ADA useful for the compositions and methods provided herein is eutE from *Dickeya zeae*. A representative eutE nucleotide sequence of *Dickeya zeae* includes accession number NC_012912.1:1110476..1111855, and SEQ ID NO: 9 as provided herein. A representative eutE protein sequence of *Dickeya zeae* includes accession number YP_003003316, and SEQ ID NO: 10 as provided herein.

[0089] ADAs also useful in the compositions and methods provided herein include those molecules which are said to be "derivatives" of any of the ADAs described herein. Such a "derivative" has the following characteristics: (1) it shares substantial homology with any of the ADAs described herein; and (2) is capable of catalyzing the conversion of acetaldehyde to acetyl-CoA. A derivative of an ADA is said to share "substantial homology" with ADA if the amino acid sequences of the derivative is at least 80%, at least 85% and more preferably at least 90%, and most preferably at least 95%, the same as that of any of the ADAs described herein.

5.5.2 Functional Disruption of the PDH-bypass

[0090] Acetyl-CoA can be formed in the mitochondria by oxidative decarboxylation of pyruvate catalyzed by the PDH complex. However, due to the inability of *S. cerevisiae* to transport acetyl-CoA out of the mitochondria, the PDH bypass has an essential role in providing acetyl-CoA in the cytosolic compartment, and provides an alternative route to the PDH reaction for the conversion of pyruvate to acetyl-CoA. The PDH bypass involves the enzymes pyruvate decarboxylase (PDC; EC 4.1.1.1), acetaldehyde dehydrogenase (ACDH; EC 1.2.1.5 and EC 1.2.1.4), and acetyl-CoA synthetase (ACS; EC 6.2.1.1). Pyruvate

decarboxylase catalyzes the decarboxylation of pyruvate to acetaldehyde and carbon dioxide. Acetaldehyde dehydrogenase oxidizes acetaldehyde to acetic acid. In *S. cerevisiae*, the family of aldehyde dehydrogenases contains five members. *ALD2* (YMR170c), *ALD3* (YMR169c), and *ALD6* (YPL061w) correspond to the cytosolic isoforms, while *ALD4* (YOR374w) and *ALD5* (YER073w) encode the mitochondrial enzyme. The main cytosolic acetaldehyde dehydrogenase isoform is encoded by *ALD6*. The formation of acetyl-CoA from acetate is catalyzed by ACS and involves hydrolysis of ATP. Two structural genes, *ACS1* and *ACS2,* encode ACS.

[0091] In some embodiments, the genetically modified host cell provided herein further comprises a functional disruption in one or more genes of the PDH-bypass pathway. In some embodiments, disruption of the one or more genes of the PDH-bypass of the host cell results in a genetically modified microbial cell that is impaired in its ability to catalyze one or more of the following reactions: (1) the decarboxylation of pyruvate into acetaldehyde by pyruvate decarboxylase; (2) the conversion of acetaldehyde into acetate by acetaldehyde dehydrogenase; and (3) the synthesis of acetyl-CoA from acetate and CoA by acetyl-CoA synthetase.

[0092] In some embodiments, compared to a parent cell, a host cell comprises a functional disruption in one or more genes of the PDH-bypass pathway, wherein the activity of the reduced-function or non-functional PDH-bypass pathway alone or in combination with a weak ADA is not sufficient to support host cell growth, viability, and/or health.

[0093] In some embodiments, the activity or expression of one or more endogenous proteins of the PDH-bypass is reduced by at least about 50%. In another embodiment, the activity or expression of one or more endogenous proteins of the PDH-bypass is reduced by at least about 60%, by at least about 65%, by at least about 70%, by at least about 75%, by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95%, or by at least about 99% as compared to a recombinant microorganism not comprising a reduction or deletion of the activity or expression of one or more endogenous proteins of the PDH-bypass.

5.5.2.1 ALD4 and ALD6

[0094] In some embodiments, one or more genes encoding aldehyde dehydrogenase (ACDH) activity are functionally disrupted in the host cell. In some embodiments, the aldehyde dehydrogenase is encoded by a gene selected from the group consisting of ALD2, ALD3, ALD4, ALD5, ALD6, and homologs and variants thereof.

[0095] In some embodiments, the genetically modified host cell comprises a functional disruption of ALD4. Representative *ALD4* nucleotide sequences of *Saccharomyces cerevisiae* include accession number NM_001183794, and SEQ ID NO:11 as provided herein. Representative Ald4 protein sequences of *Saccharomyces cerevisiae* include accession number NP_015019.1, and SEQ ID NO:12 as provided herein.

[0096] In some embodiments, the genetically modified host cell comprises a functional disruption of cytosolic aldehyde dehydrogenase (ALD6). Ald6p functions in the native PDH-bypass to convert acetaldehyde to acetate. Representative *ALD6* nucleotide sequences of *Saccharomyces cerevisiae* include accession number SCU56604, and SEQ ID NO:13 as provided herein. Representative Ald6 protein sequences of *Saccharomyces cerevisiae* include accession number AAB01219, and SEQ ID NO:14 as provided herein.

[0097] As would be understood in the art, naturally occurring homologs of aldehyde dehydrogenase in yeast other than *S. cerevisiae* can similarly be inactivated using the methods described herein.

[0098] As would be understood by one skilled in the art, the activity or expression of more than one aldehyde dehydrogenase can be reduced or eliminated. In one specific embodiment, the activity or expression of ALD4 and ALD6 or homologs or variants thereof is reduced or eliminated. In another specific embodiment, the activity or expression of ALD5 and ALD6 or homologs or variants thereof is reduced or eliminated. In yet another specific embodiment, the activity or expression of ALD4, ALD5, and ALD6 or homologs or variants thereof is reduced or eliminated. In yet another specific embodiment, the activity or expression of the cytosolically localized aldehyde dehydrogenases ALD2, ALD3, and ALD6 or homologs or variants thereof is reduced or eliminated. In yet another specific embodiment, the activity or expression of the mitochondrially localized aldehyde dehydrogenases, ALD4 and ALD5 or homologs or variants thereof, is reduced or eliminated.

5.5.2.2 ACS1 and ACS2

[0099] In some embodiments, one or more genes encoding acetyl-CoA synthetase (ACS) activity are functionally disrupted in the host cell. In some embodiments, the acetyl-CoA synthetase is encoded by a gene selected from the group consisting of ACS1, ACS2, and homologs and variants thereof.

[00100] In some embodiments, one or more genes encoding acetyl-CoA synthetase (ACS) activity is functionally disrupted in the host cell. ACS1 and ACS2 are both acetyl-CoA synthetases that can convert acetate to acetyl-CoA. ACS1 is expressed only under

respiratory conditions, whereas ACS2 is expressed constitutively. When ACS2 is knocked out, strains are able to grow on respiratory conditions (*e.g.* ethanol, glycerol, or acetate media), but die on fermentable carbon sources (*e.g.* sucrose, glucose).

[00101] In some embodiments, the genetically modified host cell comprises a functional disruption of ACS1. The sequence of the *ACS1* gene of *S. cerevisiae* has been previously described. *See, e.g.,* Nagasu *et al., Gene* 37 (1-3):247-253 (1985). Representative *ACS1* nucleotide sequences of *Saccharomyces cerevisiae* include accession number X66425, and SEQ ID NO:15 as provided herein. Representative Acs1 protein sequences of *Saccharomyces cerevisiae* include accession number AAC04979, and SEQ ID NO:16 as provided herein.

[00102] In some embodiments, the genetically modified host cell comprises a functional disruption of ACS2. The sequence of the *ACS2* gene of *S. cerevisiae* has been previously described. *See, e.g.,* Van den Berg *et al., Eur. J. Biochem.* 231(3):704-713 (1995). Representative *ACS2* nucleotide sequences of *Saccharomyces cerevisiae* include accession number S79456, and SEQ ID NO:17 as provided herein. Representative Acs2 protein sequences of *Saccharomyces cerevisiae* include accession number CAA97725, and SEQ ID NO:18 as provided herein.

[00103] As would be understood in the art, naturally occurring homologs of acetyl-CoA synthetase in yeast other than *S. cerevisiae* can similarly be inactivated using the methods described herein.

[00104] In some embodiments, the host cell comprises a cytosolic acetyl-coA synthetase activity that can convert acetate to acetyl-CoA under respiratory conditions (*i.e.*, when the host cell is grown in the presence of e.g. ethanol, glycerol, or acetate). In some such embodiments, the host cell is a yeast cell that comprises ACS1 activity. In other embodiments, the host cell compared to a parent cell comprises no or reduced endogenous acetyl-CoA synthetase activity under respiratory conditions. In some such embodiments, the host cell is a yeast cell that compared to a parent cell comprises no or reduced ACS1 activity.

[00105] In some embodiments, the host cell comprises a cytosolic acetyl-coA synthetase activity that can convert acetate to acetyl-CoA under non-respiratory conditions (*i.e.*, when the host cell is grown in the presence of fermentable carbon sources (*e.g.* sucrose, glucose)). In some such embodiments, the host cell is a yeast cell that comprises ACS2 activity. In other embodiments, the host cell compared to a parent cell comprises no or reduced endogenous acetyl-CoA synthetase activity under non-respiratory conditions. In

some such embodiments, the host cell is a yeast cell that compared to a parent cell comprises no or reduced ACS2 activity.

[00106] In some embodiments, the host cell comprises a heterologous PK and a cytosolic acetyl-coA synthetase activity (*e.g,* ACS1 and/or ACS2). In such embodiments, PK produces acetyl phosphate in the host cell. The intact cytosolic ACS activity can convert acetate that accumulates as a result of RHR2 and/or HOR2-catalyzed acetyl phosphate hydrolysis into acetyl-CoA.

5.6 MEV Pathway for Isoprenoid Production

[00107] In some embodiments, the genetically modified host cell provided herein comprises one or more heterologous enzymes of the MEV pathway. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that converts HMG-CoA to mevalonate. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate. In some embodiments, the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

[00108] In some embodiments, the one or more enzymes of the MEV pathway are selected from the group consisting of acetyl-CoA thiolase, acetoacetyl-CoA synthetase, HMG-CoA synthase, HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and mevalonate pyrophosphate decarboxylase. In some embodiments, with regard to the enzyme of the MEV pathway capable of catalyzing the formation of acetoacetyl-CoA, the genetically modified host cell comprises either an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA, *e.g.,* acetyl-CoA thiolase; or an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA, *e.g.,* acetoacetyl-CoA synthase. In some embodiments, the genetically modified host cell comprises both an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA, *e.g.,* acetyl-CoA thiolase;

and an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA, *e.g.,* acetoacetyl-CoA synthase.

[00109] In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding more than one enzyme of the MEV pathway. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding two enzymes of the MEV pathway. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding an enzyme that can convert HMG-CoA into mevalonate and an enzyme that can convert mevalonate into mevalonate 5-phosphate. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding three enzymes of the MEV pathway. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding four enzymes of the MEV pathway. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding five enzymes of the MEV pathway. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding six enzymes of the MEV pathway. In some embodiments, the host cell comprises one or more heterologous nucleotide sequences encoding seven enzymes of the MEV pathway. In some embodiments, the host cell comprises a plurality of heterologous nucleic acids encoding all of the enzymes of the MEV pathway.

[00110] In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can convert isopentenyl pyrophosphate (IPP) into dimethylallyl pyrophosphate (DMAPP). In some embodiments, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can condense IPP and/or DMAPP molecules to form a polyprenyl compound. In some embodiments, the genetically modified host cell further comprise a heterologous nucleic acid encoding an enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound.

5.6.1 Conversion of Acetyl-CoA to Acetoacetyl-CoA

[00111] In some embodiments, the genetically modified host cell comprises a heterologous nucleotide sequence encoding an enzyme that can condense two molecules of acetyl-coenzyme A to form acetoacetyl-CoA, *e.g.,* an acetyl-CoA thiolase. Illustrative examples of nucleotide sequences encoding such an enzyme include, but are not limited to: (NC_000913 REGION: 2324131.2325315; *Escherichia coli*), (D49362; *Paracoccus denitrificans*), and (L20428; *Saccharomyces cerevisiae*).

[00112] Acetyl-CoA thiolase catalyzes the reversible condensation of two molecules of acetyl-CoA to yield acetoacetyl-CoA, but this reaction is thermodynamically unfavorable; acetoacetyl-CoA thiolysis is favored over acetoacetyl-CoA synthesis. Acetoacetyl-CoA synthase (AACS) (alternately referred to as acetyl-CoA:malonyl-CoA acyltransferase; EC 2.3.1.194) condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA. In contrast to acetyl-CoA thiolase, AACS-catalyzed acetoacetyl-CoA synthesis is essentially an energy-favored reaction, due to the associated decarboxylation of malonyl-CoA. In addition, AACS exhibits no thiolysis activity against acetoacetyl-CoA, and thus the reaction is irreversible.

[00113] In host cells comprising acetyl-CoA thiolase and a heterologous ADA and/or phosphotransacetylase (PTA), the reversible reaction catalyzed by acetyl-CoA thiolase, which favors acetoacetyl-CoA thiolysis, may result in a large acetyl-CoA pool. In view of the reversible activity of ADA, this acetyl-CoA pool may in turn drive ADA towards the reverse reaction of converting acetyl-CoA to acetaldehyde, thereby diminishing the benefits provided by ADA towards acetyl-CoA production. Similarly, the activity of PTA is reversible, and thus, a large acetyl-CoA pool may drive PTA towards the reverse reaction of converting acetyl-CoA to acetyl phosphate. Therefore, in some embodiments, in order to provide a strong pull on acetyl-CoA to drive the forward reaction of ADA and PTA, the MEV pathway of the genetically modified host cell provided herein utilizes an acetoacetyl-CoA synthase to form acetoacetyl-CoA from acetyl-CoA and malonyl-CoA.

[00114] In some embodiments, the AACS is from *Streptomyces* sp. strain CL190 (Okamura *et al.*, *Proc Natl Acad Sci* USA 107(25):11265-70 (2010). Representative AACS nucleotide sequences of *Streptomyces* sp. strain CL190 include accession number AB540131.1, and SEQ ID NO:19 as provided herein. Representative AACS protein sequences of *Streptomyces* sp. strain CL190 include accession numbers D7URV0, BAJ10048, and SEQ ID NO:20 as provided herein. Other acetoacetyl-CoA synthases useful for the compositions and methods provided herein include, but are not limited to, *Streptomyces sp.* (AB183750; KO-3988 BAD86806); *S. anulatus* strain 9663 (FN178498; CAX48662); *Streptomyces sp.* KO-3988 (AB212624; BAE78983); *Actinoplanes sp.* A40644 (AB113568; BAD07381); *Streptomyces sp.* C (NZ_ACEW010000640; ZP_05511702); *Nocardiopsis dassonvillei* DSM 43111 (NZ_ABUI01000023; ZP_04335288); *Mycobacterium ulcerans* Agy99 (NC_008611; YP_907152); *Mycobacterium marinum* M (NC_010612; YP_001851502); *Streptomyces sp.* Mg1 (NZ_DS570501; ZP_05002626); *Streptomyces sp.* AA4 (NZ_ACEV01000037; ZP_05478992); *S. roseosporus* NRRL 15998

(NZ_ABYB01000295; ZP_04696763); *Streptomyces sp.* ACTE (NZ_ADFD01000030; ZP_06275834); *S. viridochromogenes* DSM 40736 (NZ_ACEZ01000031; ZP_05529691); *Frankia sp.* CcI3 (NC_007777; YP_480101); *Nocardia brasiliensis* (NC_018681; YP_006812440.1); and *Austwickia chelonae* (NZ_BAGZ01000005; ZP_10950493.1). Additional suitable acetoacetyl-CoA synthases include those described in U.S. Patent Application Publication Nos. 2010/0285549 and 2011/0281315, the contents of which are incorporated by reference in their entireties.

[00115] Acetoacetyl-CoA synthases also useful in the compositions and methods provided herein include those molecules which are said to be "derivatives" of any of the acetoacetyl-CoA synthases described herein. Such a "derivative" has the following characteristics: (1) it shares substantial homology with any of the acetoacetyl-CoA synthases described herein; and (2) is capable of catalyzing the irreversible condensation of acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA. A derivative of an acetoacetyl-CoA synthase is said to share "substantial homology" with acetoacetyl-CoA synthase if the amino acid sequences of the derivative is at least 80%, and more preferably at least 90%, and most preferably at least 95%, the same as that of acetoacetyl-CoA synthase.

5.6.2 Conversion of Acetoacetyl-CoA to HMG-CoA

[00116] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can condense acetoacetyl-CoA with another molecule of acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), *e.g.,* a HMG-CoA synthase. Illustrative examples of nucleotide sequences encoding such an enzyme include, but are not limited to: (NC_001145. complement 19061.20536; *Saccharomyces cerevisiae*), (X96617; *Saccharomyces cerevisiae*), (X83882; *Arabidopsis thaliana*), (AB037907; *Kitasatospora griseola*), (BT007302; *Homo sapiens*), and (NC_002758, Locus tag SAV2546, GeneID 1122571; *Staphylococcus aureus*).

5.6.3 Conversion of HMG-CoA to Mevalonate

[00117] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can convert HMG-CoA into mevalonate, *e.g.,* a HMG-CoA reductase. In some embodiments, HMG-CoA reductase is an NADH-using hydroxymethylglutaryl-CoA reductase-CoA reductase. HMG-CoA reductases (EC 1.1.1.34; EC 1.1.1.88) catalyze the reductive deacylation of (S)-HMG-CoA to (R)-mevalonate, and can be categorized into two classes, class I and class II HMGrs. Class I includes the enzymes from eukaryotes and most archaea, and class II includes the HMG-CoA reductases of certain

prokaryotes and archaea. In addition to the divergence in the sequences, the enzymes of the two classes also differ with regard to their cofactor specificity. Unlike the class I enzymes, which utilize NADPH exclusively, the class II HMG-CoA reductases vary in the ability to discriminate between NADPH and NADH. *See, e.g.,* Hedl *et al., Journal of Bacteriology* 186 (7): 1927-1932 (2004). Co-factor specificities for select class II HMG-CoA reductases are provided below.

[00118] **Table 1. Co-factor specificities for select class II HMG-CoA reductases**

Source	Coenzyme specificity	K_m^{NADPH} (μM)	K_m^{NADH} (μM)
P. mevalonii	NADH		80
A. fulgidus	NAD(P)H	500	160
S. aureus	NAD(P)H	70	100
E. faecalis	NADPH	30	

[00119] Useful HMG-CoA reductases for the compositions and methods provided herein include HMG-CoA reductases that are capable of utilizing NADH as a cofactor, *e.g.,* HMG-CoA reductase from *P. mevalonii*, *A. fulgidus* or *S. aureus*. In particular embodiments, the HMG-CoA reductase is capable of only utilizing NADH as a cofactor, *e.g.,* HMG-CoA reductase from *P. mevalonii* , *S. pomeroyi* or *D. acidovorans*.

[00120] In some embodiments, the NADH-using HMG-CoA reductase is from *Pseudomonas mevalonii*. The sequence of the wild-type *mvaA* gene of *Pseudomonas mevalonii*, which encodes HMG-CoA reductase (EC 1.1.1.88), has been previously described. *See* Beach and Rodwell, *J. Bacteriol.* 171:2994-3001 (1989). Representative *mvaA* nucleotide sequences of *Pseudomonas mevalonii* include accession number M24015, and SEQ ID NO: 21 as provided herein. Representative HMG-CoA reductase protein sequences of *Pseudomonas mevalonii* include accession numbers AAA25837, P13702, MVAA_PSEMV, and SEQ ID NO: 22 as provided herein.

[00121] In some embodiments, the NADH-using HMG-CoA reductase is from *Silicibacter pomeroyi*. Representative *HMG-CoA reductase* nucleotide sequences of *Silicibacter pomeroyi* include accession number NC_006569.1, and SEQ ID NO: 23 as provided herein. Representative HMG-CoA reductase protein sequences of *Silicibacter pomeroyi* include accession number YP_164994, and SEQ ID NO: 24 as provided herein.

[00122] In some embodiments, the NADH-using HMG-CoA reductase is from *Delftia acidovorans*. A representative *HMG-CoA reductase* nucleotide sequences of *Delftia acidovorans* includes NC_010002 REGION: complement(319980..321269), and SEQ ID NO: 25 as provided herein. Representative HMG-CoA reductase protein sequences of *Delftia acidovorans* include accession number YP_001561318, and SEQ ID NO: 26 as provided herein.

[00123] In some embodiments, the NADH-using HMG-CoA reductases is from *Solanum tuberosum* (Crane *et al.*, *J. Plant Physiol.* 159:1301-1307 (2002)).

[00124] NADH-using HMG-CoA reductases also useful in the compositions and methods provided herein include those molecules which are said to be "derivatives" of any of the NADH-using HMG-CoA reductases described herein, *e.g.,* from *P. mevalonii*, *S. pomeroyi* and *D. acidovorans*. Such a "derivative" has the following characteristics: (1) it shares substantial homology with any of the NADH-using HMG-CoA reductases described herein; and (2) is capable of catalyzing the reductive deacylation of (S)-HMG-CoA to (R)-mevalonate while preferentially using NADH as a cofactor. A derivative of an NADH-using HMG-CoA reductase is said to share "substantial homology" with NADH-using HMG-CoA reductase if the amino acid sequences of the derivative is at least 80%, and more preferably at least 90%, and most preferably at least 95%, the same as that of NADH-using HMG-CoA reductase.

[00125] As used herein, the phrase "NADH-using" means that the NADH-using HMG-CoA reductase is selective for NADH over NADPH as a cofactor, for example, by demonstrating a higher specific activity for NADH than for NADPH. In some embodiments, selectivity for NADH as a cofactor is expressed as a $k_{cat}^{(NADH)}/ k_{cat}^{(NADPH)}$ ratio. In some embodiments, the NADH-using HMG-CoA reductase has a $k_{cat}^{(NADH)}/ k_{cat}^{(NADPH)}$ ratio of at least 5, 10, 15, 20, 25 or greater than 25. In some embodiments, the NADH-using HMG-CoA reductase uses NADH exclusively. For example, an NADH-using HMG-CoA reductase that uses NADH exclusively displays some activity with NADH supplied as the sole cofactor *in vitro,* and displays no detectable activity when NADPH is supplied as the sole cofactor. Any method for determining cofactor specificity known in the art can be utilized to identify HMG-CoA reductases having a preference for NADH as cofactor, including those described by Kim *et al., Protein Science* 9:1226-1234 (2000); and Wilding *et al., J. Bacteriol.* 182(18):5147-52 (2000), the contents of which are hereby incorporated in their entireties.

[00126] In some embodiments, the NADH-using HMG-CoA reductase is engineered to be selective for NADH over NAPDH, for example, through site-directed mutagenesis of the cofactor-binding pocket. Methods for engineering NADH-selectivity are described in Watanabe *et al., Microbiology* 153:3044-3054 (2007), and methods for determining the cofactor specificity of HMG-CoA reductases are described in Kim *et al., Protein Sci.* 9:1226-1234 (2000), the contents of which are hereby incorporated by reference in their entireties.

[00127] In some embodiments, the NADH-using HMG-CoA reductase is derived from a host species that natively comprises a mevalonate degradative pathway, for example, a host species that catabolizes mevalonate as its sole carbon source. Within these embodiments, the NADH-using HMG-CoA reductase, which normally catalyzes the oxidative acylation of internalized (R)-mevalonate to (S)-HMG-CoA within its native host cell, is utilized to catalyze the reverse reaction, that is, the reductive deacylation of (S)-HMG-CoA to (R)-mevalonate, in a genetically modified host cell comprising a mevalonate biosynthetic pathway. Prokaryotes capable of growth on mevalonate as their sole carbon source have been described by: Anderson *et al., J. Bacteriol,* 171(12):6468-6472 (1989); Beach *et al., J. Bacteriol.* 171:2994-3001 (1989); Bensch *et al., J. Biol. Chem.* 245:3755-3762; Fimongnari *et al., Biochemistry* 4:2086-2090 (1965); Siddiqi *et al., Biochem. Biophys. Res. Commun.* 8:110-113 (1962); Siddiqi *et al., J. Bacteriol.* 93:207-214 (1967); and Takatsuji *et al., Biochem. Biophys. Res. Commun.*110:187-193 (1983), the contents of which are hereby incorporated by reference in their entireties.

[00128] In some embodiments of the compositions and methods provided herein, the host cell comprises both a NADH-using HMGr and an NADPH-using HMG-CoA reductase. Illustrative examples of nucleotide sequences encoding an NADPH-using HMG-CoA reductase include, but are not limited to: (NM_206548; *Drosophila melanogaster*), (NC_002758, Locus tag SAV2545, GeneID 1122570; *Staphylococcus aureus*), (AB015627; *Streptomyces sp.* KO 3988), (AX128213, providing the sequence encoding a truncated HMG-CoA reductase; *Saccharomyces cerevisiae*), and (NC_001145: complement (115734.118898; *Saccharomyces cerevisiae*).

5.6.4 Conversion of Mevalonate to Mevalonate-5-Phosphate

[00129] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can convert mevalonate into mevalonate 5-phosphate, *e.g.,* a mevalonate kinase. Illustrative examples of nucleotide sequences encoding such an

enzyme include, but are not limited to: (L77688; *Arabidopsis thaliana*), and (X55875; *Saccharomyces cerevisiae*).

5.6.5 Conversion of Mevalonate-5-Phosphate to Mevalonate-5-Pyrophosphate

[00130] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can convert mevalonate 5-phosphate into mevalonate 5-pyrophosphate, *e.g.,* a phosphomevalonate kinase. Illustrative examples of nucleotide sequences encoding such an enzyme include, but are not limited to: (AF429385; *Hevea brasiliensis*), (NM_006556; *Homo sapiens*), and (NC_001145. complement 712315.713670; *Saccharomyces cerevisiae*).

5.6.6 Conversion of Mevalonate-5-Pyrophosphate to IPP

[00131] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can convert mevalonate 5-pyrophosphate into isopentenyl diphosphate (IPP), *e.g.,* a mevalonate pyrophosphate decarboxylase. Illustrative examples of nucleotide sequences encoding such an enzyme include, but are not limited to: (X97557; *Saccharomyces cerevisiae*), (AF290095; *Enterococcus faecium*), and (U49260; *Homo sapiens*).

5.6.7 Conversion of IPP to DMAPP

[00132] In some embodiments, the host cell further comprises a heterologous nucleotide sequence encoding an enzyme that can convert IPP generated via the MEV pathway into dimethylallyl pyrophosphate (DMAPP), *e.g.,* an IPP isomerase. Illustrative examples of nucleotide sequences encoding such an enzyme include, but are not limited to: (NC_000913, 3031087.3031635; *Escherichia coli*), and (AF082326; *Haematococcus pluvialis*).

5.6.8 Polyprenyl Synthases

[00133] In some embodiments, the host cell further comprises a heterologous nucleotide sequence encoding a polyprenyl synthase that can condense IPP and/or DMAPP molecules to form polyprenyl compounds containing more than five carbons.

[00134] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can condense one molecule of IPP with one molecule of DMAPP to form one molecule of geranyl pyrophosphate ("GPP"), *e.g.,* a GPP synthase. Illustrative examples of nucleotide sequences encoding such an enzyme include, but are not limited to: (AF513111; *Abies grandis*), (AF513112; *Abies grandis*), (AF513113; *Abies grandis*), (AY534686; *Antirrhinum majus*), (AY534687; *Antirrhinum majus*), (Y17376;

Arabidopsis thaliana), (AE016877, Locus AP11092; *Bacillus cereus*; ATCC 14579), (AJ243739; *Citrus sinensis*), (AY534745; *Clarkia breweri*), (AY953508; *Ips pini*), (DQ286930; *Lycopersicon esculentum*), (AF182828; *Mentha x piperita*), (AF182827; *Mentha x piperita*), (MPI249453; Mentha x piperita), (PZE431697, Locus CAD24425; *Paracoccus zeaxanthinifaciens*), (AY866498; *Picrorhiza kurrooa*), (AY351862; *Vitis vinifera*), and (AF203881, Locus AAF12843; *Zymomonas mobilis*).

[00135] In some embodiments, the host cell comprises a heterologous nucleotide sequence encoding an enzyme that can condense two molecules of IPP with one molecule of DMAPP, or add a molecule of IPP to a molecule of GPP, to form a molecule of farnesyl pyrophosphate ("FPP"), *e.g.,* a FPP synthase. Illustrative examples of nucleotide sequences that encode such an enzyme include, but are not limited to: (ATU80605; *Arabidopsis thaliana*), (ATHFPS2R; *Arabidopsis thaliana*), (AAU36376; *Artemisia annua*), (AF461050; *Bos taurus*), (D00694; *Escherichia coli* K-12), (AE009951, Locus AAL95523; *Fusobacterium nucleatum subsp. nucleatum* ATCC 25586), (GFFPPSGEN; *Gibberella fujikuroi*), (CP000009, Locus AAW60034; *Gluconobacter oxydans* 621H), (AF019892; *Helianthus annuus*), (HUMFAPS; *Homo sapiens*), (KLPFPSQCR; *Kluyveromyces lactis*), (LAU15777; *Lupinus albus*), (LAU20771; *Lupinus albus*), (AF309508; *Mus musculus*), (NCFPPSGEN; *Neurospora crassa*), (PAFPS1; *Parthenium argentatum*), (PAFPS2; *Parthenium argentatum*), (RATFAPS; *Rattus norvegicus*), (YSCFPP; *Saccharomyces cerevisiae*), (D89104; *Schizosaccharomyces pombe*), (CP000003, Locus AAT87386; *Streptococcus pyogenes*), (CP000017, Locus AAZ51849; *Streptococcus pyogenes*), (NC_008022, Locus YP_598856; *Streptococcus pyogenes* MGAS10270), (NC_008023, Locus YP_600845; *Streptococcus pyogenes* MGAS2096), (NC_008024, Locus YP_602832; *Streptococcus pyogenes* MGAS10750), (MZEFPS; *Zea mays*), (AE000657, Locus AAC06913; *Aquifex aeolicus* VF5), (NM_202836; *Arabidopsis thaliana*), (D84432, Locus BAA12575; *Bacillus subtilis*), (U12678, Locus AAC28894; *Bradyrhizobium japonicum* USDA 110), (BACFDPS; *Geobacillus stearothermophilus*), (NC_002940, Locus NP_873754; *Haemophilus ducreyi* 35000HP), (L42023, Locus AAC23087; *Haemophilus influenzae* Rd KW20), (J05262; *Homo sapiens*), (YP_395294; *Lactobacillus sakei subsp. sakei* 23K), (NC_005823, Locus YP_000273; *Leptospira interrogans serovar Copenhageni str. Fiocruz* L1-130), (AB003187; *Micrococcus luteus*), (NC_002946, Locus YP_208768; *Neisseria gonorrhoeae* FA 1090), (U00090, Locus AAB91752; *Rhizobium sp.* NGR234), (J05091; *Saccharomyces cerevisae*), (CP000031, Locus AAV93568; *Silicibacter pomeroyi*

DSS-3), (AE008481, Locus AAK99890; *Streptococcus pneumoniae* R6), and (NC_004556, Locus NP 779706; *Xylella fastidiosa* Temecula1).

[00136] In some embodiments, the host cell further comprises a heterologous nucleotide sequence encoding an enzyme that can combine IPP and DMAPP or IPP and FPP to form geranylgeranyl pyrophosphate ("GGPP"). Illustrative examples of nucleotide sequences that encode such an enzyme include, but are not limited to: (ATHGERPYRS; *Arabidopsis thaliana*), (BT005328; *Arabidopsis thaliana)*, (NM_119845; *Arabidopsis thaliana*), (NZ_AAJM01000380, Locus ZP_00743052; *Bacillus thuringiensis serovar israelensis,* ATCC 35646 sq1563), (CRGGPPS; *Catharanthus roseus*), (NZ_AABF02000074, Locus ZP_00144509; *Fusobacterium nucleatum subsp. vincentii,* ATCC 49256), (GFGGPPSGN; *Gibberella fujikuroi*), (AY371321; *Ginkgo biloba*), (AB055496; *Hevea brasiliensis*), (AB017971; *Homo sapiens*), (MCI276129; *Mucor circinelloides f. lusitanicus*), (AB016044; *Mus musculus*), (AABX01000298, Locus NCU01427; *Neurospora crassa*), (NCU20940; *Neurospora crassa*), (NZ_AAKL01000008, Locus ZP_00943566; *Ralstonia solanacearum* UW551), (AB118238; *Rattus norvegicus*), (SCU31632; *Saccharomyces cerevisiae*), (AB016095; Synechococcus elongates), (SAGGPS; *Sinapis alba*), (SSOGDS; *Sulfolobus acidocaldarius*), (NC_007759, Locus YP_461832; *Syntrophus aciditrophicus* SB), (NC_006840, Locus YP_204095; *Vibrio fischeri* ES114), (NM_112315; *Arabidopsis thaliana*), (ERWCRTE; *Pantoea agglomerans*), (D90087, Locus BAA14124; *Pantoea ananatis*), (X52291, Locus CAA36538; *Rhodobacter capsulatus*), (AF195122, Locus AAF24294; *Rhodobacter sphaeroides*), and (NC_004350, Locus NP_721015; *Streptococcus mutans* UA159).

5.6.9 Terpene Synthases

[00137] In some embodiments, the host cell further comprises a heterologous nucleotide sequence encoding an enzyme that can modify a polyprenyl to form a hemiterpene, a monoterpene, a sesquiterpene, a diterpene, a triterpene, a tetraterpene, a polyterpene, a steroid compound, a carotenoid, or a modified isoprenoid compound.

[00138] In some embodiments, the heterologous nucleotide encodes a carene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (AF461460, REGION 43.1926; *Picea abies*) and (AF527416, REGION: 78.1871; *Salvia stenophylla*).

[00139] In some embodiments, the heterologous nucleotide encodes a geraniol synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited

to: (AJ457070; *Cinnamomum tenuipilum*), (AY362553; *Ocimum basilicum*), (DQ234300; *Perilla frutescens* strain 1864), (DQ234299; *Perilla citriodora* strain 1861), (DQ234298; *Perilla citriodora* strain 4935), and (DQ088667; *Perilla citriodora*).

[00140] In some embodiments, the heterologous nucleotide encodes a linalool synthase. Illustrative examples of a suitable nucleotide sequence include, but are not limited to: (AF497485; *Arabidopsis thaliana*), (AC002294, Locus AAB71482; *Arabidopsis thaliana*), (AY059757; *Arabidopsis thaliana*), (NM_104793; *Arabidopsis thaliana*), (AF154124; *Artemisia annua*), (AF067603; *Clarkia breweri*), (AF067602; *Clarkia concinna*), (AF067601; *Clarkia breweri*), (U58314; *Clarkia breweri*), (AY840091; *Lycopersicon esculentum*), (DQ263741; *Lavandula angustifolia*), (AY083653; *Mentha citrate*), (AY693647; *Ocimum basilicum*), (XM_463918; *Oryza sativa*), (AP004078, Locus BAD07605; *Oryza sativa*), (XM_463918, Locus XP_463918; *Oryza sativa*), (AY917193; *Perilla citriodora*), (AF271259; *Perilla frutescens*), (AY473623; *Picea abies*), (DQ195274; *Picea sitchensis*), and (AF444798; *Perilla frutescens* var. crispa cultivar No. 79).

[00141] In some embodiments, the heterologous nucleotide encodes a limonene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (+)-limonene synthases (AF514287, REGION: 47.1867; *Citrus limon*) and (AY055214, REGION: 48.1889; *Agastache rugosa*) and (-)-limonene synthases (DQ195275, REGION: 1.1905; *Picea sitchensis*), (AF006193, REGION: 73.1986; *Abies grandis*), and (MHC4SLSP, REGION: 29.1828; *Mentha spicata*).

[00142] In some embodiments, the heterologous nucleotide encodes a myrcene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (U87908; *Abies grandis*), (AY195609; *Antirrhinum majus*), (AY195608; *Antirrhinum majus*), (NM_127982; *Arabidopsis thaliana* TPS10), (NM_113485; *Arabidopsis thaliana* ATTPS-CIN), (NM_113483; *Arabidopsis thaliana* ATTPS-CIN), (AF271259; *Perilla frutescens*), (AY473626; *Picea abies*), (AF369919; *Picea abies*), and (AJ304839; *Quercus ilex*).

[00143] In some embodiments, the heterologous nucleotide encodes a ocimene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (AY195607; *Antirrhinum majus*), (AY195609; *Antirrhinum majus*), (AY195608; *Antirrhinum majus*), (AK221024; *Arabidopsis thaliana*), (NM_113485; *Arabidopsis thaliana* ATTPS-CIN), (NM_113483; *Arabidopsis thaliana* ATTPS-CIN), (NM_117775; *Arabidopsis thaliana* ATTPS03), (NM_001036574; *Arabidopsis thaliana* ATTPS03), (NM_127982;

Arabidopsis thaliana TPS10), (AB110642; *Citrus unshiu* CitMTSL4), and (AY575970; *Lotus corniculatus var. japonicus*).

[00144] In some embodiments, the heterologous nucleotide encodes an α-pinene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (+) α-pinene synthase (AF543530, REGION: 1.1887; *Pinus taeda*), (-)α-pinene synthase (AF543527, REGION: 32.1921; *Pinus taeda*), and (+)/(-)α-pinene synthase (AGU87909, REGION: 6111892; *Abies grandis*).

[00145] In some embodiments, the heterologous nucleotide encodes a β-pinene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (-) β-pinene synthases (AF276072, REGION: 1.1749; *Artemisia annua*) and (AF514288, REGION: 26.1834; *Citrus limon*).

[00146] In some embodiments, the heterologous nucleotide encodes a sabinene synthase. An illustrative example of a suitable nucleotide sequence includes but is not limited to AF051901, REGION: 26.1798 from *Salvia officinalis*.

[00147] In some embodiments, the heterologous nucleotide encodes a γ-terpinene synthase. Illustrative examples of suitable nucleotide sequences include: (AF514286, REGION: 30.1832 from *Citrus limon*) and (AB110640, REGION 1.1803 from *Citrus unshiu*).

[00148] In some embodiments, the heterologous nucleotide encodes a terpinolene synthase. Illustrative examples of a suitable nucleotide sequence include, but are not limited to: (AY693650 from *Oscimum basilicum*) and (AY906866, REGION: 10.1887 from *Pseudotsuga menziesii*).

[00149] In some embodiments, the heterologous nucleotide encodes an amorphadiene synthase. An illustrative example of a suitable nucleotide sequence is SEQ ID NO. 37 of U.S. Patent Publication No. 2004/0005678.

[00150] In some embodiments, the heterologous nucleotide encodes a α-farnesene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to DQ309034 from *Pyrus communis cultivar d'Anjou* (pear; gene name AFS1) and AY182241 from *Malus domestica* (apple; gene AFS1). Pechouus *et al.*, *Planta* 219(1):84-94 (2004).

[00151] In some embodiments, the heterologous nucleotide encodes a β-farnesene synthase. Illustrative examples of suitable nucleotide sequences include but is not limited to

accession number AF024615 from *Mentha x piperita* (peppermint; gene Tspa11), and AY835398 from *Artemisia annua*. Picaud *et al.*, *Phytochemistry* 66(9): 961-967 (2005).

[00152] In some embodiments, the heterologous nucleotide encodes a farnesol synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to accession number AF529266 from *Zea mays* and YDR481C from *Saccharomyces cerevisiae* (gene Pho8). Song, L., *Applied Biochemistry and Biotechnology* 128:149-158 (2006).

[00153] In some embodiments, the heterologous nucleotide encodes a nerolidol synthase. An illustrative example of a suitable nucleotide sequence includes, but is not limited to AF529266 from *Zea mays* (maize; gene tps1).

[00154] In some embodiments, the heterologous nucleotide encodes a patchouliol synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to AY508730 REGION: 1.1659 from *Pogostemon cablin*.

[00155] In some embodiments, the heterologous nucleotide encodes a nootkatone synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to AF441124 REGION: 1.1647 from *Citrus sinensis* and AY917195 REGION: 1.1653 from *Perilla frutescens*.

[00156] In some embodiments, the heterologous nucleotide encodes an abietadiene synthase. Illustrative examples of suitable nucleotide sequences include, but are not limited to: (U50768; *Abies grandis*) and (AY473621; *Picea abies*).

[00157] In some embodiments, the host cell produces a C_5 isoprenoid. These compounds are derived from one isoprene unit and are also called hemiterpenes. An illustrative example of a hemiterpene is isoprene. In other embodiments, the isoprenoid is a C_{10} isoprenoid. These compounds are derived from two isoprene units and are also called monoterpenes. Illustrative examples of monoterpenes are limonene, citranellol, geraniol, menthol, perillyl alcohol, linalool, thujone, and myrcene. In other embodiments, the isoprenoid is a C_{15} isoprenoid. These compounds are derived from three isoprene units and are also called sesquiterpenes. Illustrative examples of sesquiterpenes are periplanone B, gingkolide B, amorphadiene, artemisinin, artemisinic acid, valencene, nootkatone, epi-cedrol, epi-aristolochene, farnesol, gossypol, sanonin, periplanone, forskolin, and patchoulol (which is also known as patchouli alcohol). In other embodiments, the isoprenoid is a C_{20} isoprenoid. These compounds are derived from four isoprene units and also called diterpenes. Illustrative examples of diterpenes are casbene, eleutherobin, paclitaxel,

prostratin, pseudopterosin, and taxadiene. In yet other examples, the isoprenoid is a C_{20+} isoprenoid. These compounds are derived from more than four isoprene units and include: triterpenes (C_{30} isoprenoid compounds derived from 6 isoprene units) such as arbrusideE, bruceantin, testosterone, progesterone, cortisone, digitoxin, and squalene; tetraterpenes (C_{40} isoprenoid compounds derived from 8 isoprenoids) such as β-carotene; and polyterpenes (C_{40+} isoprenoid compounds derived from more than 8 isoprene units) such as polyisoprene. In some embodiments, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, isoprene, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpinolene and valencene. Isoprenoid compounds also include, but are not limited to, carotenoids (such as lycopene, α- and β-carotene, α- and β-cryptoxanthin, bixin, zeaxanthin, astaxanthin, and lutein), steroid compounds, and compounds that are composed of isoprenoids modified by other chemical groups, such as mixed terpene-alkaloids, and coenzyme Q-10.

5.6.10 Methods of Producing Isoprenoids

[00158] In another aspect, provided herein is a method for the production of an isoprenoid, the method comprising the steps of: (a) culturing a population of any of the genetically modified host cells described herein that are capable of producing an isoprenoid in a medium with a carbon source under conditions suitable for making an isoprenoid compound; and (b) recovering said isoprenoid compound from the medium.

[00159] In some embodiments, the genetically modified host cell comprises one or more modifications selected from the group consisting of: heterologous expression of a phosphoketolase, heterologous expression of a phosphotransacetylase, heterologous expression of one or more enzymes of the mevalonate pathway; and optionally, heterologous expression of an ADA, heterologous expression of an NADH-using HMG-CoA reductase, and heterologous expression of an AACS; and the genetically modified host cell produces an increased amount of the isoprenoid compound compared to a parent cell not comprising the one or more modifications, or a parent cell comprising only a subset of the one or more modifications of the genetically modified host cell, but is otherwise genetically identical. In some embodiments, the increased amount is at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or greater than 100%, as measured, for example, in yield, production, productivity, in grams per liter of cell culture, milligrams per gram of dry cell weight, on a per unit volume of cell culture basis,

on a per unit dry cell weight basis, on a per unit volume of cell culture per unit time basis, or on a per unit dry cell weight per unit time basis.

[00160] In some embodiments, the host cell produces an elevated level of isoprenoid that is greater than about 10 grams per liter of fermentation medium. In some such embodiments, the isoprenoid is produced in an amount from about 10 to about 50 grams, more than about 15 grams, more than about 20 grams, more than about 25 grams, or more than about 30 grams per liter of cell culture.

[00161] In some embodiments, the host cell produces an elevated level of isoprenoid that is greater than about 50 milligrams per gram of dry cell weight. In some such embodiments, the isoprenoid is produced in an amount from about 50 to about 1500 milligrams, more than about 100 milligrams, more than about 150 milligrams, more than about 200 milligrams, more than about 250 milligrams, more than about 500 milligrams, more than about 750 milligrams, or more than about 1000 milligrams per gram of dry cell weight.

[00162] In some embodiments, the host cell produces an elevated level of isoprenoid that is at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 2. 5-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, or at least about 1,000-fold, or more, higher than the level of isoprenoid produced by a parent cell, on a per unit volume of cell culture basis.

[00163] In some embodiments, the host cell produces an elevated level of isoprenoid that is at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 2. 5-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, or at least about 1,000-fold, or more, higher than the level of isoprenoid produced by the parent cell, on a per unit dry cell weight basis.

[00164] In some embodiments, the host cell produces an elevated level of an isoprenoid that is at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 2. 5-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, or at least about 1,000-fold, or more, higher than the level of isoprenoid produced by the parent cell, on a per unit volume of cell culture per unit time basis.

[00165] In some embodiments, the host cell produces an elevated isoprenoid that is at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 2-fold, at least about 2. 5-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, or at least about 1,000-fold, or more, higher than the level of isoprenoid produced by the parent cell, on a per unit dry cell weight per unit time basis.

[00166] In most embodiments, the production of the elevated level of isoprenoid by the host cell is inducible by an inducing compound. Such a host cell can be manipulated with ease in the absence of the inducing compound. The inducing compound is then added to induce the production of the elevated level of isoprenoid by the host cell. In other embodiments, production of the elevated level of isoprenoid by the host cell is inducible by changing culture conditions, such as, for example, the growth temperature, media constituents, and the like.

5.6.11 Culture Media and Conditions

[00167] Materials and methods for the maintenance and growth of microbial cultures are well known to those skilled in the art of microbiology or fermentation science (*see*, for example, Bailey *et al*., Biochemical Engineering Fundamentals, second edition, McGraw Hill, New York, 1986). Consideration must be given to appropriate culture medium, pH, temperature, and requirements for aerobic, microaerobic, or anaerobic conditions, depending on the specific requirements of the host cell, the fermentation, and the process.

[00168] The methods of producing isoprenoids provided herein may be performed in a suitable culture medium (*e.g.,* with or without pantothenate supplementation) in a suitable container, including but not limited to a cell culture plate, a flask, or a fermentor. Further, the methods can be performed at any scale of fermentation known in the art to support industrial production of microbial products. Any suitable fermentor may be used including a stirred tank fermentor, an airlift fermentor, a bubble fermentor, or any combination thereof. In particular embodiments utilizing *Saccharomyces cerevisiae* as the host cell, strains can be grown in a fermentor as described in detail by Kosaric, *et al*, in Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, Volume 12, pages 398-473, Wiley-VCH Verlag GmbH & Co. KDaA, Weinheim, Germany.

[00169] In some embodiments, the culture medium is any culture medium in which a genetically modified microorganism capable of producing an isoprenoid can subsist, *i.e.*, maintain growth and viability. In some embodiments, the culture medium is an aqueous medium comprising assimilable carbon, nitrogen and phosphate sources. Such a medium can also include appropriate salts, minerals, metals and other nutrients. In some embodiments, the carbon source and each of the essential cell nutrients, are added incrementally or continuously to the fermentation media, and each required nutrient is maintained at essentially the minimum level needed for efficient assimilation by growing cells, for example, in accordance with a predetermined cell growth curve based on the metabolic or respiratory function of the cells which convert the carbon source to a biomass.

[00170] Suitable conditions and suitable media for culturing microorganisms are well known in the art. In some embodiments, the suitable medium is supplemented with one or more additional agents, such as, for example, an inducer (*e.g.*, when one or more nucleotide sequences encoding a gene product are under the control of an inducible promoter), a repressor (*e.g.*, when one or more nucleotide sequences encoding a gene product are under the control of a repressible promoter), or a selection agent (*e.g.*, an antibiotic to select for microorganisms comprising the genetic modifications).

[00171] In some embodiments, the carbon source is a monosaccharide (simple sugar), a disaccharide, a polysaccharide, a non-fermentable carbon source, or one or more combinations thereof. Non-limiting examples of suitable monosaccharides include glucose, galactose, mannose, fructose, xylose, ribose, and combinations thereof. Non-limiting examples of suitable disaccharides include sucrose, lactose, maltose, trehalose, cellobiose, and combinations thereof. Non-limiting examples of suitable polysaccharides include starch,

glycogen, cellulose, chitin, and combinations thereof. Non-limiting examples of suitable non-fermentable carbon sources include acetate and glycerol.

[00172] The concentration of a carbon source, such as glucose, in the culture medium should promote cell growth, but not be so high as to repress growth of the microorganism used. Typically, cultures are run with a carbon source, such as glucose, being added at levels to achieve the desired level of growth and biomass, but at undetectable levels (with detection limits being about <0.1g/l). In other embodiments, the concentration of a carbon source, such as glucose, in the culture medium is greater than about 1 g/L, preferably greater than about 2 g/L, and more preferably greater than about 5 g/L. In addition, the concentration of a carbon source, such as glucose, in the culture medium is typically less than about 100 g/L, preferably less than about 50 g/L, and more preferably less than about 20 g/L. It should be noted that references to culture component concentrations can refer to both initial and/or ongoing component concentrations. In some cases, it may be desirable to allow the culture medium to become depleted of a carbon source during culture.

[00173] Sources of assimilable nitrogen that can be used in a suitable culture medium include, but are not limited to, simple nitrogen sources, organic nitrogen sources and complex nitrogen sources. Such nitrogen sources include anhydrous ammonia, ammonium salts and substances of animal, vegetable and/or microbial origin. Suitable nitrogen sources include, but are not limited to, protein hydrolysates, microbial biomass hydrolysates, peptone, yeast extract, ammonium sulfate, urea, and amino acids. Typically, the concentration of the nitrogen sources, in the culture medium is greater than about 0.1 g/L, preferably greater than about 0.25 g/L, and more preferably greater than about 1.0 g/L. Beyond certain concentrations, however, the addition of a nitrogen source to the culture medium is not advantageous for the growth of the microorganisms. As a result, the concentration of the nitrogen sources, in the culture medium is less than about 20 g/L, preferably less than about 10 g/L and more preferably less than about 5 g/L. Further, in some instances it may be desirable to allow the culture medium to become depleted of the nitrogen sources during culture.

[00174] The effective culture medium can contain other compounds such as inorganic salts, vitamins, trace metals or growth promoters. Such other compounds can also be present in carbon, nitrogen or mineral sources in the effective medium or can be added specifically to the medium.

[00175] The culture medium can also contain a suitable phosphate source. Such phosphate sources include both inorganic and organic phosphate sources. Preferred phosphate sources include, but are not limited to, phosphate salts such as mono or dibasic sodium and potassium phosphates, ammonium phosphate and mixtures thereof. Typically, the concentration of phosphate in the culture medium is greater than about 1.0 g/L, preferably greater than about 2.0 g/L and more preferably greater than about 5.0 g/L. Beyond certain concentrations, however, the addition of phosphate to the culture medium is not advantageous for the growth of the microorganisms. Accordingly, the concentration of phosphate in the culture medium is typically less than about 20 g/L, preferably less than about 15 g/L and more preferably less than about 10 g/L.

[00176] A suitable culture medium can also include a source of magnesium, preferably in the form of a physiologically acceptable salt, such as magnesium sulfate heptahydrate, although other magnesium sources in concentrations that contribute similar amounts of magnesium can be used. Typically, the concentration of magnesium in the culture medium is greater than about 0.5 g/L, preferably greater than about 1.0 g/L, and more preferably greater than about 2.0 g/L. Beyond certain concentrations, however, the addition of magnesium to the culture medium is not advantageous for the growth of the microorganisms. Accordingly, the concentration of magnesium in the culture medium is typically less than about 10 g/L, preferably less than about 5 g/L, and more preferably less than about 3 g/L. Further, in some instances it may be desirable to allow the culture medium to become depleted of a magnesium source during culture.

[00177] In some embodiments, the culture medium can also include a biologically acceptable chelating agent, such as the dihydrate of trisodium citrate. In such instance, the concentration of a chelating agent in the culture medium is greater than about 0.2 g/L, preferably greater than about 0.5 g/L, and more preferably greater than about 1 g/L. Beyond certain concentrations, however, the addition of a chelating agent to the culture medium is not advantageous for the growth of the microorganisms. Accordingly, the concentration of a chelating agent in the culture medium is typically less than about 10 g/L, preferably less than about 5 g/L, and more preferably less than about 2 g/L.

[00178] The culture medium can also initially include a biologically acceptable acid or base to maintain the desired pH of the culture medium. Biologically acceptable acids include, but are not limited to, hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid and mixtures thereof. Biologically acceptable bases include, but are not limited to,

ammonium hydroxide, sodium hydroxide, potassium hydroxide and mixtures thereof. In some embodiments, the base used is ammonium hydroxide.

[00179] The culture medium can also include a biologically acceptable calcium source, including, but not limited to, calcium chloride. Typically, the concentration of the calcium source, such as calcium chloride, dihydrate, in the culture medium is within the range of from about 5 mg/L to about 2000 mg/L, preferably within the range of from about 20 mg/L to about 1000 mg/L, and more preferably in the range of from about 50 mg/L to about 500 mg/L.

[00180] The culture medium can also include sodium chloride. Typically, the concentration of sodium chloride in the culture medium is within the range of from about 0.1 g/L to about 5 g/L, preferably within the range of from about 1 g/L to about 4 g/L, and more preferably in the range of from about 2 g/L to about 4 g/L.

[00181] In some embodiments, the culture medium can also include trace metals. Such trace metals can be added to the culture medium as a stock solution that, for convenience, can be prepared separately from the rest of the culture medium. Typically, the amount of such a trace metals solution added to the culture medium is greater than about 1 ml/L, preferably greater than about 5 mL/L, and more preferably greater than about 10 mL/L. Beyond certain concentrations, however, the addition of a trace metals to the culture medium is not advantageous for the growth of the microorganisms. Accordingly, the amount of such a trace metals solution added to the culture medium is typically less than about 100 mL/L, preferably less than about 50 mL/L, and more preferably less than about 30 mL/L. It should be noted that, in addition to adding trace metals in a stock solution, the individual components can be added separately, each within ranges corresponding independently to the amounts of the components dictated by the above ranges of the trace metals solution.

[00182] The culture media can include other vitamins, such as pantothenate, biotin, calcium, pantothenate, inositol, pyridoxine-HCl, and thiamine-HCl. Such vitamins can be added to the culture medium as a stock solution that, for convenience, can be prepared separately from the rest of the culture medium. Beyond certain concentrations, however, the addition of vitamins to the culture medium is not advantageous for the growth of the microorganisms.

[00183] The fermentation methods described herein can be performed in conventional culture modes, which include, but are not limited to, batch, fed-batch, cell recycle, continuous and semi-continuous. In some embodiments, the fermentation is carried out in fed-batch

mode. In such a case, some of the components of the medium are depleted during culture, including pantothenate during the production stage of the fermentation. In some embodiments, the culture may be supplemented with relatively high concentrations of such components at the outset, for example, of the production stage, so that growth and/or isoprenoid production is supported for a period of time before additions are required. The preferred ranges of these components are maintained throughout the culture by making additions as levels are depleted by culture. Levels of components in the culture medium can be monitored by, for example, sampling the culture medium periodically and assaying for concentrations. Alternatively, once a standard culture procedure is developed, additions can be made at timed intervals corresponding to known levels at particular times throughout the culture. As will be recognized by those in the art, the rate of consumption of nutrient increases during culture as the cell density of the medium increases. Moreover, to avoid introduction of foreign microorganisms into the culture medium, addition is performed using aseptic addition methods, as are known in the art. In addition, a small amount of anti-foaming agent may be added during the culture.

[00184] The temperature of the culture medium can be any temperature suitable for growth of the genetically modified cells and/or production of isoprenoids. For example, prior to inoculation of the culture medium with an inoculum, the culture medium can be brought to and maintained at a temperature in the range of from about 20°C to about 45°C, preferably to a temperature in the range of from about 25°C to about 40°C, and more preferably in the range of from about 28°C to about 32°C.

[00185] The pH of the culture medium can be controlled by the addition of acid or base to the culture medium. In such cases when ammonia is used to control pH, it also conveniently serves as a nitrogen source in the culture medium. Preferably, the pH is maintained from about 3.0 to about 8.0, more preferably from about 3.5 to about 7.0, and most preferably from about 4.0 to about 6.5.

[00186] In some embodiments, the carbon source concentration, such as the glucose concentration, of the culture medium is monitored during culture. Glucose concentration of the culture medium can be monitored using known techniques, such as, for example, use of the glucose oxidase enzyme test or high pressure liquid chromatography, which can be used to monitor glucose concentration in the supernatant, *e.g.*, a cell-free component of the culture medium. As stated previously, the carbon source concentration should be kept below the level at which cell growth inhibition occurs. Although such concentration may vary from

organism to organism, for glucose as a carbon source, cell growth inhibition occurs at glucose concentrations greater than at about 60 g/L, and can be determined readily by trial. Accordingly, when glucose is used as a carbon source the glucose is preferably fed to the fermentor and maintained below detection limits. Alternatively, the glucose concentration in the culture medium is maintained in the range of from about 1 g/L to about 100 g/L, more preferably in the range of from about 2 g/L to about 50 g/L, and yet more preferably in the range of from about 5 g/L to about 20 g/L. Although the carbon source concentration can be maintained within desired levels by addition of, for example, a substantially pure glucose solution, it is acceptable, and may be preferred, to maintain the carbon source concentration of the culture medium by addition of aliquots of the original culture medium. The use of aliquots of the original culture medium may be desirable because the concentrations of other nutrients in the medium (*e.g.* the nitrogen and phosphate sources) can be maintained simultaneously. Likewise, the trace metals concentrations can be maintained in the culture medium by addition of aliquots of the trace metals solution.

5.6.12 Recovery of Isoprenoids

[00187] Once the isoprenoid is produced by the host cell, it may be recovered or isolated for subsequent use using any suitable separation and purification methods known in the art. In some embodiments, an organic phase comprising the isoprenoid is separated from the fermentation by centrifugation. In other embodiments, an organic phase comprising the isoprenoid separates from the fermentation spontaneously. In other embodiments, an organic phase comprising the isoprenoid is separated from the fermentation by adding a demulsifier and/or a nucleating agent into the fermentation reaction. Illustrative examples of demulsifiers include flocculants and coagulants. Illustrative examples of nucleating agents include droplets of the isoprenoid itself and organic solvents such as dodecane, isopropyl myristrate, and methyl oleate.

[00188] The isoprenoid produced in these cells may be present in the culture supernatant and/or associated with the host cells. In embodiments where the isoprenoid is associated with the host cell, the recovery of the isoprenoid may comprise a method of permeabilizing or lysing the cells. Alternatively or simultaneously, the isoprenoid in the culture medium can be recovered using a recovery process including, but not limited to, chromatography, extraction, solvent extraction, membrane separation, electrodialysis, reverse osmosis, distillation, chemical derivatization and crystallization.

[00189] In some embodiments, the isoprenoid is separated from other products that may be present in the organic phase. In some embodiments, separation is achieved using adsorption, distillation, gas-liquid extraction (stripping), liquid-liquid extraction (solvent extraction), ultrafiltration, and standard chromatographic techniques.

5.7 Polyketides

[00190] In some embodiments, the genetically modified host cell provided herein is capable of producing a polyketide from acetyl-CoA. Polyketides are synthesized by sequential reactions catalyzed by a collection of enzyme activities called polyketide synthases (PKSs), which are large multi-enzyme protein complexes that contain a coordinated group of active sites. Polyketide biosynthesis proceeds stepwise starting from simple 2-, 3-, 4-carbon building blocks such as acetyl-CoA, propionyl CoA, butyryl-CoA and their activated derivatives, malonyl-, methylmalonyl- and ethylmalonyl-CoA, primarily through decarboxylative condensation of malonyl-CoA-derived units via Claisen condensation reactions. The PKS genes are usually organized in one operon in bacteria and in gene clusters in eukaryotes. Three types of polyketide synthases have been characterized: Type I polyketide synthases are large, highly modular proteins subdivided into two classes: 1) iterative PKSs, which reuse domains in a cyclic fashion and 2) modular PKSs, which contain a sequence of separate modules and do not repeat domains. Type II polyketide synthases are aggregates of monofunctional proteins, and Type III polyketide synthases do not use acyl carrier protein domains.

[00191] Unlike fatty acid biosynthesis, in which each successive chain elongation step is followed by a fixed sequence of ketoreduction, dehydration and enoyl, reduction as described below, the individual chain elongation intermediates of polyketide biosynthesis undergo all, some, or no functional group modifications, resulting in a large number of chemically diverse products. Additional degrees of complexity arise from the use of different starter units and chain elongation units as well as the generation of new stereo-isomers.

[00192] The order of complete polyketide-synthesis as directed by a polyketide synthase follows (in the order N-terminus to C-terminus): starting or loading the initial carbon building blocks onto an acyl carrier protein, elongation modules which catalyze the extension of the growing macrolide chain and termination modules that catalyze the release of the synthesized macrolide. Component domains or separate enzyme functionalities active in this biosynthesis include acyl-transferases for the loading of starter, extender and intermediate acyl units; acyl carrier proteins which hold the growing macrolide as a thiol

ester; β-keto-acyl synthases which catalyze chain extension; β-keto reductases responsible for the first reduction to an alcohol functionality; dehydratases which eliminate water to give an unsaturated thiolester; enoyl reductases which catalyze the final reduction to full saturation; and thiolesterases which catalyze macrolide release and cyclization.

[00193] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can condense at least one of acetyl-CoA and malonyl-CoA with an acyl carrier protein, *e.g.* an acyl-transferase.

[00194] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can condense a first reactant selected from the group consisting of acetyl-CoA and malonyl-CoA with a second reactant selected from the group consisting of malonyl-CoA or methylmalonyl-CoA to form a polyketide product, *e.g.* a β-keto-acyl synthase.

[00195] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can reduce a β-keto chemical group on a polyketide compound to a β-hydroxy group, *e.g.* a β-keto reductase.

[00196] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can dehydrate an alkane chemical group in a polyketide compound to produce an α-β-unsaturated alkene, *e.g.* a dehydratase.

[00197] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can reduce an α-β-double-bond in a polyketide compound to a saturated alkane, *e.g.* an enoyl-reductase.

[00198] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can hydrolyze a polyketide compound from an acyl carrier protein, *e.g.* a thioesterase.

[00199] In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a KS catalytic region. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising an AT catalytic region. In some embodiments, the polyketide producing cell comprises more than one heterologous nucleotide sequence encoding an enzyme comprising an AT catalytic region. In some

embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a CLF catalytic region. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising an ACP activity. In some embodiments, the polyketide producing cell comprises more than one heterologous nucleotide sequence encoding an enzyme comprising an ACP activity.

[00200] In a particular embodiment, the polyketide producing cell comprises a minimal aromatic PKS system, *e.g.,* heterologous nucleotide sequences encoding an enzyme comprising a KS catalytic region, an enzyme comprising an AT catalytic region, an enzyme comprising a CLF catalytic region, and an enzyme comprising an ACP activity, respectively. In a particular embodiment, the polyketide producing cell comprises a minimal modular PKS system, *e.g.,* heterologous nucleotide sequences encoding an enzyme comprising a KS catalytic region, an enzyme comprising an AT catalytic region, and an enzyme comprising an ACP activity, respectively. In yet another particular embodiment, the polyketide producing cell comprises a modular aromatic PKS system for *de novo* polyketide synthesis, *e.g.,* heterologous nucleotide sequences encoding an enzyme comprising a KS catalytic region, one or more enzymes comprising an AT catalytic region, and one or more enzymes comprising an ACP activity, respectively.

[00201] In some embodiments, the polyketide producing cell comprising a minimal PKS system, *e.g.,* a minimal aromatic PKS system or minimal modular PKS system, further comprises additional catalytic activities which can contribute to production of the end-product polyketide. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a cyclase (CYC) catalytic region, which facilitates the cyclization of the nascent polyketide backbone. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a ketoreductase (KR) catalytic region. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising an aromatase (ARO) catalytic region. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising an enoylreductase (ER) catalytic region. In some embodiments, the polyketide producing cell comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a thioesterase (TE) catalytic region. In some embodiments, the polyketide producing cell further comprises one

or more heterologous nucleotide sequences encoding an enzyme comprising a holo ACP synthase activity, which effects pantetheinylation of the ACP.

[00202] In some embodiments, the polyketide producing cell further comprises one or more heterologous nucleotide sequences conferring a postsynthesis polyketide modifying activity. In some embodiments, the polyketide producing cell further comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a glycosylase activity, which effects postsynthesis modifications of polyketides, for example, where polyketides having antibiotic activity are desired. In some embodiments, the polyketide producing cell further comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a hydroxylase activity. In some embodiments, the polyketide producing cell further comprises one or more heterologous nucleotide sequences encoding an enzyme comprising an epoxidase activity. In some embodiments, the polyketide producing cell further comprises one or more heterologous nucleotide sequences encoding an enzyme comprising a methylase activity.

[00203] In some embodiments, the polyketide producing cell further comprises one or more heterologous nucleotide sequences encoding a biosynthetic enzyme including, but not limited to, at least one polyketide synthesis pathway enzyme, and enzymes that can modify an acetyl-CoA compound to form a polyketide product such as a macrolide, an antibiotic, an antifungal, a cytostatic compound, an anticholesterolemic compound, an antiparasitic compound, a coccidiostatic compound, an animal growth promoter or an insecticide. In some embodiments, the HACD compound is a polyene. In some embodiments, the HACD compound is a cyclic lactone. In some embodiments, the HACD compound comprises a 14, 15, or 16-membered lactone ring. In some embodiments, the HACD compound is a polyketide selected from the group consisting of a polyketide macrolide, antibiotic, antifungal, cytostatic, anticholesterolemic, antiparasitic, a coccidiostatic, animal growth promoter and insecticide.

[00204] In some embodiments, the polyketide producing cell comprises heterologous nucleotide sequences, for example sequences encoding PKS enzymes and polyketide modification enzymes, capable of producing a polyketide selected from, but not limited to, the following polyketides: Avermectin (*see, e.g.,* U.S. Pat. No. 5,252,474; U.S. Pat. No. 4,703,009; EP Pub. No. 118,367; MacNeil *et al.*, 1993, "Industrial Microorganisms: Basic and Applied Molecular Genetics"; Baltz, Hegeman, & Skatrud, eds. (ASM), pp. 245-256, "A Comparison of the Genes Encoding the Polyketide Synthases for Avermectin, Erythromycin,

and Nemadectin"; MacNeil *et al.*, 1992, *Gene* 115: 119-125; and Ikeda and Omura, 1997, *Chem. Res.* 97: 2599-2609); Candicidin (FR008) (*see, e.g.,* Hu *et a*l., 1994, *Mol. Microbiol.* 14: 163-172); Carbomycin, Curamycin (*see, e.g.,* Bergh *et al., Biotechnol Appl Biochem.* 1992 Feb;15(1):80-9); Daunorubicin (*see, e.g., J Bacteriol.* 1994 Oct;176(20):6270-80); Epothilone (*see, e.g.,* PCT Pub. No. 99/66028; and PCT Pub. No. 00/031247); Erythromycin (*see, e.g.,* PCT Pub. No. 93/13663; U.S. Pat. No. 6,004,787; U.S. Pat. No. 5,824,513; Donadio *et al.*, 1991, *Science* 252:675-9; and Cortes *et al.*, Nov. 8, 1990, *Nature* 348:176-8); FK-506 (*see, e.g.,* Motamedi *et al.*, 1998; *Eur. J Biochem.* 256: 528-534; and Motamedi *et al.*, 1997, *Eur. J Biochem.* 244: 74-80); FK-520 (*see, e.g.,* PCT Pub. No. 00/020601; and Nielsen *et al.*, 1991, *Biochem.* 30:5789-96); Griseusin (*see, e.g.,* Yu *et al., J Bacteriol.* 1994 May;176(9):2627-34); Lovastatin (*see, e.g.,* U.S. Pat. No. 5,744,350); Frenolycin (*see, e.g.,* Khosla *et al., Bacteriol.* 1993 Apr;175(8):2197-204; and *Bibb et al., Gene* 1994 May 3;142(1):31-9); Granaticin (*see, e.g.,* Sherman *et al.,* EMBO J. 1989 Sep;8(9):2717-25; and Bechtold *et al.*, *Mol Gen Genet.* 1995 Sep 20;248(5):610-20); Medermycin (*see, e.g.,* Ichinose *et al., Microbiology* 2003 Jul;149(Pt 7):1633-45); Monensin (*see, e.g.,* Arrowsmith *et al., Mol Gen Genet.* 1992 Aug;234(2):254-64); Nonactin (*see, e.g., FEMS Microbiol Lett.* 2000 Feb 1;183(1):171-5); Nanaomycin (*see, e.g.,* Kitao *et al., J Antibiot* (Tokyo). 1980 Jul;33(7):711-6); Nemadectin (*see, e.g.,* MacNeil *et al.*, 1993, supra); Niddamycin (*see, e.g.,* PCT Pub. No. 98/51695; and Kakavas *et al.*, 1997, *J. Bacteriol.* 179: 7515-7522); Oleandomycin (*see e.g.,* Swan *et al.*, 1994, *Mol. Gen. Genet.* 242: 358-362; PCT Pub. No. 00/026349; Olano *et al.*, 1998, *Mol. Gen. Genet.* 259(3): 299-308; and PCT Pat. App. Pub. No. WO 99/05283); Oxytetracycline (*see, e.g.,* Kim *et al., Gene.* 1994 Apr 8;141(1):141-2); Picromycin (*see, e.g.,* PCT Pub. No. 99/61599; PCT Pub. No. 00/00620; Xue *et al.*, 1998, *Chemistry & Biology* 5(11): 661-667; Xue *et al.*, October 1998, *Proc. Natl. Acad. Sci.* USA 95: 12111 12116); Platenolide (*see, e.g.,* EP Pub. No. 791,656; and U.S. Pat. No. 5,945,320); Rapamycin (*see, e.g.,* Schwecke *et al.*, August 1995, *Proc. Natl. Acad. Sci.* USA 92:7839-7843; and Aparicio *et al.*, 1996, Gene 169: 9-16); Rifamycin (*see, e.g.,* PCT Pub. No. WO 98/07868; and August *et al.*, Feb. 13, 1998, *Chemistry & Biology*, 5(2): 69-79); Sorangium (*see, e.g.,* U.S. Pat. No. 6,090,601); Soraphen (*see, e.g.,* U.S. Pat. No. 5,716,849; Schupp *et a*l., 1995, *J. Bacteriology* 177: 3673-3679); Spinocyn (*see, e.g.,* PCT Pub. No. 99/46387); Spiramycin (*see, e.g.,* U.S. Pat. No. 5,098,837); Tetracenomycin (*see, e.g.,* Summers *et al., J Bacteriol.* 1992 Mar;174(6):1810-20; and Shen *et al., J Bacteriol.* 1992 Jun;174(11):3818-21); Tetracycline (*see, e.g., J Am Chem Soc.* 2009 Dec 9;131(48):17677-89); Tylosin (*see,*

e.g., U.S. Pat. No. 5,876,991; U.S. Pat. No. 5,672,497; U.S. Pat. No. 5,149,638; EP Pub. No. 791,655; EP Pub. No. 238,323; Kuhstoss *et al.*, 1996, Gene 183:231-6; and Merson-Davies and Cundliffe, 1994, *Mol. Microbiol.* 13: 349-355); and 6-methylsalicyclic acid (*see, e.g.,* Richardson *et al.*, *Metab Eng.* 1999 Apr;1(2):180-7; and Shao *et al., Biochem Biophys Res Commun.* 2006 Jun 23;345(1):133-9).

5.8 Fatty Acids

[00205] In some embodiments, the genetically modified host cell provided herein is capable of producing a fatty acid from acetyl-CoA. Fatty acids are synthesized by a series of decarboxylative Claisen condensation reactions from acetyl-CoA and malonyl-CoA catalyzed by fatty acid synthases. Similar to polyketide synthases, fatty acid synthases are not a single enzyme but an enzymatic system composed of 272 kDa multifunctional polypeptide in which substrates are handed from one functional domain to the next. Two principal classes of fatty acid synthases have been characterized: Type I fatty acid synthases are single, multifunctional polypeptides common to mammals and fungi (although the structural arrangement of fungal and mammalian synthases differ) and the CMN group of bacteria (corynebacteria, mycobacteria, and nocardia). Type II synthases, found in archaeabacteria and eubacteria, are a series of discrete, monofunctional enzymes that participate in the synthesis of fatty acids. The mechanisms fatty acid elongation and reduction is the same in the two classes of synthases, as the enzyme domains responsible for these catalytic events are largely homologous amongst the two classes.

[00206] Following each round of elongation of the fatty acid chain in the decarboxylative Claisen condensation reactions, the β-keto group is reduced to a fully saturated carbon chain by the sequential action of a ketoreductase, a dehydratase, and an enol reductase. The growing fatty acid chain moves between these active sites attached to an acyl carrier protein and is ultimately released by the action of a thioesterase upon reaching a carbon chain length of 16 (palmitidic acid).

[00207] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding a biosynthetic enzyme including, but not limited to, at least one fatty acid synthesis pathway enzyme, and enzymes that can modify an acetyl-CoA compound to form a fatty acid product such as a palmitate, palmitoyl CoA, palmitoleic acid, sapienic acid, oleic acid, linoleic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, and docosahexaenoic acid. In some embodiments, the HACD compound is a fatty acid selected from the group consisting of

palmitate, palmitoyl CoA, palmitoleic acid, sapienic acid, oleic acid, linoleic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, and docosahexaenoic acid.

[00208] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can covalently link at least one of acetyl-CoA and malonyl-CoA with an acyl carrier protein, *e.g.* an acyl-transferase.

[00209] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can condense acetyl chemical moiety and a malonyl chemical moiety, each bound to an acyl carrier protein (ACP), to form acetoacetyl-ACP, *e.g.* a β-Ketoacyl-ACP synthase.

[00210] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can reduce the double bond in acetoacetyl-ACP with NADPH to form a hydroxyl group in D-3-hydroxybutyryl hydroxylase-ACP, *e.g.* a β-Ketoacyl-ACP reductase.

[00211] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can dehydrate D-3-Hydroxybutyryl hydroxylase-ACP to create a double bond between the beta- and gamma-carbons forming crotonyl-ACP, *e.g.* a β-hydroxyacyl-ACP dehydrase.

[00212] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can reduce crotonyl ACP with NADPH to form butyryl-ACP, *e.g.* an enoyl ACP reductase.

[00213] In some embodiments, the genetically modified microorganism disclosed herein comprises a heterologous nucleotide sequence encoding an enzyme that can hydrolyze a C16 acyl compound from an acyl carrier protein to form palmitate, *e.g.* a thioesterase.

[00214] In some embodiments, the fatty acid producing cell comprises one or more heterologous nucleotide sequences encoding acetyl-CoA synthase and/or malonyl-CoA synthase, to effect increased production of one or more fatty acids as compared to a genetically unmodified parent cell.

[00215] For example, to increase acetyl-CoA production, one or more of the following genes can be expressed in the cell: *pdh*, *panK*, *aceEF* (encoding the EIp dehydrogenase component and the E2p dihydrolipoamide acyltransferase component of the pyruvate and 2-oxoglutarate dehydrogenase complexes), *fabH*, *fabD*, *fabG*, *acpP*, and *fabF*. Illustrative examples of nucleotide sequences encoding such enzymes include, but are not limited to: *pdh*

(BAB34380, AAC73227, AAC73226), *panK* (also known as coaA, AAC76952), *aceEF* (AAC73227, AAC73226), *fabH* (AAC74175), fabD (AAC74176), *fabG* (AAC74177), *acpP* (AAC74178), *fabF* (AAC74179).

[00216] In some embodiments, increased fatty acid levels can be effected in the cell by attenuating or knocking out genes encoding proteins involved in fatty acid degradation. For example, the expression levels of *fadE*, *gpsA*, *idhA*, *pflb*, *adhE*, *pta*, *poxB*, *ackA*, and/or *ackB* can be attenuated or knocked-out in an engineered host cell using techniques known in the art. Illustrative examples of nucleotide sequences encoding such proteins include, but are not limited to: *fadE* (AAC73325), *gspA* (AAC76632), *IdhA* (AAC74462), *pflb* (AAC73989), *adhE* (AAC74323), *pta* (AAC75357), *poxB* (AAC73958), *ackA* (AAC75356), and *ackB* (BAB81430). The resulting host cells will have increased acetyl-CoA production levels when grown in an appropriate environment.

[00217] In some embodiments, the fatty acid producing cell comprises a heterologous nucleotide sequence encoding an enzyme that can convert acetyl-CoA into malonyl-CoA, *e.g.,* the multisubunit AccABCD protein. An illustrative example of a suitable nucleotide sequence encoding AccABCD includes but is not limited to accession number AAC73296, EC 6.4.1.2.

[00218] In some embodiments, the fatty acid producing cell comprises a heterologous nucleotide sequence encoding a lipase. Illustrative examples of suitable nucleotide sequences encoding a lipase include, but are not limited to accession numbers CAA89087 and CAA98876.

[00219] In some embodiments, increased fatty acid levels can be effected in the cell by inhibiting PlsB, which can lead to an increase in the levels of long chain acyl-ACP, which will inhibit early steps in the fatty acid biosynthesis pathway (*e.g.*, *accABCD*, *fabH*, and *fabI*). The expression level of PlsB can be attenuated or knocked-out in an engineered host cell using techniques known in the art. An illustrative example of a suitable nucleotide sequence encoding PlsB includes but is not limited to accession number AAC77011. In particular embodiments, the *plsB* D31 IE mutation can be used to increase the amount of available acyl-CoA in the cell.

[00220] In some embodiments, increased production of monounsaturated fatty acids can be effected in the cell by overexpressing an *sfa* gene, which would result in suppression of *fabA*. An illustrative example of a suitable nucleotide sequence encoding *sfa* includes but is not limited to accession number AAN79592.

[00221] In some embodiments, increased fatty acid levels can be effected in the cell by modulating the expression of an enzyme which controls the chain length of a fatty acid substrate, *e.g.,* a thioesterase. In some embodiments, the fatty acid producing cell has been modified to overexpress a *tes* or *fat* gene. Illustrative examples of suitable *tes* nucleotide sequences include but are not limited to accession numbers: (*tesA*: AAC73596, from *E. coli,* capable of producing $C_{18:1}$ fatty acids) and (*tesB*: AAC73555 from *E. coli*). Illustrative examples of suitable *fat* nucleotide sequences include but are not limited to: (*fatB*: Q41635 and AAA34215, from *Umbellularia californica*, capable of producing $C_{12:0}$ fatty acids), (*fatB2*: Q39513 and AAC49269, from *Cuphea hookeriana*, capable of producing $C_{8:0} - C_{10:0}$ fatty acids), (*fatB3*: AAC49269 and AAC72881, from *Cuphea hookeriana*, capable of producing $C_{14:0} - C_{16:0}$ fatty acids), (*fatB*: Q39473 and AAC49151, from *Cinnamonum camphorum*, capable of producing $C_{14:0}$ fatty acids), (*fatB [M141T]:* CAA85388, from m*Arabidopsis thaliana*, capable of producing $C_{16:1}$ fatty acids), (*fatA*: NP 189147 and NP 193041, from *Arabidopsis thaliana*, capable of producing $C_{18:1}$ fatty acids), (*fatA:* CAC39106, from *Bradvrhiizobium japonicum*, capable of preferentially producing $C_{18:1}$ fatty acids), (*fatA*: AAC72883, from *Cuphea hookeriana,* capable of producing $C_{18:1}$ fatty acids), and (*fatA1*, AAL79361 from *Helianthus annus*).

[00222] In some embodiments, increased levels of C_{10} fatty acids can be effected in the cell by attenuating the expression or activity of thioesterase C_{18} using techniques known in the art. Illustrative examples of suitable nucleotide sequences encoding thioesterase C_{18} include, but are not limited to accession numbers AAC73596 and P0ADA1. In other embodiments, increased levels of C_{10} fatty acids can be effected in the cell by increasing the expression or activity of thioesterase C_{10} using techniques known in the art. An illustrative example of a suitable nucleotide sequence encoding thioesterase C_{10} includes, but is not limited to accession number Q39513.

[00223] In some embodiments, increased levels of C_{14} fatty acids can be effected in the cell by attenuating the expression or activity of endogenous thioesterases that produce non-C_{14} fatty acids, using techniques known in the art. In other embodiments, increased levels of C_{14} fatty acids can be effected in the cell by increasing the expression or activity of thioesterases that use the substrate C_{14}-ACP, using techniques known in the art. An illustrative example of a suitable nucleotide sequence encoding such a thioesterase includes, but is not limited to accession number Q39473.

[00224] In some embodiments, increased levels of C_{12} fatty acids can be effected in the cell by attenuating the expression or activity of endogenous thioesterases that produce non-C_{12} fatty acids, using techniques known in the art. In other embodiments, increased levels of C_{12} fatty acids can be effected in the cell by increasing the expression or activity of thioesterases that use the substrate C_{12}-ACP, using techniques known in the art. An illustrative example of a suitable nucleotide sequence encoding such a thioesterase includes, but is not limited to accession number Q41635.

5.9 PK/PTA for the Production of Other Compounds

[00225] In some embodiments, the genetically modified host cell provided herein (*e.g.,* a host cell comprising PK/PTA and a functional disruption of a polypeptide encoding acetyl phosphatase activity, *e.g.,* RHR2, HOR2, or homologues thereof) is engineered for the expression of biosynthetic pathways that initiate with cellular pyruvate to produce, for example, 2,3-butanediol, 2-butanol, 2-butanone, valine, leucine, lactic acid, malate, isoamyl alcohol, and isobutanol, as described in U.S. Patent Application Publication No. 20120156735. The disruption of the enzyme pyruvate decarboxylase (PDC) in recombinant host cells engineered to express a pyruvate-utilizing biosynthetic pathway has been used to increase the availability of pyruvate for product formation via the biosynthetic pathway. While PDC-KO recombinant host cells can be used to produce the products of pyruvate-utilizing biosynthetic pathways, PDC-KO recombinant host cells require exogenous carbon substrate supplementation (e.g., ethanol or acetate) for their growth. In particular, two exogenous carbon substrates are needed: one of which is converted to a desired product, the other fully or partly converted into acetyl-CoA by recombinant host cells requiring such supplementation for growth. However, expression of a heterologous phosphoketolase pathway reduces or eliminates the need for providing these exogenous carbon substrates for their growth compared to PDC-KO cells not heterologously PK/PTA. Thus, the additional functional disruption of RHR2, HOR2, or homologues thereof capable of catalzying the hydrolysis of acetyl phosphate to acetate, is expected to further improve the ability of PK/PTA to increase the supply of acetyl-CoA available as a substrate for cellular growth in these cells.

5.10 Methods of Making Genetically Modified Cells

[00226] Also provided herein are methods for producing a host cell that is genetically engineered to comprise one or more of the modifications described above, *e.g.,* one or more nucleic heterologous nucleic acids encoding PK, PTA, and/or biosynthetic pathway enzymes,

e.g., for an acetyl-CoA derived compound. Expression of a heterologous enzyme in a host cell can be accomplished by introducing into the host cells a nucleic acid comprising a nucleotide sequence encoding the enzyme under the control of regulatory elements that permit expression in the host cell. In some embodiments, the nucleic acid is an extrachromosomal plasmid. In other embodiments, the nucleic acid is a chromosomal integration vector that can integrate the nucleotide sequence into the chromosome of the host cell.

[00227] Nucleic acids encoding these proteins can be introduced into the host cell by any method known to one of skill in the art without limitation (s*ee*, for example, Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci.* USA 75:1292-3; Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376-3385; Goeddel *et al.* eds, 1990, Methods in Enzymology, vol. 185, Academic Press, Inc. , CA; Krieger, 1990, Gene Transfer and Expression -- A Laboratory Manual, Stockton Press, NY; Sambrook *et al.* , 1989, Molecular Cloning -- A Laboratory Manual, Cold Spring Harbor Laboratory, NY; and Ausubel *et al.* , eds. , Current Edition, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY). Exemplary techniques include, but are not limited to, spheroplasting, electroporation, PEG 1000 mediated transformation, and lithium acetate or lithium chloride mediated transformation.

[00228] The copy number of an enzyme in a host cell may be altered by modifying the transcription of the gene that encodes the enzyme. This can be achieved for example by modifying the copy number of the nucleotide sequence encoding the enzyme (*e.g.*, by using a higher or lower copy number expression vector comprising the nucleotide sequence, or by introducing additional copies of the nucleotide sequence into the genome of the host cell or by deleting or disrupting the nucleotide sequence in the genome of the host cell), by changing the order of coding sequences on a polycistronic mRNA of an operon or breaking up an operon into individual genes each with its own control elements, or by increasing the strength of the promoter or operator to which the nucleotide sequence is operably linked. Alternatively or in addition, the copy number of an enzyme in a host cell may be altered by modifying the level of translation of an mRNA that encodes the enzyme. This can be achieved for example by modifying the stability of the mRNA, modifying the sequence of the ribosome binding site, modifying the distance or sequence between the ribosome binding site and the start codon of the enzyme coding sequence, modifying the entire intercistronic region located "upstream of" or adjacent to the 5' side of the start codon of the enzyme coding region, stabilizing the 3'-end of the mRNA transcript using hairpins and specialized

sequences, modifying the codon usage of enzyme, altering expression of rare codon tRNAs used in the biosynthesis of the enzyme, and/or increasing the stability of the enzyme, as, for example, via mutation of its coding sequence.

[00229] The activity of an enzyme in a host cell can be altered in a number of ways, including, but not limited to, expressing a modified form of the enzyme that exhibits increased or decreased solubility in the host cell, expressing an altered form of the enzyme that lacks a domain through which the activity of the enzyme is inhibited, expressing a modified form of the enzyme that has a higher or lower Kcat or a lower or higher Km for the substrate, or expressing an altered form of the enzyme that is more or less affected by feed-back or feed-forward regulation by another molecule in the pathway.

[00230] In some embodiments, a nucleic acid used to genetically modify a host cell comprises one or more selectable markers useful for the selection of transformed host cells and for placing selective pressure on the host cell to maintain the foreign DNA.

[00231] In some embodiments, the selectable marker is an antibiotic resistance marker. Illustrative examples of antibiotic resistance markers include, but are not limited to, the *BLA, NAT1, PAT, AUR1-C, PDR4, SMR1, CAT*, mouse dhfr, *HPH, DSDA,* KAN^R, and *SH BLE* gene products. The *BLA* gene product from *E. coli* confers resistance to beta-lactam antibiotics (*e.g.* , narrow-spectrum cephalosporins, cephamycins, and carbapenems (ertapenem), cefamandole, and cefoperazone) and to all the anti-gram-negative-bacterium penicillins except temocillin; the *NAT1* gene product from *S. noursei* confers resistance to nourseothricin; the *PAT* gene product from *S. viridochromogenes* Tu94 confers resistance to bialophos; the *AUR1-C* gene product from *Saccharomyces cerevisiae* confers resistance to Auerobasidin A (AbA); the PDR4 gene product confers resistance to cerulenin; the SMR1 gene product confers resistance to sulfometuron methyl; the CAT gene product from Tn9 transposon confers resistance to chloramphenicol; the mouse dhfr gene product confers resistance to methotrexate; the *HPH* gene product of *Klebsiella pneumonia* confers resistance to Hygromycin B; the *DSDA* gene product of *E. coli* allows cells to grow on plates with D-serine as the sole nitrogen source; the KAN^R gene of the Tn903 transposon confers resistance to G418; and the *SH BLE* gene product from *Streptoalloteichus hindustanus* confers resistance to Zeocin (bleomycin). In some embodiments, the antibiotic resistance marker is deleted after the genetically modified host cell disclosed herein is isolated.

[00232] In some embodiments, the selectable marker rescues an auxotrophy (*e.g.,* a nutritional auxotrophy) in the genetically modified microorganism. In such embodiments, a

parent microorganism comprises a functional disruption in one or more gene products that function in an amino acid or nucleotide biosynthetic pathway and that when non-functional renders a parent cell incapable of growing in media without supplementation with one or more nutrients. Such gene products include, but are not limited to, the *HIS3, LEU2, LYS1, LYS2, MET15*, *TRP1, ADE2,* and *URA3* gene products in yeast. The auxotrophic phenotype can then be rescued by transforming the parent cell with an expression vector or chromosomal integration construct encoding a functional copy of the disrupted gene product, and the genetically modified host cell generated can be selected for based on the loss of the auxotrophic phenotype of the parent cell. Utilization of the *URA3, TRP1*, and *LYS2* genes as selectable markers has a marked advantage because both positive and negative selections are possible. Positive selection is carried out by auxotrophic complementation of the *URA3, TRP1*, and *LYS2* mutations, whereas negative selection is based on specific inhibitors, *i.e.,* 5-fluoro-orotic acid (FOA), 5-fluoroanthranilic acid, and aminoadipic acid (aAA), respectively, that prevent growth of the prototrophic strains but allows growth of the *URA3, TRP1*, and *LYS2* mutants, respectively. In other embodiments, the selectable marker rescues other non-lethal deficiencies or phenotypes that can be identified by a known selection method.

[00233] Described herein are specific genes and proteins useful in the methods, compositions and organisms of the disclosure; however it will be recognized that absolute identity to such genes is not necessary. For example, changes in a particular gene or polynucleotide comprising a sequence encoding a polypeptide or enzyme can be performed and screened for activity. Typically such changes comprise conservative mutations and silent mutations. Such modified or mutated polynucleotides and polypeptides can be screened for expression of a functional enzyme using methods known in the art.

[00234] Due to the inherent degeneracy of the genetic code, other polynucleotides which encode substantially the same or functionally equivalent polypeptides can also be used to clone and express the polynucleotides encoding such enzymes.

[00235] As will be understood by those of skill in the art, it can be advantageous to modify a coding sequence to enhance its expression in a particular host. The genetic code is redundant with 64 possible codons, but most organisms typically use a subset of these codons. The codons that are utilized most often in a species are called optimal codons, and those not utilized very often are classified as rare or low-usage codons. Codons can be substituted to reflect the preferred codon usage of the host, in a process sometimes called "codon optimization" or "controlling for species codon bias."

[00236] Optimized coding sequences containing codons preferred by a particular prokaryotic or eukaryotic host (Murray *et al.*, 1989, *Nucl Acids Res.* 17: 477-508) can be prepared, for example, to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced from a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, typical stop codons for *S. cerevisiae* and mammals are UAA and UGA, respectively. The typical stop codon for monocotyledonous plants is UGA, whereas insects and E. coli commonly use UAA as the stop codon (Dalphin *et al.*, 1996, *Nucl Acids Res.* 24: 216-8).

[00237] Those of skill in the art will recognize that, due to the degenerate nature of the genetic code, a variety of DNA molecules differing in their nucleotide sequences can be used to encode a given enzyme of the disclosure. The native DNA sequence encoding the biosynthetic enzymes described above are referenced herein merely to illustrate an embodiment of the disclosure, and the disclosure includes DNA molecules of any sequence that encode the amino acid sequences of the polypeptides and proteins of the enzymes utilized in the methods of the disclosure. In similar fashion, a polypeptide can typically tolerate one or more amino acid substitutions, deletions, and insertions in its amino acid sequence without loss or significant loss of a desired activity. The disclosure includes such polypeptides with different amino acid sequences than the specific proteins described herein so long as the modified or variant polypeptides have the enzymatic anabolic or catabolic activity of the reference polypeptide. Furthermore, the amino acid sequences encoded by the DNA sequences shown herein merely illustrate embodiments of the disclosure.

[00238] In addition, homologs of enzymes useful for the compositions and methods provided herein are encompassed by the disclosure. In some embodiments, two proteins (or a region of the proteins) are substantially homologous when the amino acid sequences have at least about 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity. To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In one embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, typically at least 40%, more typically at least 50%, even more typically at least 60%, and even more typically at least

70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[00239] When "homologous" is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of homology may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art (See, e.g., Pearson W. R., 1994, *Methods in Mol Biol* 25: 365-89).

[00240] The following six groups each contain amino acids that are conservative substitutions for one another: 1) Serine (S), Threonine (T); 2) Aspartic Acid (D), Glutamic Acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Alanine (A), Valine (V), and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[00241] Sequence homology for polypeptides, which is also referred to as percent sequence identity, is typically measured using sequence analysis software. A typical algorithm used comparing a molecule sequence to a database containing a large number of sequences from different organisms is the computer program BLAST. When searching a database containing sequences from a large number of different organisms, it is typical to compare amino acid sequences.

[00242] Furthermore, any of the genes encoding the foregoing enzymes (or any others mentioned herein (or any of the regulatory elements that control or modulate expression

thereof)) may be optimized by genetic/protein engineering techniques, such as directed evolution or rational mutagenesis, which are known to those of ordinary skill in the art. Such action allows those of ordinary skill in the art to optimize the enzymes for expression and activity in yeast.

[00243] In addition, genes encoding these enzymes can be identified from other fungal and bacterial species and can be expressed for the modulation of this pathway. A variety of organisms could serve as sources for these enzymes, including, but not limited to, *Saccharomyces spp.*, including *S. cerevisiae* and *S. uvarum*, *Kluyveromyces spp.*, including *K. thermotolerans*, *K. lactis*, and *K. marxianus*, *Pichia spp.*, *Hansenula spp.*, including *H. polymorpha*, *Candida spp.*, *Trichosporon spp.*, *Yamadazyma spp.*, including *Y. spp. stipitis*, *Torulaspora pretoriensis*, *Issatchenkia orientalis*, *Schizosaccharomyces spp.*, including *S. pombe, Cryptococcus spp., Aspergillus spp., Neurospora spp.*, or *Ustilago spp.* Sources of genes from anaerobic fungi include, but are not limited to, *Piromyces spp.*, *Orpinomyces spp.*, or *Neocallimastix spp.* Sources of prokaryotic enzymes that are useful include, but are not limited to, *Escherichia. coli*, *Zymomonas mobilis*, *Staphylococcus aureus*, *Bacillus spp.*, *Clostridium spp.*, *Corynebacterium spp.*, *Pseudomonas spp.*, *Lactococcus spp.*, *Enterobacter spp.*, and *Salmonella spp.*

[00244] Techniques known to those skilled in the art may be suitable to identify additional homologous genes and homologous enzymes. Generally, analogous genes and/or analogous enzymes can be identified by functional analysis and will have functional similarities. Techniques known to those skilled in the art may be suitable to identify analogous genes and analogous enzymes. For example, to identify homologous or analogous PK, PTA, RHR2 or HOR2 genes, proteins, or enzymes, techniques may include, but are not limited to, cloning a gene by PCR using primers based on a published sequence of a gene/enzyme of interest, or by degenerate PCR using degenerate primers designed to amplify a conserved region among a gene of interest. Further, one skilled in the art can use techniques to identify homologous or analogous genes, proteins, or enzymes with functional homology or similarity. Techniques include examining a cell or cell culture for the catalytic activity of an enzyme through *in vitro* enzyme assays for said activity (*e.g.* as described herein or in Kiritani, K., *Branched-Chain Amino Acids Methods Enzymology*, 1970), then isolating the enzyme with said activity through purification, determining the protein sequence of the enzyme through techniques such as Edman degradation, design of PCR primers to the likely nucleic acid sequence, amplification of said DNA sequence through PCR, and cloning

of said nucleic acid sequence. To identify homologous or similar genes and/or homologous or similar enzymes, analogous genes and/or analogous enzymes or proteins, techniques also include comparison of data concerning a candidate gene or enzyme with databases such as BRENDA, KEGG, or MetaCYC. The candidate gene or enzyme may be identified within the above mentioned databases in accordance with the teachings herein.

6. EXAMPLES

6.1 Example 1: Acetate Production in Host Cells Expressing PK and PTA

[00245] This example describes the production of acetate in yeast strains heterologously expressing phosphoketolase and phosphotransacetylase.

6.1.1 Materials and Methods

6.1.1.1 Strain Engineering

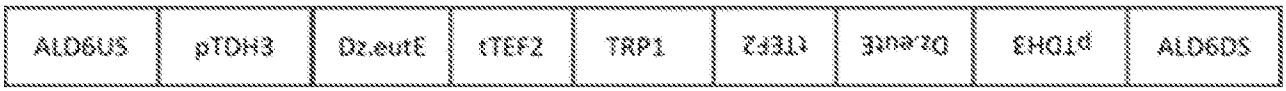
6.1.1.1.1 Y967 and Y968

[00246] Y967 and Y968 are wildtype prototrophic *Saccharomyces cerevisiae* CEN.PK2, Y967 is MatA, and Y968 is Matalpha. The starting strain for Y12869, Y12746, and all of their derivatives, was *Saccharomyces cerevisiae* strain Y003 (CEN.PK2, Mat alpha, ura3-52, trp1-289, leu2-3,122, his3^1). All DNA-mediated transformation into *S. cerevisiae* was conducted using the standard lithium acetate procedure as described by Gietz RW and Woods RA, *Guide to Yeast Genetics and Molecular and Cell Biology. Part B*. San Diego, CA: Academic Press Inc. pp. 87–96 (2002), and in all cases integration of the constructs were confirmed by PCR amplification of genomic DNA.

6.1.1.1.2 Y12869

[00247] Y12869 was generated through three successive integrations into Y003. First, the gene *ACS2* was deleted by introducing an integration construct (i2235; SEQ ID NO:27) consisting of the native *S. cerevisiae LEU2* gene, flanked by sequences consisting of upstream and downstream nucleotide sequences of the *ACS2* locus. Upon introduction of a *S. cerevisiae* host cell, this construct can integrate by homologous recombination into the *ACS2* locus of the genome, functionally disrupting *ACS2* by replacing the *ACS2* coding sequence with its integrating sequence. Transformants were plated onto CSM –leu plates containing 2% EtOH as the sole carbon source, and were confirmed by PCR amplification. The resulting strain was Y4940.

[00248] Next, ALD6 was deleted and *Dickeya zeae eutE* was introduced in Y4940 with the integration construct (i74804; SEQ ID NO:28) pictured below.



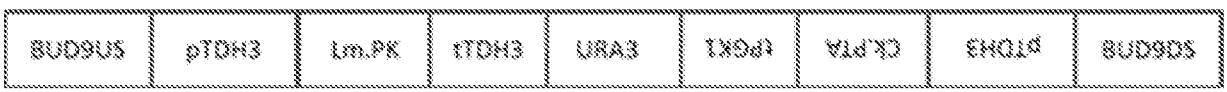
[00249] This integration construct comprises a selectable marker (TRP1), as well as two copies a yeast-codon-optimized sequence encoding the gene *eutE* from *Dickeya zeae* (NCBI Reference Sequence: YP_003003316.1) under control of the *TDH3* promoter (840 basepairs upstream of the native *S. cerevisiae TDH3* coding region), and the *TEF2* terminator (508 basepairs downstream of the native *S. cerevisiae TEF2* coding region). These components are flanked by upstream and downstream nucleotide sequences of the *ALD6* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *ALD6* by replacing the *ALD6* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. The construct was transformed into Y4940, and transformants were selected on CSM-TRP plates with 2% glucose and confirmed by PCR amplification. The resulting strain was y12602.

[00250] Next, ACS1 was deleted in Y12602 by introducing an integration construct (i76220; SEQ ID NO:29) consisting of the upstream and downstream nucleotide sequences of *ACS1*, flanking the native *S. cerevisiae HIS3* gene under its own promoter and terminator. Transformants were plated onto CSM –his plates containing 2% glucose as the sole carbon source, and were confirmed by PCR amplification. The resulting strain was Y12747.

[00251] Next, Y12747 was transformed with a PCR product amplified from the native *URA3* sequence. This sequence restores the ura3-52 mutation. *See* Rose and Winston, *Mol Gen Genet* 193:557-560 (1984). Transformants were plated onto CSM-ura plates containing 2% glucose as the sole carbon source, and were confirmed by PCR amplification. The resulting strain was Y12869.

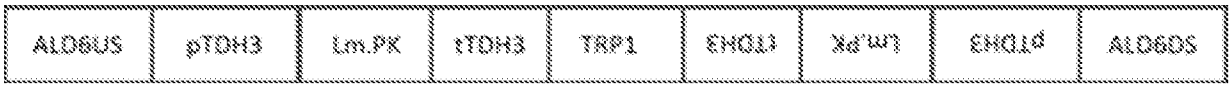
6.1.1.1.3 Y12745

[00252] Y12745 was generated through three successive integrations into Y4940. First, Y4940 was transformed with the integration construct (i73830; SEQ ID NO:30) pictured below.



[00253] This integration construct comprises a selectable marker (URA3); a yeast codon-optimized version of phosphoketolase from *Leuconostoc mesenteroides* (NCBI Reference Sequence YP_819405.1) under the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and *TDH3* terminator (259 bp downstream of the *TDH3* coding sequence);

a yeast codon-optimized version of *Clostridium kluyveri* phosphotransacetylase (NCBI Reference Sequence: YP_001394780.1) under control of the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and the *PGK1* terminator (259 bp downstream of the *PGK1* coding sequence); flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of the *S. cerevisiae BUD9* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *BUD9* by replacing the *BUD9* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose. The resulting strain was transformed with the construct (i74810; SEQ ID NO:31) shown below.

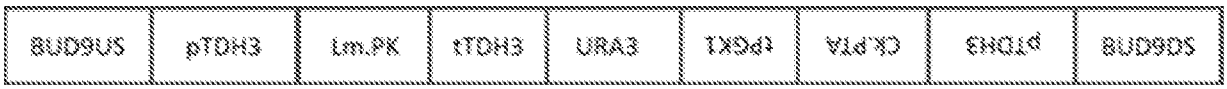


[00254] This construct comprising a selectable marker (*TRP1*); two copies of phosphoketolase from *Leuconostoc mesenteroides* under the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and *TDH3* terminator (259 bp downstream of the *TDH3* coding sequence); flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of the *ALD6* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *ALD6* by replacing the *ALD6* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose and confirmed by PCR amplification.

[00255] Next, ACS1 was deleted in by introducing an integration construct (i76220; SEQ ID NO:29) consisting of the upstream and downstream nucleotide sequences of *ACS1*, flanking the native *S. cerevisiae HIS3* gene under its own promoter and terminator. Transformants were plated onto CSM –his plates containing 2% glucose as the sole carbon source, and were confirmed by PCR amplification.

6.1.1.1.4 Y12746

[00256] Y12746 was generated through three successive integrations into Y4940. First, Y4940 was transformed with the integration construct (i73830; SEQ ID NO:30) pictured below.



[00257] This integration construct comprises a selectable marker (URA3); a yeast codon-optimized version of phosphoketolase from *Leuconostoc mesenteroides* (NCBI Reference Sequence YP_819405.1) under the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and *TDH3* terminator (259 bp downstream of the *TDH3* coding sequence); a yeast codon-optimized version of *Clostridium kluyveri* phosphotransacetylase (NCBI Reference Sequence: YP_001394780.1) under control of the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and the *PGK1* terminator (259 bp downstream of the *PGK1* coding sequence); flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of the *S. cerevisiae BUD9* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *BUD9* by replacing the *BUD9* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose.

[00258] The resulting strain was transformed with the construct (i74810; SEQ ID NO:31) shown below.

ALD6US	pTDH3	Lm.PK	tTDH3	TRP1	ENO1d	Lm.PK	ENO1d	ALD6DS

[00259] This construct comprising a selectable marker (*TRP1*); two copies of phosphoketolase from *Leuconostoc mesenteroides* under the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and *TDH3* terminator (259 bp downstream of the *TDH3* coding sequence); flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of the *ALD6* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *ALD6* by replacing the *ALD6* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose and confirmed by PCR amplification.

[00260] Finally, the resulting strain was transformed with the construct (i76221; SEQ ID NO:32) shown below.

ACS1US	pTDH3	Dz.eutE	tTEF2	HIS3	ZeEL1	Dz.eutE	ENO1d	ACS1DS

[00261] This construct comprises a selectable marker (*HIS3*); as well as two copies a yeast-codon-optimized sequence encoding the gene *eutE* from *Dickeya Zeae* (NCBI Reference Sequence: YP_003003316.1) under control of the *TDH3* promoter (840 basepairs

upstream of the native *S. cerevisiae TDH3* coding region) and the *TEF2* terminator (508 basepairs downstream of the native *S. cerevisiae TEF2* coding region). These components are flanked by upstream and downstream nucleotide sequences of the *ACS1* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *ACS1* by replacing the *ACS1* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-HIS plates with 2% glucose and confirmed by PCR amplification. The resulting strain was Y12746.

6.1.1.1.5 Y19390

[00262] Y19390 is a direct descendant of Y12869. A ura- auxotrophic derivative of Y12869 was transformed with the integration construct MS49253 (SEQ ID NO:36) shown below:

[00263] This integration construct comprises a selectable marker (URA3); two copies of a yeast codon-optimized version of phosphoketolase from *Leuconostoc mesenteroides* (NCBI Reference Sequence YP_819405.1) under the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and *TDH3* terminator (259 bp downstream of the *TDH3* coding sequence); flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of the *S. cerevisiae BUD9* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *BUD9* by replacing the *BUD9* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose.

6.1.1.1.6 Y19391

[00264] Y19391 is a direct descendant of Y12869. A ura- auxotrophic derivative of Y12869 was transformed with the integration construct MS49298 (SEQ ID NO:37) shown below:

[00265] This integration construct comprises a selectable marker (URA3); two copies of a yeast codon-optimized version of phosphotransacetylase from *Clostridium kluyveri* (NCBI Reference Sequence: YP_001394780.1) under control of the *TDH3* promoter (870 bp upstream of the *TDH3* coding sequence) and the *PGK1* terminator (259 bp downstream of the

PGK1 coding sequence); flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of the *S. cerevisiae BUD9* locus. Upon introduction into a host cell, this construct integrates by homologous recombination into the host cell genome, functionally disrupting *BUD9* by replacing the *BUD9* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose.

6.1.1.2 Culture conditions

[00266] Inoculum cultures of Y967, Y12869, Y12745, Y12746, Y19390 and Y19391 were grown from single colonies overnight in 5 ml of seed media at 30C and 200rpm (15 g/L ammonium sulfate, 8 g/L potassium phosphate, 6.1 g/L magnesium sulfate, 150 mg/L EDTA, 57.5 mg/L zinc sulfate, 4.8 mg/L cobalt chloride, 3.24 mg/L manganese chloride, 5 mg/L copper sulfate, 29.4 mg/L calcium chloride, 27.8 mg/L iron sulfate, 4.8 mg/L sodium molybdate, 0.6 mg/L biotin, 12 mg/L calcium pantothenate, 12 mg/L nicotinic acid, 30 mg/L inositol, 12 mg/L thiamin hydrochloride, 12 mg/L pyridoxine hydrochloride, 0.24 mg/L para-aminobenzoic acid) with 50 mM succinate pH 5.0, and 20 g/L sucrose. The precultures were then inoculated into a 125 ml flask carrying 25 ml of seed media with 50 mM succinate pH 5.0, and 40 g/L sucrose to an initial OD600 of 0.1, and grown at 30C and 200rpm.

6.1.1.3 Quantitation of acetate, fructose, glucose, and sucrose

[00267] Acetate and sugars (fructose, glucose, sucrose) were quantitated by transferring 1 ml of whole cell broth to a 1.5 ml eppendorf tubes, and spinning at 13,000 RPM for 1 minute using a tabletop centrifuge to clarify the supernatant. The supernatant was then diluted (1:1 v/v) in 8mM sulfuric acid, vortexed, and recentrifuged before transferring to a 1.8ml vial. Samples were analyzed with an Agilent 1200 HPLC, with variable wavelength and refractive index detection, using a BioRad Aminex HPX-87H 300mm x 7.8mm column. The mobile phase was 4mM sulfuric acid, column temperature was 40C, and the flow rate was 0.5 ml/min.

6.1.1.4 Results

[00268] **FIG. 3B** shows that wildtype Cen.PK2, Y967, produces acetate during growth in batch defined sucrose shakeflask cultures. Y12869, comprising a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ) and heterologously expressing acetaldehyde dehydrogenase acylating (Dz.eutE), produces far less acetate than the wildtype control which uses the PDH-bypass, likely due to the deletion of ALD6, the cytosolic acetaldehyde dehydrogenase that converts acetaldehyde to acetate. In the strain Y12746, comprising a deletion of the PDH-

bypass (*acs1*Δ *acs2* Δ *ald6*Δ) and heterologously expressing acetaldehyde dehydrogenase acylating (Dz.eutE) as well as phosphoketolase (Lm.PK) and phosphotransacetylase (Ck.PTA), a large increase in acetate is observed, surpassing the amount produced by wildtype Y967. The results with Y12869 indicate that the baseline level of acetate is extremely low in a strain that is *acs1*Δ *acs2* Δ *ald6*Δ and uses ADA to carry flux to cytosolic acetyl-CoA. In all cases, the rate of sugar consumption is comparable (sugars here are defined as the sum of sucrose, glucose, and fructose in the media), illustrating that the differences in acetate levels are not due to differential consumption of feedstock (**FIG. 3A**). These results suggest that the increase in acetate in Y12746 is attributable to the presence of phosphoketolase and/or phosphotransacetylase. The catalytic activity of both phosphoketolase and phosphotransacetylase produces acetyl phosphate. Therefore, acetate accumulation may arise from spontaneous or catalyzed hydrolysis of acetyl phosphate in Y12746.

[00269] To determine the source of acetate in the strain expressing ADA, PK and PTA (Y12746), we transformed a strain which uses only ADA to provide cytosolic AcCoA (Y12869, comprising a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ) and heterologously expressing acetaldehyde dehydrogenase acylating (*Dz.eutE*)) with either (1) an integration construct encoding two overexpressed copies of PK driven by the strong promoter P_{TDH3}, resulting in Y19390, or (2) an integration construct encoding two overexpressed copies of PTA driven by the strong promoter P_{TDH3}, resulting in Y19391. As shown in **FIG. 3D**, we observed an increase in acetate accumulation in strains that expressed either PK or PTA relative to the parent strain. Sugar consumption is shown in **FIG. 3C** to illustrate that acetate levels are not due to differential sugar consumption. PK converts X5P to Acetyl phosphate and G3P, whereas PTA can interconvert Acetyl CoA + Pi to Acetyl Phosphate + CoA. These observations suggest that acetyl phosphate, whether derived from X5P by PK, or derived from AcCoA by PTA, can be hydrolyzed to acetate as shown in **FIG. 1**.

6.2 Example 2: Identification of a Major Acetyl Phosphatase in *Saccharomyces cerevisiae*

[00270] This example describes the identification of an enzyme capable of hydrolyzing acetyl phosphate in yeast.

6.2.1 Materials and Methods

6.2.1.1 Cell Culture

[00271] A single colony of a given yeast strain was cultured in 5 mL Yeast Extract Peptone media with 2% dextrose (YPD) as an overnight starter culture. The following day, 50 ml YPD was inoculated with this starter culture to an OD600 of 0.2. The flasks were incubated at 30 °C by shaking at 200 RPM for 24 hours unless otherwise specified.

6.2.1.2 Cell-Free Extract Preparation

[00272] Cell culture was divided into three 15 mL falcon tubes and harvested by centrifugation at 4000 x g for 5 minutes. The supernatant was then discarded and cells were washed by resuspending in 10 mL ice cold buffer W (100 mM Tris-HCl pH 8.0, 150 mM NaCl, 10% glycerol) followed by centrifugation at 4000 x g for 5 minutes. Supernatant was discarded and cells were resuspended in 1 mL lysis buffer (100 mM Tris-HCl pH 8.0, 150 mM NaCl, 10% glycerol, 1 mM DTT, 1 EDTA free protease inhibitor tablet (Roche) per 10mL). The cells were then transferred to a 2 mL plastic screw cap microfuge tube with O ring cap (Fisher Brand 520-GRD) and cells were lysed using disruption beads (Disruption beads, 0.5Mm, Fisher) and a bead beater for 1 minute at 6 M/S. The tubes were immediately placed in an ice water bath for at least 5 minutes. The tubes were then placed back in the bead beater again for 1 minute at 6 M/S and returned to the ice bath for 5 minutes. Tubes were spun at a minimum of 16000 x g for 20 minutes to pellet cell debris. The supernatant was then transferred to a new cold tube. Protein concentration was measured using the classic Bradford assay for proteins (Bradford MM A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* 72, 248-254 (1976)).

6.2.1.3 Acetyl phosphatase reaction and quantitation of acetyl phosphate

[00273] Acetyl phosphatase activity assays were carried out at 30 °C in reaction buffer consisting of 100 mM Tris-HCl pH 7.5, 150 mM NaCl, and 1 mM $MgCl^{2+}$. Acetyl phosphate was added to a starting concentration of either 5 mM or 10 mM as indicated. The reaction was initiated by the addition of cell free extract in the amounts indicated. To test for phosphatase inhibition, sodium fluoride was added to select wells at 30 mM concentration. The reactions were carried out in a sealed 96 well plate and total reaction volume of 250 µl. Acetylphosphate concentration was measured by the method developed by Lipmann and Tuttle (Lipmann F, Tuttle LC, J. Biol. Chem. 159, 21-28 (1945)). 50 µl reaction mixture was added to 50 µl 2M hydroxylamine pH 6.8, mixed well and incubated at room temperature for at least 10 minutes. 34 µl 15% trichloroacetic acid was then added and mixed followed by 34

µl 4N HCl and 34 µl 5% $FeCl_3$ mixing well after each addition. Plates were then centrifuged in a Beckman centrifuge J-E with swinging bucket rotor JS-5.3 for 5 minutes at 3000 rpm to pellet precipitated protein. 150 µl supernatant was then transferred to a fresh 96-well clear flat bottom plate (Greiner Bio-One Cat.-No.: 655161). Plate was read by a Molecular Devices SpectraMax M5 plate reader at a wavelength of 505 nm.

6.2.1.4 Purification of Active Phosphatase Fraction

[00274] A single colony of a given yeast strain was cultured in 5 mL Yeast Extract Peptone media with 2% dextrose (YPD) as an overnight starter culture. The following day, two 2.8L Fermbach flasks with 500 ml YPD were inoculated with this starter culture to an OD600 of 0.2. The flasks were incubated at 30 °C by shaking at 160 RPM for 24 hours. The culture was harvested by centrifugation at 4000x g for 5 minutes. The cell pellet was washed with 500mL sterile water and centrifuged at 4000x g for 5 minutes. The cell pellet was then resuspended in 50 mL ice cold lysis buffer (100 mM Tris-HCl pH 8.0, 150 mM NaCl, 10% glycerol, 1 mM DTT, 1 EDTA free protease inhibitor tablet (Roche) per 10mL). Cell suspension was split into six 15 mL falcon tubes filled with 5 mL disruption beads (Disruption beads, 0.5Mm, Fisher). Tubes were then placed in a bead beater for 45 seconds at 6 M/S. The tubes were immediately placed in an ice water bath for at least 5 minutes. Bead beating was repeated 3 additional times with at least 5 minutes in an ice water bath in between each disruption segment. Tubes were spun for 30 minutes at 16,000 rpm (30,966 x g) in a Beckman centrifuge J-E in a JA-20 rotor chilled to 4 °C to pellet cell debris. Cell lysate was additionally clarified by the selective flocculation method described by Salt *et al.* (Selective flocculation of cellular contaminants from soluble proteins using polyethyleneimine: A study of several organisms and polymer molecular weights. *Enzyme and Microbial Technology* 17, 107-113(1995)) as follows: cell free lysate was adjusted to pH 7.4 by addition of 5mM NaOH stock solution. Then equal volume of PEI/Borax solution (0.5M NaCl 0.25% PEI, 100mM Borax) was added to the cell lysate and mixed well. Mixture was then centrifuged for 30 minutes at 2,500 x g at 4 °C. Protein was then precipitated by slowly adding ammonium sulfate with constant stirring until 80% of saturation concentration was reached. Stirring continued for 10 more minutes, and then precipitated protein was harvested by centrifugation at 15,000 rpm at 4 °C in a Beckman JA-20 rotor for 10 minutes. Supernatant was removed and protein was gently resuspended in Buffer A (20 mM Tris-Cl, pH 7, 10% glycerol). Protein was then added to a 0.5-3mL 3,500 Da molecular weight cut off dialysis cassette (Pierce #66300) and dialyzed overnight at 4 °C

in 1.5L buffer A. Dialyzed sample was centrifuged 16000 x g for 10 minutes to pellet precipitated protein. Protein concentration was measured using the classic Bradford assay for proteins (Bradford MM, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* 72, 248-254 (1976)). 20 mg protein was loaded onto a Source 15Q 4.6/100 PE anion exchange column on a GE ÄKTAexplorer FPLC. Protein was eluted with a 0-100% gradient of buffer B (20 mM Tris-Cl pH 7, 1M NaCl, 10% glycerol) over 30 column volumes at a flow rate of 0.5 mL/minute and 1 mL samples were collected. To assay activity of each fraction, 75 µL each fraction was added to 8 mM ACP in a 250 µL reaction containing 100mM Tris-Cl pH 7, 150 mM NaCl, 1 mM MgCl2 and assayed as described above. The active fraction from this separation was again dialyzed against buffer A overnight. The entire sample was then loaded onto the same a Source 15Q 4.6/100 PE anion exchange column and eluted with a gradient of 0-45% buffer B over 30 column volumes at a flow rate of 0.5 mL/minute and 1 mL samples were collected. Samples were assayed for activity as above.

6.2.1.5 Protein Gel Electrophoresis

[00275] Protein fractions were analyzed using a Criterion gel electrophoresis system. 10 µL of fraction was added to 10 µL of 2X Laemmli sample buffer (BioRad Cat # 161-0737) with 5% v/v 2-mercaptoethanol and boiled for 10 minutes. Samples were then briefly centrifuged and 15 µl was loaded on a 26 well 4–15% Criterion™ TGX™ Precast Gel and run in 1X Tris-Glycine-SDS buffer (prepared from BioRad 10x Tris/Glycine/SDS #161-0732) for 50 minutes at 130 volts. The gel was rinsed in 200 mL deionized water three times for 5 minutes each. SimplyBlue™ SafeStain (Life Technologies Cat # LC6060) was then added to the gel to completely cover the gel and then incubated at room temperature for 1 hour with gentle rocking. The SafeStain was then discarded and the gel was washed with 200 mL deionized water for 1 hour with rocking.

6.2.1.6 Identification of Proteins in Active Phosphatase Fraction

[00276] Proteolytic digestion and separation of peptides

[00277] 100 µg of total protein was subjected to proteolysis by trypsin for subsequent identification by LC-MS/MS. 100 µg total protein was reduced with Tris-carboxyethylphosphine (4 mM) for 30 minutes at 37 °C, then alkylated with Iodoacetamide (15 mM) for 30 minutes at RT in the dark. 5 µg Trypsin was added to the digest mixture and the entire digestion was allowed to go for 12 hours at 37 °C. The reaction was quenched with 0.1% formic acid and injected onto an Ascentis Peptide express column (5cmx2.1mm ID, 2.1

um particle size), and separated over a 90 minute gradient from low acetonitrile to high acetonitrile, with 0.1% formic acid as a modifier. The LC pumps were two Shimadzu LC20AD's operated by a Shimadzu CBM20A LC Controller.

[00278] Mass Spectrometry Parameters:

[00279] A QTRAP 4000 hybrid triple-quadrupole linear ion tram mass spectrometer was used to identify peptides being eluted from the column. IDA parameters were as follows: Select ions from 350 to 1300 da; ER Scan used for charge state determination; 1+ ions rejected, unknowns allowed; Rolling collision energy: yes (AB SCIEX standard for qtrap 4000); Max fill time for each MS/MS: 950 ms.

[00280] Peptide identification by Mascot

[00281] Mascot, by Matrix Science was used to identify peptides from a CENPK2 sequence database with the following parameters. Fixed modifications: Carbamidomethyl. Variable modifications: deamidation (NQ), oxidation (MW). Precursor mass tolerance: 0.5 da. Product mass tolerance: 1.0 da. Missed cleavages allowed: 1.

6.2.1.7 Strain Engineering

[00282] A version of Y968 lacking a functional *URA3* gene was transformed with either ms59858 to knock out *RHR2* or ms59971 to knock out *HOR2*. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose and confirmed by PCR amplification. The *URA3* marker in this construct is flanked by direct repeats, facilitating its recycling. To recycle the URA3 marker, cells were grown in YPD overnight, then plated on 5'FOA. The loopout of *URA3* was confirmed by PCR amplification and inability to grow on CSM-URA plates. The ura- version of Y968.ms59858 was then transformed with ms59971 to generate a double *RHR2* and *HOR2* knockout strain Y968.ms59858.ms59971

6.2.2 Results

6.2.2.1 Hydrolysis of acetyl phosphate is enzyme-catalyzed and inhibited by heat and a broad spectrum phosphatase inhibitor

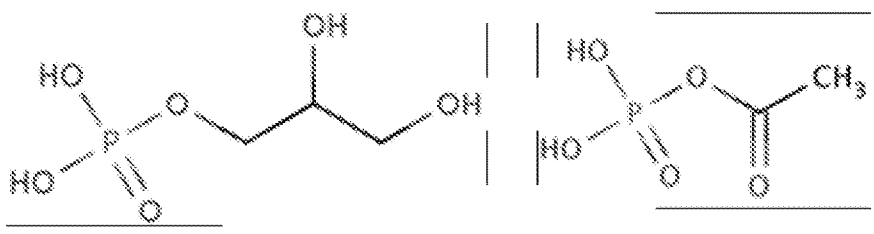
[00283] As shown in **FIG 4**, addition of cell free extract from wild type *S. cerevisiae* strain Y967 catalyzes the hydrolysis of acetyl phosphate, and the rate of hydrolysis is dependent on the amount of cell free extract added. Increasing the amount of cell free extract increases the rate of hydrolysis. When the cell free extract is boiled, the addition of increasing amounts of cell free extract no longer has an effect on the hydrolysis rate of acetyl phosphate, indicating that the responsible component has been inactivated by heat. Similarly, the addition of 30 mM sodium fluoride, a broad spectrum phosphatase inhibitor, renders the

cell free extract ineffective at hydrolyzing acetyl phosphate. These results suggest that a phosphatase is likely responsible for the catalysis of acetyl phosphate hydrolysis.

6.2.2.2 Protein fractionation isolates a single enriched active fraction

[00284] Anion exchange chromatography was used to separate soluble protein in the cell free extracts. **FIG. 5A** shows that nearly all of the phosphatase activity was concentrated in one fraction, and the remaining activity in adjacent fractions. This indicates that the enzyme responsible for this activity in the cell free extract is either a single protein or proteins with similar ionic interactions which co-elute when separated by anion exchange chromatography.

[00285] The active fraction #10 from FPLC anion exchange purification was purified a second time using a more shallow gradient 0-45% buffer B. The most active fraction from this purification, # 14, shown in **FIG. 6A**, was analyzed by mass spectrometry to determine the identity of the proteins in the fraction. Of the proteins identified in the active fraction (**FIG. 6B**), Rhr2 and its homolog Hor2, which cannot be distinguished by mass spectrometry due to significant sequence similarity, were the only proteins on the list identified as phosphatases by the SGD database. Rhr2 is a glycerol-1-phosphatase that is expressed constitutively at high levels. Hor2 catalyzes the identical reaction but is expressed only at low levels under normal conditions and is induced by osmotic stress (Norbeck *et. al.*, Purification and Characterization of Two Isoenzymes of DL-Glycerol-3-phosphatase from *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 271, 13875-13881 (1996)). Acetyl phosphate is not a metabolite that is native to yeast, therefore it is expected that the hydrolysis is caused by a promiscuous reaction of an enzyme that targets a similar substrate. Rhr2/Hor2 were top candidates for this reaction since their native substrate, glycerol-1-phosphate, is also a low molecular weight phosphorylated compound similar to acetyl phosphate, as shown below.



Glycerol-1-Phosphate **Acetyl phosphate**

6.2.2.3 Deletion of *RHR2* and/or *HOR2* reduces phosphatase activity

[00286] In order to determine whether Rhr2 and/or Hor2 were responsible for the phosphatase activity observed *in S. cerevisiae*, new strains were created lacking either *RHR2*

or *HOR2* and one strain lacking both *RHR2* and *HOR2*. These strains were cultured as described previously, and cell free extract was prepared and tested for acetyl phosphatase activity. As shown in **FIG. 7**, deletion of *RHR2* dramatically reduces phosphatase activity, while deletion of *HOR2* has no effect on the rate of hydrolysis of acetyl phosphate. Deletion of *HOR2* does however reduce hydrolysis of acetyl phosphate in a strain that already has *RHR2* deleted. This is consistent with published work that indicates that expression of Hor2 is upregulated following deletion of *RHR2* (DeLuna *et. al.*, Need-Based Up-Regulation of Protein Levels in Response to Deletion of Their Duplicate Genes, *PLOS Biol.*, 8, e10000347 (2010)). Elimination of both of these phosphatases results in near background levels of acetyl phosphate hydrolysis as shown in **FIG. 7**. These results confirm that glycerol-1-phosphatases Rhr2 and Hor2 are responsible for the majority of the acetyl phosphatase activity in *S. cerevisiae*.

6.3 Example 3: Deletion of the acetyl phosphate phosphatase reduces acetate secretion and improves production of a compound derived from Acetyl-CoA

6.3.1 Materials and Methods

6.3.1.1 Strain construction

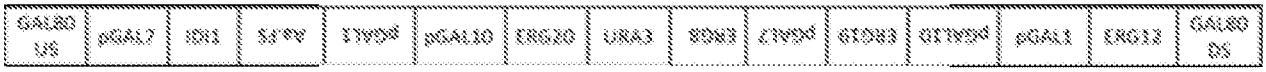
[00287] Versions of Y968, Y12869, and Y12746, lacking a functional URA3 gene, were transformed with either ms63907 or ms63909, and with ms64472, to convert them to farnesene producers.

[00288] The ms63907 integration construct (i84022; SEQ ID NO:33) is shown below.

This construct comprises nucleotide sequences that encode a selectable marker (*URA3*); a copy of the native yeast *GAL4* transcription factor under its own promoter; two native yeast enzymes of the mevalonate pathway (*ERG10* which encodes Acetoacetyl-CoA thiolase, and *ERG13*, which encodes HMG-CoA synthase), as well as two copies of a yeast codon-optimized version of *Silicibacter pomeroyi* HMG-CoA reductase, all under galactose-inducible promoters (promoters of the *S. cerevisiae* genes *GAL1* and *GAL10*, flanked by homologous sequences consisting of upstream and downstream nucleotide sequences of the *S. cerevisiae HO* endonuclease locus. Upon introduction into a host cell, the ms63907 construct integrates by homologous integration into the host cell genome, functionally disrupting *HO* by replacing the *HO* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982.

Transformants were selected on CSM-URA plates with 2% glucose and confirmed by PCR amplification. The URA3 marker in this construct is flanked by direct repeats, facilitating its recycling. To recycle the URA3 marker, cells were grown in YPD overnight, then plated on 5'FOA. The loopout of URA3 was confirmed by PCR amplification and inability to grow on CSM-URA plates. The ms63909 integration construct (i84026; SEQ ID NO:34) is identical to ms63907, with one exception: the sequences encoding *S. pomeroyi* HMG-CoA reductase are replaced by *tHMGr*, the truncated *HMG1* coding sequence which encodes the native *S. cerevisiae* HMG-CoA reductase.

[00289] The ms64472 integration construct (i85207; SEQ ID NO:35) is shown below.



This construct comprises nucleotide sequences that encode a selectable marker (*URA3)*; five native yeast enzymes of the ergosterol pathway (*ERG12* which encodes mevalonate kinase, *ERG8* which encodes phosphomevalonate kinase, *ERG19* which encodes mevalonate pyrophosphate decarboxylase, *IDI1* which encodes dimethylallyl diphosphate isomerase, and *ERG20* which encodes farnesyl pyrophosphate synthetase), as well as an evolved, yeast codon-optimized version of *Artemisia annua* farnesene synthase, all under galactose-inducible promoters (Promoters of the *S. cerevisiae* genes *GAL1, GAL10, and GAL7*). These sequences are flanked by homologous sequences consisting of the upstream and downstream nucleotide sequences of *GAL80*. Upon introduction into a host cell, the ms64472 construct integrates by homologous integration into the host cell genome, functionally disrupting *GAL80* by replacing the *GAL80* coding sequence with its integrating sequence. The construct was assembled using the methods described in U.S. Patent No. 8,221,982. Transformants were selected on CSM-URA plates with 2% glucose and confirmed by PCR amplification. The URA3 marker in this construct is flanked by direct repeats, facilitating its recycling. To recycle the URA3 marker, cells were grown in YPD overnight, then plated on 5'FOA. The loopout of URA3 was confirmed by PCR amplification and inability to grow on CSM-URA plates.

[00290] Next, ura- versions of Y968.ms63907.ms64472, Y12869.ms63907.ms64472, and Y12747.ms63907.ms64472, were transformed with ms59858 to knock out the RHR2 ORF. This integration construct consists of the upstream and downstream nucleotide sequences of *RHR2*, flanking the native *S. cerevisiae URA3* gene under its own promoter and terminator. Transformants were plated onto CSM –his plates containing 2% glucose as the sole carbon source, and were confirmed by PCR amplification.

6.3.1.2 Culture conditions

[00291] Single colonies were inoculated in wells of a 96-well plate in 360 µl of seed media (described in Example 1), and grown at 34°C for three days by shaking at 1000 rpm. Then, 14.4 µl of culture was subcultured into 360 µl of seed media with 50 mM succinate pH 5.0 and 40 g/L galactose, and grown at 34°C for two days by shaking at 1000 rpm.

6.3.1.3 Quantitation of acetate and glycerol

[00292] Acetate and glycerol were quantitated by transferring 1 ml of whole cell broth to a 1.5 ml eppendorf tubes, and spinning at 13,000 RPM for 1 minute using a tabletop centrifuge to clarify the supernatant. The supernatant was then diluted (1:1 v/v) in 8mM sulfuric acid, vortexed, and recentrifuged before transferring to a 1.8ml vial. Samples were analyzed with an Agilent 1200 HPLC, with variable wavelength and refractive index detection, using a BioRad Aminex HPX-87H 300mm x 7.8mm column. The mobile phase was 4mM sulfuric acid, column temperature was 40C, and the flow rate was 0.5 ml/min.

6.3.1.4 Quantitation of farnesene

[00293] At the end of two days incubation at 34°C, 98 µl of whole cell broth was mixed with 2 µl of Nile Red solution (100 µg/ml in DMSO) in a flat-bottom 96-well assay plate (Costar 3916), and mixed for 30 seconds on a 96-well plate shaker. The plates were then read on a Beckman M5 plate reader with excitation at 500nm and emission at 550nm.

6.3.1.5 Quantitation of optical density

[00294] In a 96-well assay plate, 8 µl of culture was mixed with 92 µl of diluent (20% PEG 200, 20% Ethanol, 2% Triton X-114) and incubated for 30 minutes at room temperature. The assay plate was vortexed before measuring OD_{600} on a Beckman M5 plate reader.

6.3.2 Results

[00295] **FIG. 8A** shows that strain Y12746.ms63909.ms64472, comprising a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), heterologously expressing acetaldehyde dehydrogenase aceylating (Dz.eutE) as well as phosphoketolase (Lm.PK) and phosphotransacetylase (Ck.PTA) and overexpressing genes in the farnesene production pathway, secretes more acetate than a version of Y12746.ms63909.ms64472 in which the *RHR2* gene has been deleted. As shown in **FIG. 8B**, deletion of RHR2 does not impact glycerol production, as glycerol levels of Y12746.ms63909.ms64472 rhr2^ are largely unchanged compared to Y12746.ms63909.ms64472. As shown in **FIG. 8C**, the substantially reduced levels of acetate in Y12746.ms63909.ms64472 rhr2^ are not due to reduced cell growth, as cell densities are similar for both RHR2+ and rhr2^ populations. These results

demonstrate that Rhr2, which was responsible for the acetyl phosphate phosphatase activity in cell free extract, is also the primary cause behind the hydrolysis of acetyl phosphate to acetate *in vivo*.

[00296] To determine whether the reduction of acetate observed upon deletion of RHR2 occurs independent of farnesene production, acetate production was measured in versions of strain 12746 with an intact or deleted *RHR2* gene, but not expressing genes in the farnesene production pathway. **FIG. 8D** shows that strain Y12746, comprising a deletion of the PDH-bypass (*acs1*Δ *acs2* Δ *ald6*Δ), heterologously expressing acetaldehyde dehydrogenase aceylating (Dz.eutE) as well as phosphoketolase (Lm.PK) and phosphotransacetylase (Ck.PTA), secretes more acetate than a version of Y12746 in which the RHR2 gene has been deleted. As shown in **FIG. 8E**, the substantially reduced levels of acetate in Y12746.ms63909.ms64472 rhr2^ are not due to reduced cell growth, as cell densities are similar for both RHR2+ and rhr2^ populations. These data illustrate that the reduction in acetate occurs regardless of the presence of an overexpressed farnesene production pathway.

[00297] **FIG. 9** shows that the deletion of rhr2 improves farnesene production in Y12746.ms63907.ms64472 by 2.1-fold, and in Y12745.ms63907.ms64472 by 1.4-fold (In each strain background, the *RHR2*+ parent is normalized to 1). Moreover, deletion of rhr2 improves the final optical density of Y12746.ms63907.ms64472 at carbon exhaustion. Both Y12745.ms63907.64472 and Y12746.ms63907.ms64472 use phosphoketolase and phosphotransacetylase, and thus acetyl phosphate as a pathway intermediate, to produce cytosolic acetyl-CoA, which is used for synthesis of farnesene. Strains Y968.ms63907.ms64472 and Y12869.ms63907.ms64472 do not express phosphoketolase or phosphotransacetylase, and do not use acetyl phosphate as a pathway intermediate. Deletion of rhr2 in these strain backgrounds has no effect on farnesene production or optical density in either strain background. This indicates that the benefit of knocking out rhr2 specifically applies to strains which use acetyl phosphate as an intermediate metabolite, *e.g.,* strains comprising heterologous PK and/or PTA.

[00298] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in

2014227811 28 Feb 2018

the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[00299] Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

[00300] The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

2014227811 13 Jun 2018

WHAT IS CLAIMED:

1. A genetically modified yeast host cell comprising:

(a) a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9);

(b) a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC 2.3.1.8); and

(c) a functional disruption of an endogenous glycerol-1-phosphatase enzyme (EC 3.1.3.21) that converts acetyl phosphate to acetate, wherein conversion from acetyl phosphate to acetate is functionally disrupted.

2. The genetically modified yeast host cell of claim 1, wherein the glycerol-1-phosphatase is selected from GPP1/RHR2, GPP2/HOR2, and homologues and variants thereof.

3. The genetically modified yeast host cell of claim 2, wherein:

(a) GPP1/RHR2, or a homologue or variant thereof, is functionally disrupted;

(b) GPP2/HOR2, or a homologue or variant thereof, is functionally disrupted; or

(c) both GPP1/RHR2 and GPP2/HOR2, or both a homologue or variant of GPP1/RHR2 and a homologue or variant of GPP2/HOR2, are functionally disrupted.

4. The genetically modified yeast host cell of any one of claims 1 to 3, wherein the genetically modified host cell further comprises a heterologous nucleic acid encoding an acylating acetylaldehyde dehydrogenase (ADA; EC 1.2.1.10).

5. The genetically modified yeast host cell of any one of claims 1 to 4, wherein the genetically modified host cell is capable of producing an isoprenoid.

6. The genetically modified yeast host cell of claim 5, wherein the genetically modified host cell comprises one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate.

2014227811 13 Jun 2018

7. The genetically modified yeast host cell of claim 6, wherein:

(a) the one or more enzymes of the MEV pathway comprise an NADH-using HMG-CoA reductase;

(b) the one or more enzymes of the MEV pathway comprise an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA;

(c) the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA;

(d) the one or more enzymes of the MEV pathway comprise an enzyme that converts HMG-CoA to mevalonate;

(e) the one or more enzymes of the MEV pathway comprise an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate;

(f) the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate;

(g) the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate;

(h) the one or more enzymes of the MEV pathway are selected from HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase and mevalonate pyrophosphate decarboxylase;

(i) the host cell comprises a plurality of heterologous nucleic acids encoding all of the enzymes of the MEV pathway;

(j) the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of a single transcriptional regulator;

(k) the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of multiple heterologous transcriptional regulators;

(l) the host cell further comprises a heterologous nucleic acid encoding an enzyme that can convert isopentenyl pyrophosphate (IPP) into dimethylallyl pyrophosphate (DMAPP);

(m) the host cell further comprises a heterologous nucleic acid encoding an enzyme that can condense IPP and/or DMAPP molecules to form a polyprenyl compound; or

(n) the host cell further comprises a heterologous nucleic acid encoding an enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound.

8. The genetically modified yeast host cell of claim 7,

wherein the enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound is selected from the group consisting of carene synthase, geraniol synthase, linalool synthase, limonene synthase, myrcene synthase, ocimene synthase, α-pinene synthase, β-pinene synthase, γ-terpinene synthase, terpinolene synthase, amorphadiene synthase, α-farnesene synthase, β-farnesene synthase, farnesol synthase, nerolidol synthase, patchouliol synthase, nootkatone synthase, and abietadiene synthase, or

wherein

(a) the isoprenoid is selected from the group consisting of a hemiterpene, monoterpene, diterpene, triterpene, tetraterpene, sesquiterpene, and polyterpene;

(b) the isoprenoid is a C5-C20 isoprenoid; or

(c) the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, isoprene, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpinolene, and valencene.

9. The genetically modified yeast host cell of any one of claims 1 to 8, wherein the genetically modified yeast host cell is *Saccharomyces cerevisiae*.

10. The genetically modified yeast host cell of any one of claims 1 to 9, wherein the genetically modified host cell produces an increased amount of an isoprenoid compared to a yeast cell not comprising a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate.

11. A method for producing an isoprenoid comprising:

(a) culturing a population of the genetically modified yeast host cells of any one of claims 4 to 9 in a medium with a carbon source under conditions suitable for making said isoprenoid compound; and

(b) recovering said isoprenoid compound from the medium.

2014227811 13 Jun 2018

12. A method for increasing the production of acetyl-CoA or an isoprenoid in a yeast host cell, the method comprising:

(a) expressing in the yeast host cell a heterologous nucleic acid encoding a phosphoketolase (PK; EC 4.1.2.9) and a heterologous nucleic acid encoding a phosphotransacetylase (PTA; EC2.3.1.8); and

(b) functionally disrupting an endogenous glycerol-1-phosphatase enzyme EC 3.1.3.21) that converts acetyl phosphate to acetate, wherein conversion from acetyl phosphate to acetate is functionally disrupted.

13. The method of claim 12, wherein the glycerol-1-phosphatase is selected from GPP1/RHR2, GPP2/HOR2, and homologues and variants thereof.

14. The method of claim 13, wherein:

(a) GPP1/RHR2, or a homologue or variant thereof, is functionally disrupted;

(b) GPP2/HOR2, or a homologue or variant thereof, is functionally disrupted; or

(c) both GPP1/RHR2 and GPP2/HOR2, or both a homologue or variant of GPP1/RHR2 and a homologue or variant of GPP2/HOR2, are functionally disrupted.

15. The method of any one of claims 12 to 14, wherein the yeast host cell is *Saccharomyces cerevisiae*.

16. The method of any one of claims 12 to 15, wherein the yeast host cell produces an increased amount of acetyl-CoA or an acetyl-CoA derived compound compared to a yeast cell not comprising a functional disruption of an endogenous glycerol-1-phosphatase enzyme (EC 3.1.3.21) that converts acetyl phosphate to acetate.

Figure 1

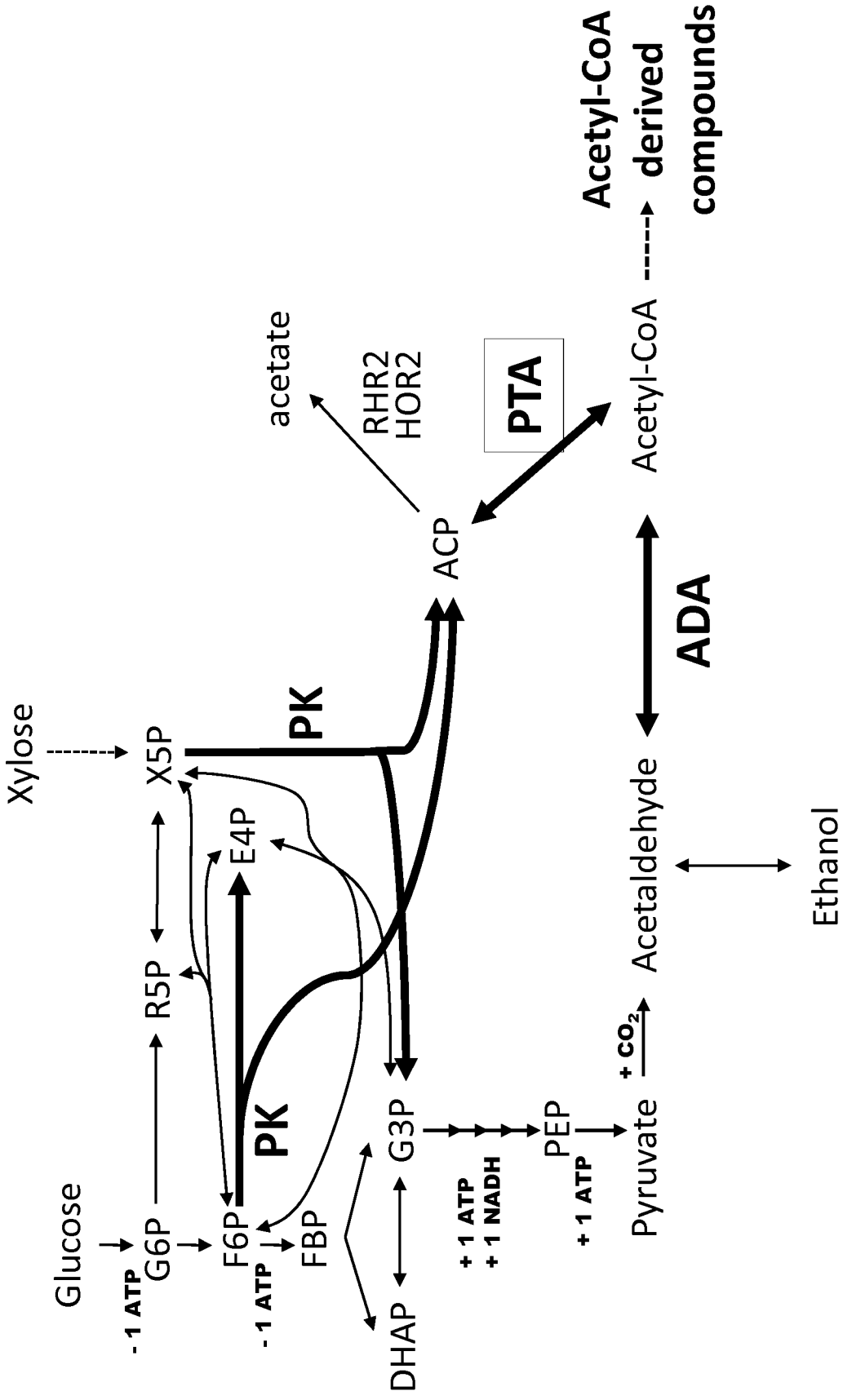

Xylose
X5P
PK
R5P
E4P
Glucose
G6P
F6P
PK
FBP
G3P
DHAP
- 1 ATP
- 1 ATP
+ 1 ATP
+ 1 NADH
PEP
+ 1 ATP
Pyruvate
+ CO2
acetate
RHR2
HOR2
ACP
PTA
Acetyl-CoA
Acetyl-CoA derived compounds
ADA
Acetaldehyde
Ethanol

Figure 2

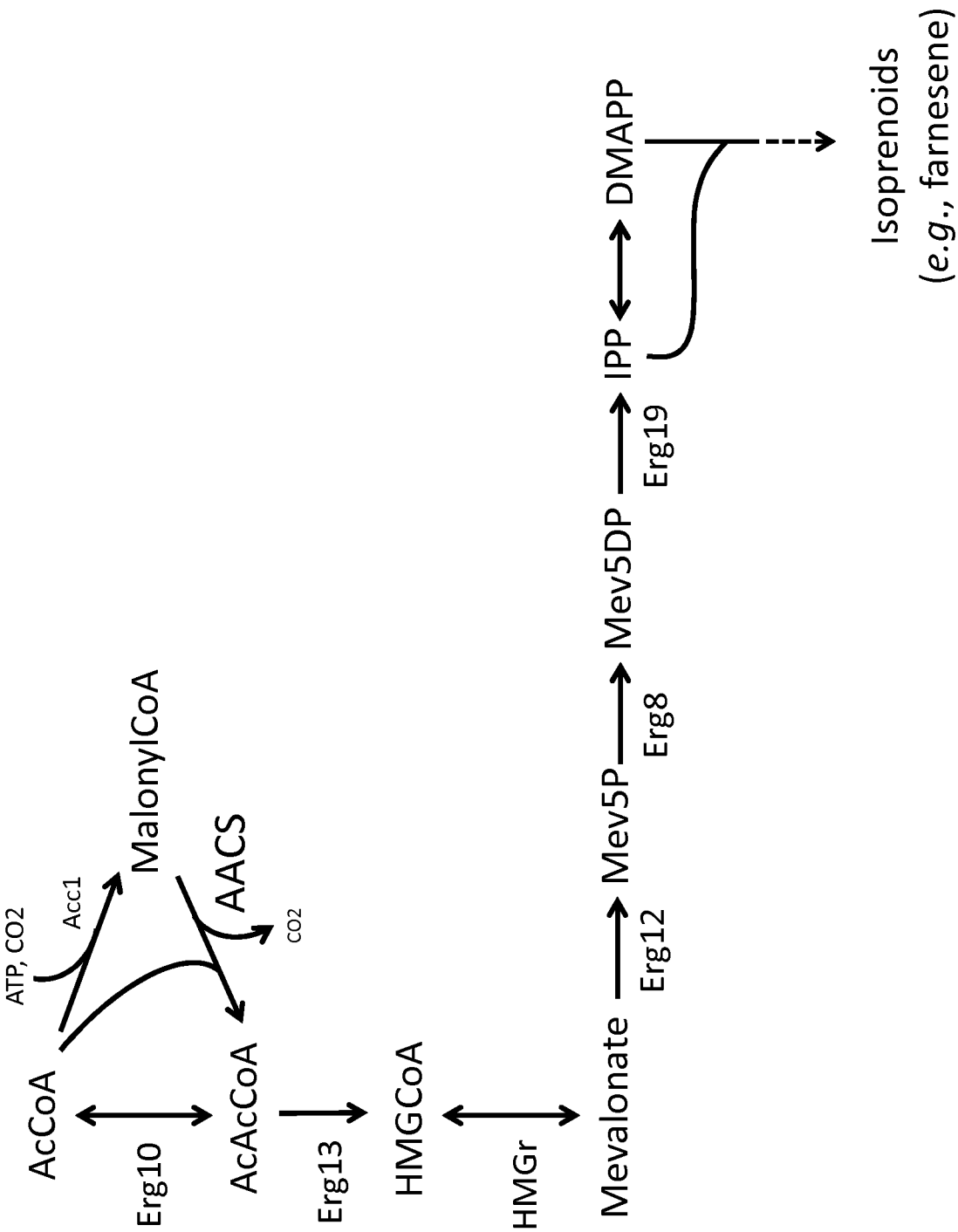
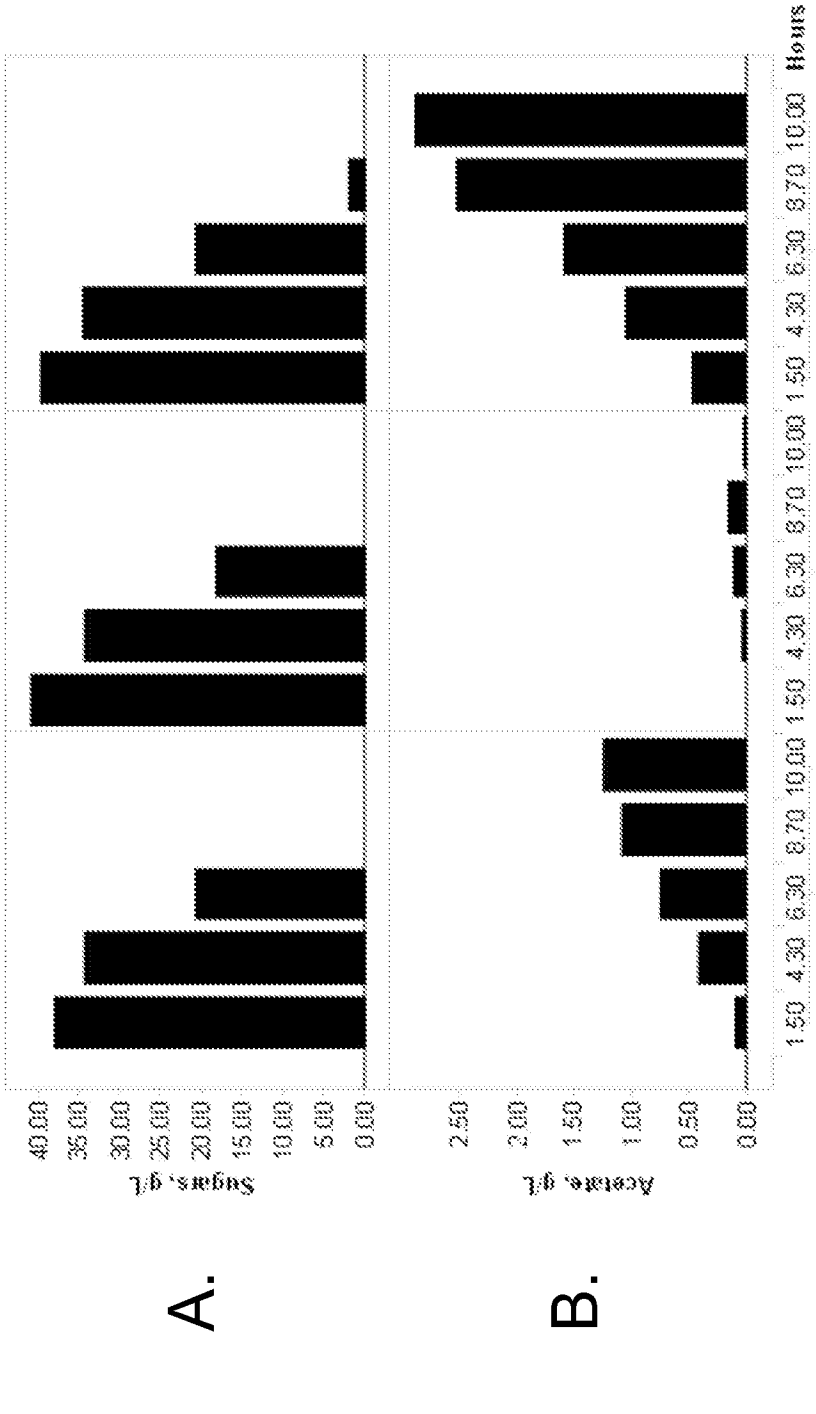
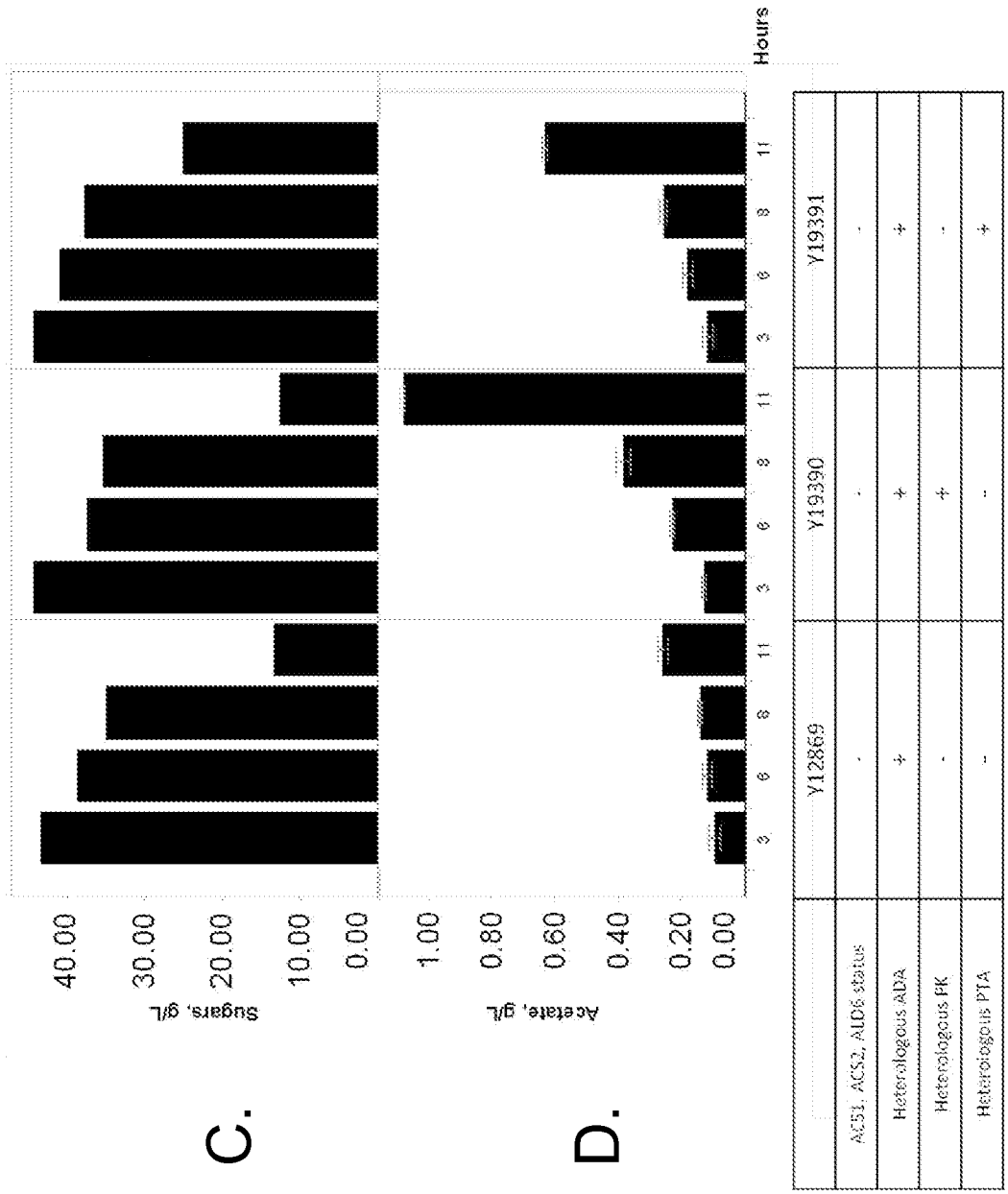

AcCoA
Erg10
AcAcCoA
Erg13
HMGCoA
HMGr
Mevalonate
Erg12
Mev5P
Erg8
Mev5DP
Erg19
IPP
DMAPP
Isoprenoids
(e.g., farnesene)
ATP, CO2
Acc1
MalonylCoA
AACS
CO2

Figure 3A & 3B



	Y967	Y12069	Y12746
ACS1, ACS2, ADE6 genes	+	-	-
Heterologous ADA	-	+	+
Heterologous PK	-	-	+
Heterologous PTA	-	-	+

Figure 3C & 3D



	Y12869	Y19390	Y19391
ACS1, ACS2, ALD6 status	-	-	-
Heterologous ADA	+	+	+
Heterologous PK	-	+	-
Heterologous PTA	-	-	+

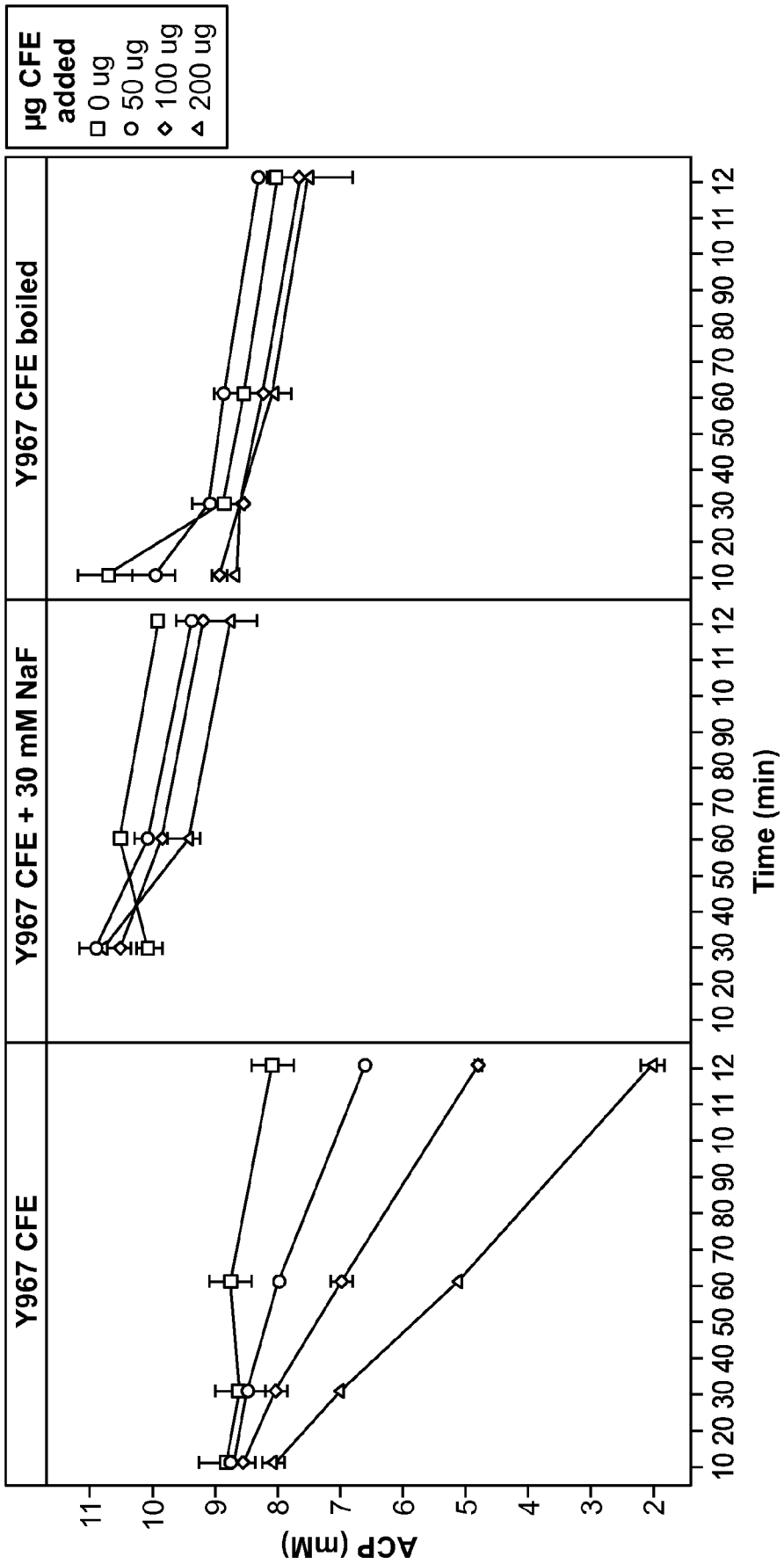

µg CFE added
0 ug
50 ug
100 ug
200 ug
Y967 CFE
Y967 CFE + 30 mM NaF
Y967 CFE boiled
ACP (mM)
Time (min)

FIG. 4

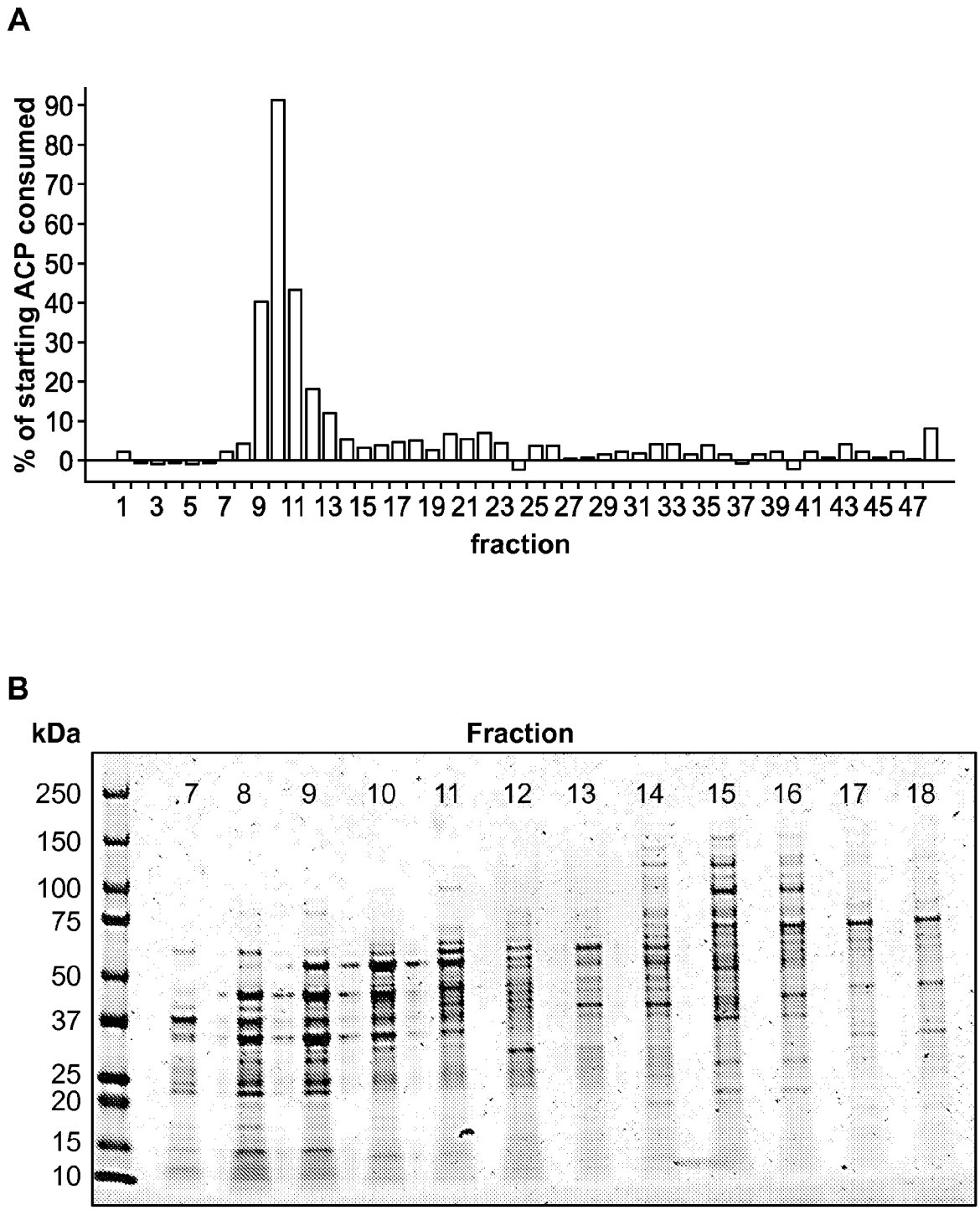
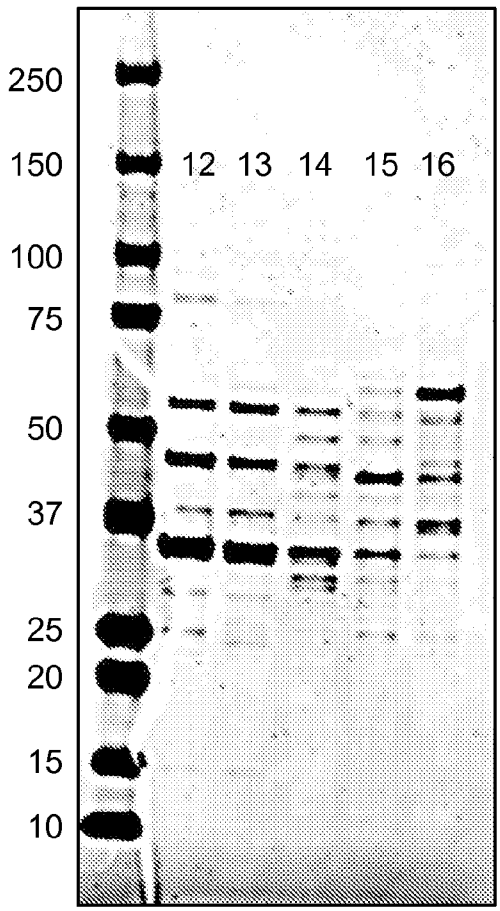

A
% of starting ACP consumed
90
80
70
60
50
40
30
20
10
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
fraction
B
kDa
Fraction
250
150
100
75
50
37
25
20
15
10
7 8 9 10 11 12 13 14 15 16 17 18

FIG. 5

A



B

prot_acc	prot_desc	MW	pI
YGR192C	TDH3	35.7	6.96
YHR174W	ENO2	46.9	5.88
YGR254W	ENO1	46.8	6.6
YJL052W	TDH1	35.7	8.59
YJR123W	RPS5	25	9.11
YGL076C	RPL7A	27.6	10.97
YPL198W	RPL7B		
YDL124W	YDL124W	35.5	6.1
YFR044C	DUG1	52.9	5.43
YIL053W	RHR2	28	5.31
YGR244C	LSC2	46.9	7.49
YBL030C	PET9	34.4	10.61
YMR099C	YMR099C	33.9	6.07
YHR183W	GND1	53.5	6.6
YLR058C	SHM2	52.2	7.44
YOR142W	LSC1	35	8.73
YBR011C	IPP1	32.3	5.33
YML126C	ERG13	55	8.23
YMR217W	GUA1	58.4	6.47
YMR226C	YMR226C	29.1	6.81
YJL167W	ERG20	40.4	5.21
YPL262W	FUM1	53.1	8.26
YOR209C	NPT1	49	6.92
YPR080W	TEF1	50	9.72
YBR118W	TEF2	50	9.72
YHR087W	RTC3	12	4.86
YOL086C	ADH1	36.8	6.66
YMR303C	ADH2	36.7	6.72
YKL085W	MDH1	35.6	8.99
YGR037C	ACB1	10	4.67
YOR265W	RBL2	12.4	4.82
YOR116C	RPO31	162.3	8.22
YLR044C	PDC1	61.4	6.12
YLR134W	PDC5	61.9	6.41
YNL095C	YNL095C	71.1	7.51
YNL327W	EGT2	108	4.43
YJL159W	HSP150	41	4.876
YBR208C	DUR1,2	201	5.39

FIG. 6

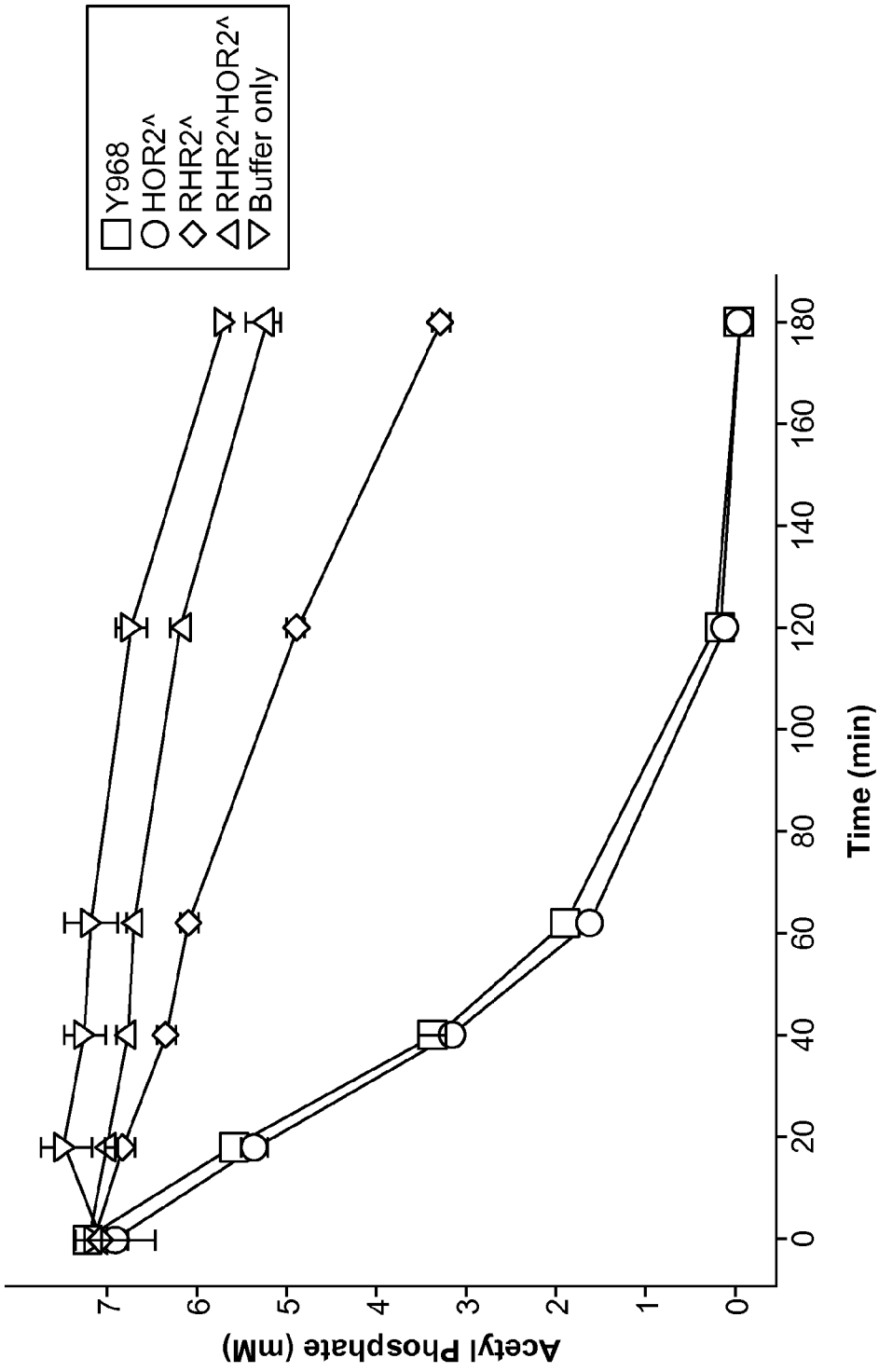

Y968
HOR2^
RHR2^
RHR2^HOR2^
Buffer only
Acetyl Phosphate (mM)
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120
140
160
180
Time (min)

FIG. 7

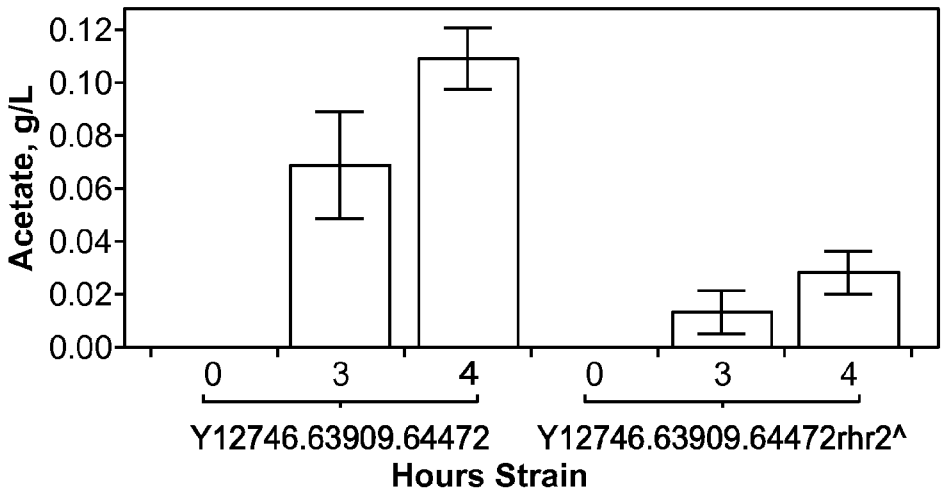


FIG. 8A

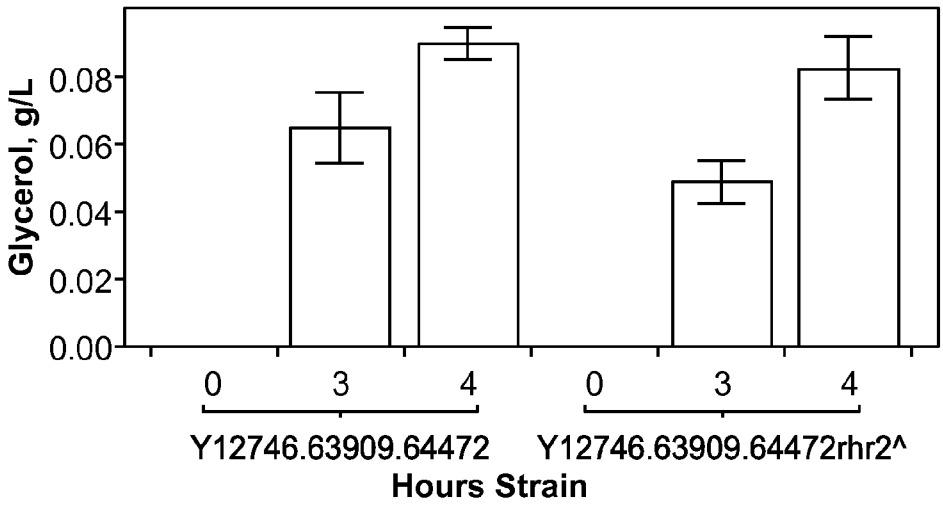


FIG. 8B

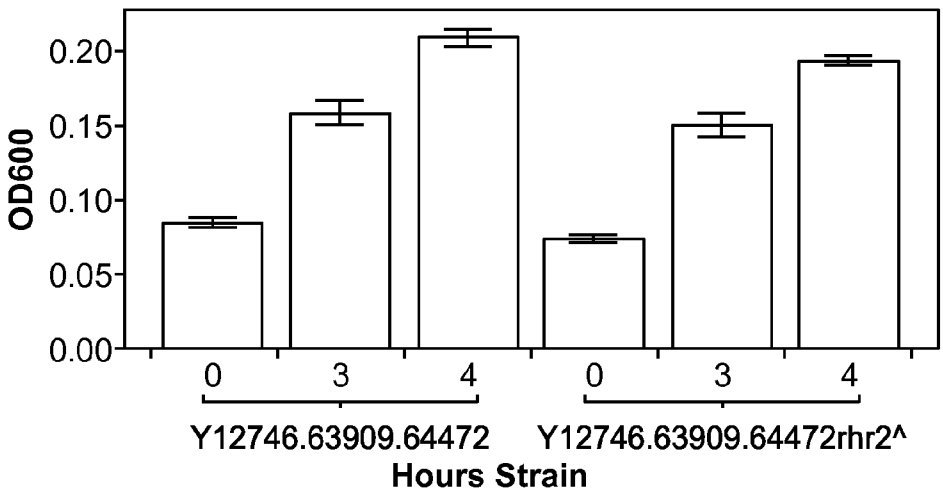


FIG. 8C

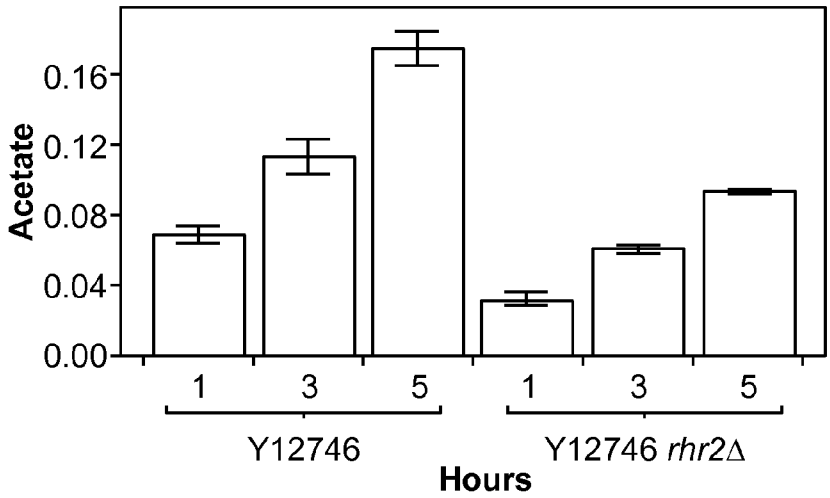

Acetate
0.16
0.12
0.08
0.04
0.00
1
3
5
1
3
5
Y12746
Y12746 rhr2Δ
Hours

FIG. 8D

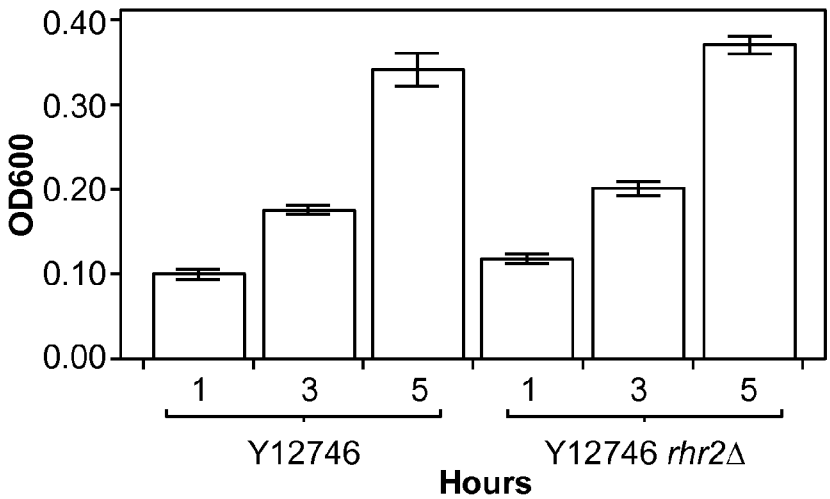
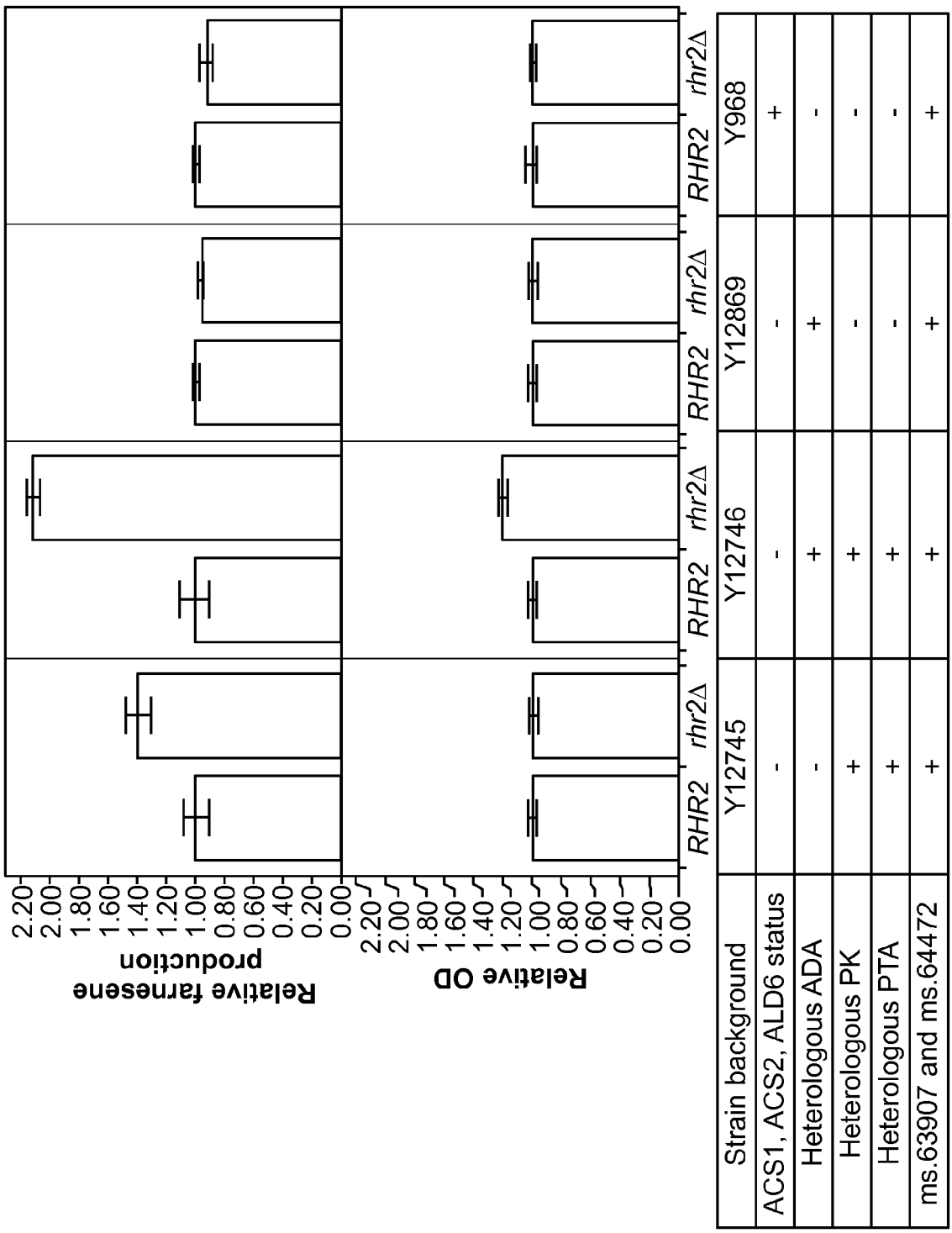

OD600
0.40
0.30
0.20
0.10
0.00
1
3
5
1
3
5
Y12746
Y12746 rhr2Δ
Hours

FIG. 8E



Strain background	Y12745		Y12746		Y12869		Y968	
ACS1, ACS2, ALD6 status	-		-		-		+	
Heterologous ADA	-		+		+		-	
Heterologous PK	+		+		-		-	
Heterologous PTA	+		+		-		-	
ms.63907 and ms.64472	+		+		+		+	

FIG. 9

SEQUENCE LISTING

<110> HAWKINS, Kristy Michelle
MEADOWS, Adam Leon
TSONG, Annie Ening
MAHATDEJKUL-MEADOWS, Tina Tipawan
PICKENS, Lauren Barbara
TAI, Anna

<120> USE OF PHOSPHOKETOLASE AND PHOSPHOTRANSACETYLASE FOR PRODUCTION OF ACETYL-COENZYME A DERIVED COMPOUNDS

<130> 107345.00466

<150> US 61/800,356
<151> 2013-03-15

<160> 37

<170> PatentIn version 3.5

<210> 1
<211> 2749
<212> DNA
<213> Leuconostoc mesenteroides

<220>
<221> misc_feature
<222> (1)..(2749)
<223> Leuconostoc mesenteroides Phosphoketolase (PK) gene sequence

<400> 1
gttacggaag aagtcgtggt ttacggtgtt tatgattctt gcaaaaaata aggagtactt 60

aatctcatgg cagatttcga ttcaaaagag tacttggaac ttgttgataa gtggtggcgc 120

gcaactaact atttgtcagc tgggatgatc tttttgaaga gcaacccatt gttctcagtt 180

actaatacac ctatcaaggc tgaagatgta aaagttaagc caatcggaca ctggggtact 240

atctcaggtc agacattctt gtatgcacat gctaaccgtt tgatcaacaa gtatggtttg 300

aacatgtttt acgttggtgg tcctggtcac ggtggccaag ttatggttac taacgcttac 360

ttagacggcg catatactga agattatcct gaaattactc aagatatcga aggtatgagc 420

cacttgttca agcgtttctc attccctggc ggtattggat cacacatgac agctcaaaca 480

cctggttcat tacacgaagg tggtgaattg ggctattcat tgagccacgc ttttggtgcc 540

gttttggaca atcctgacca agttgctttc gcagttgttg gtgatggtga agctgaaaca 600

ggtccttcaa tggcttcatg gcactcaatt aagtttttga atgctaagaa tgatggtgcc 660

gttttgcctg tcttggattt gaacggattc aagatttcaa acccaactat cttctcacgt 720

atgagtgatg aagaaatcac aaagttcttt gaaggtttgg gttattcacc tcgcttcatc 780

gaaaacgatg atattcatga ctacgcaaca tatcaccaac ttgcagcaaa cattttggat 840

caagctattg aagatattca agctattcaa aatgatgcac gtgaaaatgg taagtatcaa 900

gatggtgaaa tccctgcatg gccagtaatt attgctcgct tgccaaaggg ctggggtgga 960

ccaacgcacg atgcaagtaa caatcctatt gaaaactcat tccgtgcgca ccaagtgcca 1020

ttgcctcttg aacaacacga tcttgcaaca ttgcctgaat tcgaagactg gatgaactca 1080

tacaagcctg aagaattatt caatgctgat ggttctttga aggatgaatt gaaagctatc 1140

gctcctaagg gtgacaagcg tatgtcagct aaccctatta caaatggtgg tgctgatcgt 1200

tcagacttga agttgcctaa ctggagagaa ttcgctaacg atatcaatga tgatacacgt 1260

ggtaaggaat tcgctgatag caagcgcaat atggacatgg caacattgtc aaactacttg 1320

ggtgctgttt cacaattgaa cccaactcgt ttccgcttct tcggtcctga tgaaacaatg 1380

tcaaaccgtt tgtggggatt gttcaatgtt acaccacgtc aatggatgga agaaatcaag 1440

gaaccacaag atcaattgtt gagccctacg ggtcgcatta ttgattcaca attgtctgaa 1500

catcaagctg aaggttggct tgaaggatat actttgactg gtcgtgttgg aatcttcgca 1560

tcatacgagt cattcttgcg tgttgtcgat acaatggtta cgcaacactt caagtggttg 1620

cgtcacgctt cagaacaagc atggcgtaat gactatccat cattgaactt gattgcaact 1680

tcaactgctt tccaacaaga tcacaatgga tatactcacc aagatccagg tatgttgact 1740

cacttggctg aaaagaagtc taactttatt cgtgaatatt tgccagctga tggtaactca 1800

ttgttggctg ttcaagaacg tgctttctca gaacgtcata aggttaactt gttgattgct 1860

tctaagcaac cacgtcaaca atggtttaca gttgaagaag ctgaagtatt ggctaacgaa 1920

ggtttgaaga tcattgattg ggcttctact gcaccttcta gtgatgttga tattacattc 1980

gcatctgctg gtactgaacc aacaattgaa actttggctg ctttgtggtt gattaaccaa 2040

gcattcccag atgttaagtt ccgttatgtt aacgttgttg aattactacg tttgcaaaag 2100

aagtcagaac ctaacatgaa tgatgaacgt gaattatcag ccgaagaatt caacaagtat 2160

ttccaagctg atacaccagt tatcttcggt ttccatgctt atgaaaactt gattgaatca 2220

ttcttcttcg aacgtaagtt cacgggtgat gtatacgttc atggatatcg tgaagatggt 2280

gacatcacaa cgacatatga tatgcgtgta tattcacact tggatcgctt ccatcaagct 2340

aaggaagctg ctgaaatctt gtctgcaaat ggtaagattg atcaagctgc tgctgataca 2400

ttcatcgcta agatggatga tactttggca aagcatttcc aagttactcg taacgaaggt 2460

cgtgatatcg aagaattcac tgactggaca tggtcaccac ttaagtaatt taaaattatt 2520

ttatcaaaac caactattat ttttaatagt tggtttttt atggctaaat tgactacata 2580

ctaaacgaaa ccatgtaaaa gtgccacata gttttactta ataagttcct tttatttttt 2640

gatttgcaat gcaaaattgt aagcgtaata tgaataataa aaacccccaa ttagttagct 2700

aattgggggt tttgtaaatc accatatcag ccgctcatag tcttagacg 2749

<210> 2
<211> 813
<212> PRT
<213> Leuconostoc mesenteroides

<220>
<221> misc_feature
<222> (1)..(813)
<223> Leuconostoc mesenteroides Phosphoketolase (PK) protein sequence

<400> 2

```
Met Ala Asp Phe Asp Ser Lys Glu Tyr Leu Glu Leu Val Asp Lys Trp
1               5                  10                  15

Trp Arg Ala Thr Asn Tyr Leu Ser Ala Gly Met Ile Phe Leu Lys Ser
            20                  25                  30

Asn Pro Leu Phe Ser Val Thr Asn Thr Pro Ile Lys Ala Glu Asp Val
        35                  40                  45

Lys Val Lys Pro Ile Gly His Trp Gly Thr Ile Ser Gly Gln Thr Phe
    50                  55                  60

Leu Tyr Ala His Ala Asn Arg Leu Ile Asn Lys Tyr Gly Leu Asn Met
65                  70                  75                  80

Phe Tyr Val Gly Gly Pro Gly His Gly Gly Gln Val Met Val Thr Asn
                85                  90                  95

Ala Tyr Leu Asp Gly Ala Tyr Thr Glu Asp Tyr Pro Glu Ile Thr Gln
            100                 105                 110

Asp Ile Glu Gly Met Ser His Leu Phe Lys Arg Phe Ser Phe Pro Gly
        115                 120                 125

Gly Ile Gly Ser His Met Thr Ala Gln Thr Pro Gly Ser Leu His Glu
    130                 135                 140

Gly Gly Glu Leu Gly Tyr Ser Leu Ser His Ala Phe Gly Ala Val Leu
145                 150                 155                 160

Asp Asn Pro Asp Gln Val Ala Phe Ala Val Val Gly Asp Gly Glu Ala
                165                 170                 175

Glu Thr Gly Pro Ser Met Ala Ser Trp His Ser Ile Lys Phe Leu Asn
            180                 185                 190

Ala Lys Asn Asp Gly Ala Val Leu Pro Val Leu Asp Leu Asn Gly Phe
        195                 200                 205

Lys Ile Ser Asn Pro Thr Ile Phe Ser Arg Met Ser Asp Glu Glu Ile
    210                 215                 220

Thr Lys Phe Phe Glu Gly Leu Gly Tyr Ser Pro Arg Phe Ile Glu Asn
225                 230                 235                 240

Asp Asp Ile His Asp Tyr Ala Thr Tyr His Gln Leu Ala Ala Asn Ile
                245                 250                 255

Leu Asp Gln Ala Ile Glu Asp Ile Gln Ala Ile Gln Asn Asp Ala Arg
```

260 265 270

Glu Asn Gly Lys Tyr Gln Asp Gly Glu Ile Pro Ala Trp Pro Val Ile
275 280 285

Ile Ala Arg Leu Pro Lys Gly Trp Gly Gly Pro Thr His Asp Ala Ser
290 295 300

Asn Asn Pro Ile Glu Asn Ser Phe Arg Ala His Gln Val Pro Leu Pro
305 310 315 320

Leu Glu Gln His Asp Leu Ala Thr Leu Pro Glu Phe Glu Asp Trp Met
325 330 335

Asn Ser Tyr Lys Pro Glu Glu Leu Phe Asn Ala Asp Gly Ser Leu Lys
340 345 350

Asp Glu Leu Lys Ala Ile Ala Pro Lys Gly Asp Lys Arg Met Ser Ala
355 360 365

Asn Pro Ile Thr Asn Gly Gly Ala Asp Arg Ser Asp Leu Lys Leu Pro
370 375 380

Asn Trp Arg Glu Phe Ala Asn Asp Ile Asn Asp Asp Thr Arg Gly Lys
385 390 395 400

Glu Phe Ala Asp Ser Lys Arg Asn Met Asp Met Ala Thr Leu Ser Asn
405 410 415

Tyr Leu Gly Ala Val Ser Gln Leu Asn Pro Thr Arg Phe Arg Phe Phe
420 425 430

Gly Pro Asp Glu Thr Met Ser Asn Arg Leu Trp Gly Leu Phe Asn Val
435 440 445

Thr Pro Arg Gln Trp Met Glu Glu Ile Lys Glu Pro Gln Asp Gln Leu
450 455 460

Leu Ser Pro Thr Gly Arg Ile Ile Asp Ser Gln Leu Ser Glu His Gln
465 470 475 480

Ala Glu Gly Trp Leu Glu Gly Tyr Thr Leu Thr Gly Arg Val Gly Ile
485 490 495

Phe Ala Ser Tyr Glu Ser Phe Leu Arg Val Val Asp Thr Met Val Thr
500 505 510

Gln His Phe Lys Trp Leu Arg His Ala Ser Glu Gln Ala Trp Arg Asn
515 520 525

Asp Tyr Pro Ser Leu Asn Leu Ile Ala Thr Ser Thr Ala Phe Gln Gln

```
    530                 535                 540

Asp His Asn Gly Tyr Thr His Gln Asp Pro Gly Met Leu Thr His Leu
545                 550                 555                 560

Ala Glu Lys Lys Ser Asn Phe Ile Arg Glu Tyr Leu Pro Ala Asp Gly
                565                 570                 575

Asn Ser Leu Leu Ala Val Gln Glu Arg Ala Phe Ser Glu Arg His Lys
            580                 585                 590

Val Asn Leu Leu Ile Ala Ser Lys Gln Pro Arg Gln Gln Trp Phe Thr
        595                 600                 605

Val Glu Glu Ala Glu Val Leu Ala Asn Glu Gly Leu Lys Ile Ile Asp
    610                 615                 620

Trp Ala Ser Thr Ala Pro Ser Ser Asp Val Asp Ile Thr Phe Ala Ser
625                 630                 635                 640

Ala Gly Thr Glu Pro Thr Ile Glu Thr Leu Ala Ala Leu Trp Leu Ile
                645                 650                 655

Asn Gln Ala Phe Pro Asp Val Lys Phe Arg Tyr Val Asn Val Val Glu
            660                 665                 670

Leu Leu Arg Leu Gln Lys Lys Ser Glu Pro Asn Met Asn Asp Glu Arg
        675                 680                 685

Glu Leu Ser Ala Glu Glu Phe Asn Lys Tyr Phe Gln Ala Asp Thr Pro
    690                 695                 700

Val Ile Phe Gly Phe His Ala Tyr Glu Asn Leu Ile Glu Ser Phe Phe
705                 710                 715                 720

Phe Glu Arg Lys Phe Thr Gly Asp Val Tyr Val His Gly Tyr Arg Glu
                725                 730                 735

Asp Gly Asp Ile Thr Thr Thr Tyr Asp Met Arg Val Tyr Ser His Leu
            740                 745                 750

Asp Arg Phe His Gln Ala Lys Glu Ala Ala Glu Ile Leu Ser Ala Asn
        755                 760                 765

Gly Lys Ile Asp Gln Ala Ala Ala Asp Thr Phe Ile Ala Lys Met Asp
    770                 775                 780

Asp Thr Leu Ala Lys His Phe Gln Val Thr Arg Asn Glu Gly Arg Asp
785                 790                 795                 800

Ile Glu Glu Phe Thr Asp Trp Thr Trp Ser Pro Leu Lys
```

805 810

<210> 3
<211> 1002
<212> DNA
<213> Clostridium kluyveri

<220>
<221> misc_feature
<222> (1)..(1002)
<223> Clostridium kluyveri Phosphotransacetylase (PTA) gene sequence

<400> 3
atgaaattaa tggaaaatat ttttggttta gccaaagcag ataagaaaaa aattgttttg 60

gcagaaggag aagaagaaag gaacattaga gcttccgaag aaataataag ggatggtatt 120

gcagatataa ttttagtagg aagtgaaagt gtaataaaag agaatgcagc taaatttggg 180

gttaacttag ctggagtgga aatagtagat cctgaaactt caagtaaaac tgcaggctat 240

gccaatgctt tttatgaaat tagaaagaat aaaggagtta cactggaaaa agcagataaa 300

atagttagag atcctatata ttttgcaaca atgatggtga aacttggaga tgcagatggt 360

ttagtttcag gtgcaataca tacaacggga gatcttttga gaccaggact tcaaatagtg 420

aagacagttc caggtgcttc tgtggtttcc agtgtatttt taatgagtgt accagattgt 480

gaatatggag aagatggatt cttgttattt gctgattgtg ctgtaaatgt atgtcctact 540

gctgaagaat tatcttcaat tgcaataact acagcagaaa ctgcaaaaaa tttgtgtaaa 600

atagaaccaa gagttgccat gctttcattt tctactatgg gaagtgctag tcatgaattg 660

gtagataaag ttacaaaagc aacaaaactt gctaaagaag ctagacctga tttggatata 720

gatggagaac ttcaattgga tgcttcccta gtaaaaaaag ttgcagactt aaaagctccg 780

ggcagtaaag tggcaggaaa agccaatgta cttatattcc ctgatataca agcaggaaat 840

ataggatata agttagttca aagatttgca aaagctgagg ctataggacc tatatgtcag 900

ggatttgcaa agcctataaa tgatttatca agaggctgca gcgttgatga tatagtaaag 960

gtagtggctg taactgcagt tcaagcacag gcacagggtt ag 1002

<210> 4
<211> 333
<212> PRT
<213> Clostridium kluyveri

<220>
<221> misc_feature
<222> (1)..(333)
<223> Clostridium kluyveri Phosphotransacetylase (PTA) protein sequence

<400> 4

Met Lys Leu Met Glu Asn Ile Phe Gly Leu Ala Lys Ala Asp Lys Lys
1 5 10 15

Lys Ile Val Leu Ala Glu Gly Glu Glu Glu Arg Asn Ile Arg Ala Ser

```
            20                  25                  30

Glu Glu Ile Ile Arg Asp Gly Ile Ala Asp Ile Ile Leu Val Gly Ser
        35                  40                  45

Glu Ser Val Ile Lys Glu Asn Ala Ala Lys Phe Gly Val Asn Leu Ala
    50                  55                  60

Gly Val Glu Ile Val Asp Pro Glu Thr Ser Ser Lys Thr Ala Gly Tyr
65                  70                  75                  80

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

Lys Ala Asp Lys Ile Val Arg Asp Pro Ile Tyr Phe Ala Thr Met Met
            100                 105                 110

Val Lys Leu Gly Asp Ala Asp Gly Leu Val Ser Gly Ala Ile His Thr
        115                 120                 125

Thr Gly Asp Leu Leu Arg Pro Gly Leu Gln Ile Val Lys Thr Val Pro
    130                 135                 140

Gly Ala Ser Val Val Ser Ser Val Phe Leu Met Ser Val Pro Asp Cys
145                 150                 155                 160

Glu Tyr Gly Glu Asp Gly Phe Leu Leu Phe Ala Asp Cys Ala Val Asn
                165                 170                 175

Val Cys Pro Thr Ala Glu Glu Leu Ser Ser Ile Ala Ile Thr Thr Ala
            180                 185                 190

Glu Thr Ala Lys Asn Leu Cys Lys Ile Glu Pro Arg Val Ala Met Leu
        195                 200                 205

Ser Phe Ser Thr Met Gly Ser Ala Ser His Glu Leu Val Asp Lys Val
    210                 215                 220

Thr Lys Ala Thr Lys Leu Ala Lys Glu Ala Arg Pro Asp Leu Asp Ile
225                 230                 235                 240

Asp Gly Glu Leu Gln Leu Asp Ala Ser Leu Val Lys Lys Val Ala Asp
                245                 250                 255

Leu Lys Ala Pro Gly Ser Lys Val Ala Gly Lys Ala Asn Val Leu Ile
            260                 265                 270

Phe Pro Asp Ile Gln Ala Gly Asn Ile Gly Tyr Lys Leu Val Gln Arg
        275                 280                 285

Phe Ala Lys Ala Glu Ala Ile Gly Pro Ile Cys Gln Gly Phe Ala Lys
```

290 295 300

Pro Ile Asn Asp Leu Ser Arg Gly Cys Ser Val Asp Asp Ile Val Lys
305 310 315 320

Val Val Ala Val Thr Ala Val Gln Ala Gln Ala Gln Gly
325 330

<210> 5
<211> 753
<212> DNA
<213> S. cerevisiae

<220>
<221> misc_feature
<222> (1)..(753)
<223> Nucleotide sequence of GPP1/RHR2 of S. cerevisiae

<400> 5
atgcctttga ccacaaaacc tttatctttg aaaatcaacg ccgctctatt cgatgttgac 60

ggtaccatca tcatctctca accagccatt gctgctttct ggagagattt cggtaaagac 120

aagccttact tcgatgccga acacgttatt cacatctctc acggttggag aacttacgat 180

gccattgcca agttcgctcc agactttgct gatgaagaat acgttaacaa gctagaaggt 240

gaaatcccag aaaagtacgg tgaacactcc atcgaagttc caggtgctgt caagttgtgt 300

aatgctttga acgccttgcc aaaggaaaaa tgggctgtcg ccacctctgg tacccgtgac 360

atggccaaga aatggttcga cattttgaag atcaagagac cagaatactt catcaccgcc 420

aatgatgtca agcaaggtaa gcctcaccca gaaccatact taaagggtag aaacggtttg 480

ggtttcccaa ttaatgaaca agacccatcc aaatctaagg ttgttgtctt tgaagacgca 540

ccagctggta ttgctgctgg taaggctgct ggctgtaaaa tcgttggtat tgctaccact 600

ttcgatttgg acttcttgaa ggaaaagggt tgtgacatca ttgtcaagaa ccacgaatct 660

atcagagtcg gtgaatacaa cgctgaaacc gatgaagtcg aattgatctt tgatgactac 720

ttatacgcta aggatgactt gttgaaatgg taa 753

<210> 6
<211> 250
<212> PRT
<213> S. cerevisiae

<220>
<221> misc_feature
<222> (1)..(250)
<223> Protein sequence of Gpp1/Rhr2 of S. cerevisiae

<400> 6

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

Phe Asp Val Asp Gly Thr Ile Ile Ile Ser Gln Pro Ala Ile Ala Ala

20 25 30

Phe Trp Arg Asp Phe Gly Lys Asp Lys Pro Tyr Phe Asp Ala Glu His
35 40 45

Val Ile His Ile Ser His Gly Trp Arg Thr Tyr Asp Ala Ile Ala Lys
50 55 60

Phe Ala Pro Asp Phe Ala Asp Glu Glu Tyr Val Asn Lys Leu Glu Gly
65 70 75 80

Glu Ile Pro Glu Lys Tyr Gly Glu His Ser Ile Glu Val Pro Gly Ala
85 90 95

Val Lys Leu Cys Asn Ala Leu Asn Ala Leu Pro Lys Glu Lys Trp Ala
100 105 110

Val Ala Thr Ser Gly Thr Arg Asp Met Ala Lys Lys Trp Phe Asp Ile
115 120 125

Leu Lys Ile Lys Arg Pro Glu Tyr Phe Ile Thr Ala Asn Asp Val Lys
130 135 140

Gln Gly Lys Pro His Pro Glu Pro Tyr Leu Lys Gly Arg Asn Gly Leu
145 150 155 160

Gly Phe Pro Ile Asn Glu Gln Asp Pro Ser Lys Ser Lys Val Val Val
165 170 175

Phe Glu Asp Ala Pro Ala Gly Ile Ala Ala Gly Lys Ala Ala Gly Cys
180 185 190

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

Lys Gly Cys Asp Ile Ile Val Lys Asn His Glu Ser Ile Arg Val Gly
210 215 220

Glu Tyr Asn Ala Glu Thr Asp Glu Val Glu Leu Ile Phe Asp Asp Tyr
225 230 235 240

Leu Tyr Ala Lys Asp Asp Leu Leu Lys Trp
245 250

<210> 7
<211> 753
<212> DNA
<213> S. cerevisiae

<220>
<221> misc_feature
<222> (1)..(753)

<223> Nucleotide sequence of GPP2/HOR2 of S. cerevisiae

<400> 7
atgggattga ctactaaacc tctatctttg aaagttaacg ccgctttgtt cgacgtcgac 60

ggtaccatta tcatctctca accagccatt gctgcattct ggagggattt cggtaaggac 120

aaaccttatt tcgatgctga acacgttatc caagtctcgc atggttggag aacgtttgat 180

gccattgcta agttcgctcc agactttgcc aatgaagagt atgttaacaa attagaagct 240

gaaattccgg tcaagtacgg tgaaaaatcc attgaagtcc caggtgcagt taagctgtgc 300

aacgctttga acgctctacc aaaagagaaa tgggctgtgg caacttccgg tacccgtgat 360

atggcacaaa aatggttcga gcatctggga atcaggagac caaagtactt cattaccgct 420

aatgatgtca aacagggtaa gcctcatcca gaaccatatc tgaagggcag gaatggctta 480

ggatatccga tcaatgagca agacccttcc aaatctaagg tagtagtatt tgaagacgct 540

ccagcaggta ttgccgccgg aaaagccgcc ggttgtaaga tcattggtat tgccactact 600

ttcgacttgg acttcctaaa ggaaaaaggc tgtgacatca ttgtcaaaaa ccacgaatcc 660

atcagagttg gcggctacaa tgccgaaaca gacgaagttg aattcatttt tgacgactac 720

ttatatgcta aggacgatct gttgaaatgg taa 753

<210> 8
<211> 250
<212> PRT
<213> S. cerevisiae

<220>
<221> misc_feature
<222> (1)..(250)
<223> Protein sequence of Gpp2/Hor2 of S. cerevisiae

<400> 8

Met Gly Leu Thr Thr Lys Pro Leu Ser Leu Lys Val Asn Ala Ala Leu
1 5 10 15

Phe Asp Val Asp Gly Thr Ile Ile Ile Ser Gln Pro Ala Ile Ala Ala
20 25 30

Phe Trp Arg Asp Phe Gly Lys Asp Lys Pro Tyr Phe Asp Ala Glu His
35 40 45

Val Ile Gln Val Ser His Gly Trp Arg Thr Phe Asp Ala Ile Ala Lys
50 55 60

Phe Ala Pro Asp Phe Ala Asn Glu Glu Tyr Val Asn Lys Leu Glu Ala
65 70 75 80

Glu Ile Pro Val Lys Tyr Gly Glu Lys Ser Ile Glu Val Pro Gly Ala
85 90 95

Val Lys Leu Cys Asn Ala Leu Asn Ala Leu Pro Lys Glu Lys Trp Ala

100 105 110

Val Ala Thr Ser Gly Thr Arg Asp Met Ala Gln Lys Trp Phe Glu His
115 120 125

Leu Gly Ile Arg Arg Pro Lys Tyr Phe Ile Thr Ala Asn Asp Val Lys
130 135 140

Gln Gly Lys Pro His Pro Glu Pro Tyr Leu Lys Gly Arg Asn Gly Leu
145 150 155 160

Gly Tyr Pro Ile Asn Glu Gln Asp Pro Ser Lys Ser Lys Val Val Val
165 170 175

Phe Glu Asp Ala Pro Ala Gly Ile Ala Ala Gly Lys Ala Ala Gly Cys
180 185 190

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

Lys Gly Cys Asp Ile Ile Val Lys Asn His Glu Ser Ile Arg Val Gly
210 215 220

Gly Tyr Asn Ala Glu Thr Asp Glu Val Glu Phe Ile Phe Asp Asp Tyr
225 230 235 240

Leu Tyr Ala Lys Asp Asp Leu Leu Lys Trp
245 250

<210> 9
<211> 1380
<212> DNA
<213> Dickeya zeae

<220>
<221> misc_feature
<222> (1)..(1380)
<223> Dickeya zeae eutE gene sequence

<400> 9
atggagcatt cagttatcga accgacagtg cccatgccgc tgccagccat gtttgacgcg 60

ccatctggaa tcttttctag cctggacgat gcagtccagg cggcaaccct ggcacaacaa 120

cagttgtcgt ctgtggagtt acgccagcaa gttattaaag caattagagt tgcaggcgaa 180

cgctatgcac aggttctggc ggaaatggcg gtggctgaaa caggtatggg tcgggtagtg 240

gataaataca ttaaaaatgt ttcacaggct cgccatacac ccggcattga atgtctgagc 300

gcggaagttc tgacaggcga caatggcctg acactgattg aaaatgcccc ttggggagtg 360

gtggcttccg tgacgccaag cacgaaccca gccgccacag tcatcaataa tgcaatttcc 420

atgattgcgg cagggaattc agtcgttttt gcaccgcacc catccgccaa aaatgtgtcc 480

ttacgcacaa tatcgcttct taacaaagca attgtggcga caggtgggcc agaaaatctg 540

ctggtatccg tcgcaaatcc caacatcgaa acagctcaac gcctgttccg ttatccaggt 600

attggattac tcgtcgtaac aggtggtgag gcggtggtgg aagcggcgcg caaacacact 660

gataaacgtt taattgccgc aggcgccgga aacccccag tagtcgttga cgaaacagcg 720

gatataccga aagccgctcg cgcaatagta aagggcgctt cgtttgacaa caatattatt 780

tgtgccgacg agaaagtatt aatcgtggtt gatcgcgtag ccgacgcctt attagccgaa 840

atgcaacgca acaatgctgt tttactgacg cctgaacaga cagaacgact tctgcccgct 900

ttgctgagcg atatagatga gcaggggaag ggacgcgtga accgcgatta tgtggggagg 960

gatgccgcta aactagcggc ggccattggt ttagaagtgt cagaacacac aagattatta 1020

cttgctgaaa cagatgctga tcatcctttt gcagtaaccg aattaatgat gcccgtattg 1080

cctgttatcc gtgtaaaaaa cgttgatgac gccattgccc tcgctgtaaa acttgagagt 1140

ggttgtcgtc acactgcagc aatgcattcg acaaacatta ggaacctgaa tcggatggca 1200

aatgctataa atacatcaat ttttgttaaa aatggtccgt gtatcgctgg gctgggcctg 1260

ggtggcgagg gctggacgtc gatgactata tctacaccca caggggaagg agttacctca 1320

gcacgcacct tcgtacgttt acgtagatgt gtattggttg acatgttcag aatcgcgtaa 1380

<210> 10
<211> 459
<212> PRT
<213> Dickeya zeae

<220>
<221> misc_feature
<222> (1)..(459)
<223> Dickeya zeae eutE protein sequence

<400> 10

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

Met Phe Asp Ala Pro Ser Gly Ile Phe Ser Ser Leu Asp Asp Ala Val
20 25 30

Gln Ala Ala Thr Leu Ala Gln Gln Gln Leu Ser Ser Val Glu Leu Arg
35 40 45

Gln Gln Val Ile Lys Ala Ile Arg Val Ala Gly Glu Arg Tyr Ala Gln
50 55 60

Val Leu Ala Glu Met Ala Val Ala Glu Thr Gly Met Gly Arg Val Val
65 70 75 80

Asp Lys Tyr Ile Lys Asn Val Ser Gln Ala Arg His Thr Pro Gly Ile
85 90 95

Glu Cys Leu Ser Ala Glu Val Leu Thr Gly Asp Asn Gly Leu Thr Leu

100 105 110

Ile Glu Asn Ala Pro Trp Gly Val Val Ala Ser Val Thr Pro Ser Thr
115 120 125

Asn Pro Ala Ala Thr Val Ile Asn Asn Ala Ile Ser Met Ile Ala Ala
130 135 140

Gly Asn Ser Val Val Phe Ala Pro His Pro Ser Ala Lys Asn Val Ser
145 150 155 160

Leu Arg Thr Ile Ser Leu Leu Asn Lys Ala Ile Val Ala Thr Gly Gly
165 170 175

Pro Glu Asn Leu Leu Val Ser Val Ala Asn Pro Asn Ile Glu Thr Ala
180 185 190

Gln Arg Leu Phe Arg Tyr Pro Gly Ile Gly Leu Leu Val Val Thr Gly
195 200 205

Gly Glu Ala Val Val Glu Ala Ala Arg Lys His Thr Asp Lys Arg Leu
210 215 220

Ile Ala Ala Gly Ala Gly Asn Pro Pro Val Val Val Asp Glu Thr Ala
225 230 235 240

Asp Ile Pro Lys Ala Ala Arg Ala Ile Val Lys Gly Ala Ser Phe Asp
245 250 255

Asn Asn Ile Ile Cys Ala Asp Glu Lys Val Leu Ile Val Val Asp Arg
260 265 270

Val Ala Asp Ala Leu Leu Ala Glu Met Gln Arg Asn Asn Ala Val Leu
275 280 285

Leu Thr Pro Glu Gln Thr Glu Arg Leu Leu Pro Ala Leu Leu Ser Asp
290 295 300

Ile Asp Glu Gln Gly Lys Gly Arg Val Asn Arg Asp Tyr Val Gly Arg
305 310 315 320

Asp Ala Ala Lys Leu Ala Ala Ala Ile Gly Leu Glu Val Ser Glu His
325 330 335

Thr Arg Leu Leu Leu Ala Glu Thr Asp Ala Asp His Pro Phe Ala Val
340 345 350

Thr Glu Leu Met Met Pro Val Leu Pro Val Ile Arg Val Lys Asn Val
355 360 365

Asp Asp Ala Ile Ala Leu Ala Val Lys Leu Glu Ser Gly Cys Arg His

370 375 380

Thr Ala Ala Met His Ser Thr Asn Ile Arg Asn Leu Asn Arg Met Ala
385 390 395 400

Asn Ala Ile Asn Thr Ser Ile Phe Val Lys Asn Gly Pro Cys Ile Ala
405 410 415

Gly Leu Gly Leu Gly Gly Glu Gly Trp Thr Ser Met Thr Ile Ser Thr
420 425 430

Pro Thr Gly Glu Gly Val Thr Ser Ala Arg Thr Phe Val Arg Leu Arg
435 440 445

Arg Cys Val Leu Val Asp Met Phe Arg Ile Ala
450 455

<210> 11
<211> 1798
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(1798)
<223> Saccharomyces cerevisiae ALD4 nucleotide sequence

<400> 11
gcacccaggg acacacagca gcgaagtatt ttcagaatgt tcagtagatc tacgctctgc 60

ttaaagacgt ctgcatcctc cattgggaga cttcaattga gatatttctc acaccttcct 120

atgacagtgc ctatcaagct gcccaatggg ttggaatatg agcaaccaac ggggttgttc 180

atcaacaaca agtttgttcc ttctaaacag aacaagacct tcgaagtcat taacccttcc 240

acggaagaag aaatatgtca tatttatgaa ggtagagagg acgatgtgga agaggccgtg 300

caggccgccg accgtgcctt ctctaatggg tcttggaacg gtatcgaccc tattgacagg 360

ggtaaggctt tgtacaggtt agccgaatta attgaacagg acaaggatgt cattgcttcc 420

atcgagactt tggataacgg taaagctatc tcttcctcga gaggagatgt tgatttagtc 480

atcaactatt tgaaatcttc tgctggcttt gctgataaaa ttgatggtag aatgattgat 540

actggtagaa cccatttttc ttacactaag agacagcctt tgggtgtttg tgggcagatt 600

attccttgga atttcccact gttgatgtgg gcctggaaga ttgcccctgc tttggtcacc 660

ggtaacaccg tcgtgttgaa gactgccgaa tccaccccat tgtccgcttt gtatgtgtct 720

aaatacatcc cacaggcggg tattccacct ggtgtgatca acattgtatc cgggtttggt 780

aagattgtgg gtgaggccat tacaaaccat ccaaaaatca aaaaggttgc cttcacaggg 840

tccacggcta cgggtagaca catttaccag tccgcagccg caggcttgaa aaaagtgact 900

ttggagctgg gtggtaaatc accaaacatt gtcttcgcgg acgccgagtt gaaaaaagcc 960

gtgcaaaaca ttatccttgg tatctactac aattctggtg aggtctgttg tgcgggttca 1020

agggtgtatg ttgaagaatc tatttacgac aaattcattg aagagttcaa agccgcttct 1080

gaatccatca aggtgggcga cccattcgat gaatctactt tccaaggtgc acaaacctct 1140

caaatgcaac taaacaaaat cttgaaatac gttgacattg gtaagaatga aggtgctact 1200

ttgattaccg gtggtgaaag attaggtagc aagggttact tcattaagcc aactgtcttt 1260

ggtgacgtta aggaagacat gagaattgtc aaagaggaaa tctttggccc tgttgtcact 1320

gtaaccaaat tcaaatctgc cgacgaagtc attaacatgg cgaacgattc tgaatacggg 1380

ttggctgctg gtattcacac ctctaatatt aataccgcct taaaagtggc tgatagagtt 1440

aatgcgggta cggtctggat aaacacttat aacgatttcc accacgcagt tcctttcggt 1500

gggttcaatg catctggttt gggcagggaa atgtctgttg atgctttaca aaactacttg 1560

caagttaaag cggtccgtgc caaattggac gagtaaggtc atcaataagc ctggtgtcca 1620

atcgatgctt acatacataa aattaaatat tctgtctctg ttatatttcc acatgtcatc 1680

atttcaaata tatgtacttt aaagaaaata aaataaaaaa taaaattttt ttctcccgat 1740

aatcaatttt cttaattaat taattgcgtt acgaaacgcg atcgccgacg ccgccgat 1798

<210> 12
<211> 519
<212> PRT
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(519)
<223> Saccharomyces cerevisiae ALD4 protein sequence

<400> 12

Met Phe Ser Arg Ser Thr Leu Cys Leu Lys Thr Ser Ala Ser Ser Ile
1 5 10 15

Gly Arg Leu Gln Leu Arg Tyr Phe Ser His Leu Pro Met Thr Val Pro
20 25 30

Ile Lys Leu Pro Asn Gly Leu Glu Tyr Glu Gln Pro Thr Gly Leu Phe
35 40 45

Ile Asn Asn Lys Phe Val Pro Ser Lys Gln Asn Lys Thr Phe Glu Val
50 55 60

Ile Asn Pro Ser Thr Glu Glu Glu Ile Cys His Ile Tyr Glu Gly Arg
65 70 75 80

Glu Asp Asp Val Glu Glu Ala Val Gln Ala Ala Asp Arg Ala Phe Ser
85 90 95

Asn Gly Ser Trp Asn Gly Ile Asp Pro Ile Asp Arg Gly Lys Ala Leu
100 105 110

```
Tyr Arg Leu Ala Glu Leu Ile Glu Gln Asp Lys Asp Val Ile Ala Ser
        115                 120                 125

Ile Glu Thr Leu Asp Asn Gly Lys Ala Ile Ser Ser Ser Arg Gly Asp
    130                 135                 140

Val Asp Leu Val Ile Asn Tyr Leu Lys Ser Ser Ala Gly Phe Ala Asp
145                 150                 155                 160

Lys Ile Asp Gly Arg Met Ile Asp Thr Gly Arg Thr His Phe Ser Tyr
                165                 170                 175

Thr Lys Arg Gln Pro Leu Gly Val Cys Gly Gln Ile Ile Pro Trp Asn
            180                 185                 190

Phe Pro Leu Leu Met Trp Ala Trp Lys Ile Ala Pro Ala Leu Val Thr
        195                 200                 205

Gly Asn Thr Val Val Leu Lys Thr Ala Glu Ser Thr Pro Leu Ser Ala
    210                 215                 220

Leu Tyr Val Ser Lys Tyr Ile Pro Gln Ala Gly Ile Pro Pro Gly Val
225                 230                 235                 240

Ile Asn Ile Val Ser Gly Phe Gly Lys Ile Val Gly Glu Ala Ile Thr
                245                 250                 255

Asn His Pro Lys Ile Lys Lys Val Ala Phe Thr Gly Ser Thr Ala Thr
            260                 265                 270

Gly Arg His Ile Tyr Gln Ser Ala Ala Ala Gly Leu Lys Lys Val Thr
        275                 280                 285

Leu Glu Leu Gly Gly Lys Ser Pro Asn Ile Val Phe Ala Asp Ala Glu
    290                 295                 300

Leu Lys Lys Ala Val Gln Asn Ile Ile Leu Gly Ile Tyr Tyr Asn Ser
305                 310                 315                 320

Gly Glu Val Cys Cys Ala Gly Ser Arg Val Tyr Val Glu Glu Ser Ile
                325                 330                 335

Tyr Asp Lys Phe Ile Glu Glu Phe Lys Ala Ala Ser Glu Ser Ile Lys
            340                 345                 350

Val Gly Asp Pro Phe Asp Glu Ser Thr Phe Gln Gly Ala Gln Thr Ser
        355                 360                 365

Gln Met Gln Leu Asn Lys Ile Leu Lys Tyr Val Asp Ile Gly Lys Asn
    370                 375                 380
```

```
Glu Gly Ala Thr Leu Ile Thr Gly Gly Glu Arg Leu Gly Ser Lys Gly
385                 390                 395                 400

Tyr Phe Ile Lys Pro Thr Val Phe Gly Asp Val Lys Glu Asp Met Arg
                405                 410                 415

Ile Val Lys Glu Glu Ile Phe Gly Pro Val Val Thr Val Thr Lys Phe
            420                 425                 430

Lys Ser Ala Asp Glu Val Ile Asn Met Ala Asn Asp Ser Glu Tyr Gly
        435                 440                 445

Leu Ala Ala Gly Ile His Thr Ser Asn Ile Asn Thr Ala Leu Lys Val
    450                 455                 460

Ala Asp Arg Val Asn Ala Gly Thr Val Trp Ile Asn Thr Tyr Asn Asp
465                 470                 475                 480

Phe His His Ala Val Pro Phe Gly Gly Phe Asn Ala Ser Gly Leu Gly
                485                 490                 495

Arg Glu Met Ser Val Asp Ala Leu Gln Asn Tyr Leu Gln Val Lys Ala
            500                 505                 510

Val Arg Ala Lys Leu Asp Glu
        515
```

<210> 13
<211> 2744
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(2744)
<223> Saccharomyces cerevisiae cytosolic aldehyde dehydrogenase 6 (ALD6) nucleotide sequence

<400> 13

```
catatggcgt atccaagccg aaacccttg cctcatcccc acggaataag gcagccgaca       60

aaagaaaaac gaccgaaaag gaaccagaaa gaaaaaagag ggtgggcgcg ccgcggacgt      120

gtaaaaagat atgcatccag cttctatatc gctttaactt taccgttttg ggcatcggga      180

acgtatgtaa cattgatctc ctcttgggaa cggtgagtgc aacagatgcg atatagcacc      240

gaccatgtgg gcaaattcgt aataaattcg gggtgagggg gattcaagac aagcaacctt      300

gttagtcagc tcaaacagcg atttaacggt tgagtaacac atcaaaacac cgttcgaggt      360

caagcctggc gtgtttaaca agttcttgat atcatatata aatgtaataa gaagtttggt      420

aatattcaat tcgaagtgtt cagtctttta cttctcttgt tttatagaag aaaaaacatc      480

aagaaacatc tttaacatac acaaacacat actatcagaa tacaatgact aagctacact      540
```

ttgacactgc tgaaccagtc aagatcacac ttccaaatgg tttgacatac gagcaaccaa 600

ccggtctatt cattaacaac aagtttatga aagctcaaga cggtaagacc tatcccgtcg 660

aagatccttc cactgaaaac accgtttgtg aggtctcttc tgccaccact gaagatgttg 720

aatatgctat cgaatgtgcc gaccgtgctt tccacgacac tgaatgggct acccaagacc 780

caagagaaag aggccgtcta ctaagtaagt tggctgacga attggaaagc caaattgact 840

tggtttcttc cattgaagct ttggacaatg gtaaaacttt ggcctttaag gcccgtgggg 900

atgttaccat tgcaatcaac tgtctaagag atgctgctgc ctatgccgac aaagtcaacg 960

gtagaacaat caacaccggt gacggctaca tgaacttcac caccttagag ccaatcggtg 1020

tctgtggtca aattattcca tggaactttc caataatgat gttggcttgg aagatcgccc 1080

cagcattggc catgggtaac gtctgtatct tgaaacccgc tgctgtcaca cctttaaatg 1140

ccctatactt tgcttcttta tgtaagaagg ttggtattcc agctggtgtc gtcaacatcg 1200

ttccaggtcc tggtagaact gttggtgctg ctttgaccaa cgacccaaga atcagaaagc 1260

tggcttttac cggttctaca gaagtcggta agagtgttgc tgtcgactct tctgaatcta 1320

acttgaagaa aatcactttg gaactaggtg gtaagtccgc ccatttggtc tttgacgatg 1380

ctaacattaa gaagacttta ccaaatctag taaacggtat tttcaagaac gctggtcaaa 1440

tttgttcctc tggttctaga atttacgttc aagaaggtat ttacgacgaa ctattggctg 1500

ctttcaaggc ttacttggaa accgaaatca aagttggtaa tccatttgac aaggctaact 1560

tccaaggtgc tatcactaac cgtcaacaat tcgacacaat tatgaactac atcgatatcg 1620

gtaagaaaga aggcgccaag atcttaactg gtggcgaaaa agttggtgac aagggttact 1680

tcatcagacc aaccgttttc tacgatgtta atgaagacat gagaattgtt aaggaagaaa 1740

tttttggacc agttgtcact gtcgcaaagt tcaagacttt agaagaaggt gtcgaaatgg 1800

ctaacagctc tgaattcggt ctaggttctg gtatcgaaac agaatctttg agcacaggtt 1860

tgaaggtggc caagatgttg aaggccggta ccgtctggat caacacatac aacgattttg 1920

actccagagt tccattcggt ggtgttaagc aatctggtta cggtagagaa atgggtgaag 1980

aagtctacca tgcatacact gaagtaaaag ctgtcagaat taagttgtaa tgtaccaacc 2040

tgcatttctt tccgtcatat acacaaaata ctttcatata aacttacttg gtcttacgtc 2100

ataaataaat atgtatacat ataaattaaa aaatttggtt ttatattttt acaaaaagaa 2160

tcgtttactt catttctccc ttttaagcga tacaatccat gaaaaaagag aaaaagagag 2220

aacaggcttg tgccttcttt aaaacatccc acacaaaatc atattgaatt gaattttaca 2280

tcttaagcta gtgtacaaca actgctatat ccaaagaaaa ctaacgtgga ccgcttttag 2340

agttgagaaa aaggtttgaa aaaaatagca atacaaagac ttgtttcata tataaaatac 2400

agggagcaca ttgagctaat ataacataaa cactgcgaac caattccaat caaaaggtac 2460

acatgagagc attcccccga gtactgccat ttcgccatca gagatcatat aataacatcc 2520

ttcttcgaac agtaaggctt tttggttcat cactttcttc ttttgatttc tctaggcaaa 2580

tgcctaaggt ggaccctgac aataccgctg caatgctact acagaaaaac ttgatccaaa 2640

gaaacaacat gctctatggg tatggatcag ggacaatacg atgtactttg ctagactcaa 2700

ctggacgagc caaatcacca ttagtagaga taaaacgtga ggat 2744

<210> 14
<211> 500
<212> PRT
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(500)
<223> Saccharomyces cerevisiae cytosolic aldehyde dehydrogenase 6 (ALD6) protein sequence

<400> 14

Met Thr Lys Leu His Phe Asp Thr Ala Glu Pro Val Lys Ile Thr Leu
1 5 10 15

Pro Asn Gly Leu Thr Tyr Glu Gln Pro Thr Gly Leu Phe Ile Asn Asn
20 25 30

Lys Phe Met Lys Ala Gln Asp Gly Lys Thr Tyr Pro Val Glu Asp Pro
35 40 45

Ser Thr Glu Asn Thr Val Cys Glu Val Ser Ser Ala Thr Thr Glu Asp
50 55 60

Val Glu Tyr Ala Ile Glu Cys Ala Asp Arg Ala Phe His Asp Thr Glu
65 70 75 80

Trp Ala Thr Gln Asp Pro Arg Glu Arg Gly Arg Leu Leu Ser Lys Leu
85 90 95

Ala Asp Glu Leu Glu Ser Gln Ile Asp Leu Val Ser Ser Ile Glu Ala
100 105 110

Leu Asp Asn Gly Lys Thr Leu Ala Leu Ala Arg Gly Asp Val Thr Ile
115 120 125

Ala Ile Asn Cys Leu Arg Asp Ala Ala Ala Tyr Ala Asp Lys Val Asn
130 135 140

Gly Arg Thr Ile Asn Thr Gly Asp Gly Tyr Met Asn Phe Thr Thr Leu
145 150 155 160

Glu Pro Ile Gly Val Cys Gly Gln Ile Ile Pro Trp Asn Phe Pro Ile
165 170 175

Met Met Leu Ala Trp Lys Ile Ala Pro Ala Leu Ala Met Gly Asn Val
180 185 190

```
Cys Ile Leu Lys Pro Ala Ala Val Thr Pro Leu Asn Ala Leu Tyr Phe
        195                 200                 205

Ala Ser Leu Cys Lys Lys Val Gly Ile Pro Ala Gly Val Val Asn Ile
    210                 215                 220

Val Pro Gly Pro Gly Arg Thr Val Gly Ala Ala Leu Thr Asn Asp Pro
225                 230                 235                 240

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

Val Ala Val Asp Ser Ser Glu Ser Asn Leu Lys Lys Ile Thr Leu Glu
            260                 265                 270

Leu Gly Gly Lys Ser Ala His Leu Val Phe Asp Asp Ala Asn Ile Lys
        275                 280                 285

Lys Thr Leu Pro Asn Leu Val Asn Gly Ile Phe Lys Asn Ala Gly Gln
    290                 295                 300

Ile Cys Ser Ser Gly Ser Arg Ile Tyr Val Gln Glu Gly Ile Tyr Asp
305                 310                 315                 320

Glu Leu Leu Ala Ala Phe Lys Ala Tyr Leu Glu Thr Glu Ile Lys Val
                325                 330                 335

Gly Asn Pro Phe Asp Lys Ala Asn Phe Gln Gly Ala Ile Thr Asn Arg
            340                 345                 350

Gln Gln Phe Asp Thr Ile Met Asn Tyr Ile Asp Ile Gly Lys Lys Glu
        355                 360                 365

Gly Ala Lys Ile Leu Thr Gly Gly Glu Lys Val Gly Asp Lys Gly Tyr
    370                 375                 380

Phe Ile Arg Pro Thr Val Phe Tyr Asp Val Asn Glu Asp Met Arg Ile
385                 390                 395                 400

Val Lys Glu Glu Ile Phe Gly Pro Val Val Thr Val Ala Lys Phe Lys
                405                 410                 415

Thr Leu Glu Glu Gly Val Glu Met Ala Asn Ser Ser Glu Phe Gly Leu
            420                 425                 430

Gly Ser Gly Ile Glu Thr Glu Ser Leu Ser Thr Gly Leu Lys Val Ala
        435                 440                 445

Lys Met Leu Lys Ala Gly Thr Val Trp Ile Asn Thr Tyr Asn Asp Phe
    450                 455                 460
```

Asp Ser Arg Val Pro Phe Gly Gly Val Lys Gln Ser Gly Tyr Gly Arg
465 470 475 480

Glu Met Gly Glu Glu Val Tyr His Ala Tyr Thr Glu Val Lys Ala Val
485 490 495

Arg Ile Lys Leu
500

<210> 15
<211> 2728
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(2728)
<223> Saccharomyces cerevisiae ACS1 nucleotide sequence

<400> 15
acctcccgcg acctccaaaa tcgaactacc ttcacaatgt cgccctctgc cgtacaatca 60

tcaaaactag aagaacagtc aagtgaaatt gacaagttga aagcaaaaat gtcccagtct 120

gcctccactg cgcagcagaa gaaggaacat gagtatgaac atttgacctc ggtcaagatc 180

gtgccacaac ggcccatctc agatagactg cagcccgcaa ttgctaccca ctattctcca 240

cacttggacg ggttgcagga ctatcagcgc ttgcacaagg agtctattga agaccctgct 300

aagttcttcg gttctaaagc tacccaattt ttaaactggt ctaagccatt cgataaggtg 360

ttcatcccag actctaaaac gggtaggccc tccttccaga acaatgcatg gttcctcaac 420

ggccaattaa acgcctgtta caactgtgtt gacagacatg ccttgaagac ccctaacaag 480

aaagccatta ttttcgaagg tgacgagcct ggccaaggct attccattac ctacaaggaa 540

ctacttgaag aagtttgtca agtggcacaa gtgctgactt actctatggg cgttcgcaag 600

ggcgatactg ttgccgtgta catgcctatg gtcccagaag caatcataac cttgttggcc 660

atttcccgta tcggcgccat tcactccgta gtctttgccg ggttttcttc caactccttg 720

agagatcgta tcaacgatgg ggactctaaa gttgtcatca ctacagatga atccaacaga 780

ggtggtaaag tcattgagac taaaagaatt gttgatgacg cgctaagaga gaccccaggc 840

gtgagacacg tcttggttta tagaaagacc aacaatccat ctgttgcttt ccatgccccc 900

agagatttag attgggcaac agaaaagaag aaatacaaga cctactatcc atgcacaccc 960

gttgattctg aggatccatt attcttgttg tatacgtctg gttctactgg tgcccccaag 1020

ggtgttcaac attctaccgc aggttacttg ctgggagctt tgttgaccat gcgctacact 1080

tttgacactc accaagaaga cgttttcttc acagctggag acattggctg gattacaggc 1140

cacacttatg tggtttatgg tcccttacta tatggttgtg ccactttggt ctttgaaggg 1200

actcctgcgt acccaaatta ctcccgttat tgggatatta ttgatgaaca caaagtcacc 1260

caattttatg ttgccccaac tgctttgcgt ttgttgaaaa gagctggtga ttcctacatc 1320

gaaaatcatt ccttaaaatc tttgcgttgc ttgggttcgg tcggtgaacc aattgctgct 1380

gaagtttggg agtggtactc tgaaaaaata ggtaaaaatg aaatccccat tgtagacacc 1440

tactggcaaa cagaatctgg ttcgcatctg gtcaccccgc tggctggtgg tgtcacacca 1500

atgaaaccgg gttctgcctc attccccttc ttcggtattg atgcagttgt tcttgaccct 1560

aacactggtg aagaacttaa taccagccac gcagagggtg tccttgccgt caaagctgca 1620

tggccatcat ttgcaagaac tatttggaaa aatcatgata ggtatctaga cacttatttg 1680

aacccttacc ctggctacta tttcactggt gatggtgctg caaaggataa ggatggttat 1740

atctggattt tgggtcgtgt agacgatgtg gtgaacgtct ctggtcaccg tctgtctacc 1800

gctgaaattg aggctgctat tatcgaagat ccaattgtgg ccgagtgtgc tgttgtcgga 1860

ttcaacgatg acttgactgg tcaagcagtt gctgcatttg tggtgttgaa aaacaaatct 1920

aattggtcca ccgcaacaga tgatgaatta caagatatca agaagcattt ggtctttact 1980

gttagaaaag acatcgggcc atttgccgca ccaaaattga tcattttagt ggatgacttg 2040

cccaagacaa gatctggcaa aattatgaga cgtattttaa gaaaaatcct agcaggagaa 2100

agtgaccaac taggcgacgt ttctacattg tcaaaccctg gcattgttag acatctaatt 2160

gattcggtca agttgtaatg atgatttctt tccttttttat attgacgact ttttttttt 2220

cgtgtgtttt tgttctctta taaccgagct gcttacttat tattatttca ccttctcttt 2280

ttatttatac ttataattat ttattcttta catactgtta caagaaactc ttttctacat 2340

taattgcata aagtgtcaat cagcacatcc tctatatcgc tatcaacaac aaatttgaca 2400

aacctgccta tatcttcagg aacaactgcc gcatcgctac caccactact tgtgaagtcc 2460

ctggagttta atatgcactg aaatttacct agccgtttta cacaagacca taatccatcc 2520

atgctatcgc agtatatgat tttgtgttcg tttttcgtct tgcgaaaggc atcctcaatg 2580

gcttgtttca ttgatccatc agtgtggctc gtaggtacca gcaaaaccac ttcatcagcg 2640

gcgtactcct cccactttat gggcagtcct tgtatcgact tgctcattat aatacatttg 2700

ctctatcccc gcgtgcttgg ccggccgt 2728

<210> 16
<211> 713
<212> PRT
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(713)
<223> Saccharomyces cerevisiae ACS1 protein sequence

<400> 16

Met Ser Pro Ser Ala Val Gln Ser Ser Lys Leu Glu Glu Gln Ser Ser
1 5 10 15

Glu Ile Asp Lys Leu Lys Ala Lys Met Ser Gln Ser Ala Ser Thr Ala

20 25 30

Gln Gln Lys Lys Glu His Glu Tyr Glu His Leu Thr Ser Val Lys Ile
35 40 45

Val Pro Gln Arg Pro Ile Ser Asp Arg Leu Gln Pro Ala Ile Ala Thr
50 55 60

His Tyr Ser Pro His Leu Asp Gly Leu Gln Asp Tyr Gln Arg Leu His
65 70 75 80

Lys Glu Ser Ile Glu Asp Pro Ala Lys Phe Phe Gly Ser Lys Ala Thr
85 90 95

Gln Phe Leu Asn Trp Ser Lys Pro Phe Asp Lys Val Phe Ile Pro Asp
100 105 110

Ser Lys Thr Gly Arg Pro Ser Phe Gln Asn Asn Ala Trp Phe Leu Asn
115 120 125

Gly Gln Leu Asn Ala Cys Tyr Asn Cys Val Asp Arg His Ala Leu Lys
130 135 140

Thr Pro Asn Lys Lys Ala Ile Ile Phe Glu Gly Asp Glu Pro Gly Gln
145 150 155 160

Gly Tyr Ser Ile Thr Tyr Lys Glu Leu Leu Glu Glu Val Cys Gln Val
165 170 175

Ala Gln Val Leu Thr Tyr Ser Met Gly Val Arg Lys Gly Asp Thr Val
180 185 190

Ala Val Tyr Met Pro Met Val Pro Glu Ala Ile Ile Thr Leu Leu Ala
195 200 205

Ile Ser Arg Ile Gly Ala Ile His Ser Val Val Phe Ala Gly Phe Ser
210 215 220

Ser Asn Ser Leu Arg Asp Arg Ile Asn Asp Gly Asp Ser Lys Val Val
225 230 235 240

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

Arg Ile Val Asp Asp Ala Leu Arg Glu Thr Pro Gly Val Arg His Val
260 265 270

Leu Val Tyr Arg Lys Thr Asn Asn Pro Ser Val Ala Phe His Ala Pro
275 280 285

Arg Asp Leu Asp Trp Ala Thr Glu Lys Lys Lys Tyr Lys Thr Tyr Tyr

290 295 300

Pro Cys Thr Pro Val Asp Ser Glu Asp Pro Leu Phe Leu Leu Tyr Thr
305 310 315 320

Ser Gly Ser Thr Gly Ala Pro Lys Gly Val Gln His Ser Thr Ala Gly
325 330 335

Tyr Leu Leu Gly Ala Leu Leu Thr Met Arg Tyr Thr Phe Asp Thr His
340 345 350

Gln Glu Asp Val Phe Phe Thr Ala Gly Asp Ile Gly Trp Ile Thr Gly
355 360 365

His Thr Tyr Val Val Tyr Gly Pro Leu Leu Tyr Gly Cys Ala Thr Leu
370 375 380

Val Phe Glu Gly Thr Pro Ala Tyr Pro Asn Tyr Ser Arg Tyr Trp Asp
385 390 395 400

Ile Ile Asp Glu His Lys Val Thr Gln Phe Tyr Val Ala Pro Thr Ala
405 410 415

Leu Arg Leu Leu Lys Arg Ala Gly Asp Ser Tyr Ile Glu Asn His Ser
420 425 430

Leu Lys Ser Leu Arg Cys Leu Gly Ser Val Gly Glu Pro Ile Ala Ala
435 440 445

Glu Val Trp Glu Trp Tyr Ser Glu Lys Ile Gly Lys Asn Glu Ile Pro
450 455 460

Ile Val Asp Thr Tyr Trp Gln Thr Glu Ser Gly Ser His Leu Val Thr
465 470 475 480

Pro Leu Ala Gly Gly Val Thr Pro Met Lys Pro Gly Ser Ala Ser Phe
485 490 495

Pro Phe Phe Gly Ile Asp Ala Val Val Leu Asp Pro Asn Thr Gly Glu
500 505 510

Glu Leu Asn Thr Ser His Ala Glu Gly Val Leu Ala Val Lys Ala Ala
515 520 525

Trp Pro Ser Phe Ala Arg Thr Ile Trp Lys Asn His Asp Arg Tyr Leu
530 535 540

Asp Thr Tyr Leu Asn Pro Tyr Pro Gly Tyr Tyr Phe Thr Gly Asp Gly
545 550 555 560

Ala Ala Lys Asp Lys Asp Gly Tyr Ile Trp Ile Leu Gly Arg Val Asp

565 570 575

Asp Val Val Asn Val Ser Gly His Arg Leu Ser Thr Ala Glu Ile Glu
580 585 590

Ala Ala Ile Ile Glu Asp Pro Ile Val Ala Glu Cys Ala Val Val Gly
595 600 605

Phe Asn Asp Asp Leu Thr Gly Gln Ala Val Ala Ala Phe Val Val Leu
610 615 620

Lys Asn Lys Ser Asn Trp Ser Thr Ala Thr Asp Asp Glu Leu Gln Asp
625 630 635 640

Ile Lys Lys His Leu Val Phe Thr Val Arg Lys Asp Ile Gly Pro Phe
645 650 655

Ala Ala Pro Lys Leu Ile Ile Leu Val Asp Asp Leu Pro Lys Thr Arg
660 665 670

Ser Gly Lys Ile Met Arg Arg Ile Leu Arg Lys Ile Leu Ala Gly Glu
675 680 685

Ser Asp Gln Leu Gly Asp Val Ser Thr Leu Ser Asn Pro Gly Ile Val
690 695 700

Arg His Leu Ile Asp Ser Val Lys Leu
705 710

<210> 17
<211> 2287
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(2287)
<223> Saccharomyces cerevisiae ACS2 nucleotide sequence

<400> 17
acctcccgcg acctccaaaa tcgaactacc ttcacaatga caatcaagga acataaagta 60

gtttatgaag ctcacaacgt aaaggctctt aaggctcctc aacattttta caacagccaa 120

cccggcaagg gttacgttac tgatatgcaa cattatcaag aaatgtatca acaatctatc 180

aatgagccag aaaaattctt tgataagatg gctaaggaat acttgcattg ggatgctcca 240

tacaccaaag ttcaatctgg ttcattgaac aatggtgatg ttgcatggtt tttgaacggt 300

aaattgaatg catcatacaa ttgtgttgac agacatgcct ttgctaatcc cgacaagcca 360

gctttgatct atgaagctga tgacgaatcc gacaacaaaa tcatcacatt tggtgaatta 420

ctcagaaaag tttcccaaat cgctggtgtc ttaaaaagct ggggcgttaa gaaaggtgac 480

acagtggcta tctatttgcc aatgattcca gaagcggtca ttgctatgtt ggctgtggct 540

cgtattggtg ctattcactc tgttgtcttt gctgggttct ccgctggttc gttgaaagat 600

cgtgtcgttg acgctaattc taaagtggtc atcacttgtg atgaaggtaa aagaggtggt 660

aagaccatca acactaaaaa aattgttgac gaaggtttga acggagtcga tttggtttcc 720

cgtatcttgg ttttccaaag aactggtact gaaggtattc caatgaaggc cggtagagat 780

tactggtggc atgaggaggc cgctaagcag agaacttacc tacctcctgt ttcatgtgac 840

gctgaagatc ctctattttt attatacact tccggttcca ctggttctcc aaagggtgtc 900

gttcacacta caggtggtta tttattaggt gccgctttaa caactagata cgtttttgat 960

attcacccag aagatgttct cttcactgcc ggtgacgtcg gctggatcac gggtcacacc 1020

tatgctctat atggtccatt aaccttgggt accgcctcaa taattttcga atccactcct 1080

gcctacccag attatggtag atattggaga attatccaac gtcacaaggc tacccatttc 1140

tatgtggctc caactgcttt aagattaatc aaacgtgtag gtgaagccga aattgccaaa 1200

tatgacactt cctcattacg tgtcttgggt tccgtcggtg aaccaatctc tccagactta 1260

tgggaatggt atcatgaaaa agtgggtaac aaaaactgtg tcatttgtga cactatgtgg 1320

caaacagagt ctggttctca tttaattgct cctttggcag gtgctgtccc aacaaaacct 1380

ggttctgcta ccgtgccatt ctttggtatt aacgcttgta tcattgaccc tgttacaggt 1440

gtggaattag aaggtaatga tgtcgaaggt gtccttgccg ttaaatcacc atggccatca 1500

atggctagat ctgtttggaa ccaccacgac cgttacatgg atacttactt gaaaccttat 1560

cctggtcact atttcacagg tgatggtgct ggtagagatc atgatggtta ctactggatc 1620

aggggtagag ttgacgacgt tgtaaatgtt tccggtcata gattatccac atcagaaatt 1680

gaagcatcta tctcaaatca cgaaaacgtc tcggaagctg ctgttgtcgg tattccagat 1740

gaattgaccg gtcaaaccgt cgttgcatat gtttccctaa aagatggtta tctacaaaac 1800

aacgctactg aaggtgatgc agaacacatc acaccagata atttacgtag agaattgatc 1860

ttacaagtta ggggtgagat tggtcctttc gcctcaccaa aaaccattat tctagttaga 1920

gatctaccaa gaacaaggtc aggaaagatt atgagaagag ttctaagaaa ggttgcttct 1980

aacgaagccg aacagctagg tgacctaact actttggcca acccagaagt tgtacctgcc 2040

atcatttctg ctgtagagaa ccaatttttc tctcaaaaaa agaaataact taaatgagaa 2100

aaatttcgta atgagataaa atttcgctcc ttttctgttt tctattttct attttcccaa 2160

cttttgctct attcagttat aaattactat ttatccatca gttaaaaaac aagatctttt 2220

actggtcagc taggaaagcg aaaatacaaa gactttatgc actatccccg cgtgcttggc 2280

cggccgt 2287

<210> 18
<211> 683
<212> PRT
<213> Saccharomyces cerevisiae

<220>
<221> misc_feature
<222> (1)..(683)
<223> Saccharomyces cerevisiae ACS2 protein sequence

<400> 18

```
Met Thr Ile Lys Glu His Lys Val Val Tyr Glu Ala His Asn Val Lys
1               5                  10                  15

Ala Leu Lys Ala Pro Gln His Phe Tyr Asn Ser Gln Pro Gly Lys Gly
            20                  25                  30

Tyr Val Thr Asp Met Gln His Tyr Gln Glu Met Tyr Gln Gln Ser Ile
        35                  40                  45

Asn Glu Pro Glu Lys Phe Phe Asp Lys Met Ala Lys Glu Tyr Leu His
    50                  55                  60

Trp Asp Ala Pro Tyr Thr Lys Val Gln Ser Gly Ser Leu Asn Asn Gly
65                  70                  75                  80

Asp Val Ala Trp Phe Leu Asn Gly Lys Leu Asn Ala Ser Tyr Asn Cys
                85                  90                  95

Val Asp Arg His Ala Phe Ala Asn Pro Asp Lys Pro Ala Leu Ile Tyr
            100                 105                 110

Glu Ala Asp Asp Glu Ser Asp Asn Lys Ile Ile Thr Phe Gly Glu Leu
        115                 120                 125

Leu Arg Lys Val Ser Gln Ile Ala Gly Val Leu Lys Ser Trp Gly Val
    130                 135                 140

Lys Lys Gly Asp Thr Val Ala Ile Tyr Leu Pro Met Ile Pro Glu Ala
145                 150                 155                 160

Val Ile Ala Met Leu Ala Val Ala Arg Ile Gly Ala Ile His Ser Val
                165                 170                 175

Val Phe Ala Gly Phe Ser Ala Gly Ser Leu Lys Asp Arg Val Val Asp
            180                 185                 190

Ala Asn Ser Lys Val Val Ile Thr Cys Asp Glu Gly Lys Arg Gly Gly
        195                 200                 205

Lys Thr Ile Asn Thr Lys Lys Ile Val Asp Glu Gly Leu Asn Gly Val
    210                 215                 220

Asp Leu Val Ser Arg Ile Leu Val Phe Gln Arg Thr Gly Thr Glu Gly
225                 230                 235                 240

Ile Pro Met Lys Ala Gly Arg Asp Tyr Trp Trp His Glu Glu Ala Ala
```

245 250 255

Lys Gln Arg Thr Tyr Leu Pro Pro Val Ser Cys Asp Ala Glu Asp Pro
260 265 270

Leu Phe Leu Leu Tyr Thr Ser Gly Ser Thr Gly Ser Pro Lys Gly Val
275 280 285

Val His Thr Thr Gly Gly Tyr Leu Leu Gly Ala Ala Leu Thr Thr Arg
290 295 300

Tyr Val Phe Asp Ile His Pro Glu Asp Val Leu Phe Thr Ala Gly Asp
305 310 315 320

Val Gly Trp Ile Thr Gly His Thr Tyr Ala Leu Tyr Gly Pro Leu Thr
325 330 335

Leu Gly Thr Ala Ser Ile Ile Phe Glu Ser Thr Pro Ala Tyr Pro Asp
340 345 350

Tyr Gly Arg Tyr Trp Arg Ile Ile Gln Arg His Lys Ala Thr His Phe
355 360 365

Tyr Val Ala Pro Thr Ala Leu Arg Leu Ile Lys Arg Val Gly Glu Ala
370 375 380

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

Gly Glu Pro Ile Ser Pro Asp Leu Trp Glu Trp Tyr His Glu Lys Val
405 410 415

Gly Asn Lys Asn Cys Val Ile Cys Asp Thr Met Trp Gln Thr Glu Ser
420 425 430

Gly Ser His Leu Ile Ala Pro Leu Ala Gly Ala Val Pro Thr Lys Pro
435 440 445

Gly Ser Ala Thr Val Pro Phe Phe Gly Ile Asn Ala Cys Ile Ile Asp
450 455 460

Pro Val Thr Gly Val Glu Leu Glu Gly Asn Asp Val Glu Gly Val Leu
465 470 475 480

Ala Val Lys Ser Pro Trp Pro Ser Met Ala Arg Ser Val Trp Asn His
485 490 495

His Asp Arg Tyr Met Asp Thr Tyr Leu Lys Pro Tyr Pro Gly His Tyr
500 505 510

Phe Thr Gly Asp Gly Ala Gly Arg Asp His Asp Gly Tyr Tyr Trp Ile

515 520 525

Arg Gly Arg Val Asp Asp Val Val Asn Val Ser Gly His Arg Leu Ser
530 535 540

Thr Ser Glu Ile Glu Ala Ser Ile Ser Asn His Glu Asn Val Ser Glu
545 550 555 560

Ala Ala Val Val Gly Ile Pro Asp Glu Leu Thr Gly Gln Thr Val Val
565 570 575

Ala Tyr Val Ser Leu Lys Asp Gly Tyr Leu Gln Asn Asn Ala Thr Glu
580 585 590

Gly Asp Ala Glu His Ile Thr Pro Asp Asn Leu Arg Arg Glu Leu Ile
595 600 605

Leu Gln Val Arg Gly Glu Ile Gly Pro Phe Ala Ser Pro Lys Thr Ile
610 615 620

Ile Leu Val Arg Asp Leu Pro Arg Thr Arg Ser Gly Lys Ile Met Arg
625 630 635 640

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

Leu Thr Thr Leu Ala Asn Pro Glu Val Val Pro Ala Ile Ile Ser Ala
660 665 670

Val Glu Asn Gln Phe Phe Ser Gln Lys Lys Lys
675 680

<210> 19
<211> 2137
<212> DNA
<213> Streptomyces sp.

<220>
<221> misc_feature
<222> (1)..(2137)
<223> Streptomyces sp. CL190 nphT7 gene sequence

<400> 19
cctgcaggcc gtcgagggcg cctggaagga ctacgcggag caggacggcc ggtcgctgga 60

ggagttcgcg gcgttcgtct accaccagcc gttcacgaag atggcctaca aggcgcaccg 120

ccacctgctg aacttcaacg gctacgacac cgacaaggac gccatcgagg gcgccctcgg 180

ccagacgacg gcgtacaaca acgtcatcgg caacagctac accgcgtcgg tgtacctggg 240

cctggccgcc ctgctcgacc aggcggacga cctgacgggc cgttccatcg gcttcctgag 300

ctacggctcg ggcagcgtcg ccgagttctt ctcgggcacc gtcgtcgccg ggtaccgcga 360

gcgtctgcgc accgaggcga accaggaggc gatcgcccgg cgcaagagcg tcgactacgc 420

cacctaccgc gagctgcacg agtacacgct cccgtccgac ggcggcgacc acgccacccc 480

ggtgcagacc accggcccct tccggctggc cgggatcaac gaccacaagc gcatctacga 540

ggcgcgctag cgacacccct cggcaacggg gtgcgccact gttcggcgca ccccgtgccg 600

ggctttcgca cagctattca cgaccatttg aggggcgggc agccgcatga ccgacgtccg 660

attccgcatt atcggtacgg gtgcctacgt accggaacgg atcgtctcca acgatgaagt 720

cggcgcgccg gccggggtgg acgacgactg gatcacccgc aagaccggta tccggcagcg 780

tcgctgggcc gccgacgacc aggccacctc ggacctggcc acggccgcgg ggcgggcagc 840

gctgaaagcg gcgggcatca cgcccgagca gctgaccgtg atcgcggtcg ccacctccac 900

gccggaccgg ccgcagccgc ccacggcggc ctatgtccag caccacctcg gtgcgaccgg 960

cactgcggcg ttcgacgtca acgcggtctg ctccggcacc gtgttcgcgc tgtcctcggt 1020

ggcgggcacc ctcgtgtacc ggggcggtta cgcgctggtc atcggcgcgg acctgtactc 1080

gcgcatcctc aacccggccg accgcaagac ggtcgtgctg ttcggggacg gcgccggcgc 1140

aatggtcctc gggccgacct cgaccggcac gggccccatc gtccggcgcg tcgccctgca 1200

caccttcggc ggcctcaccg acctgatccg tgtgcccgcg ggcggcagcc gccagccgct 1260

ggacacggat ggcctcgacg cgggactgca gtacttcgcg atggacgggc gtgaggtgcg 1320

ccgcttcgtc acggagcacc tgccgcagct gatcaagggc ttcctgcacg aggccggggt 1380

cgacgccgcc gacatcagcc acttcgtgcc gcatcaggcc aacggtgtca tgctcgacga 1440

ggtcttcggc gagctgcatc tgccgcgggc gaccatgcac cggacggtcg agacctacgg 1500

caacacggga gcggcctcca tcccgatcac catggacgcg gccgtgcgcg ccggttcctt 1560

ccggccgggc gagctggtcc tgctggccgg gttcggcggc ggcatggccg cgagcttcgc 1620

cctgatcgag tggtagtcgc ccgtaccacc acagcggtcc ggcgccacct gttccctgcg 1680

ccgggccgcc ctcggggcct ttaggcccca caccgcccca gccgacggat tcagtcgcgg 1740

cagtacctca gatgtccgct gcgacggcgt cccggagagc ccgggcgaga tcgcgggccc 1800

ccttctgctc gtccccggcc cctcccgcga gcaccacccg cggcggacgg ccgccgtcct 1860

ccgcgatacg ccgggcgagg tcgcaggcga gcacgccgga cccggagaag ccccccagca 1920

ccagcgaccg gccgactccg tgcgcggcca gggcaggctg cgcgccgtcg acgtcggtga 1980

gcagcaccag gagctcctgc ggcccggcgt agaggtcggc cagccggtcg tagcaggtcg 2040

cgggcgcgcc cggcggcggg atcagacaga tcgtgcccgc ccgctcgtgc ctcgccgccc 2100

gcagcgtgac cagcggaatg tcccgcccag ctccgga 2137

<210> 20
<211> 325
<212> PRT
<213> Streptomyces sp.

<220>
<221> misc_feature

<222> (1)..(325)
<223> Streptomyces sp. CL190 acetyl-CoA:malonyl-CoA acyltransferase protein sequence

<400> 20

```
Arg Phe Arg Ile Ile Gly Thr Gly Ala Tyr Val Pro Glu Arg Ile Val
1               5                  10                  15

Ser Asn Asp Glu Val Gly Ala Pro Ala Gly Val Asp Asp Asp Trp Ile
            20                  25                  30

Thr Arg Lys Thr Gly Ile Arg Gln Arg Arg Trp Ala Ala Asp Asp Gln
        35                  40                  45

Ala Thr Ser Asp Leu Ala Thr Ala Ala Gly Arg Ala Ala Leu Lys Ala
    50                  55                  60

Ala Gly Ile Thr Pro Glu Gln Leu Thr Val Ile Ala Val Ala Thr Ser
65                  70                  75                  80

Thr Pro Asp Arg Pro Gln Pro Pro Thr Ala Ala Tyr Val Gln His His
                85                  90                  95

Leu Gly Ala Thr Gly Thr Ala Ala Phe Asp Val Asn Ala Val Cys Ser
            100                 105                 110

Gly Thr Val Phe Ala Leu Ser Ser Val Ala Gly Thr Leu Val Tyr Arg
        115                 120                 125

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

Asn Pro Ala Asp Arg Lys Thr Val Val Leu Phe Gly Asp Gly Ala Gly
145                 150                 155                 160

Ala Met Val Leu Gly Pro Thr Ser Thr Gly Thr Gly Pro Ile Val Arg
                165                 170                 175

Arg Val Ala Leu His Thr Phe Gly Gly Leu Thr Asp Leu Ile Arg Val
            180                 185                 190

Pro Ala Gly Gly Ser Arg Gln Pro Leu Asp Thr Asp Gly Leu Asp Ala
        195                 200                 205

Gly Leu Gln Tyr Phe Ala Met Asp Gly Arg Glu Val Arg Arg Phe Val
    210                 215                 220

Thr Glu His Leu Pro Gln Leu Ile Lys Gly Phe Leu His Glu Ala Gly
225                 230                 235                 240

Val Asp Ala Ala Asp Ile Ser His Phe Val Pro His Gln Ala Asn Gly
                245                 250                 255
```

```
Val Met Leu Asp Glu Val Phe Gly Glu Leu His Leu Pro Arg Ala Thr
            260                 265                 270

Met His Arg Thr Val Glu Thr Tyr Gly Asn Thr Gly Ala Ala Ser Ile
        275                 280                 285

Pro Ile Thr Met Asp Ala Ala Val Arg Ala Gly Ser Phe Arg Pro Gly
    290                 295                 300

Glu Leu Val Leu Leu Ala Gly Phe Gly Gly Gly Met Ala Ala Ser Phe
305                 310                 315                 320

Ala Leu Ile Glu Trp
                325
```

<210> 21
<211> 1287
<212> DNA
<213> Pseudomonas mevalonii

<220>
<221> misc_feature
<222> (1)..(1287)
<223> Pseudomonas mevalonii HMG-CoA reductase (mvaA) gene sequence

<400> 21

```
atgagcctcg attcccgcct gcccgctttc cgtaacctgt cccctgccgc gcgcctggac       60

cacatcggcc agttgctcgg cctgagccac gacgatgtca gcctgctggc caacgccggt      120

gccctgccga tggacatcgc caacggcatg atcgaaaacg tcatcggcac cttcgagctg      180

ccctatgccg tggccagcaa cttccagatc aatggccgtg atgtgctggt gccgctggtg      240

gtggaagagc cctcgatcgt cgccgctgct tcgtacatgg ccaagctggc ccgtgccaac      300

ggcggcttca ccacctccag cagcgccccg ctgatgcatg cccaggtaca gatcgtcggc      360

atacaggacc cgctcaatgc acgcctgagc ctgctgcgcc gcaaagacga aatcattgaa      420

ctggccaacc gcaaggacca gttgctcaac agcctcggcg gcggctgccg cgacatcgaa      480

gtgcacacct tcgccgatac cccgcgtggc ccgatgctgg tggcgcacct gatcgtcgat      540

gtacgcgatg ccatgggcgc caacaccgtc aataccatgg ccgaggccgt tgcgccgctg      600

atggaagcca tcaccggggg ccaggtacgc ctgcgcattc tgtccaacct ggccgacctg      660

cgcctggcca gggcccaggt gcggattact ccgcagcaac tggaaacggc cgaattcagt      720

ggcgaggcag tgatcgaagg catcctcgac gcctacgcct tcgctgcggt cgacccttac      780

cgcgcggcca cccacaacaa gggcatcatg aatggcatcg acccactgat cgtcgccact      840

ggcaacgact ggcgtgcagt ggaagccggc gcccatgcgt atgcctgccg cagtggtcac      900

tacggctcgc tgaccacctg ggaaaaggac aacaacggcc atttggtcgg caccctggaa      960

atgccgatgc ccgtaggcct ggtcggcggc gccaccaaaa cccatccgct ggcgcaactg     1020
```

tcgctgcgca tcctcggcgt gaaaacagcc caggcgctcg ctgagattgc cgtggccgta 1080

ggcctggcgc aaaacctcgg ggccatgcgc gccctggcca ccgaaggcat ccagcgcggc 1140

cacatggccc tgcatgcgcg caatattgcc gtggtggcgg gcgcccgagg cgatgaggtg 1200

gactgggttg cccggcagtt ggtggaatac cacgacgtgc gcgccgaccg cgccgtagca 1260

ctgctgaaac aaaagcgcgg ccaatga 1287

<210> 22
<211> 428
<212> PRT
<213> Pseudomonas mevalonii

<220>
<221> misc_feature
<222> (1)..(428)
<223> Pseudomonas mevalonii hydroxymethylglutaryl -CoA reductase protein sequence

<400> 22

Met Ser Leu Asp Ser Arg Leu Pro Ala Phe Arg Asn Leu Ser Pro Ala
1 5 10 15

Ala Arg Leu Asp His Ile Gly Gln Leu Leu Gly Leu Ser His Asp Asp
20 25 30

Val Ser Leu Leu Ala Asn Ala Gly Ala Leu Pro Met Asp Ile Ala Asn
35 40 45

Gly Met Ile Glu Asn Val Ile Gly Thr Phe Glu Leu Pro Tyr Ala Val
50 55 60

Ala Ser Asn Phe Gln Ile Asn Gly Arg Asp Val Leu Val Pro Leu Val
65 70 75 80

Val Glu Glu Pro Ser Ile Val Ala Ala Ala Ser Tyr Met Ala Lys Leu
85 90 95

Ala Arg Ala Asn Gly Gly Phe Thr Thr Ser Ser Ser Ala Pro Leu Met
100 105 110

His Ala Gln Val Gln Ile Val Gly Ile Gln Asp Pro Leu Asn Ala Arg
115 120 125

Leu Ser Leu Leu Arg Arg Lys Asp Glu Ile Ile Glu Leu Ala Asn Arg
130 135 140

Lys Asp Gln Leu Leu Asn Ser Leu Gly Gly Gly Cys Arg Asp Ile Glu
145 150 155 160

Val His Thr Phe Ala Asp Thr Pro Arg Gly Pro Met Leu Val Ala His
165 170 175

```
Leu Ile Val Asp Val Arg Asp Ala Met Gly Ala Asn Thr Val Asn Thr
            180                 185                 190

Met Ala Glu Ala Val Ala Pro Leu Met Glu Ala Ile Thr Gly Gly Gln
        195                 200                 205

Val Arg Leu Arg Ile Leu Ser Asn Leu Ala Asp Leu Arg Leu Ala Arg
    210                 215                 220

Ala Gln Val Arg Ile Thr Pro Gln Gln Leu Glu Thr Ala Glu Phe Ser
225                 230                 235                 240

Gly Glu Ala Val Ile Glu Gly Ile Leu Asp Ala Tyr Ala Phe Ala Ala
                245                 250                 255

Val Asp Pro Tyr Arg Ala Ala Thr His Asn Lys Gly Ile Met Asn Gly
            260                 265                 270

Ile Asp Pro Leu Ile Val Ala Thr Gly Asn Asp Trp Arg Ala Val Glu
        275                 280                 285

Ala Gly Ala His Ala Tyr Ala Cys Arg Ser Gly His Tyr Gly Ser Leu
    290                 295                 300

Thr Thr Trp Glu Lys Asp Asn Asn Gly His Leu Val Gly Thr Leu Glu
305                 310                 315                 320

Met Pro Met Pro Val Gly Leu Val Gly Gly Ala Thr Lys Thr His Pro
                325                 330                 335

Leu Ala Gln Leu Ser Leu Arg Ile Leu Gly Val Lys Thr Ala Gln Ala
            340                 345                 350

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

Met Arg Ala Leu Ala Thr Glu Gly Ile Gln Arg Gly His Met Ala Leu
    370                 375                 380

His Ala Arg Asn Ile Ala Val Val Ala Gly Ala Arg Gly Asp Glu Val
385                 390                 395                 400

Asp Trp Val Ala Arg Gln Leu Val Glu Tyr His Asp Val Arg Ala Asp
                405                 410                 415

Arg Ala Val Ala Leu Leu Lys Gln Lys Arg Gly Gln
            420                 425
```

<210> 23
<211> 1302
<212> DNA

<213> Silicibacter pomeroyi

<220>
<221> misc_feature
<222> (1)..(1302)
<223> Silicibacter pomeroyi hydroxymethylglutaryl-CoA reductase gene sequence

<400> 23
atgacaggca agacgggtca catcgatggt ttgaactcgc gcattgaaaa gatgcgagat 60

ctcgaccccg cacaacggct ggtgcgcgtt gccgaggcgg cgggcctcga gcccgaggcg 120

atcagcgcgc tggcgggtaa cggcgccctg cccctctcgc tggccaacgg gatgatcgag 180

aacgtcatcg gcaaattcga actgccgctg ggcgtggcca cgaatttcac tgtgaacggc 240

cgcgactatc tgatcccgat ggcggtcgaa gagccctcgg tggtggcggc cgcgtcctat 300

atggcgcgta tcgcgcgcga gaatggcgga ttcaccgcgc atggcaccgc gcccttgatg 360

cgcgcccaga tccaggtggt cgggttgggt gatcccgagg gcgcccggca gcgtctcctc 420

gcccacaagg ccgcgttcat ggaggcggcg gacgctgtcg atccggtgct tgtcgggctg 480

ggtggcggct gccgcgatat cgaggttcac gtgttccggg atacgccggt gggcgcgatg 540

gtcgtcctgc acctgatcgt cgatgtgcgc gacgcgatgg gggccaatac ggtcaacacg 600

atggccgaac ggctggcccc cgaggtcgag cggattgccg gtggcaccgt gcggctgcgc 660

atcctgtcga acctcgccga cctgcgattg gtccgggcgc gggtggaact ggccccggaa 720

acactgacaa cgcagggcta tgacggcgcc gacgtggcgc ggggcatggt cgaggcctgc 780

gcgcttgcca tcgtcgaccc ctatcgcgcg gcgacccata acaaggggat catgaacggc 840

atcgacccgg tcgtcgtcgc caccggcaat gactggcgcg cgatcgaggc gggtgcccat 900

gcctatgccg cccgcacggg tcattatacc tcgctgaccc gctgggaact ggcgaatgac 960

gggcggcttg tgggcacgat cgaactgccc ctggcgcttg gccttgtcgg cggcgcgacc 1020

aagacgcacc cgaccgcacg ggcggcgctg gccctgatgc aggtagagac tgcaaccgaa 1080

ctggcccagg tcaccgccgc cgtgggtctg gcgcagaaca tggccgccat ccgcgcgctg 1140

gcgaccgaag gcatccagcg cggtcacatg acccttcatg cgcgcaacat cgcgatcatg 1200

gccggcgcaa caggcgccga tatcgaccgc gtcacccggg tcattgtcga agcgggcgac 1260

gtcagcgtgg cccgtgcaaa acaggtgctg gaaaacacct ga 1302

<210> 24
<211> 433
<212> PRT
<213> Silicibacter pomeroyi

<220>
<221> misc_feature
<222> (1)..(433)
<223> Silicibacter pomeroyi hydroxymethylglutaryl-CoA reductase protein sequence

<400> 24

```
Met Thr Gly Lys Thr Gly His Ile Asp Gly Leu Asn Ser Arg Ile Glu
1               5                  10                  15

Lys Met Arg Asp Leu Asp Pro Ala Gln Arg Leu Val Arg Val Ala Glu
            20                  25                  30

Ala Ala Gly Leu Glu Pro Glu Ala Ile Ser Ala Leu Ala Gly Asn Gly
        35                  40                  45

Ala Leu Pro Leu Ser Leu Ala Asn Gly Met Ile Glu Asn Val Ile Gly
    50                  55                  60

Lys Phe Glu Leu Pro Leu Gly Val Ala Thr Asn Phe Thr Val Asn Gly
65                  70                  75                  80

Arg Asp Tyr Leu Ile Pro Met Ala Val Glu Glu Pro Ser Val Val Ala
                85                  90                  95

Ala Ala Ser Tyr Met Ala Arg Ile Ala Arg Glu Asn Gly Gly Phe Thr
            100                 105                 110

Ala His Gly Thr Ala Pro Leu Met Arg Ala Gln Ile Gln Val Val Gly
        115                 120                 125

Leu Gly Asp Pro Glu Gly Ala Arg Gln Arg Leu Leu Ala His Lys Ala
    130                 135                 140

Ala Phe Met Glu Ala Ala Asp Ala Val Asp Pro Val Leu Val Gly Leu
145                 150                 155                 160

Gly Gly Gly Cys Arg Asp Ile Glu Val His Val Phe Arg Asp Thr Pro
                165                 170                 175

Val Gly Ala Met Val Val Leu His Leu Ile Val Asp Val Arg Asp Ala
            180                 185                 190

Met Gly Ala Asn Thr Val Asn Thr Met Ala Glu Arg Leu Ala Pro Glu
        195                 200                 205

Val Glu Arg Ile Ala Gly Gly Thr Val Arg Leu Arg Ile Leu Ser Asn
    210                 215                 220

Leu Ala Asp Leu Arg Leu Val Arg Ala Arg Val Glu Leu Ala Pro Glu
225                 230                 235                 240

Thr Leu Thr Thr Gln Gly Tyr Asp Gly Ala Asp Val Ala Arg Gly Met
                245                 250                 255

Val Glu Ala Cys Ala Leu Ala Ile Val Asp Pro Tyr Arg Ala Ala Thr
            260                 265                 270
```

```
His Asn Lys Gly Ile Met Asn Gly Ile Asp Pro Val Val Val Ala Thr
        275                 280                 285

Gly Asn Asp Trp Arg Ala Ile Glu Ala Gly Ala His Ala Tyr Ala Ala
    290                 295                 300

Arg Thr Gly His Tyr Thr Ser Leu Thr Arg Trp Glu Leu Ala Asn Asp
305                 310                 315                 320

Gly Arg Leu Val Gly Thr Ile Glu Leu Pro Leu Ala Leu Gly Leu Val
                325                 330                 335

Gly Gly Ala Thr Lys Thr His Pro Thr Ala Arg Ala Ala Leu Ala Leu
            340                 345                 350

Met Gln Val Glu Thr Ala Thr Glu Leu Ala Gln Val Thr Ala Ala Val
        355                 360                 365

Gly Leu Ala Gln Asn Met Ala Ala Ile Arg Ala Leu Ala Thr Glu Gly
    370                 375                 380

Ile Gln Arg Gly His Met Thr Leu His Ala Arg Asn Ile Ala Ile Met
385                 390                 395                 400

Ala Gly Ala Thr Gly Ala Asp Ile Asp Arg Val Thr Arg Val Ile Val
                405                 410                 415

Glu Ala Gly Asp Val Ser Val Ala Arg Ala Lys Gln Val Leu Glu Asn
            420                 425                 430

Thr
```

<210> 25
<211> 1290
<212> DNA
<213> Delftia acidovorans

<220>
<221> misc_feature
<222> (1)..(1290)
<223> Delftia acidovorans hydroxymethylglutaryl-CoA reductase nucleotide sequence

<400> 25

```
atggttgccg attcgcgact gcccaatttc cgcgccctca caccggccca gcgccgggat       60

ttcctggccg atgcctgcgg cctgtccgat gccgagcgcg ccctgctcgc tgcccccggt      120

gccctgcccc tggcgctggc cgacggcatg atcgagaacg tgttcggcag cttcgagctg      180

ccgctgggcg tggccggcaa cttccgcgtc aacggccgcg acgtgctggt gcccatggcg      240

gtggaggagc cctcggtggt ggccgccgcc tcgtacatgg ccaagctggc gcgcgaggac      300
```

```
gggggctttc agacctcaag cacgctgccg ctgatgcgcg cccaggtcca ggtgctgggc      360

gtgaccgatc cacacggcgc gcgcctggcc gtgctgcagg cgcgtgcgca gatcatcgag      420

cgcgccaaca gccgcgacaa ggtgctgatc ggcctgggcg gcggctgcaa ggacatcgag      480

gtccatgtct tccccgacac gccgcgcggc cccatgctgg tggtccacct gatcgtggac      540

gtgcgcgacg ccatgggcgc caacaccgtc aacaccatgg ccgaatcggt ggcgcccctg      600

gtcgagaaga tcacgggcgg cagcgtgcgg ctgcgcatcc tgtccaacct ggccgacctg      660

cggctggccc gcgcccgcgt gcggctcacg ccgcagaccc tggccacgca ggatcgcagc      720

ggcgaggaga tcatcgaagg cgtgctggac gcctatacct tcgcggccat cgacccctac      780

cgcgcggcca cgcacaacaa gggaatcatg aacggcatcg accccgtcat cgtggccacg      840

ggcaacgact ggcgcgcggt cgaggccggc gcccatgcct atgccagccg cagcggcagc      900

tacacctcgc tgacgcgctg ggaaaaggat gccggcggcg ccctggtcgg cagcatcgag      960

ctgcccatgc cggtgggcct tgtcggcggc gccaccaaga cccatccgct ggcacgcctg     1020

gcgctgaaga tcatggacct gcagtccgcc cagcagctgg gcgagatcgc cgccgccgtg     1080

ggcctggcgc agaacctggg cgccctgcgc gccctggcca ccgaaggcat tcagcgcggc     1140

cacatggccc tgcacgcccg caacatcgcc ctggtggccg gcgccacggg cgacgaggtc     1200

gatgccgtgg cgcgccagct ggccgccgag cacgacgtgc gcaccgaccg cgcgctggaa     1260

gtgctggccg cgctgcgcgc cagggcctga                                      1290


<210>  26
<211>  429
<212>  PRT
<213>  Delftia acidovorans


<220>
<221>  misc_feature
<222>  (1)..(429)
<223>  Delftia acidovorans hydroxymethylglutaryl-CoA reductase protein
       sequence

<400>  26

Met Val Ala Asp Ser Arg Leu Pro Asn Phe Arg Ala Leu Thr Pro Ala
1               5                   10                  15


Gln Arg Arg Asp Phe Leu Ala Asp Ala Cys Gly Leu Ser Asp Ala Glu
            20                  25                  30


Arg Ala Leu Leu Ala Ala Pro Gly Ala Leu Pro Leu Ala Leu Ala Asp
        35                  40                  45


Gly Met Ile Glu Asn Val Phe Gly Ser Phe Glu Leu Pro Leu Gly Val
    50                  55                  60


Ala Gly Asn Phe Arg Val Asn Gly Arg Asp Val Leu Val Pro Met Ala
65                  70                  75                  80
```

```
Val Glu Glu Pro Ser Val Val Ala Ala Ala Ser Tyr Met Ala Lys Leu
                85                  90                  95

Ala Arg Glu Asp Gly Gly Phe Gln Thr Ser Ser Thr Leu Pro Leu Met
            100                 105                 110

Arg Ala Gln Val Gln Val Leu Gly Val Thr Asp Pro His Gly Ala Arg
        115                 120                 125

Leu Ala Val Leu Gln Ala Arg Ala Gln Ile Ile Glu Arg Ala Asn Ser
    130                 135                 140

Arg Asp Lys Val Leu Ile Gly Leu Gly Gly Gly Cys Lys Asp Ile Glu
145                 150                 155                 160

Val His Val Phe Pro Asp Thr Pro Arg Gly Pro Met Leu Val Val His
                165                 170                 175

Leu Ile Val Asp Val Arg Asp Ala Met Gly Ala Asn Thr Val Asn Thr
            180                 185                 190

Met Ala Glu Ser Val Ala Pro Leu Val Glu Lys Ile Thr Gly Gly Ser
        195                 200                 205

Val Arg Leu Arg Ile Leu Ser Asn Leu Ala Asp Leu Arg Leu Ala Arg
    210                 215                 220

Ala Arg Val Arg Leu Thr Pro Gln Thr Leu Ala Thr Gln Asp Arg Ser
225                 230                 235                 240

Gly Glu Glu Ile Ile Glu Gly Val Leu Asp Ala Tyr Thr Phe Ala Ala
                245                 250                 255

Ile Asp Pro Tyr Arg Ala Ala Thr His Asn Lys Gly Ile Met Asn Gly
            260                 265                 270

Ile Asp Pro Val Ile Val Ala Thr Gly Asn Asp Trp Arg Ala Val Glu
        275                 280                 285

Ala Gly Ala His Ala Tyr Ala Ser Arg Ser Gly Ser Tyr Thr Ser Leu
    290                 295                 300

Thr Arg Trp Glu Lys Asp Ala Gly Gly Ala Leu Val Gly Ser Ile Glu
305                 310                 315                 320

Leu Pro Met Pro Val Gly Leu Val Gly Gly Ala Thr Lys Thr His Pro
                325                 330                 335

Leu Ala Arg Leu Ala Leu Lys Ile Met Asp Leu Gln Ser Ala Gln Gln
            340                 345                 350
```

```
Leu Gly Glu Ile Ala Ala Ala Val Gly Leu Ala Gln Asn Leu Gly Ala
        355                 360                 365

Leu Arg Ala Leu Ala Thr Glu Gly Ile Gln Arg Gly His Met Ala Leu
    370                 375                 380

His Ala Arg Asn Ile Ala Leu Val Ala Gly Ala Thr Gly Asp Glu Val
385                 390                 395                 400

Asp Ala Val Ala Arg Gln Leu Ala Ala Glu His Asp Val Arg Thr Asp
                405                 410                 415

Arg Ala Leu Glu Val Leu Ala Ala Leu Arg Ala Arg Ala
            420                 425
```

<210> 27
<211> 3059
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i2235 integration construct

<400> 27
gacggcacgg ccacgcgttt aaaccgcctc gatatttcct gtgagaagtt taaatccact 60

aaggtttttc attgttgctg cagatgtgtt tttccattca tcctgaaata tgcactgcta 120

ttccgcattc cattccccta gtctttttta gttctttccg ttcgaccttc atcgaaaaat 180

gacaaaacgc gttaggaaca acaaccaatt gcaaacaagc agtgaaacaa aaccatcaag 240

gcccgaaaat acaagtgtgt actaatacag taagtaggtc aaatacgcaa tgaccaaaga 300

tgccgtgaat ctagatgctt acaccgtgag cttcatgcct ttctataccg agtatcaagg 360

accaaccgaa gagtttaagg attacaaatt cgaagatact atttactttc gtggcaagga 420

actgaagagg gaaaagtctg cgacgccttc cagtagcgat aacacaacta gtaatacctt 480

cagtaatggc gccatcctct cgggaaacac aataactggc aagatagttt cagtgaataa 540

ttacgaaaga gagggcactg atcgcaacga attggcgcga ttgcaagaat tgatctccct 600

catcgatgtc ataaatcagt aaatataagc tcacacgcgg ccaggggggag cccgttgagc 660

cattagtatc aatttgctta cctgtattcc tttactatcc tccttttttct ccttcttgat 720

aaatgtatgt agattgcgta tatagtttcg tctaccctat gaacatattc cattttgtaa 780

tttcgtgtcg tttctattat gaatttcatt tataaagttt atgtacaaat atcataaaaa 840

aagagaatct ttttaagcaa ggattttctt aacttcttcg gcgacagcat caccgacttc 900

ggtggtactg ttggaaccac ctaaatcacc agttctgata cctgcatcca aaacctttt 960

aactgcatct tcaatggcct taccttcttc aggcaagttc aatgacaatt tcaacatcat 1020

tgcagcagac aagatagtgg cgatagggtc aaccttattc tttggcaaat ctggagcaga 1080

accgtggcat ggttcgtaca aaccaaatgc ggtgttcttg tctggcaaag aggccaagga 1140

cgcagatggc aacaaaccca aggaacctgg gataacggag gcttcatcgg agatgatatc 1200

accaaacatg ttgctggtga ttataatacc atttaggtgg gttgggttct taactaggat 1260

catggcggca gaatcaatca attgatgttg aaccttcaat gtagggaatt cgttcttgat 1320

ggtttcctcc acagtttttc tccataatct tgaagaggcc aaaacattag ctttatccaa 1380

ggaccaaata ggcaatggtg gctcatgttg tagggccatg aaagcggcca ttcttgtgat 1440

tctttgcact tctggaacgg tgtattgttc actatcccaa gcgacaccat caccatcgtc 1500

ttcctttctc ttaccaaagt aaatacctcc cactaattct ctgacaacaa cgaagtcagt 1560

acctttagca aattgtggct tgattggaga taagtctaaa agagagtcgg atgcaaagtt 1620

acatggtctt aagttggcgt acaattgaag ttctttacgg atttttagta aaccttgttc 1680

aggtctaaca ctaccggtac cccatttagg accacccaca gcacctaaca aaacggcatc 1740

aaccttcttg gaggcttcca gcgcctcatc tggaagtggg acacctgtag catcgatagc 1800

agcaccacca attaaatgat tttcgaaatc gaacttgaca ttggaacgaa catcagaaat 1860

agctttaaga accttaatgg cttcggctgt gatttcttga ccaacgtggt cacctggcaa 1920

aacgacgatc ttcttagggg cagacatagg ggcagacatt agaatggtat atccttgaaa 1980

tatatatata tattgctgaa atgtaaaagg taagaaaagt tagaaagtaa gacgattgct 2040

aaccacctat tggaaaaaac aataggtcct taaataatat tgtcaacttc aagtattgtg 2100

atgcaagcat ttagtcatga acgcttctct attctatatg aaaagccggt tccggcctct 2160

caccttcct ttttctccca atttttcagt tgaaaaaggt atatgcgtca ggcgacctct 2220

gaaattaaca aaaaatttcc agtcatcgaa tttgattctg tgcgatagcg cccctgtgtg 2280

ttctcgttat gttgaggaaa aaaataatgg ttgctaagag attcgaactc ttgcatctta 2340

cgatacctga gtattcccac agttaactgc ggtcaagata tttcttgaat caggcgcctc 2400

gctcgtccaa cgccggcgga cctcttaaat gagaaaaatt tcgtaatgag ataaaatttc 2460

gctccttttc tgttttctat tttctatttt cccaactttt gctctattca gttataaatt 2520

actatttatc catcagttaa aaaacaagat cttttactgg tcagctagga aagcgaaaat 2580

acaaagactt tatgcactta gtgatatata tgtatagata tatccatttt tacgcactta 2640

tcatatatct tagttatcta aatacaatct agttattcgt acacaatcgc ccctgttatc 2700

cctatagtgg gaataaagta atgcactgtg acggggttct tcgcccggga tagggtaaaa 2760

ggatattgcc gtttcaagaa acttcgggga taatcgaata agataccgag aaagctattg 2820

ttcgttgtgc acgtaggatg tatattgaac aagcatgacc agaatctgat gcattacgag 2880

aaggttacgg gatgatatca gacctccgaa gtccatgttg caaaatgtgc cgactttccg 2940

cggcgctatt tggcacaaat ttcaggagaa acatcactgt cggtgttata gaattccatc 3000

tatattgttt tccccgtagg catacgtcga gcggtgttta aaccccagcg cctggcggg 3059

<210> 28
<211> 8106
<212> DNA

<213> Artificial Sequence

<220>
<223> Synthetic: i74804 integration construct

<400> 28
gacggcacgg ccacgcgttt aaaccgcccg ctcgcctcat ccccacggga ataaggcagc 60

cgacaaaaga aaaacgaccg aaaaggaacc agaaagaaaa aagagggtgg gcgcgccgcg 120

gacgtgtaaa aagatatgca tccagcttct atatcgcttt aactttaccg ttttgggcat 180

cgggaacgta tgtaacattg atctcctctt gggaacggtg agtgcaacga atgcgatata 240

gcaccgacca tgtgggcaaa ttcgtaataa attcggggtg aggggattc aagacaagca 300

accttgttag tcagctcaaa cagcgattta acggttgagt aacacatcaa aacaccgttc 360

gaggtcaagc ctggcgtgtt taacaagttc ttgatatcat atataaatgt aataagaagt 420

ttggtaatat tcaattcgaa gtgttcagtc ttttacttct cttgttttat agaagaaaaa 480

acatcaagaa acatctttaa catacacaaa cacatactat cagaatacac gctcgtccaa 540

cgccggcgga cctttcagac gcgactgcct catcagtaag acccgttgaa aagaacttac 600

ctgaaaaaaa cgaatatata ctagcgttga atgttagcgt caacaacaag aagtttaatg 660

acgcggaggc caaggcaaaa agattccttg attacgtaag ggagttagaa tcattttgaa 720

taaaaaacac gctttttcag ttcgagttta tcattatcaa tactgccatt tcaaagaata 780

cgtaaataat taatagtagt gattttccta actttattta gtcaaaaaat tagcctttta 840

attctgctgt aacccgtaca tgcccaaaat agggggcggg ttacacagaa tatataacat 900

cgtaggtgtc tgggtgaaca gtttattcct ggcatccact aaatataatg gagcccgctt 960

tttaagctgg catccagaaa aaaaaagaat cccagcacca aaatattgtt ttcttcacca 1020

accatcagtt cataggtcca ttctcttagc gcaactacag agaacagggg cacaaacagg 1080

caaaaaacgg gcacaacctc aatggagtga tgcaacctgc ctggagtaaa tgatgacaca 1140

aggcaattga cccacgcatg tatctatctc attttcttac accttctatt accttctgct 1200

ctctctgatt tggaaaaagc tgaaaaaaaa ggttgaaacc agttccctga aattattccc 1260

ctacttgact aataagtata taaagacggt aggtattgat tgtaattctg taaatctatt 1320

tcttaaactt cttaaattct acttttatag ttagtctttt ttttagtttt aaaacaccaa 1380

gaacttagtt tcgacctccc gcgacctcca aaatcgaact accttcacaa tggaacattc 1440

tgtaatcgaa ccaactgtgc ccatgccgct accagccatg tttgacgctc catctggtat 1500

ttttagctct ttggacgacg ctgtgcaagc agccacctta gcccaacaac aactaagttc 1560

agttgagttg cgtcagcaag taatcaaagc cataagagtg gccggagaaa ggtatgcaca 1620

agttttggct gaaatggcag ttgctgaaac tggtatgggt agggtggtgg ataagtacat 1680

taagaatgtc tctcaagctc gtcatacgcc tggtatagaa tgtttatcgg ccgaggttct 1740

tacgggtgat aatggcctaa cattgattga aaatgcccct tggggagtcg tagcttcagt 1800

cacgccaagc acaaatccag cagctacggt aattaataat gcaatctcaa tgattgcagc 1860

ggggaattca gtcgtgttcg caccacatcc ttctgccaaa aacgtctcac taaggactat 1920

ttctttactc aacaaggcca ttgtcgctac cggcggccca gaaaatttac tagttagtgt 1980

ggcaaaccct aacatcgaaa ctgcacagag attattcaga tatccgggta ttggattgtt 2040

agttgtgaca ggtggtgaag ccgtcgttga agccgctagg aagcatacag ataaaaggtt 2100

aattgcagcc ggcgctggta atcctcctgt tgttgtggac gaaactgctg acatacctaa 2160

agccgcaaga gcaattgtca agggtgcttc tttcgacaac aacataattt gtgctgatga 2220

aaaagttttg attgtggtag acagagttgc agatgcacta ttggcagaaa tgcaaagaaa 2280

taacgccgtc ttacttacac ccgaacagac cgaaagacta ctacccgctc ttttgtccga 2340

tattgacgaa cagggcaaag gacgtgtgaa tagagattat gttggaagag atgcggctaa 2400

attagcagcg gctattggtc tggaagttag cgaacatact cgtctactcc tggcagagac 2460

agacgctgat catccattcg ccgtgacgga gctgatgatg ccagtgttac cagtaataag 2520

agtcaagaat gtagatgatg caatcgcatt ggcagttaag ctagagtcag gctgcagaca 2580

cacagctgcg atgcactcta ctaatataag aaacttaaat agaatggcta atgccatcaa 2640

tacctctatc tttgtaaaaa atggtccatg tattgcaggt ttgggtttag gcggtgaagg 2700

ttggacttca atgactatta gcactccgac cggtgaaggt gttacaagcg ctcgtacctt 2760

tgtcagatta agaaggtgtg tcttagtcga catgtttcgg attgcttaag cggccgcgag 2820

taataattat tgcttccata taatattttt atatacctct tatttttatg tattagttaa 2880

ttaagtattt ttatctatct gcttatcatt ttcttttcat atagggggggg ttggtgtttt 2940

cttgcccatc agattgatgt cctccaactc ggcactattt tacaaagggt ttttttgtaa 3000

gagaaggaga agacagatac taaaccatac gttactcgaa acaaaaaaaa aaaaaatgga 3060

aaaagctgct atcaacaaaa gacggcctca tcaaacctaa agaaaccatg tcagcgtatg 3120

tatatacctt gtaatttacg tttccttaaa tcttctttct actaacgttt tcattattct 3180

atactctatg accaataaaa acagactgta ctttcaaaat ttacccagta ggccagcaaa 3240

taaagaaaat tataccagat tacttctgaa acacattaat cccaacaaca agtatgccat 3300

taatccgtcg ctaccccatc cccgcgtgct tggccggccg tacactgagt aatggtagtt 3360

ataagaaaga gaccgagtta gggacagtta gaggcggtgg agatattcct tatggcatgt 3420

ctggcgatga taaaactttt caaacggcag ccccgatcta aaagagctga cagggaaatg 3480

gtcagaaaaa gaaacgtgca cccgcccgtc tggacgcgcc gctcacccgc acggcagaga 3540

ccaatcagta aaaatcaacg gttaacgaca ttactatata tataatatag gaagcattta 3600

atagaacagc atcgtaatat atgtgtactt tgcagttatg acgccagatg gcagtagtgg 3660

aagatattct ttattgaaaa atagcttgtc accttacgta caatcttgat ccggagcttt 3720

tctttttttg ccgattaaga attcggtcga aaaaagaaaa ggagagggcc aagagggagg 3780

gcattggtga ctattgagca cgtgagtata cgtgattaag cacacaaagg cagcttggag 3840

tatgtctgtt attaatttca caggtagttc tggtccattg gtgaaagttt gcggcttgca 3900

gagcacagag gccgcagaat gtgctctaga ttccgatgct gacttgctgg gtattatatg 3960

tgtgcccaat agaaagagaa caattgaccc ggttattgca aggaaaattt caagtcttgt 4020

aaaagcatat aaaaatagtt caggcactcc gaaatacttg gttggcgtgt ttcgtaatca 4080

acctaaggag gatgttttgg ctctggtcaa tgattacggc attgatatcg tccaactgca 4140

tggagatgag tcgtggcaag aataccaaga gttcctcggt ttgccagtta ttaaaagact 4200

cgtatttcca aaagactgca acatactact cagtgcagct tcacagaaac ctcattcgtt 4260

tattcccttg tttgattcag aagcaggtgg gacaggtgaa cttttggatt ggaactcgat 4320

ttctgactgg gttggaaggc aagagagccc cgaaagctta cattttatgt tagctggtgg 4380

actgacgcca gaaaatgttg gtgatgcgct tagattaaat ggcgttattg gtgttgatgt 4440

aagcggaggt gtggagacaa atggtgtaaa agactctaac aaaatagcaa atttcgtcaa 4500

aaatgctaag aaataggtta ttactgagta gtatttattt aagtattgtt tgtgcacttg 4560

cctgcaggcc ttttgaaaag caagcataaa agatctaaac ataaaatctg taaaataaca 4620

agatgtaaag ataatgctaa atcatttggc tttttgattg attgtacagg aaaatataca 4680

tcgcaggggg ttgactttta ccatttcacc gcaatggaat caaacttgtt gaagagaatg 4740

ttcacaggcg catacgctac aatgacacgg ccggccaagc acgcggggat ggggtagcga 4800

cggattaatg gcatacttgt tgttgggatt aatgtgtttc agaagtaatc tggtataatt 4860

ttctttattt gctggcctac tgggtaaatt ttgaaagtac agtctgtttt tattggtcat 4920

agagtataga ataatgaaaa cgttagtaga aagaagattt aaggaaacgt aaattacaag 4980

gtatatacat acgctgacat ggtttcttta ggtttgatga ggccgtcttt tgttgatagc 5040

agctttttcc attttttttt tttttgtttc gagtaacgta tggtttagta tctgtcttct 5100

ccttctctta caaaaaaacc ctttgtaaaa tagtgccgag ttggaggaca tcaatctgat 5160

gggcaagaaa acaccaaccc cccctatatg aaaagaaaat gataagcaga tagataaaaa 5220

tacttaatta actaatacat aaaaataaga ggtatataaa aatattatat ggaagcaata 5280

attattactc gcggccgctt aagcaatccg aaacatgtcg actaagacac accttcttaa 5340

tctgacaaag gtacgagcgc ttgtaacacc ttcaccggtc ggagtgctaa tagtcattga 5400

agtccaacct tcaccgccta aacccaaacc tgcaatacat ggaccatttt ttacaaagat 5460

agaggtattg atggcattag ccattctatt taagtttctt atattagtag agtgcatcgc 5520

agctgtgtgt ctgcagcctg actctagctt aactgccaat gcgattgcat catctacatt 5580

cttgactctt attactggta acactggcat catcagctcc gtcacggcga atggatgatc 5640

agcgtctgtc tctgccagga gtagacgagt atgttcgcta acttccagac caatagccgc 5700

tgctaattta gccgcatctc ttccaacata atctctattc acacgtcctt tgccctgttc 5760

gtcaatatcg gacaaaagag cgggtagtag tctttcggtc tgttcgggtg taagtaagac 5820

ggcgttattt ctttgcattt ctgccaatag tgcatctgca actctgtcta ccacaatcaa 5880

aacttttca tcagcacaaa ttatgttgtt gtcgaaagaa gcacccttga caattgctct 5940

tgcggcttta ggtatgtcag cagtttcgtc cacaacaaca ggaggattac cagcgccggc 6000

tgcaattaac cttttatctg tatgcttcct agcggcttca acgacggctt caccacctgt 6060

cacaactaac aatccaatac ccggatatct gaataatctc tgtgcagttt cgatgttagg 6120

gtttgccaca ctaactagta aattttctgg gccgccggta gcgacaatgg ccttgttgag 6180

taaagaaata gtccttagtg agacgttttt ggcagaagga tgtggtgcga acacgactga 6240

attccccgct gcaatcattg agattgcatt attaattacc gtagctgctg gatttgtgct 6300

tggcgtgact gaagctacga ctccccaagg ggcattttca atcaatgtta ggccattatc 6360

acccgtaaga acctcggccg ataaacattc tataccaggc gtatgacgag cttgagagac 6420

attcttaatg tacttatcca ccaccctacc cataccagtt tcagcaactg ccatttcagc 6480

caaaacttgt gcataccttt ctccggccac tcttatggct ttgattactt gctgacgcaa 6540

ctcaactgaa cttagttgtt gttgggctaa ggtggctgct tgcacagcgt cgtccaaaga 6600

gctaaaaata ccagatggag cgtcaaacat ggctggtagc ggcatgggca cagttggttc 6660

gattacagaa tgttccattg tgaaggtagt tcgattttgg aggtcgcggg aggtcgaaac 6720

taagttcttg gtgttttaaa actaaaaaaa agactaacta taaaagtaga atttaagaag 6780

tttaagaaat agatttacag aattacaatc aatacctacc gtctttatat acttattagt 6840

caagtagggg aataatttca gggaactggt ttcaaccttt tttttcagct ttttccaaat 6900

cagagagagc agaaggtaat agaaggtgta agaaaatgag atagatacat gcgtgggtca 6960

attgccttgt gtcatcattt actccaggca ggttgcatca ctccattgag gttgtgcccg 7020

ttttttgcct gtttgtgccc ctgttctctg tagttgcgct aagagaatgg acctatgaac 7080

tgatggttgg tgaagaaaac aatattttgg tgctgggatt ctttttttct ctggatgcca 7140

gcttaaaaag cgggctccat tatatttagt ggatgccagg aataaactgt tcacccagac 7200

acctacgatg ttatatattc tgtgtaaccc gccccctatt ttgggcatgt acgggttaca 7260

gcagaattaa aaggctaatt ttttgactaa ataaagttag gaaaatcact actattaatt 7320

atttacgtat tctttgaaat ggcagtattg ataatgataa actcgaactg aaaaagcgtg 7380

ttttttattc aaaatgattc taactccctt acgtaatcaa ggaatctttt tgccttggcc 7440

tccgcgtcat taaacttctt gttgttgacg ctaacattca acgctagtat atattcgttt 7500

ttttcaggta agttcttttc aacgggtctt actgatgagg cagtcgcgtc tgaaaggtcc 7560

gccggcgttg gacgagcgtg taccaacctg catttctttc cgtcatatac acaaaatact 7620

ttcatataaa cttacttggt cttacgtcat aaataaatat gtatacatat aaattaaaaa 7680

atttggtttt atatttttac aaaaagaatc gtttacttca tttctccctt ttaagcgata 7740

caatccatga aaaaagagaa aaagagagaa caggcttgtg ccttctttaa aacatcccac 7800

acaaaatcat attgaattga attttacatc ttaagctagt gtacaacaac tgctatatcc 7860

aaagaaaact aacgtggacc gcttttagag ttgagaaaaa ggtttgaaaa aaatagcaat 7920

acaaagactt gtttcatata taaaatacag ggagcacatt gagctaatat aacataaaca 7980

ctgcgaacca attccaatca aaaggtacac atgagagcat tcccccgagt actgccattt 8040

cgccatcaga gatcatataa taacatcctt cttcgaacgg cggtttaaac gcgtggccgt 8100

gccgtc 8106

<210> 29
<211> 2404
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i76220 integration construct

<400> 29
gacggcacgg ccacgcgttt aaaccgccgc acgtgtatgt acggctgtgt aaatatgata 60

atcatctcgg acgaacggcg tagtactctc catcccctaa aaatgttcac gtgtgactgc 120

tccatttcgc cggatgtcga gatgaccccc ccccctcaaa aggcactcac ctgctgacat 180

gccgtggcaa atgattgggg tcatcctttt tttctgttat ctctaagatc caaagaaaag 240

taaaaaaaaa aggttggggt acgaattgcc gccgagcctc cgatgccatt attcaatggg 300

tattgcagtt ggggtatagt tcctcggtgg caaatagttc tcccttcatt ttgtatataa 360

actgggcggc tattctaagc atatttctcc cttaggttat ctggtagtac gttatatctt 420

gttcttatat tttctatcta taagcaaaac caaacatatc aaaactacta gaaagacatt 480

gccccactgt gttcgctcgt ccaacgccgg cggacctttc tcgacgtggg ccttttttctt 540

gccatatgga tccgctgcac ggtcctgttc cctagcatgt acgtgagcgt atttcctttt 600

aaaccacgac gctttgtctt cattcaacgt ttcccattgt ttttttctac tattgctttg 660

ctgtgggaaa aacttatcga aagatgacga ctttttctta attctcgttt taagagcttg 720

gtgagcgcta ggagtcactg ccaggtatcg tttgaacacg gcattagtca gggaagtcat 780

aacacagtcc tttcccgcaa ttttcttttt ctattactct tggcctcctc tagtacactc 840

tatatttttt tatgcctcgg taatgatttt catttttttt tttccaccta gcggatgact 900

ctttttttttt cttagcgatt ggcattatca cataatgaat tatacattat ataaagtaat 960

gtgatttctt cgaagaatat actaaaaaat gagcaggcaa gataaacgaa ggcaaagatg 1020

acagagcaga aagccctagt aaagcgtatt acaaatgaaa ccaagattca gattgcgatc 1080

tctttaaagg gtggtcccct agcgatagag cactcgatct tcccagaaaa agaggcagaa 1140

gcagtagcag aacaggccac acaatcgcaa gtgattaacg tccacacagg tatagggttt 1200

ctggaccata tgatacatgc tctggccaag cattccggct ggtcgctaat cgttgagtgc 1260

attggtgact tacacataga cgaccatcac accactgaag actgcgggat tgctctcggt 1320

caagctttta aagaggccct aggggccgtg cgtggagtaa aaaggtttgg atcaggattt 1380

gcgcctttgg atgaggcact ttccagagcg gtggtagatc tttcgaacag gccgtacgca 1440

gttgtcgaac ttggtttgca aagggagaaa gtaggagatc tctcttgcga gatgatcccg 1500

cattttcttg aaagctttgc agaggctagc agaattaccc tccacgttga ttgtctgcga 1560

ggcaagaatg atcatcaccg tagtgagagt gcgttcaagg ctcttgcggt tgccataaga 1620

gaagccacct cgcccaatgg taccaacgat gttccctcca ccaaaggtgt tcttatgtag 1680

tgacaccgat tatttaaagc tgcagcatac gatatatata catgtgtata tatgtatacc 1740

tatgaatgtc agtaagtatg tatacgaaca gtatgatact gaagatgaca aggtaatgca 1800

tcattctata cgtgtcattc tgaacgaggc gcgctttcct tttttctttt tgctttttct 1860

tttttttttct cttgaactcg aggtccgccg gcgttggacg agcgtgatga tttctttcct 1920

ttttatattg acgacttttt ttttttcgtg tgtttttgtt ctcttataac cgagctgctt 1980

acttattatt atttcacctt ctcttttttat ttatacttat aattatttat tctttacata 2040

ctgttacaag aaactctttt ctacattaat tgcataaagt gtcaatcagc acatcctcta 2100

tatcgctatc aacaacaaat ttgacaaacc tgcctatatc ttcaggaaca actgccgcat 2160

cgctaccacc actacttgtg aagtccctgg agtttaatat gcactgaaat ttacctagcc 2220

gttttacaca agaccataat ccatccatgc tatcgcagta tatgattttg tgttcgtttt 2280

tcgtcttgcg aaaggcatcc tcaatggctt gtttcattga tccatcagtg tggctcgtag 2340

gtaccagcaa aaccacttca tcagcggcgt actcctggcg gtttaaacgc gtggccgtgc 2400

cgtc 2404

<210> 30
<211> 8536
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i73830 integration construct

<400> 30
gacggcacgg ccacgcgttt aaaccgccac ccagccaagg tagtctaaaa gctaatttct 60

ctaaaaggga gaaagttggt gatttttttat ctcgcattat tatatatgca agaatagtta 120

aggtatagtt ataaagtttt atcttaattg ccacatacgt acattgacac gtagaaggac 180

tccattattt ttttcattct agcatactat tattccttgt aacgtcccag agtattccat 240

ttaattgtcc tccatttctt aacggtgacg aaggatcacc atacaacaac tactaaagat 300

tatagtacac tctcaccttg caactattta tctgacattt gccttacttt tatctccagc 360

ttcccctcga ttttattttt caatttgatt tctaaagctt tttgcttagg cataccaaac 420

catccactca tttaacacct tatttttttt ttcgaagaca gcatccaact ttatacgttc 480

actacctttt tttttacaac aatttcattc ttcatcctat gaacgctcgt ccaacgccgg 540

cggacctttc agacgcgact gcctcatcag taagacccgt tgaaaagaac ttacctgaaa 600

aaaacgaata tatactagcg ttgaatgtta gcgtcaacaa caagaagttt aatgacgcgg 660

aggccaaggc aaaaagattc cttgattacg taagggagtt agaatcattt tgaataaaaa 720

acacgctttt tcagttcgag tttatcatta tcaatactgc catttcaaag aatacgtaaa 780

taattaatag tagtgatttt cctaacttta tttagtcaaa aaattagcct tttaattctg 840

ctgtaacccg tacatgccca aaataggggg cgggttacac agaatatata acatcgtagg 900

tgtctgggtg aacagtttat tcctggcatc cactaaatat aatggagccc gctttttaag 960

ctggcatcca gaaaaaaaaa gaatcccagc accaaaatat tgttttcttc accaaccatc 1020

agttcatagg tccattctct tagcgcaact acagagaaca ggggcacaaa caggcaaaaa 1080

acgggcacaa cctcaatgga gtgatgcaac ctgcctggag taaatgatga cacaaggcaa 1140

ttgacccacg catgtatcta tctcattttc ttacaccttc tattaccttc tgctctctct 1200

gatttggaaa aagctgaaaa aaaaggttga aaccagttcc ctgaaattat tcccctactt 1260

gactaataag tatataaaga cggtaggtat tgattgtaat tctgtaaatc tatttcttaa 1320

acttcttaaa ttctactttt atagttagtc tttttttag ttttaaaaca ccaagaactt 1380

agtttcgacc tcccgcgacc tccaaaatcg aactaccttc acaatggctg atttcgattc 1440

taaagaatac ttggagttag ttgacaagtg gtggcgtgcc accaactact tgtccgctgg 1500

tatgattttc ttgaagtcca acccattatt ctctgttact aataccccaa tcaaggccga 1560

agatgtcaaa gttaaaccaa ttggtcactg gggtactatt tccggtcaaa ctttcttata 1620

cgcccacgct aaccgtttga ttaacaagta cggtctcaac atgttttacg ttggtggtcc 1680

aggtcacggt ggtcaagtca tggttactaa cgcctactta gacggtgcct acaccgaaga 1740

ttacccagaa attactcaag acatcgaagg tatgtctcat ttgttcaagc gtttctcttt 1800

ccctggtggt attggttccc atatgaccgc tcaaactcca ggttccttgc acgaaggtgg 1860

tgaattgggt tactctttgt cccatgcttt cggtgctgtt ttggacaacc cagaccaagt 1920

tgcttttgct gtcgttggtg atggtgaagc tgaaactggt ccatctatgg cctcttggca 1980

ttccattaag ttcttaaatg ccaagaacga tggtgccgtt ttgccagttt tggatttaaa 2040

cggtttcaag atttccaatc caaccatttt ttctagaatg tctgatgaag aaattactaa 2100

gttcttcgaa ggtttgggtt attcccctag attcattgaa aatgatgaca ttcacgacta 2160

cgccacctac caccaattgg ccgctaacat cttagatcaa gccatcgaag acattcaagc 2220

tattcaaaat gacgccagag agaatggtaa atatcaagat ggtgaaattc cagcttggcc 2280

tgttattatc gctagattgc caaagggttg gggtggtcca acccacgatg cttctaataa 2340

tccaattgaa aactctttca gagctcacca agttccatta ccattggaac aacacgattt 2400

ggccaccttg ccagaattcg aagattggat gaactcttac aagccagaag aattattcaa 2460

cgctgatggt tccttgaagg atgagttgaa agctattgcc ccaaagggtg ataagagaat 2520

gtctgctaac ccaatcacca acggtggtgc tgacagatcc gacttgaaat tgccaaattg 2580

gagagaattc gctaacgaca tcaacgacga taccagaggt aaggaattcg ctgactctaa 2640

gagaaacatg gatatggcta ctttatccaa ctatttaggt gccgtttctc aattgaaccc 2700

aaccagattc agattcttcg gtccagatga aaccatgtcc aacagattgt ggggtttgtt 2760

taatgttacc ccacgtcaat ggatggaaga aatcaaggaa ccacaagatc aattgttgtc 2820

tccaactggt cgtatcatcg attcccaatt gtctgaacac caagctgaag gttggttgga 2880

aggttacact ttgactggta gagttggtat ctttgcctct tacgaatctt tcttgagagt 2940

tgttgatacc atggtcactc aacatttcaa gtggttgcgt cacgcttccg aacaagcttg 3000

gagaaatgac tatccatcct taaatttgat cgctacctct accgctttcc aacaagatca 3060

taacggttat actcaccaag accctggtat gttaactcat ttggccgaga agaagtctaa 3120

cttcattaga gaatatttgc cagccgacgg taactctttg ttagccgttc aagagagagc 3180

tttctctgaa agacataagg ttaacttatt gatcgcttct aaacaaccaa gacaacaatg 3240

gttcactgtt gaagaagctg aagtcttagc taacgaaggt ttgaagatta tcgattgggc 3300

ttctactgct ccatcttccg atgttgatat tacttttgct tctgccggta ctgaaccaac 3360

cattgagact ttggccgcct tatggttgat taatcaagct ttccctgacg ttaagtttag 3420

atacgttaac gttgttgaat tgttaagatt gcaaaagaaa tctgaaccaa acatgaacga 3480

cgaaagagaa ttatctgccg aagaatttaa taagtacttc caagccgaca ctccagttat 3540

cttcggtttc cacgcttacg aaaacttgat tgaatctttc tttttcgaga gaaagttcac 3600

cggtgatgtc tatgttcacg gttatagaga agatggtgat atcactacca cctacgatat 3660

gagagtctat tcccacttgg atcgtttcca tcaagccaag gaagccgccg aaatcttgtc 3720

tgctaacggt aaaatcgacc aagccgctgc cgacaccttt attgctaaga tggacgacac 3780

tttggccaaa cacttccaag ttactagaaa tgaaggtaga gatattgaag aattcactga 3840

ctggacttgg tctccattga agtaagtgaa tttactttaa atcttgcatt taaataaatt 3900

ttctttttat agctttatga cttagtttca atttatatac tattttaatg acattttcga 3960

ttcattgatt gaaagctttg tgtttttttct tgatgcgcta ttgcattgtt cttgtctttt 4020

tcgccacatg taatatctgt agtagatacc tgatacattg tggatgctga gtgaaatttt 4080

agttaataat ggaggcgctc ttaataattt tggggatatt ggcttatccc cgcgtgcttg 4140

gccggccgta cgaaaatcgt tattgtcttg aaggtgaaat ttctactctt attaatggtg 4200

aacgttaagc tgatgctatg atggaagctg attggtctta acttgcttgt catcttgcta 4260

atggtcattg gctcgtgtta ttacttaagt tatttgtact cgttttgaac gtaatgctaa 4320

tgatcatctt atggaataat agtgagtggt ttcagggtcc ataaagcttt tcaattcatc 4380

ttttttttttt ttgttctttt ttttgattcc ggtttctttg aaatttttttt gattcggtaa 4440

tctccgagca gaaggaagaa cgaaggaagg agcacagact tagattggta tatatacgca 4500

tatgtggtgt tgaagaaaca tgaaattgcc cagtattctt aacccaactg cacagaacaa 4560

aaacctgcag gaaacgaaga taaatcatgt cgaaagctac atataaggaa cgtgctgcta 4620

ctcatcctag tcctgttgct gccaagctat ttaatatcat gcacgaaaag caaacaaact 4680

tgtgtgcttc attggatgtt cgtaccacca aggaattact ggagttagtt gaagcattag 4740

gtcccaaaat ttgtttacta aaaacacatg tggatatctt gactgatttt tccatggagg 4800

gcacagttaa gccgctaaag gcattatccg ccaagtacaa ttttttactc ttcgaagaca 4860

gaaaatttgc tgacattggt aatacagtca aattgcagta ctctgcgggt gtatacagaa 4920

tagcagaatg ggcagacatt acgaatgcac acggtgtggt gggcccaggt attgttagcg 4980

gtttgaagca ggcggcggaa gaagtaacaa aggaacctag aggccttttg atgttagcag 5040

aattgtcatg caagggctcc ctagctactg gagaatatac taagggtact gttgacattg 5100

cgaagagtga caaagatttt gttatcggct ttattgctca aagagacatg ggtggaagag 5160

atgaaggtta cgattggttg attatgacac ccggtgtggg tttagatgac aagggagacg 5220

cattgggtca acagtataga accgtggatg atgtggtctc tacaggatct gacattatta 5280

ttgttggaag aggactattt gcaaagggaa gggatgctaa ggtagagggt gaacgttaca 5340

gaaaagcagg ctgggaagca tatttgagaa gatgcggcca gcaaaactaa aaaactgtat 5400

tataagtaaa tgcatgtata ctaaactcac aaattagagc ttcaatttaa ttatatcagt 5460

tattaccacg aaaatcgtta ttgtcttgaa ggtgaaattt ctactcttat taatggtgaa 5520

cgttaagctg atgctatgat ggaagctgat tggtcttaac ttgcttgtca tcttgctaat 5580

ggtcatatgg ctcgtgttat tacttaagtt atttgtactc gttttgaacg taatgctaat 5640

gatcatctta tggaataata gtgaacggcc ggccaagcac gcggggatgg gatgagcttg 5700

gagcaggaag aatacactat actggatcta aagagtacaa tagatggata agaatattgg 5760

cagcgcaaaa aggcttcaag cttacacaac acggtttatt tcgaaataat atccttctcg 5820

aaagctttaa cgaacgcaga attttcgagt tattaaactt aaaatacgct gaacccgaac 5880

atagaaatat cgaatgggaa aaaaaaactg cataaaggca ttaaaagagg agcgaatttt 5940

tttttaataa aaatcttaat aatcattaaa agataaataa tagtctatat atacgtatat 6000

aaataaaaaa tattcaaaaa ataaaataaa ctattatttt agcgtaaagg atggggaaag 6060

agaaaagaaa aaaattgatc tatcgatttc aattcaattc aatagatctt tatccttgtg 6120

cttgtgcctg aactgcggta acggcaacaa ctttgacgat gtcgtcgact gaacatcccc 6180

ttgacaaatc gttgataggt ttggcaaatc cctgacatat aggaccgatg gcttcggcct 6240

ttgcgaatct ttggaccaac ttgtatccga tgtttcctgc ctggatgtct gggaagatca 6300

agacatttgc cttaccagcg actttagatc caggggcttt caaatctgcg accttcttaa 6360

ccaatgaggc gtctaactgc aattcaccgt cgatgtctaa gtcaggccta gcctccttag 6420

ccaattttgt tgcctttgta accttgtcga ctaattcatg tgaggctgat cccatggttg 6480

agaatgacaa catggctacc cttggctcga tcttgcacaa attctttgca gtctcagcag 6540

tggtaattgc gattgaagat aactcttcag cggtaggaca aacatttaca gcgcagtcag 6600

cgaataacaa aaaaccgtcc tctccatact cgcagtcagg tactgacatc aagaagactg 6660

atgagacgac agatgcacct ggtactgttt tgacaatctg caaaccaggc cttaacaagt 6720

ctcctgtagt atgtatagca ccagatacca aaccgtcagc gtcacctaac ttgaccatca 6780

ttgttgcgaa gtagattggg tccctgacga ttttgtcagc cttctccaag gtgactccct 6840

tgtttttttct gatctcgtag aaagcgttgg cgtaaccggc ggtcttagaa gaagtttctg 6900

ggtcgactat ctctactccg gccaaattta ctccgaattt tgcggcgttt tccttaatga 6960

cagactctga accgaccaag attatgtcgg caataccgtc cctaataatc tcctctgaag 7020

ccctgatgtt cctctcttcc tcaccctctg ccaaaacgat tttcttcttg tcggccttgg 7080

ccaatccgaa gatattctcc atcaatttca ttgtgaaggt agttcgattt tggaggtcgc 7140

gggaggtcga aactaagttc ttggtgtttt aaaactaaaa aaaagactaa ctataaaagt 7200

agaatttaag aagtttaaga aatagattta cagaattaca atcaatacct accgtcttta 7260

tatacttatt agtcaagtag gggaataatt tcagggaact ggtttcaacc tttttttca 7320

gctttttcca aatcagagag agcagaaggt aatagaaggt gtaagaaaat gagatagata 7380

catgcgtggg tcaattgcct tgtgtcatca tttactccag gcaggttgca tcactccatt 7440

gaggttgtgc ccgttttttg cctgtttgtg cccctgttct ctgtagttgc gctaagagaa 7500

tggacctatg aactgatggt tggtgaagaa aacaatattt tggtgctggg attctttttt 7560

tttctggatg ccagcttaaa aagcgggctc cattatattt agtggatgcc aggaataaac 7620

tgttcaccca gacacctacg atgttatata ttctgtgtaa cccgccccct attttgggca 7680

tgtacgggtt acagcagaat taaaaggcta atttttgac taaataaagt taggaaaatc 7740

actactatta attatttacg tattctttga aatggcagta ttgataatga taaactcgaa 7800

ctgaaaaagc gtgtttttta ttcaaaatga ttctaactcc cttacgtaat caaggaatct 7860

ttttgccttg gcctccgcgt cattaaactt cttgttgttg acgctaacat tcaacgctag 7920

tatatattcg ttttttcag gtaagttctt ttcaacgggt cttactgatg aggcagtcgc 7980

gtctgaaagg tccgccggcg ttggacgagc gctccatgct ggacttactc gtcgaagatt 8040

tcctgctact ctctatataa ttagacaccc atgttataga tttcagaaaa caatgtaata 8100

atatatggta gcctcctgaa actaccaagg gaaaaatctc aacaccaaga gctcatattc 8160

gttggaatag cgataatatc tctttacctc aatcttatat gcatgttatt tgctcttata 8220

attggtctct atttagggaa aaaagtcggt ttgagagctt ctcgcgatgt gaaatctcaa 8280

tttgaactgc acgccaaagc tagcccattt cacgaacacc agaaagaaga aatccccaag 8340

gatcgcatga cagagtatgc tctctcatat cgttgagtat gaatgccaat acactgatca 8400

gctttacaag aaacgtaaaa tctggcacga tggtagactg aaatactttc agttaaacaa 8460

cagattcatg ctttatacgg aaaaggataa cgttttgtta gctagtgagg cggtttaaac 8520

gcgtggccgt gccgtc 8536

<210> 31
<211> 9734
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i74810 integration construct

<400> 31
gacggcacgg ccacgcgttt aaaccgcccg ctcgcctcat ccccacggga ataaggcagc 60

cgacaaaaga aaaacgaccg aaaaggaacc agaaagaaaa aagagggtgg gcgcgccgcg 120

gacgtgtaaa aagatatgca tccagcttct atatcgcttt aactttaccg ttttgggcat 180

cgggaacgta tgtaacattg atctcctctt gggaacggtg agtgcaacga atgcgatata 240

gcaccgacca tgtgggcaaa ttcgtaataa attcggggtg agggggattc aagacaagca 300

accttgttag tcagctcaaa cagcgattta acggttgagt aacacatcaa aacaccgttc 360

gaggtcaagc ctggcgtgtt taacaagttc ttgatatcat atataaatgt aataagaagt 420

ttggtaatat tcaattcgaa gtgttcagtc ttttacttct cttgttttat agaagaaaaa 480

acatcaagaa acatctttaa catacacaaa cacatactat cagaatacac gctcgtccaa 540

cgccggcgga cctttcagac gcgactgcct catcagtaag acccgttgaa aagaacttac 600

ctgaaaaaaa cgaatatata ctagcgttga atgttagcgt caacaacaag aagtttaatg 660

acgcggaggc caaggcaaaa agattccttg attacgtaag ggagttagaa tcattttgaa 720

taaaaaacac gctttttcag ttcgagttta tcattatcaa tactgccatt tcaaagaata 780

cgtaaataat taatagtagt gattttccta actttattta gtcaaaaaat tagcctttta 840

attctgctgt aacccgtaca tgcccaaaat agggggcggg ttacacagaa tatataacat 900

cgtaggtgtc tgggtgaaca gtttattcct ggcatccact aaatataatg gagcccgctt 960

tttaagctgg catccagaaa aaaaaagaat cccagcacca aaatattgtt ttcttcacca 1020

accatcagtt cataggtcca ttctcttagc gcaactacag agaacagggg cacaaacagg 1080

caaaaaacgg gcacaacctc aatggagtga tgcaacctgc ctggagtaaa tgatgacaca 1140

aggcaattga cccacgcatg tatctatctc attttcttac accttctatt accttctgct 1200

ctctctgatt tggaaaaagc tgaaaaaaaa ggttgaaacc agttccctga aattattccc 1260

ctacttgact aataagtata taaagacggt aggtattgat tgtaattctg taaatctatt 1320

tcttaaactt cttaaattct acttttatag ttagtctttt ttttagtttt aaaacaccaa 1380

gaacttagtt tcgacctccc gcgacctcca aaatcgaact accttcacaa tggctgattt 1440

cgattctaaa gaatacttgg agttagttga caagtggtgg cgtgccacca actacttgtc 1500

cgctggtatg attttcttga agtccaaccc attattctct gttactaata ccccaatcaa 1560

ggccgaagat gtcaaagtta aaccaattgg tcactggggt actatttccg gtcaaacttt 1620

cttatacgcc cacgctaacc gtttgattaa caagtacggt ctcaacatgt tttacgttgg 1680

tggtccaggt cacggtggtc aagtcatggt tactaacgcc tacttagacg gtgcctacac 1740

cgaagattac ccagaaatta ctcaagacat cgaaggtatg tctcatttgt tcaagcgttt 1800

ctctttccct ggtggtattg gttcccatat gaccgctcaa actccaggtt ccttgcacga 1860

aggtggtgaa ttgggttact ctttgtccca tgctttcggt gctgttttgg acaacccaga 1920

ccaagttgct tttgctgtcg ttggtgatgg tgaagctgaa actggtccat ctatggcctc 1980

ttggcattcc attaagttct taaatgccaa gaacgatggt gccgttttgc cagttttgga 2040

tttaaacggt ttcaagattt ccaatccaac catttttct agaatgtctg atgaagaaat 2100

tactaagttc ttcgaaggtt tgggttattc ccctagattc attgaaaatg atgacattca 2160

cgactacgcc acctaccacc aattggccgc taacatctta gatcaagcca tcgaagacat 2220

tcaagctatt caaaatgacg ccagagagaa tggtaaatat caagatggtg aaattccagc 2280

ttggcctgtt attatcgcta gattgccaaa gggttggggt ggtccaaccc acgatgcttc 2340

taataatcca attgaaaact ctttcagagc tcaccaagtt ccattaccat tggaacaaca 2400

cgatttggcc accttgccag aattcgaaga ttggatgaac tcttacaagc cagaagaatt 2460

attcaacgct gatggttcct tgaaggatga gttgaaagct attgccccaa agggtgataa 2520

gagaatgtct gctaacccaa tcaccaacgg tggtgctgac agatccgact tgaaattgcc 2580

aaattggaga gaattcgcta acgacatcaa cgacgatacc agaggtaagg aattcgctga 2640

ctctaagaga aacatggata tggctacttt atccaactat ttaggtgccg tttctcaatt 2700

gaacccaacc agattcagat tcttcggtcc agatgaaacc atgtccaaca gattgtgggg 2760

tttgtttaat gttaccccac gtcaatggat ggaagaaatc aaggaaccac aagatcaatt 2820

gttgtctcca actggtcgta tcatcgattc ccaattgtct gaacaccaag ctgaaggttg 2880

gttggaaggt tacactttga ctggtagagt tggtatcttt gcctcttacg aatctttctt 2940

gagagttgtt gataccatgg tcactcaaca tttcaagtgg ttgcgtcacg cttccgaaca 3000

agcttggaga aatgactatc catccttaaa tttgatcgct acctctaccg ctttccaaca 3060

agatcataac ggttatactc accaagaccc tggtatgtta actcatttgg ccgagaagaa 3120

gtctaacttc attagagaat atttgccagc cgacggtaac tctttgttag ccgttcaaga 3180

gagagctttc tctgaaagac ataaggttaa cttattgatc gcttctaaac aaccaagaca 3240

acaatggttc actgttgaag aagctgaagt cttagctaac gaaggtttga agattatcga 3300

ttgggcttct actgctccat cttccgatgt tgatattact tttgcttctg ccggtactga 3360

accaaccatt gagactttgg ccgccttatg gttgattaat caagctttcc ctgacgttaa 3420

gtttagatac gttaacgttg ttgaattgtt aagattgcaa aagaaatctg aaccaaacat 3480

gaacgacgaa agagaattat ctgccgaaga atttaataag tacttccaag ccgacactcc 3540

agttatcttc ggtttccacg cttacgaaaa cttgattgaa tctttctttt tcgagagaaa 3600

gttcaccggt gatgtctatg ttcacggtta tagagaagat ggtgatatca ctaccaccta 3660

cgatatgaga gtctattccc acttggatcg tttccatcaa gccaaggaag ccgccgaaat 3720

cttgtctgct aacggtaaaa tcgaccaagc cgctgccgac acctttattg ctaagatgga 3780

cgacactttg gccaaacact tccaagttac tagaaatgaa ggtagagata ttgaagaatt 3840

cactgactgg acttggtctc cattgaagta agtgaattta ctttaaatct tgcatttaaa 3900

taaattttct ttttatagct ttatgactta gtttcaattt atatactatt ttaatgacat 3960

tttcgattca ttgattgaaa gctttgtgtt ttttcttgat gcgctattgc attgttcttg 4020

tctttttcgc cacatgtaat atctgtagta gatacctgat acattgtgga tgctgagtga 4080

aattttagtt aataatggag gcgctcttaa taattttggg gatattggct tatccccgcg 4140

tgcttggccg gccgtacact gagtaatggt agttataaga aagagaccga gttagggaca 4200

gttagaggcg gtggagatat tccttatggc atgtctggcg atgataaaac ttttcaaacg 4260

gcagccccga tctaaaagag ctgacaggga aatggtcaga aaaagaaacg tgcacccgcc 4320

cgtctggacg cgccgctcac ccgcacggca gagaccaatc agtaaaaatc aacggttaac 4380

gacattacta tatatataat ataggaagca tttaatagaa cagcatcgta atatatgtgt 4440

actttgcagt tatgacgcca gatggcagta gtggaagata ttctttattg aaaaatagct 4500

tgtcacctta cgtacaatct tgatccggag cttttctttt tttgccgatt aagaattcgg 4560

tcgaaaaaag aaaaggagag ggccaagagg gagggcattg gtgactattg agcacgtgag 4620

tatacgtgat taagcacaca aaggcagctt ggagtatgtc tgttattaat ttcacaggta 4680

gttctggtcc attggtgaaa gtttgcggct tgcagagcac agaggccgca gaatgtgctc 4740

tagattccga tgctgacttg ctgggtatta tatgtgtgcc caatagaaag agaacaattg 4800

acccggttat tgcaaggaaa atttcaagtc ttgtaaaagc atataaaaat agttcaggca 4860

ctccgaaata cttggttggc gtgtttcgta atcaacctaa ggaggatgtt ttggctctgg 4920

tcaatgatta cggcattgat atcgtccaac tgcatggaga tgagtcgtgg caagaatacc 4980

aagagttcct cggtttgcca gttattaaaa gactcgtatt tccaaaagac tgcaacatac 5040

tactcagtgc agcttcacag aaacctcatt cgtttattcc cttgtttgat tcagaagcag 5100

gtgggacagg tgaacttttg gattggaact cgatttctga ctgggttgga aggcaagaga 5160

gccccgaaag cttacatttt atgttagctg gtggactgac gccagaaaat gttggtgatg 5220

cgcttagatt aaatggcgtt attggtgttg atgtaagcgg aggtgtggag acaaatggtg 5280

taaaagactc taacaaaata gcaaatttcg tcaaaaatgc taagaaatag gttattactg 5340

agtagtattt atttaagtat tgtttgtgca cttgcctgca ggccttttga aaagcaagca 5400

taaaagatct aaacataaaa tctgtaaaat aacaagatgt aaagataatg ctaaatcatt 5460

tggctttttg attgattgta caggaaaata tacatcgcag ggggttgact tttaccattt 5520

caccgcaatg gaatcaaact tgttgaagag aatgttcaca ggcgcatacg ctacaatgac 5580

acggccggcc aagcacgcgg ggataagcca atatccccaa aattattaag agcgcctcca 5640

ttattaacta aaatttcact cagcatccac aatgtatcag gtatctacta cagatattac 5700

atgtggcgaa aaagacaaga acaatgcaat agcgcatcaa gaaaaaacac aaagctttca 5760

atcaatgaat cgaaaatgtc attaaaatag tatataaatt gaaactaagt cataaagcta 5820

taaaaagaaa atttatttaa atgcaagatt taaagtaaat tcacttactt caatggagac 5880

caagtccagt cagtgaattc ttcaatatct ctaccttcat ttctagtaac ttggaagtgt 5940

ttggccaaag tgtcgtccat cttagcaata aaggtgtcgg cagcggcttg gtcgatttta 6000

ccgttagcag acaagatttc ggcggcttcc ttggcttgat ggaaacgatc caagtgggaa 6060

tagactctca tatcgtaggt ggtagtgata tcaccatctt ctctataacc gtgaacatag 6120

acatcaccgg tgaactttct ctcgaaaaag aaagattcaa tcaagttttc gtaagcgtgg 6180

aaaccgaaga taactggagt gtcggcttgg aagtacttat taaattcttc ggcagataat 6240

tctctttcgt cgttcatgtt tggttcagat ttcttttgca atcttaacaa ttcaacaacg 6300

ttaacgtatc taaacttaac gtcagggaaa gcttgattaa tcaaccataa ggcggccaaa 6360

gtctcaatgg ttggttcagt accggcagaa gcaaaagtaa tatcaacatc ggaagatgga 6420

gcagtagaag cccaatcgat aatcttcaaa ccttcgttag ctaagacttc agcttcttca 6480

acagtgaacc attgttgtct tggttgttta gaagcgatca ataagttaac cttatgtctt 6540

tcagagaaag ctctctcttg aacggctaac aaagagttac cgtcggctgg caaatattct 6600

ctaatgaagt tagacttctt ctcggccaaa tgagttaaca taccagggtc ttggtgagta 6660

taaccgttat gatcttgttg gaaagcggta gaggtagcga tcaaatttaa ggatggatag 6720

tcatttctcc aagcttgttc ggaagcgtga cgcaaccact tgaaatgttg agtgaccatg 6780

gtatcaacaa ctctcaagaa agattcgtaa gaggcaaaga taccaactct accagtcaaa 6840

gtgtaacctt ccaaccaacc ttcagcttgg tgttcagaca attgggaatc gatgatacga 6900

ccagttggag acaacaattg atcttgtggt tccttgattt cttccatcca ttgacgtggg 6960

gtaacattaa acaaacccca caatctgttg gacatggttt catctggacc gaagaatctg 7020

aatctggttg ggttcaattg agaaacggca cctaaatagt tggataaagt agccatatcc 7080

atgtttctct tagagtcagc gaattcctta cctctggtat cgtcgttgat gtcgttagcg 7140

aattctctcc aatttggcaa tttcaagtcg gatctgtcag caccaccgtt ggtgattggg 7200

ttagcagaca ttctcttatc acccttttggg gcaatagctt tcaactcatc cttcaaggaa 7260

ccatcagcgt tgaataattc ttctggcttg taagagttca tccaatcttc gaattctggc 7320

aaggtggcca aatcgtgttg ttccaatggt aatggaactt ggtgagctct gaaagagttt 7380

tcaattggat tattagaagc atcgtgggtt ggaccacccc aacccttttgg caatctagcg 7440

ataataacag gccaagctgg aatttcacca tcttgatatt taccattctc tctggcgtca 7500

ttttgaatag cttgaatgtc ttcgatggct tgatctaaga tgttagcggc caattggtgg 7560

taggtggcgt agtcgtgaat gtcatcattt tcaatgaatc taggggaata acccaaacct 7620

tcgaagaact tagtaatttc ttcatcagac attctagaaa aaatggttgg attggaaatc 7680

ttgaaaccgt ttaaatccaa aactggcaaa acggcaccat cgttcttggc atttaagaac 7740

ttaatggaat gccaagaggc catagatgga ccagtttcag cttcaccatc accaacgaca 7800

gcaaaagcaa cttggtctgg gttgtccaaa acagcaccga aagcatggga caaagagtaa 7860

cccaattcac caccttcgtg caaggaacct ggagtttgag cggtcatatg ggaaccaata 7920

ccaccaggga aagagaaacg cttgaacaaa tgagacatac cttcgatgtc ttgagtaatt 7980

tctgggtaat cttcggtgta ggcaccgtct aagtaggcgt tagtaaccat gacttgacca 8040

ccgtgacctg gaccaccaac gtaaaacatg ttgagaccgt acttgttaat caaacggtta 8100

gcgtgggcgt ataagaaagt ttgaccggaa atagtacccc agtgaccaat tggtttaact 8160

ttgacatctt cggccttgat tggggtatta gtaacagaga ataatgggtt ggacttcaag 8220

aaaatcatac cagcggacaa gtagttggtg gcacgccacc acttgtcaac taactccaag 8280

tattctttag aatcgaaatc agccattgtg aaggtagttc gattttggag gtcgcgggag 8340

gtcgaaacta agttcttggt gttttaaaac taaaaaaaag actaactata aaagtagaat 8400

ttaagaagtt taagaaatag atttacagaa ttacaatcaa tacctaccgt ctttatatac 8460

ttattagtca agtaggggaa taatttcagg gaactggttt caacctttt tttcagcttt 8520

ttccaaatca gagagagcag aaggtaatag aaggtgtaag aaaatgagat agatacatgc 8580

gtgggtcaat tgccttgtgt catcatttac tccaggcagg ttgcatcact ccattgaggt 8640

tgtgcccgtt ttttgcctgt ttgtgcccct gttctctgta gttgcgctaa gagaatggac 8700

ctatgaactg atggttggtg aagaaaacaa tattttggtg ctgggattct ttttttttct 8760

ggatgccagc ttaaaaagcg ggctccatta tatttagtgg atgccaggaa taaactgttc 8820

acccagacac ctacgatgtt atatattctg tgtaacccgc cccctatttt gggcatgtac 8880

gggttacagc agaattaaaa ggctaatttt ttgactaaat aaagttagga aaatcactac 8940

tattaattat ttacgtattc tttgaaatgg cagtattgat aatgataaac tcgaactgaa 9000

aaagcgtgtt ttttattcaa aatgattcta actcccttac gtaatcaagg aatcttttg 9060

ccttggcctc cgcgtcatta aacttcttgt tgttgacgct aacattcaac gctagtatat 9120

attcgttttt ttcaggtaag ttcttttcaa cgggtcttac tgatgaggca gtcgcgtctg 9180

aaaggtccgc cggcgttgga cgagcgtgta ccaacctgca tttctttccg tcatatacac 9240

aaaatacttt catataaact tacttggtct tacgtcataa ataaatatgt atacatataa 9300

attaaaaaat ttggttttat atttttacaa aaagaatcgt ttacttcatt tctccctttt 9360

aagcgataca atccatgaaa aaagagaaaa agagagaaca ggcttgtgcc ttctttaaaa 9420

catcccacac aaaatcatat tgaattgaat tttacatctt aagctagtgt acaacaactg 9480

ctatatccaa agaaaactaa cgtggaccgc ttttagagtt gagaaaaagg tttgaaaaaa 9540

atagcaatac aaagacttgt ttcatatata aaatacaggg agcacattga gctaatataa 9600

cataaacact gcgaaccaat tccaatcaaa aggtacacat gagagcattc ccccgagtac 9660

tgccatttcg ccatcagaga tcatataata acatccttct tcgaacggcg gtttaaacgc 9720

gtggccgtgc cgtc 9734

<210> 32
<211> 7980
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i76221 integration construct

<400> 32
gacggcacgg ccacgcgttt aaaccgccgc acgtgtatgt acggctgtgt aaatatgata 60

atcatctcgg acgaacggcg tagtactctc catcccctaa aaatgttcac gtgtgactgc 120

tccatttcgc cggatgtcga gatgaccccc ccccctcaaa aggcactcac ctgctgacat 180

gccgtggcaa atgattgggg tcatcctttt tttctgttat ctctaagatc caaagaaaag 240

taaaaaaaaa aggttggggt acgaattgcc gccgagcctc cgatgccatt attcaatggg 300

tattgcagtt ggggtatagt tcctcggtgg caaatagttc tcccttcatt ttgtatataa 360

actgggcggc tattctaagc atatttctcc cttaggttat ctggtagtac gttatatctt 420

gttcttatat tttctatcta taagcaaaac caaacatatc aaaactacta gaaagacatt 480

gccccactgt gttcgctcgt ccaacgccgg cggacctttc agacgcgact gcctcatcag 540

taagacccgt tgaaaagaac ttacctgaaa aaaacgaata tatactagcg ttgaatgtta 600

gcgtcaacaa caagaagttt aatgacgcgg aggccaaggc aaaaagattc cttgattacg 660

taagggagtt agaatcattt tgaataaaaa acacgctttt tcagttcgag tttatcatta 720

tcaatactgc catttcaaag aatacgtaaa taattaatag tagtgatttt cctaacttta 780

tttagtcaaa aaattagcct tttaattctg ctgtaacccg tacatgccca aaataggggg 840

cgggttacac agaatatata acatcgtagg tgtctgggtg aacagtttat tcctggcatc 900

cactaaatat aatggagccc gctttttaag ctggcatcca gaaaaaaaaa gaatcccagc 960

accaaaatat tgttttcttc accaaccatc agttcatagg tccattctct tagcgcaact 1020

acagagaaca ggggcacaaa caggcaaaaa acgggcacaa cctcaatgga gtgatgcaac 1080

ctgcctggag taaatgatga cacaaggcaa ttgacccacg catgtatcta tctcattttc 1140

ttacaccttc tattaccttc tgctctctct gatttggaaa aagctgaaaa aaaaggttga 1200

aaccagttcc ctgaaattat tcccctactt gactaataag tatataaaga cggtaggtat 1260

tgattgtaat tctgtaaatc tatttcttaa acttcttaaa ttctactttt atagttagtc 1320

tttttttag ttttaaaaca ccaagaactt agtttcgacc tcccgcgacc tccaaaatcg 1380

aactaccttc acaatggaac attctgtaat cgaaccaact gtgcccatgc cgctaccagc 1440

catgtttgac gctccatctg gtatttttag ctctttggac gacgctgtgc aagcagccac 1500

cttagcccaa caacaactaa gttcagttga gttgcgtcag caagtaatca aagccataag 1560

agtggccgga gaaaggtatg cacaagtttt ggctgaaatg gcagttgctg aaactggtat 1620

gggtagggtg gtggataagt acattaagaa tgtctctcaa gctcgtcata cgcctggtat 1680

agaatgttta tcggccgagg ttcttacggg tgataatggc ctaacattga ttgaaaatgc 1740

cccttgggga gtcgtagctt cagtcacgcc aagcacaaat ccagcagcta cggtaattaa 1800

taatgcaatc tcaatgattg cagcggggaa ttcagtcgtg ttcgcaccac atccttctgc 1860

caaaaacgtc tcactaagga ctatttcttt actcaacaag gccattgtcg ctaccggcgg 1920

cccagaaaat ttactagtta gtgtggcaaa ccctaacatc gaaactgcac agagattatt 1980

cagatatccg ggtattggat tgttagttgt gacaggtggt gaagccgtcg ttgaagccgc 2040

taggaagcat acagataaaa ggttaattgc agccggcgct ggtaatcctc ctgttgttgt 2100

ggacgaaact gctgacatac ctaaagccgc aagagcaatt gtcaagggtg cttctttcga 2160

caacaacata atttgtgctg atgaaaaagt tttgattgtg gtagacagag ttgcagatgc 2220

actattggca gaaatgcaaa gaaataacgc cgtcttactt acacccgaac agaccgaaag 2280

actactaccc gctcttttgt ccgatattga cgaacagggc aaaggacgtg tgaatagaga 2340

ttatgttgga agagatgcgg ctaaattagc agcggctatt ggtctggaag ttagcgaaca 2400

tactcgtcta ctcctggcag agacagacgc tgatcatcca ttcgccgtga cggagctgat 2460

gatgccagtg ttaccagtaa taagagtcaa gaatgtagat gatgcaatcg cattggcagt 2520

taagctagag tcaggctgca gacacacagc tgcgatgcac tctactaata taagaaactt 2580

aaatagaatg gctaatgcca tcaatacctc tatctttgta aaaaatggtc catgtattgc 2640

aggtttgggt ttaggcggtg aaggttggac ttcaatgact attagcactc cgaccggtga 2700

aggtgttaca agcgctcgta cctttgtcag attaagaagg tgtgtcttag tcgacatgtt 2760

tcggattgct taagcggccg cgagtaataa ttattgcttc catataatat ttttatatac 2820

ctcttatttt tatgtattag ttaattaagt atttttatct atctgcttat cattttcttt 2880

tcatataggg ggggttggtg ttttcttgcc catcagattg atgtcctcca actcggcact 2940

attttacaaa gggtttttt gtaagagaag gagaagacag atactaaacc atacgttact 3000

cgaaacaaaa aaaaaaaaaa tggaaaaagc tgctatcaac aaaagacggc ctcatcaaac 3060

ctaaagaaac catgtcagcg tatgtatata ccttgtaatt tacgtttcct taaatcttct 3120

ttctactaac gttttcatta ttctatactc tatgaccaat aaaaacagac tgtactttca 3180

aaatttaccc agtaggccag caaataaaga aaattatacc agattacttc tgaaacacat 3240

taatcccaac aacaagtatg ccattaatcc gtcgctaccc catccccgcg tgcttggccg 3300

gccgtttctc gacgtgggcc tttttcttgc catatggatc cgctgcacgg tcctgttccc 3360

tagcatgtac gtgagcgtat ttccttttaa accacgacgc tttgtcttca ttcaacgttt 3420

cccattgttt ttttctacta ttgctttgct gtgggaaaaa cttatcgaaa gatgacgact 3480

ttttcttaat tctcgtttta agagcttggt gagcgctagg agtcactgcc aggtatcgtt 3540

tgaacacggc attagtcagg gaagtcataa cacagtcctt tcccgcaatt ttcttttttct 3600

attactcttg gcctcctcta gtacactcta tatttttta tgcctcggta atgattttca 3660

ttttttttt tccacctagc ggatgactct tttttttct tagcgattgg cattatcaca 3720

taatgaatta tacattatat aaagtaatgt gatttcttcg aagaatatac taaaaaatga 3780

gcaggcaaga taaacgaagg caaagatgac agagcagaaa gccctagtaa agcgtattac 3840

aaatgaaacc aagattcaga ttgcgatctc tttaaagggt ggtcccctag cgatagagca 3900

ctcgatcttc ccagaaaaag aggcagaagc agtagcagaa caggccacac aatcgcaagt 3960

gattaacgtc cacacaggta tagggtttct ggaccatatg atacatgctc tggccaagca 4020

ttccggctgg tcgctaatcg ttgagtgcat tggtgactta cacatagacg accatcacac 4080

cactgaagac tgcgggattg ctctcggtca agcttttaaa gaggccctag gggccgtgcg 4140

tggagtaaaa aggtttggat caggatttgc gcctttggat gaggcacttt ccagagcggt 4200

ggtagatctt tcgaacaggc cgtacgcagt tgtcgaactt ggtttgcaaa gggagaaagt 4260

aggagatctc tcttgcgaga tgatcccgca ttttcttgaa agctttgcag aggctagcag 4320

aattaccctc cacgttgatt gtctgcgagg caagaatgat catcaccgta gtgagagtgc 4380

gttcaaggct cttgcggttg ccataagaga agccacctcg cccaatggta ccaacgatgt 4440

tccctccacc aaaggtgttc ttatgtagtg acaccgatta tttaaagctg cagcatacga 4500

tatatataca tgtgtatata tgtataccta tgaatgtcag taagtatgta tacgaacagt 4560

atgatactga agatgacaag gtaatgcatc attctatacg tgtcattctg aacgaggcgc 4620

gctttccttt tttcttttg ctttttcttt ttttttctct tgaactcgac ggccggccaa 4680

gcacgcgggg atggggtagc gacggattaa tggcatactt gttgttggga ttaatgtgtt 4740

tcagaagtaa tctggtataa ttttctttat ttgctggcct actgggtaaa ttttgaaagt 4800

acagtctgtt tttattggtc atagagtata gaataatgaa aacgttagta gaaagaagat 4860

ttaaggaaac gtaaattaca aggtatatac atacgctgac atggtttctt taggtttgat 4920

gaggccgtct tttgttgata gcagcttttt ccatttttt ttttttgtt tcgagtaacg 4980

tatggtttag tatctgtctt ctccttctct tacaaaaaaa ccctttgtaa aatagtgccg 5040

agttggagga catcaatctg atgggcaaga aaacaccaac ccccctata tgaaaagaaa 5100

atgataagca gatagataaa aatacttaat taactaatac ataaaaataa gaggtatata 5160

aaaatattat atggaagcaa taattattac tcgcggccgc ttaagcaatc cgaaacatgt 5220

cgactaagac acaccttctt aatctgacaa aggtacgagc gcttgtaaca ccttcaccgg 5280

tcggagtgct aatagtcatt gaagtccaac cttcaccgcc taaacccaaa cctgcaatac 5340

atggaccatt ttttacaaag atagaggtat tgatggcatt agccattcta tttaagtttc 5400

ttatattagt agagtgcatc gcagctgtgt gtctgcagcc tgactctagc ttaactgcca 5460

atgcgattgc atcatctaca ttcttgactc ttattactgg taacactggc atcatcagct 5520

ccgtcacggc gaatggatga tcagcgtctg tctctgccag gagtagacga gtatgttcgc 5580

taacttccag accaatagcc gctgctaatt tagccgcatc tcttccaaca taatctctat 5640

tcacacgtcc tttgccctgt tcgtcaatat cggacaaaag agcgggtagt agtctttcgg 5700

tctgttcggg tgtaagtaag acggcgttat ttctttgcat ttctgccaat agtgcatctg 5760

caactctgtc taccacaatc aaaacttttt catcagcaca aattatgttg ttgtcgaaag 5820

aagcaccctt gacaattgct cttgcggctt taggtatgtc agcagtttcg tccacaacaa 5880

caggaggatt accagcgccg gctgcaatta acctttttatc tgtatgcttc ctagcggctt 5940

caacgacggc ttcaccacct gtcacaacta acaatccaat acccggatat ctgaataatc 6000

tctgtgcagt ttcgatgtta gggtttgcca cactaactag taaattttct gggccgccgg 6060

tagcgacaat ggccttgttg agtaaagaaa tagtccttag tgagacgttt ttggcagaag 6120

gatgtggtgc gaacacgact gaattccccg ctgcaatcat tgagattgca ttattaatta 6180

ccgtagctgc tggatttgtg cttggcgtga ctgaagctac gactccccaa ggggcatttt 6240

caatcaatgt taggccatta tcacccgtaa gaacctcggc cgataaacat tctataccag 6300

gcgtatgacg agcttgagag acattcttaa tgtacttatc caccacccta cccataccag 6360

tttcagcaac tgccatttca gccaaaactt gtgcatacct ttctccggcc actcttatgg 6420

ctttgattac ttgctgacgc aactcaactg aacttagttg ttgttgggct aaggtggctg 6480

cttgcacagc gtcgtccaaa gagctaaaaa taccagatgg agcgtcaaac atggctggta 6540

gcggcatggg cacagttggt tcgattacag aatgttccat tgtgaaggta gttcgatttt 6600

ggaggtcgcg ggaggtcgaa actaagttct tggtgtttta aaactaaaaa aaagactaac 6660

tataaaagta gaatttaaga agtttaagaa atagatttac agaattacaa tcaataccta 6720

ccgtctttat atacttatta gtcaagtagg ggaataattt cagggaactg gtttcaacct 6780

ttttttcag ctttttccaa atcagagaga gcagaaggta atagaaggtg taagaaaatg 6840

agatagatac atgcgtgggt caattgcctt gtgtcatcat ttactccagg caggttgcat 6900

cactccattg aggttgtgcc cgttttttgc ctgtttgtgc ccctgttctc tgtagttgcg 6960

ctaagagaat ggacctatga actgatggtt ggtgaagaaa acaatatttt ggtgctggga 7020

ttctttttt ttctggatgc cagcttaaaa agcgggctcc attatattta gtggatgcca 7080

ggaataaact gttcacccag acacctacga tgttatatat tctgtgtaac ccgccccta 7140

ttttgggcat gtacgggtta cagcagaatt aaaaggctaa ttttttgact aaataaagtt 7200

aggaaaatca ctactattaa ttatttacgt attctttgaa atggcagtat tgataatgat 7260

aaactcgaac tgaaaaagcg tgtttttttat tcaaaatgat tctaactccc ttacgtaatc 7320

aaggaatctt tttgccttgg cctccgcgtc attaaacttc ttgttgttga cgctaacatt 7380

caacgctagt atatattcgt ttttttcagg taagttcttt tcaacgggtc ttactgatga 7440

ggcagtcgcg tctgaaaggt ccgccggcgt tggacgagcg tgatgatttc tttccttttt 7500

atattgacga ctttttttttt ttcgtgtgtt tttgttctct tataaccgag ctgcttactt 7560

attattattt caccttctct ttttatttat acttataatt atttattctt tacatactgt 7620

tacaagaaac tcttttctac attaattgca taaagtgtca atcagcacat cctctatatc 7680

gctatcaaca acaaatttga caaacctgcc tatatcttca ggaacaactg ccgcatcgct 7740

accaccacta cttgtgaagt ccctggagtt taatatgcac tgaaatttac ctagccgttt 7800

tacacaagac cataatccat ccatgctatc gcagtatatg attttgtgtt cgtttttcgt 7860

cttgcgaaag gcatcctcaa tggcttgttt cattgatcca tcagtgtggc tcgtaggtac 7920

cagcaaaacc acttcatcag cggcgtactc ctggcggttt aaacgcgtgg ccgtgccgtc 7980

<210> 33
<211> 13266
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i84022 integration construct

<400> 33
gacggcacgg ccacgcgttt aaaccgccaa gtgatgtaac taaatacacg attaccatgg 60

aaattaacgt acctttttttg tgcgtgtatt gaaatattat gacatattac agaaagggtt 120

cgcaagtcct gtttctatgc ctttctctta gtaattcacg aaataaacct atggtttacg 180

aaatgatcca cgaaaatcat gttattattt acatcaacat atcgcgaaaa ttcatgtcat 240

gtccacatta acatcattgc agagcaacaa ttcattttca tagagaaatt tgctactatc 300

acccactagt actaccattg gtacctacta ctttgaattg tactaccgct gggcgttatt 360

aggtgtgaaa ccacgaaaag ttcaccataa cttcgaataa agtcgcggaa aaaagtaaac 420

agctattgct actcaaatga ggtttgcaga agcttgttga agcatgatga agcgttctaa 480

acgcactatt catcattaaa tatttaaagc tcataaaatt gtattcaatt cctattctaa 540

atggctttta tttctattac aactattagc tctaaatcca tatcctcata agcagcaatc 600

aattctatct atactttaaa cgctcgtcca acgccggcgg acctgatgtg tattactagt 660

gtcgacgaca gcattcgccc agtatttttt ttattctaca aaccttctat aatttcaaag 720

tatttacata attctgtatc agtttaatca ccataatatc gttttctttg tttagtgcaa 780

ttaatttttc ctattgttac ttcgggcctt tttctgtttt atgagctatt ttttccgtca 840

tccttccgga tccagatttt cagcttcatc tccagattgt gtctacgtaa tgcacgccat 900

cattttaaga gaggacctcc cgcgacctcc aaaatcgaac taccttcaca atgaaacttc 960

ttagtagtat tgagcaggcg tgtgacatct gtagattgaa gaaattgaaa tgtagtaagg 1020

agaagcccaa atgtgcgaaa tgccttaaaa ataattggga atgcagatat agtccgaaga 1080

cgaagcgcag tcccccttacc cgcgcgcacc ttacggaggt cgagagtcgc cttgagcgcc 1140

ttgagcaact tttccttctt atcttcccca gagaggattt ggatatgatc cttaagatgg 1200

acagtcttca agacattaag gcgcttctta cggggctttt cgtgcaggac aacgtcaaca 1260

aggacgcggt gacggaccgc cttgccagtg tcgaaaccga catgcccctt acgcttcgcc 1320

aacaccgcat ttccgccacg agtagtagtg aggaatcctc caataagggg cagcgccaac 1380

ttaccgtgag tatcgatagt gcggcccacc acgacaatag tacgatcccc cttgacttca 1440

tgccgcgcga cgccttgcac gggttcgact ggagtgagga agacgatatg agtgacggtc 1500

ttccgtttct taagaccgat ccgaataaca acggtttttt cggtgatggg agtttgcttt 1560

gcatcttgag aagtatcggt ttcaagcccg agaactatac caatagtaat gtcaatcgct 1620

tgcccacgat gatcaccgac cgctataccc ttgccagtcg cagtacgacg agtagacttt 1680

tgcagtccta cttgaacaac ttccatccgt attgtcccat tgtccatagt cccaccctta 1740

tgatgcttta caacaatcaa atcgagattg ccagtaaaga ccagtggcag attttgttca 1800

attgtattct tgcgatcggg gcgtggtgca ttgaaggtga gagtaccgac attgacgtct 1860

tctattacca gaacgccaag agtcacctta cctccaaagt gtttgaaagt gggagtatta 1920

tccttgtcac ggcgcttcac ttgcttagta gatacacgca atggcgccaa aagacgaaca 1980

cctcctacaa cttccattcc ttcagtattc gcatggcgat tagtcttggt cttaaccgcg 2040

atttgccgag tagtttttcc gactcctcca tccttgagca gcgcagaaga atctggtgga 2100

gtgtgtatag ttgggaaatt cagcttagtc ttttgtacgg gagaagtatt caattgagtc 2160

aaaacacgat tagttttccc agtagtgtgg atgacgtcca aagaacgacg acggggccga 2220

cgatttacca cggtattatc gagacggcgc gcttgcttca ggtctttacg aagatttacg 2280

agcttgataa gacggtgacc gcggagaagt cccccatttg cgcgaagaag tgtcttatga 2340

tctgcaacga aatcgaagaa gtcagtcgcc aagcgccgaa attccttcag atggacatca 2400

gtacgacggc ccttacgaac cttcttaaag agcatccctg gcttagtttc acgcgctttg 2460

agcttaaatg gaagcaactt agtttgatta tctacgtgct tcgcgacttc tttaccaact 2520

tcacgcaaaa gaaaagtcag cttgagcaag accagaacga ccaccagtcc tacgaggtca 2580

agagatgtag tattatgctt tccgacgcgg cgcagcgcac cgtcatgagt gtgtcctcct 2640

acatggataa ccacaacgtg acgccgtact tcgcgtggaa ctgcagttac tatcttttta 2700

acgcggtgct tgtgccgatt aaaacccttt tgagtaatag taagagtaac gccgaaaaca 2760

atgaaacggc gcagcttctt cagcagatca ataccgtcct tatgcttctt aagaagcttg 2820

cgaccttcaa gattcaaacc tgcgagaagt atatccaggt gcttgaggaa gtgtgcgccc 2880

ccttccttct tagtcaatgc gcgattccgc ttccccacat ttcctacaat aactccaacg 2940

ggtccgcgat caagaacatc gtggggagtg cgaccattgc gcagtatccc accttgcccg 3000

aagagaacgt gaataacatt tccgtcaagt acgtcagtcc cggtagtgtg ggtcccagtc 3060

ccgtcccgct taagagtggg gcgtcctttt ccgaccttgt gaaacttctt agtaatagac 3120

cgccgagtag aaatagtccg gtcacgattc cgcgctccac gcccagtcac agaagtgtga 3180

ccccccttcct tggtcagcaa cagcaacttc agagtcttgt cccgcttacg cccagtgccc 3240

ttttcggggg tgcgaacttc aaccagtccg gtaacatcgc cgactccagt cttagtttta 3300

cctttaccaa ttcctccaat gggcccaatt tgattacgac ccagacgaac agtcaggcct 3360

tgagtcagcc gatcgcgagt agtaatgtcc acgacaattt tatgaacaac gagattaccg 3420

cctccaagat cgacgacggg aacaacagta agccgcttag tcccgggtgg accgatcaga 3480

ccgcctacaa tgccttcggg attaccacgg gtatgttcaa cacgaccacg atggacgacg 3540

tgtacaatta ccttttgac gacgaggaca cgccgccgaa tccgaagaag gaatgagcca 3600

attggtgcgg caattgataa taacgaaaat gtcttttaat gatctgggta taatgaggaa 3660

ttttccgaac gtttttactt tatatatata tatacatgta acatatattc tatacgctat 3720

atcgagaaaa cgcgatggtg gggtgacttt caactcggcg tatccccgcg tgcttggccg 3780

gccgtagtta tgacaattac aacaacagaa ttctttctat atatgcacga acttgtaata 3840

tggaagaaat tatgacgtac aaactataaa gtaaatattt tacgtaacac atggtgctgt 3900

tgtgcttctt tttcaagaga ataccaatga cgtatgacta agtttatgta ttttccaaaa 3960

cctgtttagc cctggcgaca gatacgtctc cggcttcaac gatgaccctg gtgaccctgt 4020

caatgtcggc tccggtggca ccggccatga ttgcgatgtt ccttgcgtgt aaggtcatgt 4080

gtccccttg gattccctcg gttgccaagg ccctaattgc ggccatattc tgagccaaac 4140

caacggcggc agtaacctgg gccaactcag tagcggtttc gacctgcatt aaggccaaag 4200

cggccctagc tgtagggtga gtcttggtgg ctcctcctac caaacccaag gccaaaggca 4260

attcaatggt accgaccaac ctaccgtcgt tggccaactc ccaccttgtc aaagaggtgt 4320

aatgtccggt cctggcggcg taggcgtggg ctccagcttc gatggccctc cagtcgttac 4380

ctgttgcgac gacgactggg tcaattccgt tcataattcc cttgttatgg gttgcggccc 4440

tgtaagggtc gactattgct aaggcgcagg cttcaaccat tccccttgca acgtcggcac 4500

catcgtatcc ctgggtggtc aaagtctcag gggctaactc aaccctggct cttaccaacc 4560

tcaagtcggc caagttagac aaaatcctca acctgacggt tccaccagcg atcctctcta 4620

cctctggagc taacctttca gccatggtgt taactgtgtt ggcacccatg gcgtctctga 4680

catcaacaat caagtgcaat acgaccattg caccaacagg ggtgtcccta aaaacatgga 4740

cctcaatgtc tctgcaacca ccacctaaac caaccaaaac tggatctacg gcatctgctg 4800

cttccatgaa agcagcctta tgggccaaca acctttgcct agctccttct gggtctccta 4860

atccgacaac ttggatttgg gccctcatta aaggtgcagt tccgtgtgcg gtgaatccac 4920

cgttctctct agctatcctt gccatatatg aggctgcggc aacaacagat ggttcctcga 4980

ctgccatagg tattaagtag tcccttccgt tgacggtgaa gttggtggcg acacccaatg 5040

gcaactcaaa ttttccgata acattctcga tcataccgtt ggccaatgac aaaggcaaag 5100

caccgttacc ggccaatgca gaaatggctt caggttccaa tcctgcggct tcggcaaccc 5160

taactaacct ctgagcagga tccaagtccc tcatcttctc gatccttgag ttcaatccgt 5220

cgatgtgacc tgtctttcca gtcattgtaa agttagttgg ttgcgcgact tcgggtgggg 5280

taagtataga ggtatattaa caatttttg ttgatacttt tatgacattt gaataagaag 5340

taatacaaac cgaaaatgtt gaaagtatta gttaaagtgg ttatgcagct tttgcattta 5400

tatatctgtt aatagatcaa aaatcatcgc ttcgctgatt aattacccca gaaataaggc 5460

taaaaaacta atcgcattat tatcctatgg ttgttaattt gattcgttga tttgaaggtt 5520

tgtggggcca ggttactgcc aatttttcct cttcataacc ataaaagcta gtattgtaga 5580

atctttattg ttcggagcag tgcggcgcga ggcacatctg cgtttcagga acgcgaccgg 5640

tgaagaccag gacgcacgga ggagagtctt ccgtcggagg gctgtcgccc gctcggcggc 5700

ttctaatccg tacttcaata tagcaatgag cagttaagcg tattactgaa agttccaaag 5760

agaaggtttt tttaggctaa gataatgggg ctctttacat ttccacaaca tataagtaag 5820

attagatatg gatatgtata tggtggtatt gccatgtaat atgattatta aacttctttg 5880

cgtccatcca aaaaaaaagt aacgcacgca cactcccgac agacaactag cttgataatg 5940

tctcagaacg tttacattgt atcgactgcc agaaccccaa ttggttcatt ccagggttct 6000

ctatcctcca agacagcagt ggaattgggt gctgttgctt taaaaggcgc cttggctaag 6060

gttccagaat tggatgcatc caaggatttt gacgaaatta tttttggtaa cgttctttct 6120

gccaatttgg gccaagctcc ggccagacaa gttgctttgg ctgccggttt gagtaatcat 6180

atcgttgcaa gcacagttaa caaggtctgt gcatccgcta tgaaggcaat cattttgggt 6240

gctcaatcca tcaaatgtgg taatgctgat gttgtcgtag ctggtggttg tgaatctatg 6300

actaacgcac catactacat gccagcagcc cgtgcgggtg ccaaatttgg ccaaactgtt 6360

cttgttgatg gtgtcgaaag agatgggttg aacgatgcgt acgatggtct agccatgggt 6420

gtacacgcag aaaagtgtgc ccgtgattgg gatattacta gagaacaaca agacaatttt 6480

gccatcgaat cctaccaaaa atctcaaaaa tctcaaaagg aaggtaaatt cgacaatgaa 6540

attgtacctg ttaccattaa gggatttaga ggtaagcctg atactcaagt cacgaaggac 6600

gaggaacctg ctagattaca cgttgaaaaa ttgagatctg caaggactgt tttccaaaaa 6660

gaaaacggta ctgttactgc cgctaacgct tctccaatca acgatggtgc tgcagccgtc 6720

atcttggttt ccgaaaaagt tttgaaggaa aagaatttga agcctttggc tattatcaaa 6780

ggttggggtg aggccgctca tcaaccagct gattttacat gggctccatc tcttgcagtt 6840

ccaaaggctt tgaaacatgc tggcatcgaa gacatcaatt ctgttgatta ctttgaattc 6900

aatgaagcct tttcggttgt cggtttggtg aacactaaga ttttgaagct agacccatct 6960

aaggttaatg tatatggtgg tgctgttgct ctaggtcacc cattgggttg ttctggtgct 7020

agagtggttg ttacactgct atccatctta cagcaagaag gaggtaagat cggtgttgcc 7080

gccatttgta atggtggtgg tggtgcttcc tctattgtca ttgaaaagat atgattacgt 7140

tctgcgattt tctcatgatc tttttcataa aatacataaa tatataaatg gctttatgta 7200

taacaggcat aatttaaagt tttatttgcg attcatcgtt tttcaggtac tcaaacgctg 7260

aggtgtgcct tttgacttac ttttccgcct tggcaagctg gccgaacctg caggccgcga 7320

gcgccgatac gaaaatcgtt attgtcttga aggtgaaatt tctactctta ttaatggtga 7380

acgttaagct gatgctatga tggaagctga ttggtcttaa cttgcttgtc atcttgctaa 7440

tggtcattgg ctcgtgttat tacttaagtt atttgtactc gttttgaacg taatgctaat 7500

gatcatctta tggaataata gtgagtggtt tcagggtcca taaagctttt caattcatct 7560

ttttttttt tgttcttttt tttgattccg gtttctttga aatttttttg attcggtaat 7620

ctccgagcag aaggaagaac gaaggaagga gcacagactt agattggtat atatacgcat 7680

atgtggtgtt gaagaaacat gaaattgccc agtattctta acccaactgc acagaacaaa 7740

aacctgcagg aaacgaagat aaatcatgtc gaaagctaca tataaggaac gtgctgctac 7800

tcatcctagt cctgttgctg ccaagctatt taatatcatg cacgaaaagc aaacaaactt 7860

gtgtgcttca ttggatgttc gtaccaccaa ggaattactg gagttagttg aagcattagg 7920

tcccaaaatt tgtttactaa aaacacatgt ggatatcttg actgattttt ccatggaggg 7980

cacagttaag ccgctaaagg cattatccgc caagtacaat tttttactct tcgaagacag 8040

aaaatttgct gacattggta atacagtcaa attgcagtac tctgcgggtg tatacagaat 8100

agcagaatgg gcagacatta cgaatgcaca cggtgtggtg ggcccaggta ttgttagcgg 8160

tttgaagcag gcggcggaag aagtaacaaa ggaacctaga ggccttttga tgttagcaga 8220

attgtcatgc aagggctccc tagctactgg agaatatact aagggtactg ttgacattgc 8280

gaagagtgac aaagattttg ttatcggctt tattgctcaa agagacatgg gtggaagaga 8340

tgaaggttac gattggttga ttatgacacc cggtgtgggt ttagatgaca agggagacgc 8400

attgggtcaa cagtatagaa ccgtggatga tgtggtctct acaggatctg acattattat 8460

tgttggaaga ggactatttg caaagggaag ggatgctaag gtagagggtg aacgttacag 8520

aaaagcaggc tgggaagcat atttgagaag atgcggccag caaaactaaa aaactgtatt 8580

ataagtaaat gcatgtatac taaactcaca aattagagct tcaatttaat tatatcagtt 8640

attaccacga aaatcgttat tgtcttgaag gtgaaatttc tactcttatt aatggtgaac 8700

gttaagctga tgctatgatg gaagctgatt ggtcttaact tgcttgtcat cttgctaatg 8760

gtcatatggc tcgtgttatt acttaagtta tttgtactcg ttttgaacgt aatgctaatg 8820

atcatcttat ggaataatag tgaacggccg gccaagcacg cggggattga atgagaaaaa 8880

aaatcggttg ggcttaactt taaagaaaaa agttgagatt agatttattg tgttataaat 8940

atagatatac aattctttat aaaaaaaata tatatatata tcattgttat taaataaaga 9000

gttttcctag tatatagatt aaaaaactac tctattaaat gagagctaaa aaaagcaggc 9060

tgccaaaaaa ataaagcatt tatgaagggg gttcagcaag atgcaatcga tgggggaaga 9120

ttatttttta acatcgtaag atcttctaaa tttgtcatcg atgttggtca agtagtaaac 9180

accactttgc aaatgctcaa tggaaccttg aggtttgaag ttcttcttca aatgggcatt 9240

ttctctcaat tcgatggcag cttcgtaatc ctttggagtt tcggtgattc tcttggctaa 9300

tttgttagta atatctaatt ccttgataat atgttggacg tcaccaacaa ttttgcaaga 9360

atatagagat gcagctaaac cggaaccgta agaaaataaa ccaacacgct tgccttgtaa 9420

gtcgtcagat ccaacatagt ttaatagaga tgcaaaggcg gcataaacag atgcggtgta 9480

catgttacct gtgtttgttg gaacaatcaa agattgggca actctctctt tgtggaatgg 9540

cttagcaaca ttaacaaaag ttttttcaat gttcttatcg gttaaagatt cgtcataatc 9600

gcgagtagct aattcggcgt caacttctgg gaacaattga ggattggctc tgaaatcgtt 9660

atatagtaat ctaccgtatg attttgtgac caatttacag gttggaacat ggaaaacgtt 9720

gtagtcgaaa tatttcaaaa cgttcaaagc atccgaacca gcgggatcgc taaccaaccc 9780

tttagaaata gccttcttgg aataactctt gtaaacttga tcaagagcct tgacgtaaca 9840

agttaatgaa aaatgaccat cgacgtaagg atattcgctg gtgaaatctg gcttgtaaaa 9900

atcgtaggcg tgttccatgt aagaagctct tacagagtca aatacaattg gagcatcagg 9960

accgatccac atagcaacag taccggcacc accggttggt cttgcggcac ccttatcgta 10020

gatggcaata tcaccgcaaa ctacaatggc gtctctacca tcccatgcgt tagattcaat 10080

ccagttcaaa gagttgaaca acgcgttggt accaccgtaa caggcattaa gcgtgtcaat 10140

accttcgacg tcagtgtttt caccaaacaa ttgcatcaag acagacttga cagacttgga 10200

cttgtcaatc agagtttcag taccgacttc taatctacca attttgttgg tgtcgatgtt 10260

gtaactcttg atcaacttag acaaaacagt tagggacatc gagtagatat cttctctgtc 10320

attgacaaaa gacatgttgg tttggcccag accaattgtg tatttacctt gagaaacgcc 10380

atcaaatttc tctagctcag attggttgac acattgagtt gggatgtaaa tttggatacc 10440

tttaataccg acattttgag gtctggtttt ttgttcagcg gtcttttgtt tttttagttc 10500

agtcatttgc aagtttgtat tgtgtaattg ttgttgcttt tgcggcctaa gtcttccttt 10560

aataccacac caacaaagtt tagttgagag tttcattgtg aaggtagttc gattttggag 10620

gtcgcgggag gttacttttt ttttggatgg acgcaaagaa gtttaataat catattacat 10680

ggcaatacca ccatatacat atccatatct aatcttactt atatgttgtg gaaatgtaaa 10740

gagccccatt atcttagcct aaaaaaacct tctctttgga actttcagta atacgcttaa 10800

ctgctcattg ctatattgaa gtacggatta gaagccgccg agcgggcgac agccctccga 10860

cggaagactc tcctccgtgc gtcctggtct tcaccggtcg cgttcctgaa acgcagatgt 10920

gcctcgcgcc gcactgctcc gaacaataaa gattctacaa tactagcttt tatggttatg 10980

aagaggaaaa attggcagta acctggcccc acaaaccttc aaatcaacga atcaaattaa 11040

caaccatagg ataataatgc gattagtttt ttagccttat ttctggggta attaatcagc 11100

gaagcgatga tttttgatct attaacagat atataaatgc aaaagctgca taaccacttt 11160

aactaatact ttcaacattt tcggtttgta ttacttctta ttcaaatgtc ataaaagtat 11220

caacaaaaaa ttgttaatat acctctatac ttaccccacc cgaagtcgcg caaccaacta 11280

actttacaat gactggaaag acaggtcaca tcgacggatt gaactcaagg atcgagaaga 11340

tgagggactt ggatcctgct cagaggttag ttagggttgc cgaagccgca ggattggaac 11400

ctgaagccat ttctgcattg gccggtaacg gtgctttgcc tttgtcattg gccaacggta 11460

tgatcgagaa tgttatcgga aaatttgagt tgccattggg tgtcgccacc aacttcaccg 11520

tcaacggaag ggactactta atacctatgg cagtcgagga accatctgtt gttgccgcag 11580

cctcatatat ggcaaggata gctagagaga acggtggatt caccgcacac ggaactgcac 11640

ctttaatgag ggcccaaatc caagttgtcg gattaggaga cccagaagga gctaggcaaa 11700

ggttgttggc ccataaggct gctttcatgg aagcagcaga tgccgtagat ccagttttgg 11760

ttggtttagg tggtggttgc agagacattg aggtccatgt ttttagggac acccctgttg 11820

gtgcaatggt cgtattgcac ttgattgttg atgtcagaga cgccatgggt gccaacacag 11880

ttaacaccat ggctgaaagg ttagctccag aggtagagag gatcgctggt ggaaccgtca 11940

ggttgaggat tttgtctaac ttggccgact tgaggttggt aagagccagg gttgagttag 12000

cccctgagac tttgaccacc cagggatacg atggtgccga cgttgcaagg ggaatggttg 12060

aagcctgcgc cttagcaata gtcgaccctt acagggccgc aacccataac aagggaatta 12120

tgaacggaat tgacccagtc gtcgtcgcaa caggtaacga ctggagggcc atcgaagctg 12180

gagcccacgc ctacgccgcc aggaccggac attacacctc tttgacaagg tgggagttgg 12240

ccaacgacgg taggttggtc ggtaccattg aattgccttt ggccttgggt ttggtaggag 12300

gagccaccaa gactcaccct acagctaggg ccgctttggc cttaatgcag gtcgaaaccg 12360

ctactgagtt ggcccaggtt actgccgccg ttggtttggc tcagaatatg gccgcaatta 12420

gggccttggc aaccgaggga atccaaaggg gacacatgac cttacacgca aggaacatcg 12480

caatcatggc cggtgccacc ggagccgaca ttgacagggt caccagggtc atcgttgaag 12540

ccggagacgt atctgtcgcc agggctaaac aggttttgga aaatacataa acttagtcat 12600

acgtcattgg tattctcttg aaaaagaagc acaacagcac catgtgttac gtaaaatatt 12660

tactttatag tttgtacgtc ataatttctt ccatattaca agttcgtgca tatatagaaa 12720

gaattctgtt gttgtaattg tcataactag gtccgccggc gttggacgag cgaatgtgta 12780

tattagttta aaaagttgta tgtaataaaa gtaaaattta atattttgga tgaaaaaaac 12840

cattttaga ctttttctta actagaatgc tggagtagaa atacgccatc tcaagataca 12900

aaaagcgtta ccggcactga tttgtttcaa ccagtatata gattattatt gggtcttgat 12960

caactttcct cagacatatc agtaacagtt atcaagctaa atatttacgc gaaagaaaaa 13020

caaatatttt aattgtgata cttgtgaatt ttattttatt aaggatacaa agttaagaga 13080

aaacaaaatt tatatacaat ataagtaata ttcatatata tgtgatgaat gcagtcttaa 13140

cgagaagaca tggccttggt gacaactctc ttcaaaccaa cttcagcctt tctcaattca 13200

tcagcagatg ggtcttcgat ttgcaaagca gccaaagcgg cggtttaaac gcgtggccgt 13260

gccgtc 13266

<210> 34
<211> 13964
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i84026 integration construct

<400> 34
gacggcacgg ccacgcgttt aaaccgccaa gtgatgtaac taaatacacg attaccatgg 60

aaattaacgt acctttttg tgcgtgtatt gaaatattat gacatattac agaaagggtt 120

cgcaagtcct gtttctatgc ctttctctta gtaattcacg aaataaacct atggtttacg 180

aaatgatcca cgaaaatcat gttattattt acatcaacat atcgcgaaaa ttcatgtcat 240

gtccacatta acatcattgc agagcaacaa ttcattttca tagagaaatt tgctactatc 300

acccactagt actaccattg gtacctacta ctttgaattg tactaccgct gggcgttatt 360

aggtgtgaaa ccacgaaaag ttcaccataa cttcgaataa agtcgcggaa aaaagtaaac 420

agctattgct actcaaatga ggtttgcaga agcttgttga agcatgatga agcgttctaa 480

acgcactatt catcattaaa tatttaaagc tcataaaatt gtattcaatt cctattctaa 540

atggctttta tttctattac aactattagc tctaaatcca tatcctcata agcagcaatc 600

aattctatct atactttaaa cgctcgtcca acgccggcgg acctgatgtg tattactagt 660

gtcgacgaca gcattcgccc agtatttttt ttattctaca aaccttctat aatttcaaag 720

tatttacata attctgtatc agtttaatca ccataatatc gttttctttg tttagtgcaa 780

```
ttaatttttc ctattgttac ttcgggcctt tttctgtttt atgagctatt ttttccgtca      840
tccttccgga tccagatttt cagcttcatc tccagattgt gtctacgtaa tgcacgccat      900
cattttaaga gaggacctcc cgcgacctcc aaaatcgaac taccttcaca atgaaacttc      960
ttagtagtat tgagcaggcg tgtgacatct gtagattgaa gaaattgaaa tgtagtaagg     1020
agaagcccaa atgtgcgaaa tgccttaaaa ataattggga atgcagatat agtccgaaga     1080
cgaagcgcag tcccccttacc cgcgcgcacc ttacggaggt cgagagtcgc cttgagcgcc     1140
ttgagcaact tttccttctt atcttcccca gagaggattt ggatatgatc cttaagatgg     1200
acagtcttca agacattaag gcgcttctta cggggctttt cgtgcaggac aacgtcaaca     1260
aggacgcggt gacggaccgc cttgccagtg tcgaaaccga catgcccctt acgcttcgcc     1320
aacaccgcat ttccgccacg agtagtagtg aggaatcctc caataagggg cagcgccaac     1380
ttaccgtgag tatcgatagt gcggcccacc acgacaatag tacgatcccc cttgacttca     1440
tgccgcgcga cgccttgcac gggttcgact ggagtgagga agacgatatg agtgacggtc     1500
ttccgtttct taagaccgat ccgaataaca acggtttttt cggtgatggg agtttgcttt     1560
gcatcttgag aagtatcggt ttcaagcccg agaactatac caatagtaat gtcaatcgct     1620
tgcccacgat gatcaccgac cgctataccc ttgccagtcg cagtacgacg agtagacttt     1680
tgcagtccta cttgaacaac ttccatccgt attgtcccat tgtccatagt cccaccctta     1740
tgatgcttta caacaatcaa atcgagattg ccagtaaaga ccagtggcag attttgttca     1800
attgtattct tgcgatcggg gcgtggtgca ttgaaggtga gagtaccgac attgacgtct     1860
tctattacca gaacgccaag agtcacctta cctccaaagt gtttgaaagt gggagtatta     1920
tccttgtcac ggcgcttcac ttgcttagta gatacacgca atggcgccaa aagacgaaca     1980
cctcctacaa cttccattcc ttcagtattc gcatggcgat tagtcttggt cttaaccgcg     2040
atttgccgag tagtttttcc gactcctcca tccttgagca gcgcagaaga atctggtgga     2100
gtgtgtatag ttgggaaatt cagcttagtc ttttgtacgg gagaagtatt caattgagtc     2160
aaaacacgat tagttttccc agtagtgtgg atgacgtcca aagaacgacg acggggccga     2220
cgatttacca cggtattatc gagacggcgc gcttgcttca ggtctttacg aagatttacg     2280
agcttgataa gacggtgacc gcggagaagt cccccatttg cgcgaagaag tgtcttatga     2340
tctgcaacga aatcgaagaa gtcagtcgcc aagcgccgaa attccttcag atggacatca     2400
gtacgacggc ccttacgaac cttcttaaag agcatccctg gcttagtttc acgcgctttg     2460
agcttaaatg gaagcaactt agtttgatta tctacgtgct tcgcgacttc tttaccaact     2520
tcacgcaaaa gaaaagtcag cttgagcaag accagaacga ccaccagtcc tacgaggtca     2580
agagatgtag tattatgctt tccgacgcgg cgcagcgcac cgtcatgagt gtgtcctcct     2640
acatggataa ccacaacgtg acgccgtact tcgcgtggaa ctgcagttac tatcttttta     2700
acgcggtgct tgtgccgatt aaaacccttt tgagtaatag taagagtaac gccgaaaaca     2760
atgaaacggc gcagcttctt cagcagatca ataccgtcct tatgcttctt aagaagcttg     2820
```

cgaccttcaa gattcaaacc tgcgagaagt atatccaggt gcttgaggaa gtgtgcgccc 2880

ccttccttct tagtcaatgc gcgattccgc ttccccacat ttcctacaat aactccaacg 2940

ggtccgcgat caagaacatc gtggggagtg cgaccattgc gcagtatccc accttgcccg 3000

aagagaacgt gaataacatt tccgtcaagt acgtcagtcc cggtagtgtg ggtcccagtc 3060

ccgtcccgct taagagtggg gcgtcctttt ccgaccttgt gaaacttctt agtaatagac 3120

cgccgagtag aaatagtccg gtcacgattc cgcgctccac gcccagtcac agaagtgtga 3180

ccccccttcct tggtcagcaa cagcaacttc agagtcttgt cccgcttacg cccagtgccc 3240

ttttcggggg tgcgaacttc aaccagtccg gtaacatcgc cgactccagt cttagtttta 3300

cctttaccaa ttcctccaat gggcccaatt tgattacgac ccagacgaac agtcaggcct 3360

tgagtcagcc gatcgcgagt agtaatgtcc acgacaattt tatgaacaac gagattaccg 3420

cctccaagat cgacgacggg aacaacagta agccgcttag tcccgggtgg accgatcaga 3480

ccgcctacaa tgccttcggg attaccacgg gtatgttcaa cacgaccacg atggacgacg 3540

tgtacaatta ccttttttgac gacgaggaca cgccgccgaa tccgaagaag gaatgagcca 3600

attggtgcgg caattgataa taacgaaaat gtcttttaat gatctgggta taatgaggaa 3660

ttttccgaac gtttttactt tatatatata tatacatgta acatatattc tatacgctat 3720

atcgagaaaa cgcgatggtg gggtgacttt caactcggcg tatccccgcg tgcttggccg 3780

gccgtccgca tgactcaaga gaagcatgtg gtttttgagt ttttttcgtt gaattttcag 3840

gtaaagctca atagttatga caattacaac aacagaattc tttctatata tgcacgaact 3900

tgtaatatgg aagaaattat gacgtacaaa ctataaagta aatattttac gtaacacatg 3960

gtgctgttgt gcttcttttt caagagaata ccaatgacgt atgactaagt ttaggattta 4020

atgcaggtga cggacccatc tttcaaacga tttatatcag tggcgtccaa attgttaggt 4080

tttgttggtt cagcaggttt cctgttgtgg gtcatatgac tttgaaccaa atggccggct 4140

gctagggcag cacataagga taattcacct gccaagacgg cacaggcaac tattcttgct 4200

aattgacgtg cgttggtacc aggagcggta gcatgtgggc ctcttacacc taataagtcc 4260

aacatggcac cttgtggttc tagaacagta ccaccaccga tggtacctac ttcgatggat 4320

ggcatggata cggaaattct caaatcaccg tccacttctt tcatcaatgt tatacagttg 4380

gaactttcga cattttgtgc aggatcttgt cctaatgcca agaaaacagc tgtcactaaa 4440

ttagctgcat gtgcgttaaa tccaccaaca gacccagcca ttgcagatcc aaccaaattc 4500

ttagcaatgt tcaactcaac caatgcggaa acatcacttt ttaacacttt tctgacaaca 4560

tcaccaggaa tagtagcttc tgcgacgaca ctcttaccac gaccttcgat ccagttgatg 4620

gcagctggtt ttttgtcggt acagtagtta ccagaaacgg agacaacctc catatcttcc 4680

cagccatact cttctaccat ttgctttaat gagtattcga cacccttaga aatcatattc 4740

atacccattg cgtcaccagt agttgttcta aatctcatga agagtaaatc tcctgctaga 4800

caagtttgaa tatgttgcag acgtgcaaat cttgatgtag agttaaaagc ttttttaatt 4860

gcgttttgtc cctcttctga gtctaaccat atcttacagg caccagatct tttcaaagtt 4920

gggaaacgga ctactgggcc tcttgtcata ccatccttag ttaaaacagt tgttgcacca 4980

ccgccagcat tgattgcctt acagccacgc atggcagaag ctaccaaaca accctctgta 5040

gttgccattg gtatatgata agatgtacca tcgataacca aggggcctat aacaccaacg 5100

ggcaaaggca tgtaacctat aacattttca caacaagcgc caaatacgcg gtcgtagtca 5160

taatttttat atggtaaacg atcagatgct aatacaggag cttctgccaa aattgaaaga 5220

gccttcctac gtaccgcaac cgctctcgta gtatcaccta atttttttctc caaagcgtac 5280

aaaggtaact taccgtgaat aaccaaggca gcgacctctt tgttcttcaa ttgttttgta 5340

tttccactac ttaataatgc ttctaattct tctaaaggac gtattttctt atccaagctt 5400

tcaatatcgc gggaatcatc ttcctcacta gatgatgaag gtcctgatga gctcgattgc 5460

gcagatgata aacttttgac tttcgatcca gaaatgactg ttttattggt taaaactggt 5520

gtagaagcct tttgtacagg agcagtaaaa gacttcttgg tgacttcagt cttcaccaat 5580

tggtctgcag ccattgtaaa gttagttggt tgcgcgactt cgggtggggt aagtatagag 5640

gtatattaac aatttttgt tgatactttt atgacatttg aataagaagt aatacaaacc 5700

gaaaatgttg aaagtattag ttaaagtggt tatgcagctt ttgcatttat atatctgtta 5760

atagatcaaa aatcatcgct tcgctgatta attaccccag aaataaggct aaaaaactaa 5820

tcgcattatt atcctatggt tgttaatttg attcgttgat ttgaaggttt gtggggccag 5880

gttactgcca atttttcctc ttcataacca taaaagctag tattgtagaa tctttattgt 5940

tcggagcagt gcggcgcgag gcacatctgc gtttcaggaa cgcgaccggt gaagaccagg 6000

acgcacggag gagagtcttc cgtcggaggg ctgtcgcccg ctcggcggct tctaatccgt 6060

acttcaatat agcaatgagc agttaagcgt attactgaaa gttccaaaga gaaggttttt 6120

ttaggctaag ataatggggc tctttacatt tccacaacat ataagtaaga ttagatatgg 6180

atatgtatat ggtggtattg ccatgtaata tgattattaa acttctttgc gtccatccaa 6240

aaaaaaagta acgcacgcac actcccgaca gacaactagc ttgataatgt ctcagaacgt 6300

ttacattgta tcgactgcca gaaccccaat tggttcattc cagggttctc tatcctccaa 6360

gacagcagtg gaattgggtg ctgttgcttt aaaaggcgcc ttggctaagg ttccagaatt 6420

ggatgcatcc aaggattttg acgaaattat ttttggtaac gttctttctg ccaatttggg 6480

ccaagctccg gccagacaag ttgctttggc tgccggtttg agtaatcata tcgttgcaag 6540

cacagttaac aaggtctgtg catccgctat gaaggcaatc attttgggtg ctcaatccat 6600

caaatgtggt aatgctgatg ttgtcgtagc tggtggttgt gaatctatga ctaacgcacc 6660

atactacatg ccagcagccc gtgcgggtgc caaatttggc caaactgttc ttgttgatgg 6720

tgtcgaaaga gatgggttga acgatgcgta cgatggtcta gccatgggtg tacacgcaga 6780

aaagtgtgcc cgtgattggg atattactag agaacaacaa gacaattttg ccatcgaatc 6840

ctaccaaaaa tctcaaaaat ctcaaaagga aggtaaattc gacaatgaaa ttgtacctgt 6900

taccattaag ggatttagag gtaagcctga tactcaagtc acgaaggacg aggaacctgc 6960

tagattacac gttgaaaaat tgagatctgc aaggactgtt ttccaaaaag aaaacggtac 7020

tgttactgcc gctaacgctt ctccaatcaa cgatggtgct gcagccgtca tcttggtttc 7080

cgaaaaagtt ttgaaggaaa agaatttgaa gcctttggct attatcaaag gttggggtga 7140

ggccgctcat caaccagctg attttacatg ggctccatct cttgcagttc caaaggcttt 7200

gaaacatgct ggcatcgaag acatcaattc tgttgattac tttgaattca atgaagcctt 7260

ttcggttgtc ggtttggtga acactaagat tttgaagcta gacccatcta aggttaatgt 7320

atatggtggt gctgttgctc taggtcaccc attgggttgt tctggtgcta gagtggttgt 7380

tacactgcta tccatcttac agcaagaagg aggtaagatc ggtgttgccg ccatttgtaa 7440

tggtggtggt ggtgcttcct ctattgtcat tgaaaagata tgattacgtt ctgcgatttt 7500

ctcatgatct ttttcataaa atacataaat atataaatgg ctttatgtat aacaggcata 7560

atttaaagtt ttatttgcga ttcatcgttt ttcaggtact caaacgctga ggtgtgcctt 7620

ttgacttact tttccgcctt ggcaagctgg ccgaacctgc aggccgcgag cgccgatacg 7680

aaaatcgtta ttgtcttgaa ggtgaaattt ctactcttat taatggtgaa cgttaagctg 7740

atgctatgat ggaagctgat tggtcttaac ttgcttgtca tcttgctaat ggtcattggc 7800

tcgtgttatt acttaagtta tttgtactcg ttttgaacgt aatgctaatg atcatcttat 7860

ggaataatag tgagtggttt cagggtccat aaagcttttc aattcatctt ttttttttt 7920

gttctttttt ttgattccgg tttctttgaa attttttga ttcggtaatc tccgagcaga 7980

aggaagaacg aaggaaggag cacagactta gattggtata tatacgcata tgtggtgttg 8040

aagaaacatg aaattgccca gtattcttaa cccaactgca cagaacaaaa acctgcagga 8100

aacgaagata aatcatgtcg aaagctacat ataaggaacg tgctgctact catcctagtc 8160

ctgttgctgc caagctattt aatatcatgc acgaaaagca aacaaacttg tgtgcttcat 8220

tggatgttcg taccaccaag gaattactgg agttagttga agcattaggt cccaaaattt 8280

gtttactaaa aacacatgtg gatatcttga ctgatttttc catggagggc acagttaagc 8340

cgctaaaggc attatccgcc aagtacaatt ttttactctt cgaagacaga aaatttgctg 8400

acattggtaa tacagtcaaa ttgcagtact ctgcgggtgt atacagaata gcagaatggg 8460

cagacattac gaatgcacac ggtgtggtgg gcccaggtat tgttagcggt ttgaagcagg 8520

cggcggaaga agtaacaaag gaacctagag gccttttgat gttagcagaa ttgtcatgca 8580

agggctccct agctactgga gaatatacta agggtactgt tgacattgcg aagagtgaca 8640

aagattttgt tatcggcttt attgctcaaa gagacatggg tggaagagat gaaggttacg 8700

attggttgat tatgacaccc ggtgtgggtt tagatgacaa gggagacgca ttgggtcaac 8760

agtatagaac cgtggatgat gtggtctcta caggatctga cattattatt gttggaagag 8820

gactatttgc aaagggaagg gatgctaagg tagagggtga acgttacaga aaagcaggct 8880

gggaagcata tttgagaaga tgcggccagc aaaactaaaa aactgtatta taagtaaatg 8940

catgtatact aaactcacaa attagagctt caatttaatt atatcagtta ttaccacgaa 9000

aatcgttatt gtcttgaagg tgaaatttct actcttatta atggtgaacg ttaagctgat 9060

gctatgatgg aagctgattg gtcttaactt gcttgtcatc ttgctaatgg tcatatggct 9120

cgtgttatta cttaagttat ttgtactcgt tttgaacgta atgctaatga tcatcttatg 9180

gaataatagt gaacggccgg ccaagcacgc ggggattgaa tgagaaaaaa aatcggttgg 9240

gcttaacttt aaagaaaaaa gttgagatta gatttattgt gttataaata tagatataca 9300

attctttata aaaaaaatat atatatatat cattgttatt aaataaagag ttttcctagt 9360

atatagatta aaaaactact ctattaaatg agagctaaaa aaagcaggct gccaaaaaaa 9420

taaagcattt atgaaggggg ttcagcaaga tgcaatcgat gggggaagat tatttttaa 9480

catcgtaaga tcttctaaat ttgtcatcga tgttggtcaa gtagtaaaca ccactttgca 9540

aatgctcaat ggaaccttga ggtttgaagt tcttcttcaa atgggcattt tctctcaatt 9600

cgatggcagc ttcgtaatcc tttggagttt cggtgattct cttggctaat ttgttagtaa 9660

tatctaattc cttgataata tgttggacgt caccaacaat tttgcaagaa tatagagatg 9720

cagctaaacc ggaaccgtaa gaaaataaac caacacgctt gccttgtaag tcgtcagatc 9780

caacatagtt taatagagat gcaaaggcgg cataaacaga tgcggtgtac atgttacctg 9840

tgtttgttgg aacaatcaaa gattgggcaa ctctctcttt gtggaatggc ttagcaacat 9900

taacaaaagt tttttcaatg ttcttatcgg ttaaagattc gtcataatcg cgagtagcta 9960

attcggcgtc aacttctggg aacaattgag gattggctct gaaatcgtta tatagtaatc 10020

taccgtatga ttttgtgacc aatttacagg ttggaacatg gaaaacgttg tagtcgaaat 10080

atttcaaaac gttcaaagca tccgaaccag cgggatcgct aaccaaccct ttagaaatag 10140

ccttcttgga ataactcttg taaacttgat caagagcctt gacgtaacaa gttaatgaaa 10200

aatgaccatc gacgtaagga tattcgctgg tgaaatctgg cttgtaaaaa tcgtaggcgt 10260

gttccatgta agaagctctt acagagtcaa atacaattgg agcatcagga ccgatccaca 10320

tagcaacagt accggcacca ccggttggtc ttgcggcacc cttatcgtag atggcaatat 10380

caccgcaaac tacaatggcg tctctaccat cccatgcgtt agattcaatc cagttcaaag 10440

agttgaacaa cgcgttggta ccaccgtaac aggcattaag cgtgtcaata ccttcgacgt 10500

cagtgttttc accaaacaat tgcatcaaga cagacttgac agacttggac ttgtcaatca 10560

gagtttcagt accgacttct aatctaccaa ttttgttggt gtcgatgttg taactcttga 10620

tcaacttaga caaaacagtt agggacatcg agtagatatc ttctctgtca ttgacaaaag 10680

acatgttggt ttggcccaga ccaattgtgt atttaccttg agaaacgcca tcaaatttct 10740

ctagctcaga ttggttgaca cattgagttg ggatgtaaat ttggatacct ttaataccga 10800

cattttgagg tctggttttt tgttcagcgg tcttttgttt ttttagttca gtcatttgca 10860

agtttgtatt gtgtaattgt tgttgctttt gcggcctaag tcttccttta ataccacacc 10920

aacaaagttt agttgagagt ttcattgtga aggtagttcg attttggagg tcgcgggagg 10980

ttacttttt tttggatgga cgcaaagaag tttaataatc atattacatg gcaataccac 11040

catatacata tccatatcta atcttactta tatgttgtgg aaatgtaaag agccccatta 11100

tcttagccta aaaaaacctt ctctttggaa ctttcagtaa tacgcttaac tgctcattgc 11160

tatattgaag tacggattag aagccgccga gcgggcgaca gccctccgac ggaagactct 11220

cctccgtgcg tcctggtctt caccggtcgc gttcctgaaa cgcagatgtg cctcgcgccg 11280

cactgctccg aacaataaag attctacaat actagctttt atggttatga agaggaaaaa 11340

ttggcagtaa cctggcccca caaaccttca aatcaacgaa tcaaattaac aaccatagga 11400

taataatgcg attagttttt tagccttatt tctggggtaa ttaatcagcg aagcgatgat 11460

ttttgatcta ttaacagata tataaatgca aaagctgcat aaccacttta actaatactt 11520

tcaacatttt cggtttgtat tacttcttat tcaaatgtca taaaagtatc aacaaaaaat 11580

tgttaatata cctctatact taccccaccc gaagtcgcgc aaccaactaa ctttacaatg 11640

gctgcagacc aattggtgaa gactgaagtc accaagaagt cttttactgc tcctgtacaa 11700

aaggcttcta caccagtttt aaccaataaa acagtcattt ctggatcgaa agtcaaaagt 11760

ttatcatctg cgcaatcgag ctcatcagga ccttcatcat ctagtgagga agatgattcc 11820

cgcgatattg aaagcttgga taagaaaata cgtcctttag aagaattaga agcattatta 11880

agtagtggaa atacaaaaca attgaagaac aaagaggtcg ctgccttggt tattcacggt 11940

aagttacctt tgtacgcttt ggagaaaaaa ttaggtgata ctacgagagc ggttgcggta 12000

cgtaggaagg ctctttcaat tttggcagaa gctcctgtat tagcatctga tcgtttacca 12060

tataaaaatt atgactacga ccgcgtattt ggcgcttgtt gtgaaaatgt tataggttac 12120

atgcctttgc ccgttggtgt tataggcccc ttggttatcg atggtacatc ttatcatata 12180

ccaatggcaa ctacagaggg ttgtttggta gcttctgcca tgcgtggctg taaggcaatc 12240

aatgctggcg gtggtgcaac aactgtttta actaaggatg gtatgacaag aggcccagta 12300

gtccgtttcc caactttgaa aagatctggt gcctgtaaga tatggttaga ctcagaagag 12360

ggacaaaacg caattaaaaa agcttttaac tctacatcaa gatttgcacg tctgcaacat 12420

attcaaactt gtctagcagg agatttactc ttcatgagat ttagaacaac tactggtgac 12480

gcaatgggta tgaatatgat ttctaagggt gtcgaatact cattaaagca aatggtagaa 12540

gagtatggct gggaagatat ggaggttgtc tccgtttctg gtaactactg taccgacaaa 12600

aaaccagctg ccatcaactg gatcgaaggt cgtggtaaga gtgtcgtcgc agaagctact 12660

attcctggtg atgttgtcag aaaagtgtta aaaagtgatg tttccgcatt ggttgagttg 12720

aacattgcta agaatttggt tggatctgca atggctgggt ctgttggtgg atttaacgca 12780

catgcagcta atttagtgac agctgttttc ttggcattag gacaagatcc tgcacaaaat 12840

gtcgaaagtt ccaactgtat aacattgatg aaagaagtgg acggtgattt gagaatttcc 12900

gtatccatgc catccatcga agtaggtacc atcggtggtg gtactgttct agaaccacaa 12960

ggtgccatgt tggacttatt aggtgtaaga ggcccacatg ctaccgctcc tggtaccaac 13020

gcacgtcaat tagcaagaat agttgcctgt gccgtcttgg caggtgaatt atccttatgt 13080

gctgccctag cagccggcca tttggttcaa agtcatatga cccacaacag gaaacctgct 13140

gaaccaacaa aacctaacaa tttggacgcc actgatataa atcgtttgaa agatgggtcc 13200

gtcacctgca ttaaatccta aacttagtca tacgtcattg gtattctctt gaaaaagaag 13260

cacaacagca ccatgtgtta cgtaaaatat ttactttata gtttgtacgt cataatttct 13320

tccatattac aagttcgtgc atatatagaa agaattctgt tgttgtaatt gtcataacta 13380

ttgagcttta cctgaaaatt caacgaaaaa aactcaaaaa ccacatgctt ctcttgagtc 13440

atgcggaggt ccgccggcgt tggacgagcg aatgtgtata ttagtttaaa aagttgtatg 13500

taataaaagt aaaatttaat attttggatg aaaaaaacca tttttagact ttttcttaac 13560

tagaatgctg gagtagaaat acgccatctc aagatacaaa aagcgttacc ggcactgatt 13620

tgtttcaacc agtatataga ttattattgg gtcttgatca actttcctca gacatatcag 13680

taacagttat caagctaaat atttacgcga aagaaaaaca aatattttaa ttgtgatact 13740

tgtgaatttt attttattaa ggatacaaag ttaagagaaa acaaaattta tatacaatat 13800

aagtaatatt catatatatg tgatgaatgc agtcttaacg agaagacatg gccttggtga 13860

caactctctt caaaccaact tcagcctttc tcaattcatc agcagatggg tcttcgattt 13920

gcaaagcagc caaagcggcg gtttaaacgc gtggccgtgc cgtc 13964

<210> 35
<211> 13963
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: i85207 integration construct

<400> 35
gacggcacgg ccacgcgttt aaaccgccag ggcaaggttg gcctctactt actccatcga 60

caattcaaga tacagaacct cctccagatg gaatcccttc catagagaga aggagcaagc 120

aactgaccca atattgactg ccactggacc tgaagacatg caacaaagtg caagcatagt 180

ggggccttct tccaatgcta atccggtcac tgccactgct gctacggaaa accaacctaa 240

aggtattaac ttcttcacta taagaaaatc acacgagcgc ccggacgatg tctctgttta 300

aatggcgcaa gttttccgct ttgtaatata tatttatacc cctttcttct ctcccctgca 360

atataatagt ttaattctaa tattaataat atcctatatt ttcttcattt accggcgcac 420

tctcgcccga acgacctcaa aatgtctgct acattcataa taaccaaaag ctcataactt 480

tttttttga acctgaatat atatacatca catgtcactg ctggtccttg ccgaccagcg 540

tatacaatct cgatagttgg tttcccgttc tttccactcc cgtccgctcg tccaacgccg 600

gcggaccttc acatgtaggg accgaattgt ttacaagttc tctgtaccac catggagaca 660

tcaaagattg aaaatctatg gaaagatatg gacggtagca acaagaatat agcacgagcc 720

gcgaagttca tttcgttact tttgatatcg ctcacaacta ttgcgaagcg cttcagtgaa 780

aaaatcataa ggaaaagttg taaatattat tggtagtatt cgtttggtaa agtagagggg 840

gtaatttttc ccctttattt tgttcataca ttcttaaatt gctttgcctc tccttttgga 900

aagctatact tcggagcact gttgagcgaa ggctcattag atatattttc tgtcattttc 960

cttaacccaa aaataaggga aagggtccaa aaagcgctcg gacaactgtt gaccgtgatc 1020

cgaaggactg gctatacagt gttcacaaaa tagccaagct gaaaataatg tgtagctatg 1080

ttcagttagt ttggctagca aagatataaa agcaggtcgg aaatatttat gggcattatt 1140

atgcagagca tcaacatgat aaaaaaacct cccgcgacct ccaaaatcga actaccttca 1200

caatgactgc cgacaacaat agtatgcccc atggtgcagt atctagttac gccaaattag 1260

tgcaaaacca aacacctgaa gacattttgg aagagtttcc tgaaattatt ccattacaac 1320

aaagacctaa tacccgatct agtgagacgt caaatgacga aagcggagaa acatgttttt 1380

ctggtcatga tgaggagcaa attaagttaa tgaatgaaaa ttgtattgtt ttggattggg 1440

acgataatgc tattggtgcc ggtaccaaga aagtttgtca tttaatggaa aatattgaaa 1500

agggtttact acatcgtgca ttctccgtct ttattttcaa tgaacaaggt gaattacttt 1560

tacaacaaag agccactgaa aaaataactt tccctgatct ttggactaac acatgctgct 1620

ctcatccact atgtattgat gacgaattag gtttgaaggg taagctagac gataagatta 1680

agggcgctat tactgcggcg gtgagaaaac tagatcatga attaggtatt ccagaagatg 1740

aaactaagac aaggggtaag tttcactttt taaacagaat ccattacatg gcaccaagca 1800

atgaaccatg gggtgaacat gaaattgatt acatcctatt ttataagatc aacgctaaag 1860

aaaacttgac tgtcaaccca aacgtcaatg aagttagaga cttcaaatgg gtttcaccaa 1920

atgatttgaa aactatgttt gctgacccaa gttacaagtt tacgccttgg tttaagatta 1980

tttgcgagaa ttacttattc aactggtggg agcaattaga tgacctttct gaagtggaaa 2040

atgacaggca aattcataga atgctataac aacgcgtcaa taatataggc tacataaaaa 2100

tcataataac tttgttatca tagcaaaatg tgatataaaa cgtttcattt cacctgaaaa 2160

atagtaaaaa taggcgacaa aaatccttag taatatgtaa actttatttt ctttatttat 2220

ttacagaact ctgaatatac attgattgtt cacatttttt ttttctcttc tcaatttccc 2280

ttgattatat tcaaaaggtt attggcctct tgaatgtttc ccactgaatc cccgcgtgct 2340

tggccggccg tggagcgacc tcatgctata cctgagaaag caacctgacc tacaggaaag 2400

agttactcaa gaataagaat tttcgtttta aaacctaaga gtcactttaa aatttgtata 2460

cacttatttt ttttataact tatttaataa taaaaatcat aaatcataag aaattcgctc 2520

aaacgaccat tggatggaca aagaaggact tcatgtaaga tttcatgtca ccttcggcgt 2580

gagtgaaacc atcgttaaca gagtataaaa cttcacacat tctagccaag ttgatagctg 2640

gcattaacaa agggaatgga acggcggttg gtctcaaaga ttctctgtta ataaccttcc 2700

aggcgtcttc gacttttcta gagatgtatt cacaggcttc ttcttcagaa gcaccggatt 2760

ccttagaata acattcgatg gaggaggcaa catgacctct ttcttgttct tctttatgag 2820

agacaatatc atccatcaat ctaatgataa cacaagaagc ttcaacaata ggtgggtagg 2880

aagaaaccca tttaaaagtg tcctcgttaa caatgtcacc tctaccaacg taagatctag 2940

cagtgatcaa accgtaggta ccggtaacca tggaaacaga catgtactct tccaaagtag 3000

gcatgtaacc ttctttcaac catctggctt caaccaagta gtttctgacc aattccttag 3060

ccatttcctt aacgtagtgg atttgataag ccttaccttc cttttctaaa gattcttcca 3120

tttcaacgtg caagttaacc aattcttggt agatcaactt catgtattct ggcaacatgt 3180

ccaaacaaga aatggaccac ttctcaacgg cttgagtgaa aatttccaat tcttcgtagg 3240

taccgtagtt gtcgaaggta tcatccaaaa cgaccaacca catacaagac ttcatcaaga 3300

acattctggt tctggcatgt tgtggttcat agtaaataga caaaatccag aagtaacctt 3360

cgacaactct atcacgaacg aatggcaatt tgttttgcaa gtctaaatct ttccaccact 3420

tgcagatgtg agacaattct ttcttatgca tggattgcaa aacagagaaa tctaacttag 3480

ccaacttcaa caaaacctcg tcgtgagaag tttcttgttg gtaaattggc atatagtgta 3540

aagcttcgat tctggccaat cttcttctca atggttgctt caaggcttgg tggatttggg 3600

ttcttaagga agagtcacaa gatggatcct tggcaataat gtccaagtga accttagaga 3660

attccaaagc gttgtccaag atggtttcat cttcgactct catgaaagca gcttcgtaca 3720

aggccaagat accttgagcg tcgttacaca aagattcctt aaatttacct ttttcgtcca 3780

taaagtcctt gaaaacacca gaggagacat tgaaaccttg ttgacgcaac aaacgaaacc 3840

acaaggagat agattgtaaa ttttccttat cgacccattg ttcaccgtaa gtgacatgga 3900

tatgttgtaa agcttcttcg atttcttctt caaaatggta agcaatacct aaacgttgaa 3960

cagcattgat taattcgatc aacttaacat gttgcatagg ttcgttagaa cccttaatag 4020

taatcaattc cttcttaact tcctccttta actcttcgac taattgcttc ttcataacca 4080

agtcctctgg ttcatcgtaa gtcaaaaatt gatcacccca aatggaagcg ttgaagttag 4140

cggtatgtct aataacgtct ggcttggtag aatccttatc atcgacaaca attggggaag 4200

tagatggaga ggaagaaaca gaggaaatag gcaaagtgga cattgtaaag ttagttggtt 4260

gcgcgacttc gggtggggta agtatagagg tatattaaca atttttgtt gatactttta 4320

tgacatttga ataagaagta atacaaaccg aaaatgttga aagtattagt taaagtggtt 4380

atgcagcttt tgcatttata tatctgttaa tagatcaaaa atcatcgctt cgctgattaa 4440

ttaccccaga aataaggcta aaaaactaat cgcattatta tcctatggtt gttaatttga 4500

ttcgttgatt tgaaggtttg tggggccagg ttactgccaa tttttcctct tcataaccat 4560

aaaagctagt attgtagaat ctttattgtt cggagcagtg cggcgcgagg cacatctgcg 4620

tttcaggaac gcgaccggtg aagaccagga cgcacggagg agagtcttcc gtcggagggc 4680

tgtcgcccgc tcggcggctt ctaatccgta cttcaatata gcaatgagca gttaagcgta 4740

ttactgaaag ttccaaagag aaggtttttt taggctaaga taatggggct ctttacattt 4800

ccacaacata taagtaagat tagatatgga tatgtatatg gtggtattgc catgtaatat 4860

gattattaaa cttctttgcg tccatccaaa aaaaaagtaa cgcacgcaca ctcccgacag 4920

acaactagct tgataatggc ttcagaaaaa gaaattagga gagagagatt cttgaacgtt 4980

ttccctaaat tagtagagga attgaacgca tcgcttttgg cttacggtat gcctaaggaa 5040

gcatgtgact ggtatgccca ctcattgaac tacaacactc caggcggtaa gttaaataga 5100

ggtttgtccg ttgtggacac gtatgctatt ctctccaaca agaccgttga acaattgggg 5160

caagaagaat acgaaaaggt tgctattcta ggttggtgca ttgagttgtt gcaggcttac 5220

ttcttggtcg ccgatgatat gatggacaag tccattacca gaagaggcca accatgttgg 5280

tacaaggttc ctgaagttgg ggaaattgcc atcaatgacg cattcatgtt agaggctgct 5340

atctacaagc ttttgaaatc tcacttcaga aacgaaaaat actacataga tatcaccgaa 5400

ttgttccatg aagtcacctt ccaaaccgaa ttgggccaat tgatggactt aatcactgca 5460

cctgaagaca aagtcgactt gagtaagttc tccctaaaga agcactcctt catagttact 5520

ttcaagactg cttactattc tttctacttg cctgtcgcat tggctatgta cgttgccggt 5580

atcacagatg aaaaggattt gaaacaagcc agagatgtct tgattccatt gggtgaatat 5640

ttccaaattc aagatgacta cttagactgc ttcggtaccc cagaacagat cggtaagatc 5700

ggtacagata tccaagataa caaatgttct tgggtaatca acaaggcatt agaacttgct 5760

tccgcagaac aaagaaagac tttagacgaa aattacggta agaaggactc agtcgcagaa 5820

gccaaatgca aaaagatttt caatgacttg aaaatcgacc agttatacca cgaatatgaa 5880

gagtctgttg ccaaggattt gaaggccaag atctcccaag tcgacgagtc tcgtggcttc 5940

aaagccgacg tcttaactgc gtttttgaac aaggtttaca agagaagtaa atagaactaa 6000

cgctaatcga taaaacatta gatttcagat tagataagga ccatgtataa gaaatatata 6060

cttccactat aatatagtat aagcttacag atagtatctc tcgatctacc gttccacgtg 6120

actagtccaa gaacctgcag gccgcgagcg ccgatacgaa aatcgttatt gtcttgaagg 6180

tgaaatttct actcttatta atggtgaacg ttaagctgat gctatgatgg aagctgattg 6240

gtcttaactt gcttgtcatc ttgctaatgg tcattggctc gtgttattac ttaagttatt 6300

tgtactcgtt ttgaacgtaa tgctaatgat catcttatgg aataatagtg agtggtttca 6360

gggtccataa agcttttcaa ttcatctttt ttttttttgt tctttttttt gattccggtt 6420

tctttgaaat ttttttgatt cggtaatctc cgagcagaag gaagaacgaa ggaaggagca 6480

cagacttaga ttggtatata tacgcatatg tggtgttgaa gaaacatgaa attgcccagt 6540

attcttaacc caactgcaca gaacaaaaac ctgcaggaaa cgaagataaa tcatgtcgaa 6600

agctacatat aaggaacgtg ctgctactca tcctagtcct gttgctgcca agctatttaa 6660

tatcatgcac gaaaagcaaa caaacttgtg tgcttcattg gatgttcgta ccaccaagga 6720

attactggag ttagttgaag cattaggtcc caaaatttgt ttactaaaaa cacatgtgga 6780

tatcttgact gatttttcca tggagggcac agttaagccg ctaaaggcat tatccgccaa 6840

gtacaatttt ttactcttcg aagacagaaa atttgctgac attggtaata cagtcaaatt 6900

gcagtactct gcgggtgtat acagaatagc agaatgggca gacattacga atgcacacgg 6960

tgtggtgggc ccaggtattg ttagcggttt gaagcaggcg gcggaagaag taacaaagga 7020

acctagaggc cttttgatgt tagcagaatt gtcatgcaag ggctccctag ctactggaga 7080

atatactaag ggtactgttg acattgcgaa gagtgacaaa gattttgtta tcggctttat 7140

tgctcaaaga gacatgggtg gaagagatga aggttacgat tggttgatta tgacacccgg 7200

tgtgggttta gatgacaagg gagacgcatt gggtcaacag tatagaaccg tggatgatgt 7260

ggtctctaca ggatctgaca ttattattgt tggaagagga ctatttgcaa agggaaggga 7320

tgctaaggta gagggtgaac gttacagaaa agcaggctgg gaagcatatt tgagaagatg 7380

cggccagcaa aactaaaaaa ctgtattata agtaaatgca tgtatactaa actcacaaat 7440

tagagcttca atttaattat atcagttatt accacgaaaa tcgttattgt cttgaaggtg 7500

aaatttctac tcttattaat ggtgaacgtt aagctgatgc tatgatggaa gctgattggt 7560

cttaacttgc ttgtcatctt gctaatggtc atatggctcg tgttattact taagttattt 7620

gtactcgttt tgaacgtaat gctaatgatc atcttatgga ataatagtga atcggcgctc 7680

gcggcctgca ggtttcctca tcctagtatg tatagcttgt acccattaaa cgaattttat 7740

catgccgccg aaaggaacaa tttcaagtac tatcggaaga tgaatggtta gatgttaagc 7800

gcggtcactt caaacttcac atttataaag atgtcacatg gaccactatt atctacctta 7860

agttatttat caagataagt ttccggatct ttttctttcc taacacccca gtcagcctga 7920

gttacatcca gccattgaac cttagaaaat cttttgtcat cagcggtttg agccctaaga 7980

tcaacatctt gcttagcaat cactgcaatg gcgtcataac caccagcacc aggtattaag 8040

caagtaagaa ctccttttaa ggtctggcaa tcatccaata agctagtttg tacgggaggt 8100

tcgatatcgg caccagattc tttagttatt tttctaaagg aacgtctaat tgtggcaact 8160

gcatctctaa cttctgtgat ctcaggatac ttttgacagg tacagtcatt cctctcaaga 8220

gactcaaata tctgatcgct gtaatcgtca tgagtctcgt gtaagcgatc tagtttagat 8280

agtccatcca taaatctaga atttgcatga tcgagttctg tatatatttt caagctttcc 8340

ggcatatgcg aatcatacca atttttttacc ttctggacca gttttactgt ttctgaacca 8400

ttcttaatat cgcccatcca taaagttaat cccgaaggta aatggttact tttaatcgtt 8460

atattccagt cttcttcatt aaccaaatgc gccagtttac tgccgtaagt agcacttcca 8520

atatctggca aattagagat taatgcgggt gggaatcttc tatatctgat agatccatat 8580

gctgccgccg ctacatcaaa cccgcttcca attttaccct gagcttgaca atgagcaact 8640

tgtgataaat tatgaataac ttctctatat ttgtctacat tattttccag gtccgataca 8700

aaaaaggagg ccaaagctgt agttaaaact gtgactaaac ctgccgagga gcccagccct 8760

gttttgggaa cttcttcaat tctgtgcgaa tgaaaactca atcttctgtt gccacgatgt 8820

tcggtaacgc tgtcctcctg agaatggtag gcatcatcag agaaaatatc aataacgaac 8880

aagtttctat tgcagtagtc gtccatgtta ggcttaaagt agctaaatac gttagcgata 8940

acttttttcaa tgaaagggtt cttagatccg cctatcgaaa caggaatgaa gccagtttta 9000
ggacttatat ggtacagcca ctccccatct ttaaattgtt tacttttcac acgcacttca 9060
aacttatcag actcttgcaa tgaaccgtaa ggatgggcta cagcatgcat tcttgccgat 9120
aatccgacta caaatgcttc atatttcgga tctaaaacta aatatccacc agctagtaac 9180
gctttccctg gggcactgaa ggctctcaac tctgacatta tcaagctagt tgtctgtcgg 9240
gagtgtgcgt gcgtttttt atcatgttga tgctctgcat aataatgccc ataaatattt 9300
ccgacctgct tttatatctt tgctagccaa actaactgaa catagctaca cattattttc 9360
agcttggcta ttttgtgaac actgtatagc cagtccttcg gatcacggtc aacagttgtc 9420
cgagcgcttt ttggaccctt tcccttattt ttgggttaag gaaaatgaca gaaaatatat 9480
ctaatgagcc ttcgctcaac agtgctccga agtatagctt tccaaaagga gaggcaaagc 9540
aatttaagaa tgtatgaaca aaataaaggg gaaaaattac cccctctact ttaccaaacg 9600
aatactacca ataatattta caacttttcc ttatgatttt ttcactgaag cgcttcgcaa 9660
tagttgtgag cgatatcaaa agtaacgaaa tgaacttcgc ggctcgtgct atattcttgt 9720
tgctaccgtc catatctttc catagatttt caatctttga tgtctccatg gtggtacaga 9780
gaacttgtaa acaattcggt ccctacatgt gaacggccgg ccaagcacgc ggggatccga 9840
agcatgtagg gaggtcatga tatgaaaaag caaaagagta ggcatcaaaa agtttctcat 9900
tcaagtggta actgctgtta aaattaagat atttataaat tgaagcttgg tcgttccgac 9960
caataccgta gggaaacgta aattagctat tgtaaaaaaa ggaaaagaaa agaaaagaaa 10020
aatgttacat atcgaattga tcttattcct ttggtagacc agtctttgcg tcaatcaaag 10080
attcgtttgt ttcttgtggg cctgaaccga cttgagttaa aatcactctg gcaacatcct 10140
tttgcaactc aagatccaat tcacgtgcag taaagttaga tgattcaaat tgatggttga 10200
aagcctcaag ctgctcagta gtaaatttct tgtcccatcc aggaacagag ccaaacaatt 10260
tatagataaa tgcaaagagt ttcgactcat tttcagctaa gtagtacaac acagcatttg 10320
gacctgcatc aaacgtgtat gcaacgattg tttctccgta aaactgatta atggtgtggc 10380
accaactgat gatacgcttg gaagtgtcat tcatgtagaa tattggaggg aaagagtcca 10440
aacatgtggc atggaaagag ttggaatcca tcattgtttc ctttgcaaag gtggcgaaat 10500
ctttttcaac aatggcttta cgcatgactt caaatctctt tggtacgaca tgttcaattc 10560
tttctttaaa tagttcggag gttgccacgg tcaattgcat accctgagtg gaactcacat 10620
ccttttttaat atcgctgaca actaggacac aagctttcat ctgaggccag tcagagctgt 10680
ctgcgatttg tactgccatg gaatcatgac catcttcagc ttttcccatt tcccaggcca 10740
cgtatccgcc aaacaacgat ctacaagctg aaccagaccc ctttcttgct attctagata 10800
tttctgaagt tgactgtggt aattggtata acttagcaat tgcagagacc aatgcagcaa 10860
agccagcagc ggaggaagct aaaccagctg ctgtaggaaa gttattttcg gagacaatgt 10920
ggagtttcca ttgagataat gtgggcaatg aggcgtcctt cgattccatt tcctttctta 10980

attggcgtag gtcgcgcaga caattttgag ttctttcatt gtcgatgctg tgtggttctc 11040

catttaacca caaagtgtcg cgttcaaact caggtgcagt agccgcagag gtcaacgttc 11100

tgaggtcatc ttgcgataaa gtcactgata tggacgaatt ggtgggcaga ttcaacttcg 11160

tgtccctttt cccccaatac ttaagggttg cgatgttgac gggtgcggta acggatgctg 11220

tgtaaacggt cattgtgaag gtagttcgat tttggaggtc gcgggaggtt actttttttt 11280

tggatggacg caaagaagtt taataatcat attacatggc aataccacca tatacatatc 11340

catatctaat cttacttata tgttgtggaa atgtaaagag ccccattatc ttagcctaaa 11400

aaaaccttct ctttggaact ttcagtaata cgcttaactg ctcattgcta tattgaagta 11460

cggattagaa gccgccgagc gggcgacagc cctccgacgg aagactctcc tccgtgcgtc 11520

ctggtcttca ccggtcgcgt tcctgaaacg cagatgtgcc tcgcgccgca ctgctccgaa 11580

caataaagat tctacaatac tagcttttat ggttatgaag aggaaaaatt ggcagtaacc 11640

tggccccaca aaccttcaaa tcaacgaatc aaattaacaa ccataggata ataatgcgat 11700

tagtttttta gccttatttc tggggtaatt aatcagcgaa gcgatgattt ttgatctatt 11760

aacagatata taaatgcaaa agctgcataa ccactttaac taatactttc aacattttcg 11820

gtttgtatta cttcttattc aaatgtcata aaagtatcaa caaaaaattg ttaatatacc 11880

tctatactta ccccacccga agtcgcgcaa ccaactaact ttacaatgtc attaccgttc 11940

ttaacttctg caccgggaaa ggttattatt tttggtgaac actctgctgt gtacaacaag 12000

cctgccgtcg ctgctagtgt gtctgcgttg agaacctacc tgctaataag cgagtcatct 12060

gcaccagata ctattgaatt ggacttcccg gacattagct ttaatcataa gtggtccatc 12120

aatgatttca atgccatcac cgaggatcaa gtaaactccc aaaaattggc caaggctcaa 12180

caagccaccg atggcttgtc tcaggaactc gttagtcttt tggatccgtt gttagctcaa 12240

ctatccgaat ccttccacta ccatgcagcg ttttgtttcc tgtatatgtt tgtttgccta 12300

tgcccccatg ccaagaatat taagttttct ttaaagtcta ctttacccat cggtgctggg 12360

ttgggctcaa gcgcctctat ttctgtatca ctggccttag ctatggccta cttgggggg 12420

ttaataggat ctaatgactt ggaaaagctg tcagaaaacg ataagcatat agtgaatcaa 12480

tgggccttca taggtgaaaa gtgtattcac ggtacccctt caggaataga taacgctgtg 12540

gccacttatg gtaatgccct gctatttgaa aaagactcac ataatggaac aataaacaca 12600

aacaatttta agttcttaga tgatttccca gccattccaa tgatcctaac ctatactaga 12660

attccaaggt ctacaaaaga tcttgttgct cgcgttcgtg tgttggtcac cgagaaattt 12720

cctgaagtta tgaagccaat tctagatgcc atgggtgaat gtgccctaca aggcttagag 12780

atcatgacta agttaagtaa atgtaaaggc accgatgacg aggctgtaga aactaataat 12840

gaactgtatg aacaactatt ggaattgata agaataaatc atggactgct tgtctcaatc 12900

ggtgtttctc atcctggatt agaacttatt aaaaatctga gcgatgattt gagaattggc 12960

tccacaaaac ttaccggtgc tggtggcggc ggttgctctt tgactttgtt acgaagagac 13020

attactcaag agcaaattga cagtttcaaa aagaaattgc aagatgattt tagttacgag 13080

acatttgaaa cagacttggg tgggactggc tgctgtttgt taagcgcaaa aaatttgaat 13140

aaagatctta aaatcaaatc cctagtattc caattatttg aaaataaaac taccacaaag 13200

caacaaattg acgatctatt attgccagga aacacgaatt taccatggac ttcataagct 13260

aatttgcgat aggcattatt tattagttgt ttttaatctt aactgtgtat gaagttttat 13320

gtaataaaga tagaaagaga aacaaaaaaa aatttttcgt agtatcaatt cagctttcga 13380

agacagaatg aaatttaagc agaccatagt atccttgata cattgactca ggtccgccgg 13440

cgttggacga gcgaagcatc ttgccctgtg cttggccccc agtgcagcga acgttataaa 13500

aacgaatact gagtatatat ctatgtaaaa caaccatatc atttcttgtt ctgaactttg 13560

tttacctaac tagttttaaa tttccctttt tcgtgcatgc gggtgttctt atttattagc 13620

atactacatt tgaaatatca aatttcctta gtagaaaagt gagagaaggt gcactgacac 13680

aaaaaataaa atgctacgta taactgtcaa aactttgcag cagcgggcat ccttccatca 13740

tagcttcaaa catattagcg ttcctgatct tcatacccgt gctcaaaatg atcaaacaaa 13800

ctgttattgc caagaaataa acgcaaggct gccttcaaaa actgatccat tagatcctca 13860

tatcaagctt cctcatagaa cgcccaatta caataagcat gttttgctgt tatcaccggg 13920

tgataggttt gctcaggcgg tttaaacgcg tggccgtgcc gtc 13963

<210> 36
<211> 10209
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: MS49253 integration construct

<400> 36
gacggcacgg ccacgcgttt aaaccgccac ccagccaagg tagtctaaaa gctaatttct 60

ctaaaaggga gaaagttggt gatttttttat ctcgcattat tatatatgca agaatagtta 120

aggtatagtt ataaagtttt atcttaattg ccacatacgt acattgacac gtagaaggac 180

tccattattt ttttcattct agcatactat tattccttgt aacgtcccag agtattccat 240

ttaattgtcc tccatttctt aacggtgacg aaggatcacc atacaacaac tactaaagat 300

tatagtacac tctcaccttg caactattta tctgacattt gccttacttt tatctccagc 360

ttcccctcga ttttattttt caatttgatt tctaaagctt tttgcttagg cataccaaac 420

catccactca tttaacacct tatttttttt ttcgaagaca gcatccaact ttatacgttc 480

actacctttt tttttacaac aatttcattc ttcatcctat gaacgctcgt ccaacgccgg 540

cggacctttc agacgcgact gcctcatcag taagacccgt tgaaaagaac ttacctgaaa 600

aaaacgaata tatactagcg ttgaatgtta gcgtcaacaa caagaagttt aatgacgcgg 660

aggccaaggc aaaaagattc cttgattacg taagggagtt agaatcattt tgaataaaaa 720

acacgctttt tcagttcgag tttatcatta tcaatactgc catttcaaag aatacgtaaa 780

taattaatag tagtgatttt cctaacttta tttagtcaaa aaattagcct tttaattctg 840

ctgtaacccg tacatgccca aaataggggg cgggttacac agaatatata acatcgtagg 900

tgtctgggtg aacagtttat tcctggcatc cactaaatat aatggagccc gctttttaag 960

ctggcatcca gaaaaaaaaa gaatcccagc accaaaatat tgttttcttc accaaccatc 1020

agttcatagg tccattctct tagcgcaact acagagaaca ggggcacaaa caggcaaaaa 1080

acgggcacaa cctcaatgga gtgatgcaac ctgcctggag taaatgatga cacaaggcaa 1140

ttgacccacg catgtatcta tctcattttc ttacaccttc tattaccttc tgctctctct 1200

gatttggaaa aagctgaaaa aaaaggttga aaccagttcc ctgaaattat tcccctactt 1260

gactaataag tatataaaga cggtaggtat tgattgtaat tctgtaaatc tatttcttaa 1320

acttcttaaa ttctactttt atagttagtc tttttttag ttttaaaaca ccaagaactt 1380

agtttcgacc tcccgcgacc tccaaaatcg aactaccttc acaatgacat ctccagttat 1440

cggaactcct tggaagaagt tgaacgcccc agtatctgaa gaggcaatag agggtgtcga 1500

caagtattgg agggctgcta attacttgtc aatcggacaa atctacttga ggtcaaaccc 1560

attaatgaag gaacctttca ccagggaaga tgttaagcat aggttggtcg gtcactgggg 1620

aaccacacca ggattaaact ttttgattgg acacatcaac agattgattg cagatcacca 1680

acagaacacc gtcataatca tgggtccagg acatggaggt ccagctggta ccgcccagtc 1740

ttatttggat ggtacctata ccgagtattt cccaaatatt acaaaggacg aggcaggttt 1800

acagaagttt ttcagacagt tctcttaccc aggaggaatc ccttcacact acgctccaga 1860

aacaccaggt tctattcatg agggtggtga attaggatat gccttatcac atgcttacgg 1920

agcagtcatg aacaatcctt ctttgttcgt cccagccata gtaggtgatg gagaagccga 1980

gaccggtcct ttagccacag gttggcaatc taacaagtta gtaaacccta ggactgacgg 2040

aattgtcttg cctattttgc atttgaacgg atacaagatc gctaacccaa ccatcttgtc 2100

taggatatct gacgaggagt tgcatgagtt tttccacggt atgggatacg agccttatga 2160

gttcgtcgca ggatttgaca acgaagacca tttgtcaatc cacagaaggt tcgccgagtt 2220

gtttgagacc gtctttgacg agatctgtga cattaaggca gctgctcaga cagacgacat 2280

gaccagacct ttctatccta tgataatctt caggacacct aagggttgga cttgccctaa 2340

gtttatagac ggaaaaaaga ctgagggatc atggagatct catcaagtac ctttggcatc 2400

tgcaagagat acagaagctc acttcgaggt tttgaaaaat tggttggagt catataagcc 2460

tgaagaattg ttcgatgcaa atggagctgt taagccagag gtaactgctt ttatgcctac 2520

cggagagtta agaatcggag agaatcctaa cgctaatggt ggtagaatca gagaggaatt 2580

gaatttgcct gcattggagg attacgaggt aaaagaggtt gctgaatatg gtcatggatg 2640

gggtcagttg gaagcaacca gaagattagg tgtttacacc agggacatta taaagaacaa 2700

cccagactca tttaggatct ttggaccaga tgaaaccgca tcaaataggt tacaggctgc 2760

atatgacgtt actaataagc aatgggacgc tggttactta tcagcccaag tagacgaaca 2820

tatggccgtt acaggtcaag ttacagagca attgtctgaa catcaaatgg aaggattctt 2880

ggaagcttat ttgttgaccg gaaggcatgg aatttggtca tcatatgagt cattcgtaca 2940

tgtcatcgac tcaatgttaa atcagcatgc taagtggtta gaagccactg taagagagat 3000

cccatggagg aaaccaattt cttcaatgaa cttattggtt tcatctcacg tctggaggca 3060

ggatcataat ggattttctc atcaggaccc aggtgtcaca tcagttttat tgaacaagtg 3120

cttcaacaac gatcacgtta tcggaattta ctttcctgtc gattctaaca tgttgttagc 3180

tgttgccgag aagtgctaca agtctacaga catgataaac gccatcattg ccggaaagca 3240

gccagccgcc acctggttga ccttggatga ggcaagggct gaattggaga aaggagcagc 3300

cgaatgggag tgggcctcaa cagccaagtc aaatgatgaa gcacagatag tattggcttc 3360

agccggtgat gttcctgctc aagaaatcat ggctgctgcc gataagttag atgctatggg 3420

tatcaagttc aaggttgtca acgtagtcga cttggtcaaa ttgcagtcta ccaaagaaaa 3480

tgacgaggcc atctctgacg ctgacttcgc agacttattt accgaagaca agccagtatt 3540

attcgcctac cattcatacg ccagagatgt taggggattg atctatgaca ggcctaacca 3600

tgacaacttc aacgtccacg gatacgaaga acagggttca accactaccc cttatgacat 3660

ggtcagagtc aacaatattg acaggtacga gttggtcgct gaagcattga gaatgatcga 3720

tgcagacaaa tacgcagata aaatcgacga attggaggcc ttcagaaagg aagcattcca 3780

gtttgcagtt gataacggtt acgaccatcc tgactacacc gactgggtct attcaggagt 3840

aaataccaac aagcagggtg ctgtttcagc taccgctgca actgctggtg acaatgaata 3900

aagatctatt gaattgaatt gaaatcgata gatcaatttt tttcttttct ctttccccat 3960

cctttacgct aaaataatag tttattttat tttttgaata ttttttattt atatacgtat 4020

atatagacta ttatttatct tttaatgatt attaagattt ttattaaaaa aaaattcgct 4080

cctcttttaa tgcctttatg cagtttttt ttcccattcg atatttctat gttcgggttc 4140

agcgtatttt aagtttaata actcgaaaat tctgcgttcg ttaaagcttt cgagaaggat 4200

attatttcga aataaaccgt gttgtgtaag cttgaagcct ttttgcgctg ccaatattct 4260

tatccatcta ttgtactctt tagatccagt atagtgtatt cttcctgctc caagctcatc 4320

ccatccccgc gtgcttggcc ggccgtacga aaatcgttat tgtcttgaag gtgaaatttc 4380

tactcttatt aatggtgaac gttaagctga tgctatgatg gaagctgatt ggtcttaact 4440

tgcttgtcat cttgctaatg gtcattggct cgtgttatta cttaagttat ttgtactcgt 4500

tttgaacgta atgctaatga tcatcttatg gaataatagt gagtggtttc agggtccata 4560

aagcttttca attcatcttt ttttttttg ttctttttt tgattccggt ttctttgaaa 4620

ttttttgat tcggtaatct ccgagcagaa ggaagaacga aggaaggagc acagacttag 4680

attggtatat atacgcatat gtggtgttga agaaacatga aattgcccag tattcttaac 4740

ccaactgcac agaacaaaaa cctgcaggaa acgaagataa atcatgtcga aagctacata 4800

taaggaacgt gctgctactc atcctagtcc tgttgctgcc aagctattta atatcatgca 4860

cgaaaagcaa acaaacttgt gtgcttcatt ggatgttcgt accaccaagg aattactgga 4920

gttagttgaa gcattaggtc ccaaaatttg tttactaaaa acacatgtgg atatcttgac 4980

tgatttttcc atggagggca cagttaagcc gctaaaggca ttatccgcca agtacaattt 5040

tttactcttc gaagacagaa aatttgctga cattggtaat acagtcaaat tgcagtactc 5100

tgcgggtgta tacagaatag cagaatgggc agacattacg aatgcacacg gtgtggtggg 5160

cccaggtatt gttagcggtt tgaagcaggc ggcggaagaa gtaacaaagg aacctagagg 5220

ccttttgatg ttagcagaat tgtcatgcaa gggctcccta gctactggag aatatactaa 5280

gggtactgtt gacattgcga agagtgacaa agattttgtt atcggcttta ttgctcaaag 5340

agacatgggt ggaagagatg aaggttacga ttggttgatt atgacacccg gtgtgggttt 5400

agatgacaag ggagacgcat tgggtcaaca gtatagaacc gtggatgatg tggtctctac 5460

aggatctgac attattattg ttggaagagg actatttgca aagggaaggg atgctaaggt 5520

agagggtgaa cgttacagaa aagcaggctg ggaagcatat ttgagaagat gcggccagca 5580

aaactaaaaa actgtattat aagtaaatgc atgtatacta aactcacaaa ttagagcttc 5640

aatttaatta tatcagttat taccacgaaa atcgttattg tcttgaaggt gaaatttcta 5700

ctcttattaa tggtgaacgt taagctgatg ctatgatgga agctgattgg tcttaacttg 5760

cttgtcatct tgctaatggt catatggctc gtgttattac ttaagttatt tgtactcgtt 5820

ttgaacgtaa tgctaatgat catcttatgg aataatagtg aacggccggc caagcacgcg 5880

gggatgggat gagcttggag caggaagaat acactatact ggatctaaag agtacaatag 5940

atggataaga atattggcag cgcaaaaagg cttcaagctt acacaacacg gtttatttcg 6000

aaataatatc cttctcgaaa gctttaacga acgcagaatt ttcgagttat taaacttaaa 6060

atacgctgaa cccgaacata gaaatatcga atgggaaaaa aaaactgcat aaaggcatta 6120

aaagaggagc gaatttttt ttaataaaaa tcttaataat cattaaaaga taaataatag 6180

tctatatata cgtatataaa taaaaaatat tcaaaaaata aaataaacta ttattttagc 6240

gtaaaggatg gggaaagaga aaagaaaaaa attgatctat cgatttcaat tcaattcaat 6300

agatctttat tcattgtcac cagcagttgc agcggtagct gaaacagcac cctgcttgtt 6360

ggtatttact cctgaataga cccagtcggt gtagtcagga tggtcgtaac cgttatcaac 6420

tgcaaactgg aatgcttcct ttctgaaggc ctccaattcg tcgattttat ctgcgtattt 6480

gtctgcatcg atcattctca atgcttcagc gaccaactcg tacctgtcaa tattgttgac 6540

tctgaccatg tcataagggg tagtggttga accctgttct tcgtatccgt ggacgttgaa 6600

gttgtcatgg ttaggcctgt catagatcaa tcccctaaca tctctggcgt atgaatggta 6660

ggcgaataat actggcttgt cttcggtaaa taagtctgcg aagtcagcgt cagagatggc 6720

ctcgtcattt tctttggtag actgcaattt gaccaagtcg actacgttga caaccttgaa 6780

cttgataccc atagcatcta acttatcggc agcagccatg atttcttgag caggaacatc 6840

accggctgaa gccaatacta tctgtgcttc atcatttgac ttggctgttg aggcccactc 6900

ccattcggct gctcctttct ccaattcagc ccttgcctca tccaaggtca accaggtggc 6960

ggctggctgc tttccggcaa tgatggcgtt tatcatgtct gtagacttgt agcacttctc 7020

ggcaacagct aacaacatgt tagaatcgac aggaaagtaa attccgataa cgtgatcgtt 7080

gttgaagcac ttgttcaata aaactgatgt gacacctggg tcctgatgag aaaatccatt 7140

atgatcctgc ctccagacgt gagatgaaac caataagttc attgaagaaa ttggtttcct 7200

ccatgggatc tctcttacag tggcttctaa ccacttagca tgctgattta acattgagtc 7260

gatgacatgt acgaatgact catatgatga ccaaattcca tgccttccgg tcaacaaata 7320

agcttccaag aatccttcca tttgatgttc agacaattgc tctgtaactt gacctgtaac 7380

ggccatatgt tcgtctactt gggctgataa gtaaccagcg tcccattgct tattagtaac 7440

gtcatatgca gcctgtaacc tatttgatgc ggtttcatct ggtccaaaga tcctaaatga 7500

gtctgggttg ttctttataa tgtccctggt gtaaacacct aatcttctgg ttgcttccaa 7560

ctgaccccat ccatgaccat attcagcaac ctcttttacc tcgtaatcct ccaatgcagg 7620

caaattcaat tcctctctga ttctaccacc attagcgtta ggattctctc cgattcttaa 7680

ctctccggta ggcataaaag cagttacctc tggcttaaca gctccatttg catcgaacaa 7740

ttcttcaggc ttatatgact ccaaccaatt tttcaaaacc tcgaagtgag cttctgtatc 7800

tcttgcagat gccaaaggta cttgatgaga tctccatgat ccctcagtct tttttccgtc 7860

tataaactta gggcaagtcc aacccttagg tgtcctgaag attatcatag gatagaaagg 7920

tctggtcatg tcgtctgtct gagcagctgc cttaatgtca cagatctcgt caaagacggt 7980

ctcaaacaac tcggcgaacc ttctgtggat tgacaaatgg tcttcgttgt caaatcctgc 8040

gacgaactca taaggctcgt atcccatacc gtggaaaaac tcatgcaact cctcgtcaga 8100

tatcctagac aagatggttg ggttagcgat cttgtatccg ttcaaatgca aaataggcaa 8160

gacaattccg tcagtcctag ggtttactaa cttgttagat tgccaacctg tggctaaagg 8220

accggtctcg gcttctccat cacctactat ggctgggacg aacaaagaag gattgttcat 8280

gactgctccg taagcatgtg ataaggcata tcctaattca ccaccctcat gaatagaacc 8340

tggtgtttct ggagcgtagt gtgaagggat tcctcctggg taagagaact gtctgaaaaa 8400

cttctgtaaa cctgcctcgt cctttgtaat atttgggaaa tactcggtat aggtaccatc 8460

caaataagac tgggcggtac cagctggacc tccatgtcct ggacccatga ttatgacggt 8520

gttctgttgg tgatctgcaa tcaatctgtt gatgtgtcca atcaaaaagt ttaatcctgg 8580

tgtggttccc cagtgaccga ccaacctatg cttaacatct tccctggtga aaggttcctt 8640

cattaatggg tttgacctca agtagatttg tccgattgac aagtaattag cagccctcca 8700

atacttgtcg acaccctcta ttgcctcttc agatactggg gcgttcaact tcttccaagg 8760

agttccgata actggagatg tcattgtgaa ggtagttcga ttttggaggt cgcgggaggt 8820

cgaaactaag ttcttggtgt tttaaaacta aaaaaaagac taactataaa agtagaattt 8880

aagaagttta agaaatagat ttacagaatt acaatcaata cctaccgtct ttatatactt 8940

attagtcaag taggggaata atttcaggga actggtttca acctttttt tcagcttttt 9000

ccaaatcaga gagagcagaa ggtaatagaa ggtgtaagaa aatgagatag atacatgcgt 9060

gggtcaattg ccttgtgtca tcatttactc caggcaggtt gcatcactcc attgaggttg 9120

tgcccgtttt ttgcctgttt gtgcccctgt tctctgtagt tgcgctaaga gaatggacct 9180

atgaactgat ggttggtgaa gaaaacaata ttttggtgct gggattcttt ttttttctgg 9240

atgccagctt aaaaagcggg ctccattata tttagtggat gccaggaata aactgttcac 9300

ccagacacct acgatgttat atattctgtg taacccgccc cctattttgg gcatgtacgg 9360

gttacagcag aattaaaagg ctaatttttt gactaaataa agttaggaaa atcactacta 9420

ttaattattt acgtattctt tgaaatggca gtattgataa tgataaactc gaactgaaaa 9480

agcgtgtttt ttattcaaaa tgattctaac tcccttacgt aatcaaggaa tctttttgcc 9540

ttggcctccg cgtcattaaa cttcttgttg ttgacgctaa cattcaacgc tagtatatat 9600

tcgtttttt caggtaagtt cttttcaacg ggtcttactg atgaggcagt cgcgtctgaa 9660

aggtccgccg gcgttggacg agcgctccat gctggactta ctcgtcgaag atttcctgct 9720

actctctata taattagaca cccatgttat agatttcaga aaacaatgta ataatatatg 9780

gtagcctcct gaaactacca agggaaaaat ctcaacacca agagctcata ttcgttggaa 9840

tagcgataat atctctttac ctcaatctta tatgcatgtt atttgctctt ataattggtc 9900

tctatttagg gaaaaaagtc ggtttgagag cttctcgcga tgtgaaatct caatttgaac 9960

tgcacgccaa agctagccca tttcacgaac accagaaaga agaaatcccc aaggatcgca 10020

tgacagagta tgctctctca tatcgttgag tatgaatgcc aatacactga tcagctttac 10080

aagaaacgta aaatctggca cgatggtaga ctgaaatact ttcagttaaa caacagattc 10140

atgctttata cggaaaagga taacgttttg ttagctagtg aggcggttta aacgcgtggc 10200

cgtgccgtc 10209

<210> 37
<211> 7257
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: MS49298 integration construct

<400> 37
gacggcacgg ccacgcgttt aaaccgccac ccagccaagg tagtctaaaa gctaatttct 60

ctaaaaggga gaaagttggt gatttttttat ctcgcattat tatatatgca agaatagtta 120

aggtatagtt ataaagtttt atcttaattg ccacatacgt acattgacac gtagaaggac 180

tccattattt ttttcattct agcatactat tattccttgt aacgtcccag agtattccat 240

ttaattgtcc tccatttctt aacggtgacg aaggatcacc atacaacaac tactaaagat 300

tatagtacac tctcaccttg caactattta tctgacattt gccttacttt tatctccagc 360

ttcccctcga ttttatttttt caatttgatt tctaaagctt tttgcttagg cataccaaac 420

catccactca tttaacacct tatttttttt ttcgaagaca gcatccaact ttatacgttc 480

actacctttt tttttacaac aatttcattc ttcatcctat gaacgctcgt ccaacgccgg 540

cggacctttc agacgcgact gcctcatcag taagacccgt tgaaaagaac ttacctgaaa 600

aaaacgaata tatactagcg ttgaatgtta gcgtcaacaa caagaagttt aatgacgcgg 660

aggccaaggc aaaaagattc cttgattacg taagggagtt agaatcattt tgaataaaaa 720

acacgctttt tcagttcgag tttatcatta tcaatactgc catttcaaag aatacgtaaa 780

taattaatag tagtgatttt cctaacttta tttagtcaaa aaattagcct tttaattctg 840

ctgtaacccg tacatgccca aaataggggg cgggttacac agaatatata acatcgtagg 900

tgtctgggtg aacagtttat tcctggcatc cactaaatat aatggagccc gctttttaag 960

ctggcatcca gaaaaaaaaa gaatcccagc accaaaatat tgttttcttc accaaccatc 1020

agttcatagg tccattctct tagcgcaact acagagaaca ggggcacaaa caggcaaaaa 1080

acgggcacaa cctcaatgga gtgatgcaac ctgcctggag taaatgatga cacaaggcaa 1140

ttgacccacg catgtatcta tctcattttc ttacaccttc tattaccttc tgctctctct 1200

gatttggaaa aagctgaaaa aaaaggttga aaccagttcc ctgaaattat tcccctactt 1260

gactaataag tatataaaga cggtaggtat tgattgtaat tctgtaaatc tatttcttaa 1320

acttcttaaa ttctactttt atagttagtc tttttttttag ttttaaaaca ccaagaactt 1380

agtttcgacc tcccgcgacc tccaaaatcg aactaccttc acaatgaaat tgatggagaa 1440

tatcttcgga ttggccaagg ccgacaagaa gaaaatcgtt ttggcagagg gtgaggaaga 1500

gaggaacatc agggcttcag aggagattat tagggacggt attgccgaca taatcttggt 1560

cggttcagag tctgtcatta aggaaaacgc cgcaaaattc ggagtaaatt tggccggagt 1620

agagatagtc gacccagaaa cttcttctaa gaccgccggt tacgccaacg ctttctacga 1680

gatcagaaaa aacaagggag tcaccttgga gaaggctgac aaaatcgtca gggacccaat 1740

ctacttcgca acaatgatgg tcaagttagg tgacgctgac ggtttggtat ctggtgctat 1800

acatactaca ggagacttgt taaggcctgg tttgcagatt gtcaaaacag taccaggtgc 1860

atctgtcgtc tcatcagtct tcttgatgtc agtacctgac tgcgagtatg gagaggacgg 1920

ttttttgtta ttcgctgact gcgctgtaaa tgtttgtcct accgctgaag agttatcttc 1980

aatcgcaatt accactgctg agactgcaaa gaatttgtgc aagatcgagc caagggtagc 2040

catgttgtca ttctcaacca tgggatcagc ctcacatgaa ttagtcgaca aggttacaaa 2100

ggcaacaaaa ttggctaagg aggctaggcc tgacttagac atcgacggtg aattgcagtt 2160

agacgcctca ttggttaaga aggtcgcaga tttgaaagcc cctggatcta aagtcgctgg 2220

taaggcaaat gtcttgatct tcccagacat ccaggcagga aacatcggat acaagttggt 2280

ccaaagattc gcaaaggccg aagccatcgg tcctatatgt cagggatttg ccaaacctat 2340

caacgatttg tcaaggggat gttcagtcga cgacatcgtc aaagttgttg ccgttaccgc 2400

agttcaggca caagcacaag gataaagatc tattgaattg aattgaaatc gatagatcaa 2460

```
tttttttctt ttctctttcc ccatccttta cgctaaaata atagtttatt ttatttttg     2520
aatatttttt atttatatac gtatatatag actattattt atcttttaat gattattaag     2580
atttttatta aaaaaaaatt cgctcctctt ttaatgcctt tatgcagttt ttttttccca     2640
ttcgatattt ctatgttcgg gttcagcgta ttttaagttt aataactcga aaattctgcg     2700
ttcgttaaag ctttcgagaa ggatattatt tcgaaataaa ccgtgttgtg taagcttgaa     2760
gccttttttgc gctgccaata ttcttatcca tctattgtac tctttagatc cagtatagtg     2820
tattcttcct gctccaagct catcccatcc ccgcgtgctt ggccggccgt acgaaaatcg     2880
ttattgtctt gaaggtgaaa tttctactct tattaatggt gaacgttaag ctgatgctat     2940
gatggaagct gattggtctt aacttgcttg tcatcttgct aatggtcatt ggctcgtgtt     3000
attacttaag ttatttgtac tcgttttgaa cgtaatgcta atgatcatct tatggaataa     3060
tagtgagtgg tttcagggtc cataaagctt ttcaattcat ctttttttttt tttgttcttt     3120
tttttgattc cggtttcttt gaaatttttt tgattcggta atctccgagc agaaggaaga     3180
acgaaggaag gagcacagac ttagattggt atatatacgc atatgtggtg ttgaagaaac     3240
atgaaattgc ccagtattct taacccaact gcacagaaca aaaacctgca ggaaacgaag     3300
ataaatcatg tcgaaagcta catataagga acgtgctgct actcatccta gtcctgttgc     3360
tgccaagcta tttaatatca tgcacgaaaa gcaaacaaac ttgtgtgctt cattggatgt     3420
tcgtaccacc aaggaattac tggagttagt tgaagcatta ggtcccaaaa tttgtttact     3480
aaaaacacat gtggatatct tgactgattt ttccatggag ggcacagtta agccgctaaa     3540
ggcattatcc gccaagtaca atttttttact cttcgaagac agaaaatttg ctgacattgg     3600
taatacagtc aaattgcagt actctgcggg tgtatacaga atagcagaat gggcagacat     3660
tacgaatgca cacggtgtgg tgggcccagg tattgttagc ggtttgaagc aggcggcgga     3720
agaagtaaca aaggaaccta gaggcctttt gatgttagca gaattgtcat gcaagggctc     3780
cctagctact ggagaatata ctaagggtac tgttgacatt gcgaagagtg acaaagattt     3840
tgttatcggc tttattgctc aaagagacat gggtggaaga gatgaaggtt acgattggtt     3900
gattatgaca cccggtgtgg gtttagatga caagggagac gcattgggtc aacagtatag     3960
aaccgtggat gatgtggtct ctacaggatc tgacattatt attgttggaa gaggactatt     4020
tgcaaaggga agggatgcta aggtagaggg tgaacgttac agaaaagcag gctgggaagc     4080
atatttgaga agatgcggcc agcaaaacta aaaaactgta ttataagtaa atgcatgtat     4140
actaaactca caaattagag cttcaattta attatatcag ttattaccac gaaaatcgtt     4200
attgtcttga aggtgaaatt tctactctta ttaatggtga acgttaagct gatgctatga     4260
tggaagctga ttggtcttaa cttgcttgtc atcttgctaa tggtcatatg gctcgtgtta     4320
ttacttaagt tatttgtact cgttttgaac gtaatgctaa tgatcatctt atggaataat     4380
agtgaacggc cggccaagca cgcggggatg ggatgagctt ggagcaggaa gaatacacta     4440
tactggatct aaagagtaca atagatggat aagaatattg gcagcgcaaa aaggcttcaa     4500
```

gcttacacaa cacggtttat ttcgaaataa tatccttctc gaaagcttta acgaacgcag 4560
aattttcgag ttattaaact taaaatacgc tgaacccgaa catagaaata tcgaatggga 4620
aaaaaaaact gcataaaggc attaaaagag gagcgaattt ttttttaata aaaatcttaa 4680
taatcattaa aagataaata atagtctata tatacgtata taaataaaaa atattcaaaa 4740
aataaaataa actattattt tagcgtaaag gatggggaaa gagaaaagaa aaaaattgat 4800
ctatcgattt caattcaatt caatagatct ttatccttgt gcttgtgcct gaactgcggt 4860
aacggcaaca actttgacga tgtcgtcgac tgaacatccc cttgacaaat cgttgatagg 4920
tttggcaaat ccctgacata taggaccgat ggcttcggcc tttgcgaatc tttggaccaa 4980
cttgtatccg atgtttcctg cctggatgtc tgggaagatc aagacatttg ccttaccagc 5040
gactttagat ccaggggctt tcaaatctgc gaccttctta accaatgagg cgtctaactg 5100
caattcaccg tcgatgtcta agtcaggcct agcctcctta gccaattttg ttgcctttgt 5160
aaccttgtcg actaattcat gtgaggctga tcccatggtt gagaatgaca acatggctac 5220
ccttggctcg atcttgcaca aattctttgc agtctcagca gtggtaattg cgattgaaga 5280
taactcttca gcggtaggac aaacatttac agcgcagtca gcgaataaca aaaaaccgtc 5340
ctctccatac tcgcagtcag gtactgacat caagaagact gatgagacga cagatgcacc 5400
tggtactgtt ttgacaatct gcaaaccagg ccttaacaag tctcctgtag tatgtatagc 5460
accagatacc aaaccgtcag cgtcacctaa cttgaccatc attgttgcga agtagattgg 5520
gtccctgacg attttgtcag ccttctccaa ggtgactccc ttgtttttc tgatctcgta 5580
gaaagcgttg gcgtaaccgg cggtcttaga agaagtttct gggtcgacta tctctactcc 5640
ggccaaattt actccgaatt ttgcggcgtt ttccttaatg acagactctg aaccgaccaa 5700
gattatgtcg gcaataccgt ccctaataat ctcctctgaa gccctgatgt tcctctcttc 5760
ctcaccctct gccaaaacga ttttcttctt gtcggccttg gccaatccga agatattctc 5820
catcaatttc attgtgaagg tagttcgatt ttggaggtcg cgggaggtcg aaactaagtt 5880
cttggtgttt taaaactaaa aaaaagacta actataaaag tagaatttaa gaagtttaag 5940
aaatagattt acagaattac aatcaatacc taccgtcttt atatacttat tagtcaagta 6000
ggggaataat ttcagggaac tggtttcaac ctttttttc agcttttcc aaatcagaga 6060
gagcagaagg taatagaagg tgtaagaaaa tgagatagat acatgcgtgg gtcaattgcc 6120
ttgtgtcatc atttactcca ggcaggttgc atcactccat tgaggttgtg cccgtttttt 6180
gcctgtttgt gcccctgttc tctgtagttg cgctaagaga atggacctat gaactgatgg 6240
ttggtgaaga aaacaatatt ttggtgctgg gattcttttt ttttctggat gccagcttaa 6300
aaagcgggct ccattatatt tagtggatgc caggaataaa ctgttcaccc agacacctac 6360
gatgttatat attctgtgta acccgccccc tattttgggc atgtacgggt tacagcagaa 6420
ttaaaaggct aatttttga ctaaataaag ttaggaaaat cactactatt aattatttac 6480
gtattctttg aaatggcagt attgataatg ataaactcga actgaaaaag cgtgtttttt 6540

attcaaaatg attctaactc ccttacgtaa tcaaggaatc tttttgcctt ggcctccgcg 6600

tcattaaact tcttgttgtt gacgctaaca ttcaacgcta gtatatattc gttttttca 6660

ggtaagttct tttcaacggg tcttactgat gaggcagtcg cgtctgaaag gtccgccggc 6720

gttggacgag cgctccatgc tggacttact cgtcgaagat ttcctgctac tctctatata 6780

attagacacc catgttatag atttcagaaa acaatgtaat aatatatggt agcctcctga 6840

aactaccaag ggaaaaatct caacaccaag agctcatatt cgttggaata gcgataatat 6900

ctctttacct caatcttata tgcatgttat ttgctcttat aattggtctc tatttaggga 6960

aaaaagtcgg tttgagagct tctcgcgatg tgaaatctca atttgaactg cacgccaaag 7020

ctagcccatt tcacgaacac cagaaagaag aaatccccaa ggatcgcatg acagagtatg 7080

ctctctcata tcgttgagta tgaatgccaa tacactgatc agctttacaa gaaacgtaaa 7140

atctggcacg atggtagact gaaatacttt cagttaaaca acagattcat gctttatacg 7200

gaaaaggata acgttttgtt agctagtgag gcggtttaaa cgcgtggccg tgccgtc 7257