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

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

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

WIPO | PCT

(10) International Publication Number
WO 2014/146047 A9

(51) International Patent Classification:
C12N 1/21 (2006.01) *C12P 7/52* (2006.01)
C12N 15/52 (2006.01)

(21) International Application Number:
PCT/US2014/030923

(22) International Filing Date:
17 March 2014 (17.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/852,387 15 March 2013 (15.03.2013) US
61/791,743 15 March 2013 (15.03.2013) US

(71) Applicant: **OPX BIOTECHNOLOGIES, INC.** [US/US]; 2425 55th Street, Suite 100, Boulder, CO 80301 (US).

(72) Inventors: **LIAO, Hans**; 2157 Holyoke Lane, Superior, CO 80027 (US). **MERCOGLIANO, Christopher, Patrick**; 48 Groveland Terrace, Unit 212, Minneapolis, MN 55403 (US). **WOLTER, Travis, Robert**; 2522 W. 33rd Avenue, #2, Denver, CO 80211 (US). **LOUIE, Michael, Tai Man**; 4597 Winona Place, Broomfield, CO 80020 (US). **RIBBLE, Wendy, Kathleen**; 5827 West 80th Circle, Arvada, CO 80003 (US). **LIPSCOMB, Tanya**; 5333 Euclid Avenue, Boulder, CO 80303 (US). **SPINDLER, Eileen, Colie**; 10449 Isabelle Road, Lafayette, CO 80026 (US). **LYNCH, Michael**; 136 Hudson Hall, Campus Box 90281, Durham, NC 27708 (US).

(74) Agents: **ANDRES, JR., Charles** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050 (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.

[Continued on next page]

(54) Title: MONOFUNCTIONAL MCR + 3-HP DEHYDROGENASE

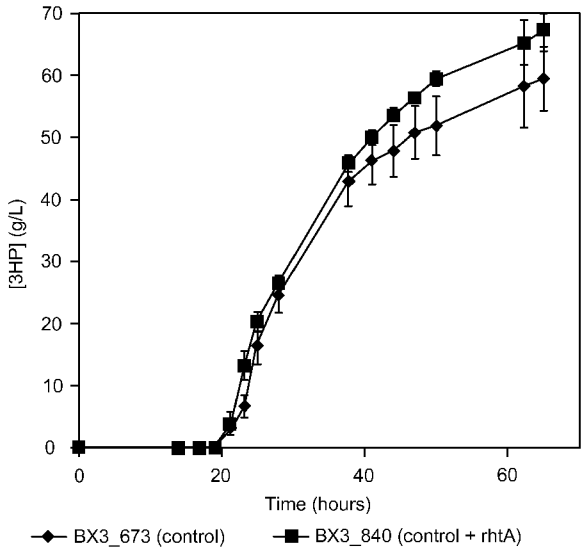


FIG. 7

(57) Abstract: The present invention provides various combinations of genetic modifications to a transformed host cell that provide increase conversion of carbon to a chemical product. The present invention also provides methods of fermentation and methods of making various chemical products.

WO 2014/146047 A9

(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).

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))*

(48) Date of publication of this corrected version:
6 November 2014

(15) Information about Correction:
see Notice of 6 November 2014

MONOFUNCTIONAL MCR + 3-HP DEHYDROGENASE

[001] This application claims priority to U.S. Provisional Patent Application No. 61/852,387, filed on March 15, 2013; and U.S. Provisional Patent Application No. 61/791,743, filed on March 15, 2013; which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[002] There is a need for alternative production methods of industrial chemicals used for various consumer products and fuels that are currently made from petroleum. One alternative method is the use of engineered microorganisms to produce industrial chemicals. Currently, in the field of bioproduced chemicals there is a need to improve microbial enzyme performance, enhanced production rate in order to reach the goal of becoming an at-cost replacement basis for petro-based chemicals.

[003] A common challenge faced in field of bio-produced chemicals in microorganisms is that any one modification to a host cell may require coordination with other modifications in order to successfully enhance chemical bioproduction.

[004] Provided herein are, *e.g.*, methods, systems of fermentation, genetically modified microorganisms, and modified enhanced enzymes for chemical production, all of which may be used *e.g.*, in various combinations to increase chemical production of a desired chemical product.

INCORPORATION BY REFERENCE

[005] All publications, patents, and patent applications herein are incorporated by reference in their entireties. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

SUMMARY OF THE INVENTION

[006] The inventive embodiments provided in this Summary of the Invention are meant to be illustrative only and to provide an overview of selected embodiments disclosed herein. The Summary of the Invention, being illustrative and selective, does not limit the scope of any claim, does not provide the entire scope of inventive embodiments disclosed or contemplated herein, and should not be construed as limiting or constraining the scope of this disclosure or any claimed inventive embodiment.

[007] Provided herein are genetically modified organisms capable of producing an industrial chemical product of interest, wherein the genetic modification can include introduction of nucleic acid sequences coding for polynucleotides encoding one or more of the following: (1) an acetyl-CoA carboxylase gene with one or more of its subunits fused together in the genetic structure of the organism; and/or (2) an acetyl-CoA carboxylase gene having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another.

[008] Also provided herein are methods of producing a chemical product using a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetic modification can include introduction of nucleic acid sequences coding for polynucleotides encoding one or more of the following: (1) an acetyl-CoA carboxylase gene with one or more of its subunits fused together in the genetic structure of the organism; and/or (2) an acetyl-CoA carboxylase gene having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another.

[009] Further provided herein are products made from a genetically modified organisms capable of producing an industrial chemical product of interest, wherein the genetic modification includes introduction of nucleic acid sequences coding for polynucleotides encoding one or more of the following: (1) an acetyl-CoA carboxylase gene with one or more of its subunits fused together in the genetic structure of the organism; and/or (2) an acetyl-CoA carboxylase gene having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another. The present invention also relates to products made from such methods.

[0010] Provided herein are genetically modified organisms (GMOs) capable of producing an industrial chemical product of interest, wherein the GMOs comprise a genetic modification including introduction of one or more nucleic acid sequences encoding one or more of the following: (1) an acetyl-CoA carboxylase (ACCase) with one or more of its subunits fused together; and/or (2) an acetyl-CoA carboxylase having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another.

[0011] Also provided herein are methods of producing a chemical product using a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetic modification includes introduction of at least one nucleic acid sequence encoding one or more enzymes selected from the group consisting of: (1) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures; (2) a salt-tolerant enzyme; (3) a polypeptide that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from the cell; and/or (4) a polypeptide that facilitates the importation into a cell of a reactant, precursor, and/or metabolite used for producing a chemical product of interest.

[0012] Further provided herein are products made from a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetic modification includes introduction of at least one nucleic acid sequence encoding one or more enzymes selected from the group consisting of: (1) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures; (2) a salt-tolerant enzyme; (3) a polypeptide that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from the cell;

and/or (4) a polypeptide that facilitates the importation into a cell of a reactant, precursor, and/or metabolite used for producing a chemical product of interest.

[0013] Provided herein are (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and/or nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0014] Also provided herein are genetically modified organisms capable of producing an industrial chemical product of interest, wherein the genetically modified organisms comprise (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0015] Further provided herein are methods of producing a chemical product using a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetically modified organism can comprise (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD,

rutE, and/or nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0016] Further provided herein are products made from a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetically modified organism can comprise (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and/or nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0017] Provided herein are genetically modified organisms ("GMOs") capable of producing an industrial chemical product of interest, wherein the genetic modification can include introduction of at least one nucleic acid sequence encoding one or more enzymes selected from the group consisting of: (1) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures; (2) a salt-tolerant enzyme; (3) a polypeptide that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from the cell; and/or (4) a polypeptide that facilitates the importation into a cell of a reactant, precursor, and/or metabolite used for producing a chemical product of interest.

[0018] Also provided herein are methods of producing a chemical product using the genetically modified organisms herein. Further provided herein are products made from these methods. In certain embodiments, the products can be acetyl-CoA, malonyl-CoA, malonate semialdehyde, 3-hydroxypropionic acid (3-HP), acrylic acid, 1,3 propanediol, malonic acid, ethyl 3-HP, propiolactone, acrylonitrile, acrylamide, methyl acrylate, a polymer including super absorbent polymers and polyacrylic acid, and/or a consumer product.

[0019] Provided herein are methods of producing a chemical product from a renewable carbon source through a bioproduction process that can comprise a controlled multi-phase production process wherein the initiation and/or completion of one or more phases of the production process can be controlled by genetic modifications to the organism producing the chemical product and/or can be controlled by changes made to the cell environment. The bioproduction process may include two or more of the following phases: (1) growth phase; (2) induction phase; and (3) production phase. Also provided are products made from these methods.

[0020] Also provided herein are methods of making GMOs herein.

[0021] Additionally, provided herein are methods of producing a chemical product using a GMO herein.

[0022] Further provided herein are products produced using a GMO herein which can be, *e.g.*, capable of producing an industrial chemical product of interest.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0024] **FIG. 1** Depicts some embodiments of the metabolic pathways to produce 3-hydroxypropionic acid ("3-HP").

[0025] **FIG. 2** Depicts some embodiments of the of various equilibrium states in the malonate semialdehyde to 3-HP reaction in a cell environment.

[0026] **FIG. 3** Depicts some embodiments of the reaction catalyzed by acetyl-CoA carboxylase (ACCase)

[0027] **FIG. 4** Shows the inhibition of ACCase enzyme activity by high salt concentration

[0028] **FIG. 5** Depicts some embodiments of the fusion ACCase subunit gene constructs overexpressed in *E. coli.* CAT = chloramphenicol resistance marker; p15a rep = replication origin; arrow = promoter.

[0029] **FIG. 6** Show improved production of 3-HP by genetically modified organism with DA fusion ACCase.

[0030] **FIG. 7** Shows improved production of 3-HP by genetically modified organism with overexpression of rhtA exporter.

[0031] **FIG. 8** Shows various embodiments of the genetic modules used for optimizing expression in host cells.

[0032] **FIG. 9** Shows various chemical products that can made from various embodiments of the invention.

[0033] **FIG. 10** Shows an enzymatic reaction of the transport of homoserine (threonine and homoserine exporters).

[0034] **FIG. 11** Shows results from the overexpression of 33 transporters (adapted from Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H. (2006))

[0035] **FIG. 12** Shows the average OD after 24 hours of cultures grown in M9 in the presence of different levels of 3-HP at different pHs.

[0036] **FIGs. 13A-D** Shows multiple sequence alignments for rhtA.

[0037] **FIGs. 14A-K** Shows pileup sequence comparisons for rhtA.

[0038] **FIG. 15**. Shows improved production of 3-HP in fermentors by biocatalyst with DA fusion ACCase.

[0039] **Table 1.** Lists the accession numbers for genes encoding ACCase subunits from *Halomonas elongate* ("*H. elongate*").

[0040] **Table 2.** Depicts some embodiments of the RBS sequences used to enhance expression of *H. elongate* ACCase subunits.

[0041] **Table 3.** Shows improvement in 3-HP production by RBS-optimized expression of *H. elongata* ACCase subunits.

[0042] **Table 4.** Shows some embodiments of the ACCase subunit fusions that increase and ACCase enzyme complex activity.

DETAILED DESCRIPTION OF THE INVENTION

[0043] **DEFINITIONS**

[0044] As used herein, the term "homology" can refer to the optimal alignment of sequences (either nucleotides or amino acids), which may be conducted by computerized implementations of algorithms. "Homology", with regard to polynucleotides, for example, may be determined by analysis with BLASTN version 2.0 using the default parameters. "Homology", with respect to polypeptides (*i.e.*, amino acids), may be determined using a program, such as BLASTP version

2.2.2 with the default parameters, which aligns the polypeptide or fragments (and can also align nucleotide fragments) being compared and determines the extent of amino acid identity or similarity between them. It will be appreciated that amino acid "homology" includes conservative substitutions, *i.e.* those that substitute a given amino acid in a polypeptide by another amino acid of similar characteristics. Typically seen as conservative substitutions are the following replacements: replacements of an aliphatic amino acid such as Ala, VaI, Leu and Ile with another aliphatic amino acid; replacement of a Ser with a Thr or vice versa; replacement of an acidic residue such as Asp or Glu with another acidic residue; replacement of a residue bearing an amide group, such as Asn or Gln, with another residue bearing an amide group; exchange of a basic residue such as Lys or Arg with another basic residue; and replacement of an aromatic residue such as Phe or Tyr with another aromatic residue. For example, homologs can have about: 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%; or at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% overall amino acid or nucleotide identity to the gene or proteins of the invention; or can have about: 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, or 70% overall amino acid or nucleotide identity to the essential protein functional domains of the gene or proteins of the invention; or at least: 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, or 70%, overall amino acid or nucleotide to the essential binding amino acids within an essential functional domain of the gene or proteins of the invention.

[0045] The above descriptions and methods for sequence homology are intended to be exemplary and it is recognized that this concept is well-understood in the art. Further, it is appreciated that nucleic acid sequences may be varied and still provide a functional enzyme, and such variations are within the scope of the present invention. The term “enzyme homolog” can also mean a functional variant.

[0046] The term “functional homolog” can describe a polypeptide that is determined to possess an enzymatic activity and specificity of an enzyme of interest but which has an amino acid sequence different from such enzyme of interest. A corresponding “homolog nucleic acid sequence” may be constructed that is determined to encode such an identified enzymatic functional variant.

[0047] The term “3-HP” means 3-hydroxypropionic acid.

[0048] The term "heterologous DNA," "heterologous nucleic acid sequence," and the like as used herein can refer to a nucleic acid sequence wherein at least one of the following is true: (a) the sequence of nucleic acids is foreign to (*i.e.*, not naturally found in) a given host microorganism; (b) the sequence may be naturally found in a given host microorganism, but in an unnatural (*e.g.*, greater than expected) amount; and/or (c) the sequence of nucleic acids comprises two or more subsequences that are not found in the same relationship to each other in nature. For example, regarding instance (c), a heterologous nucleic acid sequence that is recombinantly produced can *e.g.*, have two or more sequences from unrelated genes arranged to make a new functional nucleic acid. Embodiments of the present invention may result from introduction of an expression vector into a host microorganism, wherein the expression vector contains a nucleic acid sequence coding for an enzyme that is, or is not, normally found in a host microorganism. With reference to the host microorganism's genome prior to the introduction of the heterologous nucleic acid sequence, then, the nucleic acid sequence that codes for the enzyme is heterologous (whether or not the heterologous nucleic acid sequence is introduced into that genome). The term "heterologous" is intended to include the term "exogenous" as the latter term is generally used in the art as well as "endogenous".

[0049] As used herein and unless otherwise indicated, the singular forms "a," "an," and "the" include plural referents (*e.g.*, mean one or more). Thus, for example, reference to an "expression vector" includes a single expression vector as well as a plurality of expression vectors, either the same (*e.g.*, the same operon) or different; reference to "microorganism" includes a single microorganism as well as a plurality of microorganisms; and the like.

[0050] As used herein and unless otherwise indicated, the term "organism" refers to any contiguous living system. Examples of organisms can include, but are not limited to, animals, fungus, microorganisms, and/or plants. The term organism is meant to encompass unicellular and/or multicellular entities, including but not limited to, prokaryotes (including but not limited to bacteria and fungus) and/or eukaryotes (including, but not limited to, viruses).

[0051] As used herein and unless otherwise indicated, terms such as "contain", "containing", "include", "including", and the like mean comprising.

[0052] Some embodiments herein contemplate numerical ranges. When ranges are provided, the ranges include the range endpoints unless otherwise indicated. Unless otherwise indicated, numerical ranges include all values and subranges therein as if explicitly written out.

[0053] Some values herein are modified by the term "about." In some instances, the term *"about"* in relation to a reference numerical value can include a range of values plus or minus 10% from that value. For example the amount *"about 10"* can include amounts from 9 to 11. In

other embodiments, the term "about" in relation to a reference numerical value can include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[0054] Carbon sources can include *e.g.*, sugars such as *e.g.,* glucose, fructose, galactose, dextrose, maltose, glycerol, and/or a solution containing equal to or less than 50% glycerol.

[0055] A disruption of gene function may also be effectuated, in which the normal encoding of a functional enzyme by a nucleic acid sequence has been altered so that the production of the functional enzyme in a microorganism cell has been reduced or eliminated. A disruption may broadly include a gene deletion, and also includes, but is not limited to gene modification (e.g., introduction of stop co dons, frame shift mutations, introduction or removal of portions of the gene, introduction of a degradation signal), affecting mRNA transcription levels and/or stability, and altering the promoter or repressor upstream of the gene encoding the polypeptide. In some embodiments, a gene disruption is taken to mean any genetic modification to the DNA, mRNA encoded from the DNA, and the amino acid sequence resulting there from that result in at least a 50 percent reduction of enzyme function of the encoded gene in the microorganism cell.

[0056] The term "overexpress" and its equivalents, can mean to increase the amount of a nucleic acid (*e.g.*, mRNA, DNA, chromosomes, plasmids, etc.) and/or protein (*e.g.*, enzymes and other polypeptides) in the cell to an amount that is greater than the amount that is produced in an unmodified host cell. A nucleic acid and/or protein that is overexpressed may be endogenous to the host cell or exogenous to the host cell.

[0057] I. INTRODUCTION

[0058] Provided herein are, *e.g.*, genetically modified microorganisms, methods for making the same, and use of the same in making industrial products. Any and all of the microorganisms herein may include a combination of genetic alterations as described herein. The present invention contemplates, for example, a genetically modified microorganism having one or more of the following genetic modifications: (i) an alteration that affects the stoichiometric ratio, expression or production of one or more ACCase enzyme genes (ii) a recombinant ACCase gene having at least: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence homology to an ACCase gene from a salt tolerant organism and/or (iii) a genetic alteration in one or more non-ACCase genes.

[0059] The present invention contemplates, for example, a genetically modified microorganism having one or more genetic alterations that encode for one or more exporters capable of exporting 3-HP out of a cell.

[0060] The present invention also relates to methods of fermentation. The genetically modified microorganisms can be cultured under conditions that optimized a host cell for increase

chemical production. The bio-production process may include two or more of the following phases of fermentation: (1) growth phase where the culture organism replicates itself and the carbon intermediate product is built up; (2) the induction phase, where the expression of key enzymes critical to the chemical production is induced and the enzymes accumulate within the organism to carry out the engineered pathway reactions required to further produce the chemical product (3) production phase is where the organism expresses proteins that provide for continuously production the desired chemical product. The above phases can be further controlled by (1) addition and amount of the initiating reactant added to the reaction vessel and (2) key enzymes engineered into the organism using promoters that are sensitive to (*e.g.*, activated by) the depletion of the initiating reactant. Addition details about the fermentation process of the invention are disclosed below.

[0061] Provided herein are, *e.g.*, genetically modified microorganisms, methods for making the same, and use of the same in making industrial products. Any and all of the microorganisms herein may include a combination of genetic alterations as described herein. The present invention contemplates, for example, (i) new hybrid molecules or co-expressed molecules of a mono-functional malonyl-CoA reductase enzyme with various 3-HP dehydrogenase proteins that: (a) exhibit less inhibition by high 3-HP concentrations (b) can catalyze a reaction pathway to 3-HP that is less reversible, substantially irreversible and/or irreversible (c) can utilize NADH (d) enzymes that utilize and/or can utilize flavin; and (ii) can have one or more genetic alterations that can be used to channel a carbon within in standard cellular metabolic pathway into a pathway engineered to produce a desired chemical.

[0062] When utilizing certain organisms to create certain products, it may be advantageous to control each phase discretely. For example, depending on the pathway involved, reactions, reactants, intermediates and byproducts created during cell growth can inhibit enzyme induction and/or the organism's ability to produce the desired chemical product. Similarly, reactions, reactants, intermediates and byproducts created as part of the production pathway can impact cell growth, and even the increased concentration of the chemical product as it is produced can impede cell replication.

[0063] II. ACETYL-CoA CARBOXYLASE

[0064] Malonyl-CoA Flux

[0065] One of the steps in the biosynthesis of 3-HP involves the reaction catalyzed by acetyl-CoA carboxylase enzyme. ACCase is a primary control point in the 3-HP pathway shown in **FIG. 1** (previously described in) for the converting acetyl-CoA to malonyl-CoA and hence to malonate semialdehyde and 3-HP. Inventive embodiments herein contemplate the use of genetic

modifications that increase activity of ACCase complex enzymes to thereby increase 3-HP production in a host cell.

[0066] Fused Subunits

[0067] The acetyl-CoA carboxylase complex is a multi-subunit protein. Prokaryotes and plants have multi-subunit acetyl-CoA carboxylase complexes composed of several polypeptides encoded by distinct genes. However, humans and most other eukaryotes, such as yeast, have evolved an acetyl-CoA carboxylase complex with CT and BC catalytic domains and biotin carboxyl carrier domains on a single polypeptide. The biotin carboxylase (BC) activity, biotin carboxyl carrier protein (BCCP), and carboxyl transferase (CT) activity are each contained on a different subunit. In *E. coli* the ACCase complex is derived from multi polypeptide transcribed by distinct, separable protein components known as accA, accB, accC, and accD.

[0068] Acetyl-CoA carboxylase is a biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic activities, biotin carboxylase and carboxyltransferase. The first reaction is carried out by BC and involves the ATP-dependent carboxylation of biotin with bicarbonate. The carboxyl group is transferred from biotin to acetyl-CoA to form malonyl-CoA in the second reaction, which is catalyzed by CT. The main function of ACCase complex in the cell is to provide the malonyl-CoA substrate for the biosynthesis of fatty acids.

[0069] The conversion of acetyl-CoA to malonyl-CoA is an important step in the bioconversion of a renewable carbon source (such as, for example, sugar, cellulosic material, switch grass, or natural gas or any combination thereof) to a useful industrial chemical (such as, for example, 3-hydroxypropionic acid (3-HP)). In certain organisms, such as *E. coli* or yeast, the native ACCase expression from the chromosome alone is insufficient to enable the organism to produce chemicals such as 3-HP at a rate to support a commercial scale operation. Overexpression of the ACCase complex has been shown to provide some advantage. *See, e.g.*, US Patent Application Nos.: 12/891,760; 12/891,790; and 13/055,138.

[0070] Applicants have discovered that the introduction of an acetyl-CoA carboxylase gene with one or more of its subunits fused into *e.g.*, an organism or GMO is beneficial to the production of a chemical product in a host cell (*e.g.*, the organism or GMO). In any embodiment herein, fusion can be of two gene products produced from a single polynucleotide controlled by a single promoter, which *e.g.*, can further enhance an organism's bioproduction of an industrial chemical. In any embodiment herein, fusion can be of two gene products, enhanced by at least one promoter, which can further enhance an organism's bioproduction of an industrial chemical. In any embodiment herein, fusion can be of two gene products produced from a single

polynucleotide controlled by at least one inducible promoter, which can further enhance an organism's bioproduction of an industrial chemical. Keeping components of the ACCase complex fused together in the genetic structure of an organism can be advantageous because *e.g.*, it can enhance the stability of the non-native ACCase genetic modification and it can facilitate equimolar expression of the fused acc subunits.

[0071] In any embodiment herein, the subunit-fused ACCase (*e.g.*, in the organism, cell or GMO) *e.g.*, may be or contain an accA-accB, accA-accC, accA-accD, accB-accC, accB-accD, accC-accD, accA-accB-accC, accA-accB-accD, accA-accC-accD, accB-accC-accD, or accA-accB-accC-accD fused subunit having *e.g.*, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence homology to the same combination of *E.coli* accA, accB, accC and accD, or their functional homologs. In addition, in any embodiment herein, the organism may include any combination of these fused subunits, or any combination of these fused subunits together with one or more of the four non-fused subunits. When such combinations are used, the subunits (fused and non-fused) may be expressed on the same plasmid or on different plasmids or on one or more of the organism's chromosome.

[0072] In any embodiment herein, the subunit-fused ACCase (*e.g.*, in the organism, cell or GMO) *e.g.*, may be or contain an accA-accB, accA-accC, accA-accD, accB-accC, accB-accD, accC-accD, accA-accB-accC, accA-accB-accD, accA-accC-accD, accB-accC-accD, or accA-accB-accC-accD fused subunit having *e.g.*, about: 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence homology to the same

combination of *E.coli* accA, accB, accC and accD, or their functional homologs. In addition, in any embodiment herein, the organism may include any combination of these fused subunits, or any combination of these fused subunits together with one or more of the four non-fused subunits. When such combinations are used, the subunits (fused and non-fused) may be expressed on the same plasmid or on different plasmids or on one or more of the organism's chromosome. Further, in any embodiment herein, the organism, cell, or GMO which can include the subunit-fused ACCase can be capable of reducing malonyl-COA. Further, the organism, cell, or GMO can comprise a polynucleotide encoding a protein that reduces or is capable of reducing malonly-CoA. In any embodiment herein, the protein that reduces or is capable of reducing malonyl-CoA can be NADPH dependent, NADH dependent, or a combination of these.

[0073] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase, wherein the encoded protein comprises at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0074] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase complex, wherein the encoded protein comprises at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0075] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase domain, wherein the encoded protein comprises at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0076] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase subunit, wherein the encoded protein comprises at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0077] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous polynucleotide residues from any one of SEQ ID NOs: 2, 4, 6, 8, or 10.

[0078] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous polynucleotide residues from any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase.

[0079] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous polynucleotide residues from any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase complex.

[0080] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous polynucleotide residues from any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase domain.

[0081] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous polynucleotide residues from any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase subunit.

[0082] In any embodiment herein, an accA-accD fused subunit can be introduced into an organism or GMO either alone or in combination with the accB-accC fused subunit, the accB gene, and/or the accC gene. In any embodiment herein, the organism or GMO can be a bacteria, *e.g.*, *E. coli* or *Cupriavidus necator*.

[0083] **Composition stoichiometry**

[0084] Composition stoichiometry can be, *e.g.*, the quantitative relationships among elements or parts that comprise a compound or thing (*e.g.*, such as an enzyme or enzyme complex, for example an ACCase or ACCase complex). Although stoichiometric terms are traditionally reserved for chemical compounds, theoretical consideration of stoichiometry are relevant when considering the optimal function of heterologous multi-subunit protein in a host cell.

[0085] For example, in any embodiment herein, a stoichiometric ratio of each of the four ACCase subunits relative to one another can be, for example, between or from: 0 and about 10, 0 and about 9, 0 and about 8, 0 and about 7, 0 and about 6, 0 and about 5, 0 and about 4, 0 and about 3, 0 and about 2, 0 and about 1, about 1 and about 10, about 2 and about 10, about 3 and about 10, about 4 and about 10, about 5 and about 10, about 6 and about 10, about 7 and about 10, about 8 and about 10, about 9 and about 10, or between or from about 0.5 to about 2, or about 7 to about 9. For example, the stoichiometric ratios, in any embodiment herein, for the protein subunits accA:accB:accC:accD can be about: 1:2:1:1. In any embodiment herein, an organism can be genetically modified to include an accA-accD fused subunit, an accB non-fused subunit, and an accC non-fused subunit, with the ratios or molar ratios of the accDA fusion:accB:accC being about 1:2:1, which can be close to the optimum for enzymatic activity.

[0086] In any embodiment herein, where an organism can be engineered to make 3-HP, in order to get optimal function in a host cell of a heterologous ACCase enzyme complex it can be important to engineer the stoichiometry of these subunits in such a way that provides maximal production of 3-HP such that the subunit can make a more stable enzyme complex when overexpressed in the cell.

[0087] In any embodiment herein can be provided for the controlled expression of the natural accA, accB, accC, and accD subunits of *E.coli* or having at least: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology to *E.coli* accA, accB, accC and accD. In any embodiment herein can be provided for the inducible expression of the natural accA, accB, accC, and accD subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC, and accD. In certain aspects the invention provides for the low, medium, high and/or inducible expression of the natural accA, accB, accC, and accD subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC and accD.

[0088] In any embodiment herein can be provided for the expression of the natural accC and accD subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC and accD in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the expression of the natural accB and accA subunits of *E.coli* or having at least

80% sequence homology to *E.coli* accA, accB, accC, and accD in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the expression of the natural accC and accD subunits with the accA subunit of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC, and accD in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the expression of the natural accC and accD subunits with the accB subunit of *E.coli* or having at least about: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology to *E.coli* accA, accB, accC, and accD in low, medium, high or inducible expression vectors.

[0089] In any embodiment herein can be provided for the expression of a fusion of two, three, or all of the four ACCase subunits in one polypeptide in low, medium, high or inducible expression vectors. Such fusion may include any of the following combinations of the ACCase subunits: accA-accB, accA-accC, accA-accD, accB-accC, accB-accD, accC-accD, accA-accB-accC, accA-accB-accD, accA-accC-accD, accB-accC-accD, and accA-accB-accC-accD having at least 80% sequence homology to the same combinations of *E.coli* accA, accB, accC and accD or functional homologs thereof.

[0090] In any embodiment herein can be provided for an ACC complex in the stoichiometry of these subunits of the accCB and accDA in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provide for an ACC complex in the stoichiometry of these subunits of the accCB and accDA in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for an ACC complex in the stoichiometry of these subunits of the accDA and accCB in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for an ACC complex in the stoichiometry of these subunits of the accDA and accCB in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8,

5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors.

[0091] In any embodiment herein can be provided for the stoichiometry of the accD-A subunits in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the stoichiometry of the accD-A subunits in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the stoichiometry of the accC-B subunits in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the stoichiometry of the accC-A subunits in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the stoichiometry of the accC-A subunits in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the stoichiometry of the accC-B subunits in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the stoichiometry of the accC-B subunits in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5,

7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors.

[0092] Bioproduction Methods

[0093] In any inventive embodiment herein, the host cell can be genetically modified for increased malonyl-CoA flux by at least one heterologous ACCase complex, such as **Table 4** to further increase chemical bio-production in host cell.

[0094] III. CONVERSION OF MALONYL-COA TO MALONATE SEMIALDEHYDE

[0095] One of the steps in the biosynthesis of 3-HP involves the conversion of malonyl-CoA (MCA) to malonate semialdehyde (MSA) and the conversion of malonate semialdehyde (MSA) to 3-HP (*see, e.g.*, WO2011/038364). Inventive embodiments herein contemplate the use of novel enzymes and/or combinations of enzymes to catalyze the reaction in a microorganism from MCA to MSA, which results in enhanced cellular bioproduction of 3-HP in the host cell.

[0096] In some embodiments, the invention provides novel enzyme compositions or co-expression of a combinations of enzyme compositions to catalyze the conversion of malonyl-CoA to 3-HP. A general overview of the enzymes and the relevant reaction pathways methods are shown in **FIG.1**. Any and all combinations of the relevant enzymes shown in **FIG.1** are contemplated in this invention.

[0097] In one embodiment, malonyl-CoA is converted to malonate semialdehyde by a malonyl-CoA reductase and malonate semialdehyde is converted to 3-HP through separate or both alternative pathways.

[0098] In another embodiment, malonyl-CoA is converted to malonate semialdehyde by a monofunctional malonyl-CoA reductase that catalyzes the malonyl-CoA conversion, but does not catalyze the malonate semialdehyde conversion.

[0099] In one embodiment, the microorganism herein comprises a monofunctional malonyl-CoA reductase derived from, *e.g.*, *Sulfolobus tokodaii* (stMCR) (SEQ ID NOs. 15 and 16); a functional homolog of stMCR; an enzyme with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity thereto.

[00100] In some embodiments, the microorganism herein comprises a bi-functional malonyl-CoA reductase having at least two protein fragments, with one fragment having malonyl-CoA reductase activity and the other fragment having malonate semialdehyde dehydrogenase activity derived from, *e.g.*, *Chloroflexus aurantiacus* (caMCR).

[00101] MCR-Dehydrogenase Enzymes for Conversion of 3-HP ions

[00102] Following the conversion of the malonyl-CoA to malonate semialdehyde, the malonate semialdehyde is converted to 3-HP through separate or both of pathways. Malonate

semialdehyde may exist in at least three states; the keto form, the enol form, and hydrate form, as shown in **FIG. 2**. Malonate semialdehyde in the enol form, which will stabilize this form when compared to other aldehydes where the enol form is highly unfavored in the equilibrium among the three forms.

[00103] The malonate semialdehyde keto form can be converted to 3-HP utilizing a 3-hydroxy acid dehydrogenase enzyme (*e.g., ydfG*; SEQ ID NOs. 21 and 22), a 3-hydroxyisobutyrate dehydrogenase enzyme (*e.g., Pseudomonas aeruginosa mmsB*; SEQ ID NOs. 23 and 24), and/or NAD+-dependent serine dehydrogenase (*e.g., Pseudomonas NDSD*; SEQ ID NOs. 25 and 26). In an embodiment, *Pseudomonas mmsB*, *Pseudomonas NDSD*, and/or *E. coli ydfG* are used. The gene, *ydfG* from *E. coli* can be largely NADPH dependent, whereas *mmsB* and *NDSD* from *Pseudomonas* can utilize either NADPH or NADH.

[00104] The malonate semialdehyde enol form can be converted to 3-HP utilizing an N-ethylmaleimide reductase (*e.g., nemA*; SEQ ID NOs. 17 and 18), and/or a malonic semialdehyde reductase (*e.g., rutE*, SEQ ID NOs. 19 and 20) from *E. coli*. These enzymes do not directly utilize NADPH or NADH. Instead, these enzymes utilize a flavin mononucleotide that is cycled between oxidized and reduced states by NADPH or NADH. The enol pathway also has advantages over the keto pathway in that the equilibrium between the malonate semialdehyde enol form and 3-HP significantly favors the formation of 3-HP. Thus, the reaction may be considered less reversible, and/or essentially irreversible (*e.g.*, drives the reaction toward the formation of 3-HP).

[00105] The malonate semialdehyde hydrated form may also be converted to 3-HP by either 3-HP dehydrogenase or malonate semialdehyde reductase enzymes, although the hydrated form is more likely to be converted to the enol form as the equilibrium continuously re-adjusts.

[00106] In one embodiment, the microorganism comprises a polynucleotide encoding: (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; and (2) one or more genes encoding for at least one enzymes selected from the group consisting of: *ydfG*, *mmsB*, *NDSD*, *rutE*, and *nemA* or a functional homolog or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity .

[00107] In accordance with another aspect of the invention, there is provided an organism that is genetically modified to make 3-HP, wherein the genetic modification includes a polynucleotide encoding: (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; (2) one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP;

and/or (3) one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form or the malonate semialdehyde hydrated form to 3-HP.

[00108] In certain aspects, the invention provides a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible.

[00109] In certain aspects the invention also provides a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is primarily not NADPH-dependent or primarily NADH-dependent .

[00110] Suitable 3-HP dehydrogenase enzymes that are largely NADH-dependent that can be used with the claimed invention include, but are not limited to, *mmsB* or *NDSD*. Suitable malonate reductase enzymes that are flavin-dependent include, but are not limited to, *rutE* and *nemA*. Suitable 3-HP dehydrogenase enzymes that are less susceptible 3-HP inhibition at high concentration that can be used with the claimed invention include, but are not limited to, *ydfG* and *NDSD*. Suitable 3-HP dehydrogenase or malonate semialdehyde dehydrogenase enzymes that catalyze a reaction pathway to 3-HP that is substantially irreversible are *rutE* and *nemA*.

[00111] In certain aspects the invention provides a monofunctional malonyl-CoA reductase enzyme fused to one or more dehydrogenase enzymes. Malonate semialdehyde, which is the intermediate product in the conversion of malonyl-CoA to 3-HP can be very reactive. Therefore, it can be advantageous to have a reaction pathway wherein the residence time of malonate semialdehyde within the cell is minimized, *e.g.,* its conversion to 3-HP occurs quickly. By fusing the malonyl-CoA reductase with the malonate semialdehyde dehydrogenase to create a multi-domain protein (*e.g.*, two domain protein) and having the MCR and dehydrogenase domains adjacent in the sequence, the the malonate semialdehyde can be quickly converted to 3-HP.

[00112] In certain aspects, the invention provides a first monofunctional malonyl-CoA reductase enzyme fused to a first dehydrogenase enzyme of one type and second monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme of a different type than the first dehydrogenase enzyme. Suitable different dehydrogenase enzymes include, but are not limited to, enzymes that use as a substrate, different forms of malonate semialdehyde.

[00113] In some embodiments, the invention provides for microorganisms comprising a genetic modification that include, but are not limited to, a malonyl-CoA reductase from *S. tokadaii* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme

may include any of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA – rutE,* functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00114] In certain aspects the invention provides for microorganisms comprising a genetic modification that include, but are not limited to, malonyl-CoA reductase from *C. aggregans* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme may include any of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr* - nemA, mcr - ydfG - mmsB, mcr - *ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA – rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity thereof.

[00115] In certain aspects the invention provides for microorganisms comprising a genetic modification that include, but are not limited to, a malonyl-CoA reductase from *O. trichoides* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme may include, but are not limited to, any of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA – rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00116] Enhanced Mutated Monofunctional MCR for Bioproduction

[00117] In certain aspects, the invention provides for microorganisms comprising a genetic modification having a mutated form of *stMCR* that has enhanced activity at about 0 °C to about 10°C, at about 8°C to about 15°C, at about 12°C to about 21°C, at 20°C to about 44°C, at about

30°C to about 37°C, at about 32°C to about 38°C, at about 35°C to about 42°C, at about 40°C to about 50°C, at about 50°C to about 60°C, and/or at about 59°C to less than equal to about 72°C. Such mutated forms may be designed based on the crystal structure for *stMCR*; *see* Demmer *et al.*, J. Biol. Chem. 288:6363-6370, 2013.

[00118] The carboxylase domains of the malonyl-CoA reductase derived from *Chloroflexus aggregans* and/or *Oscillochloris trichoides* can be enhanced by mutations in the carboxylase binding domain to provide increased 3-HP production over the natural occurring enzyme.

[00119] The carboxylase activity of the malonyl-CoA reductase derived from *Chloroflexus aurantiacus* can be enhanced to increase activity. In certain aspects the invention provides for a mutated form of *Chloroflexus aurantiacus* malonyl-CoA reductase having a mutated carboxylase domain to provide increased 3-HP production over the natural occurring enzyme.

[00120] In certain aspects the invention provides for microorganisms comprising a genetic modification that include carboxylase domains of the malonyl-CoA reductase derived from *C. aggregans* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). It is contemplated that the any of the enhanced MCR by mutation, as provide above, may be fused in any of the following configurations including, but not limited to, *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA* - ydfG, *mcr - nemA - mmsB*, mcr - *nemA - NDSD*, *mcr - nemA – rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00121] IV. SALT-TOLERANT ENZYMES

[00122] The growth of engineered microorganisms for enhanced production of a chemical product, such as *E. coli* can be inhibited by high salt concentrations, *e.g.*, when the chemical product accumulates when the organism produces large amounts of the chemical product.

[00123] Dose-dependent studies with increasing amounts of NaCl and Na-3-HP show that salt can have inhibitory effects on ACCase activity which can be essential to fatty acid biosynthesis of membranes required for growth and propagation and for the production of 3-HP (**see EXAMPLE 1**). Thus, the use of salt-tolerant enzymes in 3-HP production should increase 3-HP production in a host cell.

[00124] A. Enzymes from Halophilic Organisms

[00125] Halophiles are characterized as organisms with a great affinity for salt. In some embodiments, a halophilic organism can require at least 0.01M, 0.05M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1.0M, 1.25M, or 1.5M concentrations of salt (NaCl) for growth. Halophiles can live in hypersaline environments that are generally defined occurring to their salt concentration of their habitats. Halophilic organisms that are defined as having "slight salt affinity" can grow optimally at 2-5% NaCl, moderate halophiles can grow optimally at 5-20% NaCl, and extreme halophiles can grow optimally at 20-30% NaCl.

[00126] In an embodiment, a genetically engineered microorganism can be made to express homologous enzymes of a specific halophile, for example, from a moderate halophile or an extreme halophile.

[00127] In an embodiment, microorganisms can comprise a genetic modification that includes or codes for enzymes from slight halophile organisms. In an embodiment, microorganisms can comprise a genetic modification that includes or codes for enzymes from moderate halophile organisms. In an embodiment, microorganisms can comprise a genetic modification that includes or codes for enzymes from extreme halophile organisms.

[00128] Homology with genes may be determined by analysis with BLASTN version 2.0 provided through the NCBI website. Homology with proteins may be determined by analysis with BLASTP version 2.2.2 provided through the NCBI website. These programs can align disclosed fragments being compared and can determine the extent of identity or similarity between them.

[00129] To date there are many sequenced halophilic organisms that can be used with the claimed invention. Examples of some sequenced halophilic organisms include, but are not limited to, *Chromohalobacter salexigens, Flexistipes sinusarabici strain (MAS10T), Halobacterium sp. NRC-1*, *Haloarcula marismortui*, *Natronomonas pharaonis*, *Haloquadratum walsbyi*, *Haloferax volcanii*, *Halorubrum lacusprofundi*, *Halobacterium sp. R-1*, *Halomicrobium mukohataei*, *Halorhabdus utahensis*, *Halogeometricum borinquense*, *Haloterrigena turkmenica*, *Natronobacterium gregoryi SP2*, *Halalkalicoccus jeotgali*, *Natrialba magadii*, *Haloarcula hispanica*, *Halopiger xanaduensis*, *Halophilic archaeon DL31*, *Haloferax mediterranei*, *Halovivax ruber*, *Natronococcus gregoryi*, and *Natronococcus occultus*.

[00130] Examples of suitable moderate halophilic organisms in which homologous enzymes of the invention can be derived from include, but are not limited to, eukaryotes such as crustaceans (*e.g.*, *Artemia salina*), insects (*e.g.*, *Ephydra hians*), certain plants from the genera *Salicornia spp*, algae (*e.g.*, *Dunaliella viridis*), fungi, and protozoa (*e.g.*, *Fabrea salina*), phototrophic organisms, such as planktonic and microbial mat-formers cyanobacteria as well as other anaerobic red and

green sulphur bacteria from the genera *Ectothiorhodospira spp.*) and non-sulphur bacteria from the genera *Chromatium spp.*; gram-negative anaerobic bacteria, for example from the genera *Haloanaerobacter spp.* some of which are methanogenic, for example from the genera *Methanohalophilus* spp. and either aerobic or facultative such as species from the genera *Halomonas*, *Chromohalobacter*, *Salinovibrio*, *Pseudomonas*, for example (*e.g.*, *Halomonase elongate*); gram-positive bacteria from genera such as *Halobacillus*, *Bacillus*, *Marinococcus*, etc. as well as some actinomycetes, for example, *Actinopolyspora halophila*.

[00131] Genomic and Proteomic Hallmarks of Halophilic Organisms

[00132] Comparative genomic and proteomic studies of halophiles and non-halophiles can reveal some common trends in the genomes and proteomes of halophiles. At the protein level, halophilic organisms can be characterized by low hydrophobicity, over-representation of acidic residues, especially Asp, under-representation of Cys, lower propensities for helix formation and higher propensities for coil structure.

[00133] At the DNA level, halophilic organisms can be characterized by the dinucleotide abundant profiles of halophilic genomes that bear some common characteristics, which are distinct from non-halophiles, and hence may be regarded as specific genomic signatures for salt-adaptation. The synonymous codon usage in halophiles may also exhibit similar patterns regardless of their long-term evolutionary history.

[00134] In one embodiment, microorganisms can be synthesized to comprise proteins that are modified for salt tolerance such that they have low hydrophobicity, over-representation of acidic residues, especially Asp, under-representation of Cys, lower propensities for helix formation and higher propensities for coil structure.

[00135] Suitable salt-tolerant enzymes can include enzymes from salt-tolerant organisms. Salt-tolerant organisms (such as, for example, halophiles) can include any living organisms that are adapted to living in conditions of high salinity. Suitable salt-tolerant enzymes can include enzymes from salt-tolerant organisms that are homologs of the following enzymes: Sucrose-6-phosphate hydrolase (cscA from *E. coli*), glucose-6-phosphate isomerase (pgi from *E. coli*), fructokinase (cscK from *E. coli*), fructose-1,6-bisphosphatase (yggF from *E. coli*), fructose 1,6-bisphosphatase (ybhA from *E. coli*), fructose 1,6-bisphosphatase II (glpX from *E. coli*), fructose-1,6-bisphosphatase monomer (fbp from *E. coli*), 6-phosphofructokinase-1 monomer (pfkA from *E. coli*), 6-phosphofructokinase-2 monomer (pfkB from *E. coli*), fructose bisphosphate aldolase monomer (fbaB from *E. coli*), fructose bisphosphate aldolase monomer (fbaA from *E. coli*), triose phosphate isomerase monomer (tpiA), glyceraldehyde 3-phosphate dehydrogenase-A monomer (gapA from *E. coli*), phosphoglycerate kinase (pgk), 2,3-bisphosphoglycerate-

independent phosphoglycerate mutase (gpmM from *E. coli*), 2,3-bisphosphoglycerate-dependent or tdcE (from *E. coli*), phosphoglycerate mutase (gpmA), enolase (eno from *E. coli*), phosphoenolpyruvate carboxylase (ppc from *E. coli*), malate dehydrogenase (mdh), fumarase A (fum from *E. coli*), fumarase B (fumB), fumarase C (fumC from *E. coli*), phosphoenolpyruvate synthetase (ppsA from *E. coli*), pyruvate kinase I monomer (pykF from *E. coli*), pyruvate kinase II monomer (pykA from *E. coli*), fumarate reductase (frdABCD from *E. coli*), lipoamide dehydrogenase (lpd from *E. coli*), pyruvate dehydrogenase (aceE from *E. coli*), pyruvate dehydrogenase (aceF from *E. coli*), pyruvate formate-lyase (pflB from *E. coli*), acetyl-CoA carboxylase (accABCD from *E. coli*), malonyl CoA reductase (mcr), 3HP dehydrogenase (mmsB, NDSD, or ydfG), malonate semialdehyde reductase (nemA, rutE from *E. coli*), or a combination thereof.

[00136] Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% overall amino acid or nucleotide identity and/or homology to the above enzymes. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% amino acid or nucleotide identity and/or homology to the essential protein function domains of the enzymes above. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least or at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% overall amino acid or nucleotide identity and/or homology to the essential binding amino acids within an essential protein function domain of the enzymes above.

[00137] In an embodiment, suitable salt-tolerant enzyme homologs can be enzymes from one of the following organisms: *Halomonas elongata*, *Salinibacter rubu*r, or *Halobacterium* species (Archaea).

[00138] In an embodiment, there can be provided a non-salt-tolerant organism that can be genetically modified to make 3-HP, wherein the genetic modification can include introducing into the organism a polynucleotide encoding for an acetyl-CoA carboxylase from a salt-tolerant organism. In an embodiment, the acetyl-CoA carboxylase subunits accA, accB, accC and accD can be from *Halomonas elongata.*

[00139] V. CHEMICAL TRANSPORTER

[00140] In one aspect of this disclosure, any of the microorganisms may be genetically modified to introduce a nucleic acid sequence encoding a polypeptide that: (1) facilitates the exportation of the chemical of interest or the export of an inhibitory chemical from the cell; and/or (2) facilitates the importation into the cell, a reactant, precursor, and/or metabolite used in the organism's production pathway for producing the chemical of interest.

[00141] 3-HP Exporter

[00142] In one aspect of this disclosure, 3-HP can be produced using a genetically modified *E. coli* organism. Thus, the present invention contemplates a host cell genetically modified to express or increase expression of an exporter that can function to transfer 3HP from the cellular environment extracellularly.

[00143] Bacterial cells, such as *E. coli*, have at least five different types of exporters: the major facilitator superfamily (MFS); the ATP-binding cassette superfamily (ABC); the small multidrug resistance family (SMR); the resistance-nodulation-cell division superfamily (RND); and the multi antimicrobial extrusion protein family (MATE). In addition, amino acid exporters, which are common to almost all host cells, may be made to export 3-HP. Additionally, solvent tolerant transporters, for example, bromoacetate, butanol, isobutanol, and the like, may be used to export 3-HP.

[00144] In certain aspects the invention provides a host cell with a recombinant exporter wherein the exporter is an MFS exporter, ABC exporter, SMR exporter, RND exporter, MATE exporter, amino acid exporter, solvent tolerance transporter, or a combination thereof.

[00145] Suitable exporters that can be used include, but are not limited to, *acrD*, *bcr*, *cusA*, *dedA*, *eamA*, *eamB*, *eamH*, *emaA*, *emaB*, *emrB*, *emrD*, *emrKY*, *emrY*, *garP*, *gudP*, *hsrA*, *leuE*, *mdlB*, *mdtD*, *mdtG*, *mdtL*, mdtM, *mhpT*, *rhtA*, *rhtB*, *rhtC*, *thtB*, *yahN*, *yajR*, *ybbP*, *ybiF*, *ybjJ*, *ycaP*, *ydcO*, *yddG*, *ydeD*, *ydgE*, *yddG*, *ydhC*, *ydhP*, *ydiN*, *ydiM*, *ydjE*, *ydjI*, *ydjK*, *yeaS*, *yedA*, *yeeO*, *yegH*, *yggA*, *yfcJ*, *yfiK*, *yhjE*, *yidE*, *yigK*, *yigJ*, *yijE*, *yjiI*, *yjiJ*, *yjiO*, *ykgH*, *ypjD*, *ytfF*, *ytfL*, or functional homolog or homolog having at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% sequence identity and/or homology. Other potential transporter proteins may be identified using topology analysis as illustrated in [Daley *et al.*, Science 308: 1321-1323, 2005].

[00146] In certain aspects the invention can provide the exporter to be improved for binding to 3-HP. In certain aspects the invention can provide the exporters named to be further enhance by genetic modification of the predicted cytoplasmic domain to increase 3-HP binding. In certain aspects the invention can provide the exporter to be improved for binding to 3-HP. In certain

aspects the invention can provide the exporters named to be further enhance by genetic modification of the predicted transmembrane binding domain to increase 3-HP binding or incorporation into the host cell membrane.

[00147] Suitable exporter homologs that can be used with the claimed invention can have at least or about at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% overall amino acid or nucleotide identity and/or homology to the previously mentioned exporters. Suitable exporter homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% amino acid or nucleotide identity and/or homology to the essential protein function domains of the exporters above. Suitable exporter homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% amino acid or nucleotide identity and/or homology to the essential binding amino acids within an essential exporter domain of the enzymes above.

[00148] In certain aspects the invention can provide for at least one of the exporters provided herein to be expressed in a host cell to increase the chemical production of 3-HP. In certain aspects the invention can provide for at least one of the exporters provided herein to be expressed in a host cell and with a genetic modification of *tig* to increase the chemical production of 3-HP.

[00149] In certain aspects the invention can provide for one exporter to be further modified by one or more genetic modulates so that the expression level and timing of expression of the exporter can be controlled in the host cell. In certain aspects the invention can provide for one exporter to be further modified by an inducible promoter, RBS, high, multicopy plasmid or combination thereof.

[00150] In certain aspects the invention can provide exporters to be expressed in a host cell in an equal ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 1:2 ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 1:3 ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 1:4 ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 2:3 ratio.

[00151] In certain aspects the invention can provide for the exporter to maintain the host cell at pH 7.0-7.4 during the continuous production phase. In certain aspects the invention provides for

an exporter and a base importer, wherein the cell can maintain a pH 7.0-7.4 during the continuous production phase. In certain aspects the invention can provide for the exporter to maintain the host cell at pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, pH 9.0 to pH 10.0, and/or pH 7.0-7.3 during the continuous production phase. In certain aspects the invention provides for an exporter and base importer, wherein the cell can maintain a pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, pH 9.0 to pH 10.0, and/or pH 7.0-7.3 during the continuous production phase.

[00152] As a carboxylic acid group without a strong electronegative group nearby, 3-HP has a typical pKa ~4.5. Therefore diffusion across the membrane should be limited at pHs much above this pH. In some embodiments, exporters are used for one or more fermentations that occur at pH above about 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, and/or above 10.0. In some embodiments, exporters are used for one or more fermentations that occur at pH above about 4.5.

[00153] In some embodiments, additional modifications to the host cell may be made to further enhance the transporter's function. In particular, deletion of the tig gene from the genome of the host cell may enhance expression and total activity of integral membrane proteins such as exporters and importers.

[00154] Bicarbonate Importer

[00155] One of the steps in the conversion of biomass to 3-HP can be the conversion of acetyl-CoA to malonyl-CoA, which is illustrated in **FIG. 3**.

[00156] As shown in **FIG. 3**, this reaction can be catalyzed by the acetyl-CoA carboxylase, and bicarbonate is a reactant that can drive the reaction. One of the primary sources of bicarbonate to drive this reaction can be carbon dioxide within the cell. Carbon dioxide can readily diffuse through a cell's membrane, and a natural equilibrium will be reached between the intracellular and extracellular carbon dioxide. As a cell produces carbon dioxide it can migrate through the cell, and since it tends not to be very soluble in the media, it may bubble out of the system and more intracellular carbon dioxide may migrate out of the cell to maintain the equilibrium. This process can impede the production of 3-HP since bicarbonate (which is in equilibrium with the dissolved carbon dioxide in the form of carbonic acid) can be needed to drive the acetyl-CoA → malonyl-CoA reaction, and the intracellular carbon dioxide can be the primary source for intracellular bicarbonate.

[00157] In some embodiments , an organism can be provided that can include a heterologous gene encoding for a polypeptide that can act as a carbon dioxide importer (*e.g.*, it enhances the

importation of carbon dioxide into the cell or inhibits the exportation of carbon dioxide from the cell), which can result in increased intracellular carbon dioxide. Use of a CO_2 importer can mitigate against the natural outflow of carbon dioxide.

[00158] In some embodiments, there can be provided an organism that can be genetically modified, wherein the genetic modification can include a polynucleotide encoding a polypeptide that is capable of importing extracellular carbon dioxide from the media to within the cell membrane or inhibiting the exportation of intracellular carbon dioxide from within the cell membrane to the media. In some embodiments, a microorganism can be genetically modified to encode one or more of the following heterologous genes: *bicA* from *Synechococcus* species, *ychM* gene product of *E. coli*, *yidE* gene product of *E. coli*, any of the bicarbonate transporters as described in [Felce and Saier, J. Mol. Microbiol. Biotechnol. 8: 169-176, 2004] or any amino acid sequences homologous thereof (*e.g.*, at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% homologous and/or identical to the amino acid or nucleotide sequences of the CO2 importer/exporters described herein].

[00159] Bioproduction Methods

[00160] In some embodiments, the host cell can be genetically modified by at least one heterologous gene and/or salt tolerant heterologous gene of **FIG. 1** or **Table 1** and at least one 3-HP exporter provided herein to further increase chemical bioproduction in a host cell.

[00161] In some embodiments, the host cell can be genetically modified with a heterologous gene for increased malonyl-CoA flux, 3-HP export, and to increase chemical bio-production. In some embodiments, the host cell can be genetically modified to increase malonyl-CoA flux, 3-HP export to increase chemical bioproduction.

VI. MULTI- PHASE FERMENTATION

[00162] In accordance with another aspect of the present invention, there is provided a method of producing a chemical product from a carbon source through a bioproduction process that comprises a controlled multi-phase production process. The multi-phase production process includes an *e.g.,* an initiation phase and a production phase, wherein the production phase and/or process can be controlled by genetic modifications to the organism producing the chemical product and/or can be controlled by changes made to the cell environment.

[00163] In one aspect, the bioproduction process may include two or more of the following phases: (1) growth phase; (2) induction phase; and (3) production phase. During the growth phase, the organism replicates itself and the biocatalyst needed to produce the chemical product is built up. During the induction phase, expression of key enzymes critical to the production of

the chemical is induced and the enzymes accumulate within the biocatalyst to carry out the reactions required to produce the product. During the production phase organism produces the desired chemical product.

[00164] The initiation and/or completion of the growth, induction and/or production phases can be controlled. In accordance with the present invention, the growth phase can be dependent on the presence of a critical external reactant that will initiate growth. The initiation and completion of the growth phase can be controlled by the addition and amount of the initiating reactant added to the reaction vessel.

[00165] In accordance with certain embodiments, the chemical product can be 3-HP and the production organism can be *E. coli* or yeast. The critical external reactant may be phosphate, which may be needed for replication of *E. coli* cells. In one embodiment, the growth phase can be initiated by the addition of phosphate to a reaction vessel (together with a carbon source such as sugar and the *E. coli* cells), and the duration of the growth phase can be controlled by the amount of phosphate added to the system.

[00166] The induction phase can be controlled by genetic modifications to the producing organism. The key enzymes triggered during this phase can be engineered into the organism using promoters that can be sensitive to (*e.g.*, activated by) the depletion of the initiating reactant. As a result, once the initiating reactant is depleted, the growth phase ends, the key enzymes are activated and the induction phase begins.

[00167] In another embodiment, the induction phase can be controlled by key genes that encode for enzymes in the biosynthetic pathway for the product into the production organism using promoters that are activated by phosphate depletion. In one embodiment where the chemical product is 3-HP and the production organism is *E. coli*, the key genetic modifications may include one or more of the following: mcr, mmsB, ydfG, rutE, nemA and NDSD; genes that encode individual or fused subunits of ACCase, such as accA, accB, accC, accD, accDA fusion, and accCB fusion, and the promoters may include one or more of the promoters that direct expression of the following *E. coli* genes: amn, tktB, xasA, yibD, ytfK, pstS, phoH, phnC, or other phosphate-regulated genes as described in Baek and Lee, FEMS Microbiol Lett 264: 104-109, 2006. In accordance with this embodiment, once the phosphate is depleted, expression of the key enzymes can be activated by their promoters and the induction phase begins.

[00168] The production phase may also be controlled by genetic modifications. For example, the organism can be engineered to included mutated forms of enzymes critical to the initiation of production of the chemical product. These initiation enzymes may facilitate initiation of production either by: (1) becoming active and serving a key function in the production pathway;

and/or (2) becoming inactive and/or disrupted thereby turning off a branch pathway or other competitive pathway that prevents or limits the production pathway leading to the chemical product. In accordance with an embodiment, initiation enzymes can be mutated to form temperature sensitive variants of the enzymes that can be either activated by or deactivated at certain temperatures. As a result, the production phase can be initiated by changing the changing the temperature within the reaction vessel.

[00169] In one embodiment, the production phase can be controlled by genetically modifying the microorganism with a heterologous nucleotide sequence encoding one or more of the following temperature sensitive enzymes: $fabI^{ts}$ (SEQ ID NO. 27), $fabB^{ts}$ (SEQ ID NO.28) and $fabD^{ts}$ (SEQ ID NO. 29). These enzymes can be deactivated or shut-off or altered to have decreased activity at the desired temperature for production of the chemical product. These enzymes can play a key role shuttling carbon atoms into the fatty acid synthesis pathway. Although fatty acid synthesis pathway can be critical during the growth phase, it can inhibit production of the chemical product. Once the reaction vessel temperature is changed, the temperature sensitive enzymes are deactivated and the fatty acid synthesis pathway shuts down thereby allowing the production pathway of the chemical product to ramp up.

[00170] In accordance with the present invention, the growth phase can last be between 10 to 40 hours, or about 15 to about 35 hours, or about 20 to about 30 hours. The induction phase may be for about 1 to about 6 hours, about 1 to about 5 hours, or about 2 to about 4 hours. The production phase may be greater than about: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 hours depending on the amount of chemical product that is desired.

[00171] In accordance with the present invention, the growth phase and induction phase can be conducted at a temperature of about 25°C to about 35°C, about 28°C to about 32°C, or about 30 °C. The production phase can be conducted at a temperature of about 35°C to about 45°C, about 35°C to about 40°C, or about 36°C to about 38 °C. The production phase temperature can be higher than the induction phase temperature, and the increase in temperature that initiates the production phase can occur over a period of about 1 to about 5 hours, about 1 to about 3 hours, about 2 hours, or about 1 hour.

[00172] In accordance with the present invention, there is provided a method of producing a chemical product from a renewable carbon source through a bioproduction process comprising:

(1) constructing a genetically modified organism capable of converting said renewable carbon source to said chemical product, wherein said genetically modified organism requires inorganic phosphate for growth and comprises: (a) at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels

within a culture system, wherein said gene provides a critical function in converting said carbon source to said chemical product and is not required for the genetically modified organism to replicate; and (b) a gene encoding a temperature-sensitive enzyme;

(2) forming a culture system comprising said carbon source in an aqueous medium and said genetically modified microorganism;

(3) maintaining the culture system under conditions that allow the genetically modified microorganism to replicate comprising maintaining a sufficient level of inorganic phosphate within said culture system;

(4) allowing the inorganic phosphate to deplete thereby triggering the expression of the gene regulated by a promoter sensitive to inorganic phosphate levels; and

(5) changing the temperature of the culture system thereby activating or deactivating said temperature-sensitive enzyme and initiating the production of said chemical product.

[00173] In accordance with the present invention, there is provided a method of producing 3-hydropropionic acid (3-HP) from a renewable carbon source, comprising:

(1) constructing a genetically modified organism capable of converting said renewable carbon source to 3-HP, wherein said genetically modified organism requires inorganic phosphate for growth and comprises: (a) at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system, wherein said gene is selected from the group consisting of mcr, mmsB, ydfG, rutE, nemA, NDSD, accA, accB, accC, accD, accDA fusion, and accCB fusion; and (b) a gene encoding a temperature-sensitive enzyme selected from the group consisting of fabI, fabB, fabD, and combinations thereof;

(2) forming a culture system comprising said carbon source in an aqueous medium, phosphate and said genetically modified microorganism, and thereby initiating a growth phase during which the genetically modified microorganism replicates;

(3) maintaining a sufficient level of inorganic phosphate within said culture system until the desired level of cell growth is achieved;

(4) allowing the inorganic phosphate to deplete thereby initiating an induction phase which begins the expression of said gene regulated by a promoter sensitive to inorganic phosphate levels; and

(5) changing the temperature of the culture system thereby activating or deactivating said temperature-sensitive enzyme and initiating a growth phase during which said genetically modified microorganism produces 3-HP.

[00174] **Fermentation Conditions**

[00175] Depending on the host cell fermentation may be performed under aerobic, microaerobic, or anaerobic conditions, with or without agitation. The operation of culture systems to achieve aerobic, microaerobic and anaerobic conditions are well known to those of ordinary skill in the art.

[00176] Suitable pH ranges for fermentation depend on the multiple factors such as the host cell. In some applications of the invention fermentation can occur between various pH ranges for example, pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, or pH 9.0 to pH 10.0. However, the actual pH conditions for a particular application are not meant to be limited by these ranges and can be between the expressed pH ranges if it provides more optimal production of the fermentation process, such as increased 3-HP production.

[00177] VII. GENES AND PROTEINS FOR THE BIOPRODUCTION OF CHEMICALS

[00178] An overview of the engineered pathways provided by the invention in a host cell is shown in **FIG.1**. Various combinations of the pathways shown can be carried out by various combinations of genetic modifications to key enzymes either in the intrinsic pathways or supplied through the transformation of a heterologous gene.

[00179] In some applications of the genetically modified microorganism of the invention may comprise a single genetic modification, or one or more genetic modifications. Various types of genetic modifications that can be used with the invention are disclosed herein.

[00180] In some embodiments the genetic modified organism of the invention can comprise a genetic modification to the following gene/proteins or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homolog/identity to or a functional homolog of: bifunctional malonyl-CoA reductase (MCR from *Chloroflexus aurantiacus*), monofunctional malonyl-CoA reductase (caMCR from *Chloroflexus aurantiacus*), malonyl-CoA reductase (stMCR from *Sulfolobus tokodaii*.), Enzyme: malonyl-CoA reductase (cgMCR from *Chloroflexus aggregans*), Enzyme: malonyl-CoA reductase (otMCR from *Oscillochloris trichoides)*, Polypeptide: host restriction; endonuclease R (hsdR from *E. coli*), lactose metabolism (lac from *E. coli*), L-rhamnulose kinase (rhaB from *E. coli*), rhamnulose-1-phosphate aldolase (rhaD from *E. coli*), Enzyme: β-galactosidase (lacZ from *E. coli*), L-ribulose 5-phosphate 4-epimerase (araD from *E. coli*), L-ribulokinase (araB from *E. coli*), Enzyme: D-lactate dehydrogenase - fermentative (ldhA from *E. coli*), enzyme: pyruvate formate-lyase (pflB from *E. coli*), Enzyme: phosphate acetyltransferase / phosphate propionyltransferase (pta from *E. coli*), Enzyme: pyruvate oxidase (poxB from *E. coli*), Enzyme: methylglyoxal synthase (mgsA from *E. coli*), enzyme: Acetate kinase (ackA from *E. coli*), enzymes:

phosphotransacetylase-acetate kinase (pta-ack from *E. coli*), Enzyme: enoyl-[acyl-carrier-protein] reductase (fabI from *E. coli*), Protein: zeocin binding protein (zeoR from *Streptoalloteichus Hindustanus*), Enzyme: carboxytransferase moiety of acetyl-CoA carboxylase (accAD from *E. coli*), Enzyme: triose phosphate isomerase (tpiA from *E. coli*), Enzyme: biotoin carboxylase moiety of acetyl-CoA carboxylase (accBC from *E. coli*), Enzyme: transhydrogenase (pntAB from *E. coli*), Polypeptide: LacI DNA-binding transcriptional repressor (lacI from *E. coli*), Enzyme: β-ketoacyl-ACP synthases I (fabB from *E. coli*), Enzyme: β-ketoacyl-ACP synthases II (fabF from *E. coli*), Enzyme: malonyl-CoA-ACP transacylase (fabD from *E. coli*), Enzyme: pantothenate kinase (coaA from *E. coli*), Enzyme: pyruvate dehydrogenase complex (aceEF from *E. coli*), Enzyme: 3-hydroxyisobutyrate/3-HP dehydrogenase (mmsB from *Pseudomonas aeruginosa*), Enzyme: lipoamide dehydrogenase (lpd from *E. coli*), Enzyme: γ-glutamyl-γ-aminobutyraldehyde dehydrogenase (puuC from *E. coli*), Enzyme: malate synthase A (aceB from *E. coli*), Enzyme: isocitrate lyase (aceA from *E. coli*), Enzyme: isocitrate dehydrogenase phosphatase/kinase (aceK from *E. coli*), Enzyme: 3-hydroxy acid dehydrogenase (ydfG from *E. coli*), Enzyme: acetyl CoA carboxylase (accADBC from *E. coli*), Polypeptide: predicted transcriptional regulator (yieP from *E. coli*), Blastocyin resistance gene (BSD from *Schizosaccharomyces pombe*), Enzyme: pyridine nucleotide transhydrogenase (udha from *E. coli*), Protein: Cra DNA-binding transcriptional dual regulator (fruR from *E. coli*), (SCB from *E. coli*), enzyme: aldehyde dehydrogenase B (aldB from *E. coli*), Enzyme: carbonic anhydrase (cynT from *E. coli*), Enzyme: cyanase (cynS from *E. coli*), DNA gyrase toxin-antitoxin system (ccdAB from *E. coli*), Enzyme: phosphoglycerate mutase (pgi from *E. coli*), ArcA transcriptional dual regulator or Aerobic respiration control (arcA from *E. coli*), Enzyme: 6-phosphofructokinase (pfk from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase-A complex (gapA from *E. coli*), aldehyde dehydrogenase A (alda from *E. coli*), Enzyme: glutamate dehydrogenase (gdhA from *E. coli*), Enzyme: NADH-dependent serine dehydrogenase (NDSD from *Pseudomonas aeruginosa*), Protein: threonine/homoserine efflux transporter (rhtA from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase (gapN from *E. coli*), Phosphotransferase system (pts from *E. coli*), Enzyme: 6-phosphofructokinase II (pfkB from *E. coli*), Enzyme: methylmalonate-semialdehyde dehydrogenase (mmsA from *Pseudomonas aeruginosa*), Oxaloacetate:beta-alanine aminotransferase (OAT-1 from *Bacillus cereus*), Enzyme: aspartate 1-decarboxylase (panD from *E. coli*), Gene that confers resistance to valine (ValR from *E. coli*), Enzyme: glucokinase (glk from *E. coli*), Polypeptide: 30 S ribosomal sununit protein S12 (rpsL from *E. coli*), Polypeptide: CynR DNA-binding transcriptional repressor (cynR from *E. coli*), Transporter: galactose:H+ symporter (galP from *E. coli*), aspartate aminotransferase

(aspC from *E. coli*), Enzyme: alpha-ketoglutarate reductase (serA from *E. coli*), Enzyme: 6-phosphofructokinase I (pfkA from *E. coli*), Enzyme: phosphoenolpyruvate carboxylase (ppc from *E. coli*), Enzyme: succinate-semialdehyde dehydrogenase (NADP+) (gabD from *E. coli*), Enzyme: pyruvate kinase (pyk from *E. coli*), Enzyme: oxaloacetate 4-decarboxylase (OAD from *Leuconostoc mesenteroides*), Enzyme: trigger factor; a molecular chaperone involved in cell division (tig from *E. coli*), Transcription Unit (ptsHIcrr from *E. coli*), Enzyme: acetyl-CoA acetaldehyde dehydrogenase/alcohol dehydrogenase (adhE from *E. coli*), Enzyme: fattyacyl thioesterase I (tesA from *E. coli*), Enzyme: guanosine 3'-diphosphate 5'-triphosphate 3'-diphosphatase (spoT from *E. coli*), combination of genes encoding accABCD subunits (from *E. coli* and *Halomonas elongata*), pol (from *E. coli*), Enzyme: GDP pyrophosphokinase / GTP pyrophosphokinase (relA from *E. coli*), [Enzyme Name] (me from *E. coli*), Enzyme: citrate synthase (gltA from *E. coli*), Polypeptide: DNA gyrase, subunit A (gyrA from *E. coli*), Enzyme: multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase (eda from *E. coli*), thiamin biosynthesis (thi from *E. coli*), Polypeptide: acetolactate synthase II (ilvG from *E. coli*), acetyl CoA carboxylase (accDACB from *E. coli*), Citrate synthase (ArCS from *Arthrobacter aurescens*), Acetyl-CoA carboxylase from Corynebacter glutamicum (CgACC from *Corynebacter glutamicum*), Polypeptide: ferrichrome / phage / antibiotic outer membrane porin FhuA (fhuA from *E. coli*), Transporter: phosphate:H+ symporter PitA (pitA from *E. coli*), Transporter: uracil:H+ symporter (uraA from *E. coli*), Enzyme: uracil phosphoribosyltransferase (upp from *E. coli*), Enzyme: acylphosphatase (yccX from *E. coli*), acetyl-CoA synthetase (acsA from *E. coli*), Polypeptide: restriction of methylated adenine (mrr from *E. coli*), Protein: TrpR transcriptional repressor (trpR from *E. coli*), Enzymes: glutamate 5-semialdehyde dehydrogenase / gamma-glutamyl kinase (proAB from *E. coli*), methylcytosine restriction system (mcrBC from *E. coli*), Protein: citrate lyase, citrate-ACP transferase component (citF from *E. coli*), Enzyme: thioesterase II (tesB from *E. coli*), Enzyme: DNA-specific endonuclease I (endA from *E. coli*), Enzyme: phosphate acetyltransferase (eutD from *E. coli*), Enzyme: propionate kinase (tdcD from *E. coli*), tRNA: tRNA glnV (supE from *E. coli*), Enzyme: DNA-binding, ATP-dependent protease La (lon from *E. coli*), Polypeptide: DNA strand exchange and recombination protein with protease and nuclease activity (recA from *E. coli*), Transcription Unit: restriction endonulease component of EcoKI restriction-modification system (hsdRMS from *E. coli*), Enzyme: restriction of DNA at 5-methylcytosine residues (mcrA from *E. coli*) araD (from *E. coli),* araB (from *E. coli),* rhaD (from *E. coli),* rhaB (from *E. coli),* ack (from *E. coli),* fruR (from *E. coli*), gapA (from *E. coli*), lacI (from *E. coli*), lacZ (from *E. coli*), ldhA (from *E. coli*), mgsA (from *E. coli*), pfkA (from *E. coli*),

pflB (from *E. coli)*, pgi (from *E. coli)*, poxB (from *E. coli)*, pta-ack (from *E. coli)*, ptsH (from *E. coli)*, glut1 (from *E. coli)* and/ or ack (from *E. coli)* or any combination thereof.

[00181] The use of genetic modifications in genetic elements, genes, proteins or the use of compounds, such as siRNA technology, anti-sense technology, and small molecule inhibitors supplied to the host cell that modulate the expression of gene and proteins provided by the present invention are also contemplated.

[00182] In some embodiments the genetic modified organism of the invention uses genetic elements such as siRNA or equivalent (including but not limited to shRNA, RNAi, miRNA, etc.), genes, proteins or compounds supplied to the host cell to modulate one or more of the following: bifunctional malonyl-CoA reductase (MCR from *Chloroflexus aurantiacus*), monofunctional malonyl-CoA reductase (caMCR from *Chloroflexus aurantiacus*),malonyl-CoA reductase (stMCR from *Sulfolobus tokodaii*.), Enzyme: malonyl-CoA reductase (cgMCR from *Chloroflexus aggregans*), Enzyme: malonyl-CoA reductase (otMCR from *Oscillochloris trichoides)*, Polypeptide: host restriction; endonuclease R (hsdR from *E. coli*), lactose metabolism (lac from *E. coli*), L-rhamnulose kinase (rhaB from *E. coli*), rhamnulose-1-phosphate aldolase (rhaD from *E. coli*), Enzyme: β-galactosidase (lacZ from *E. coli*), L-ribulose 5-phosphate 4-epimerase (araD from *E. coli*), L-ribulokinase (araB from *E. coli*), Enzyme: D-lactate dehydrogenase - fermentative (ldhA from *E. coli*), enzyme: pyruvate formate-lyase (pflB from *E. coli*), Enzyme: phosphate acetyltransferase / phosphate propionyltransferase (pta from *E. coli*), Enzyme: pyruvate oxidase (poxB from *E. coli*), Enzyme: methylglyoxal synthase (mgsA from *E. coli*), enzyme: Acetate kinase (ackA from *E. coli*), enzymes: phosphotransacetylase-acetate kinase (pta-ack from *E. coli*), Enzyme: enoyl-[acyl-carrier-protein] reductase (fabI from *E. coli*), Protein: zeocin binding protein (zeoR from *Streptoalloteichus Hindustanus*), Enzyme: carboxytransferase moiety of acetyl-CoA carboxylase (accAD from *E. coli*), Enzyme: triose phosphate isomerase (tpiA from *E. coli*), Enzyme: biotoin carboxylase moiety of acetyl-CoA carboxylase (accBC from *E. coli*), Enzyme: transhydrogenase (pntAB from *E. coli*), Polypeptide: LacI DNA-binding transcriptional repressor (lacI from *E. coli*), Enzyme: β-ketoacyl-ACP synthases I (fabB from *E. coli*), Enzyme: β-ketoacyl-ACP synthases II (fabF from *E. coli*), Enzyme: malonyl-CoA-ACP transacylase (fabD from *E. coli*), Enzyme: pantothenate kinase (coaA from *E. coli*), Enzyme: pyruvate dehydrogenase complex (aceEF from *E. coli*), Enzyme: 3-hydroxyisobutyrate/3-HP dehydrogenase (mmsB from *Pseudomonas aeruginosa*), Enzyme: lipoamide dehydrogenase (lpd from *E. coli*), Enzyme: γ-glutamyl-γ-aminobutyraldehyde dehydrogenase (puuC from *E. coli*), Enzyme: malate synthase A (aceB from *E. coli*), Enzyme: isocitrate lyase (aceA from *E. coli*), Enzyme: isocitrate dehydrogenase

phosphatase/kinase (aceK from *E. coli*), Enzyme: 3-hydroxy acid dehydrogenase (ydfG from *E. coli*), Enzyme: acetyl CoA carboxylase (accADBC from *E. coli*), Polypeptide: predicted transcriptional regulator (yieP from *E. coli*), Blastocyin resistance gene (BSD from *Schizosaccharomyces pombe*), Enzyme: pyridine nucleotide transhydrogenase (udha from *E. coli*), Protein: Cra DNA-binding transcriptional dual regulator (fruR from *E. coli*), (SCB from *E. coli*), enzyme: aldehyde dehydrogenase B (aldB from *E. coli*), Enzyme: carbonic anhydrase (cynT from *E. coli*), Enzyme: cyanase (cynS from *E. coli*), DNA gyrase toxin-antitoxin system (ccdAB from *E. coli*), Enzyme: phosphoglycerate mutase (pgi from *E. coli*), ArcA transcriptional dual regulator or Aerobic respiration control (arcA from *E. coli*), Enzyme: 6-phosphofructokinase (pfk from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase-A complex (gapA from *E. coli*), aldehyde dehydrogenase A (alda from *E. coli*), Enzyme: glutamate dehydrogenase (gdhA from *E. coli*), Enzyme: NADH-dependent serine dehydrogenase (NDSD from *Pseudomonas aeruginosa*), Protein: threonine/homoserine efflux transporter (rhtA from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase (gapN from *E. coli*), Phosphotransferase system (pts from *E. coli*), Enzyme: 6-phosphofructokinase II (pfkB from *E. coli*), Enzyme: methylmalonate-semialdehyde dehydrogenase (mmsA from *Pseudomonas aeruginosa*), Oxaloacetate:beta-alanine aminotransferase (OAT-1 from *Bacillus cereus*), Enzyme: aspartate 1-decarboxylase (panD from *E. coli*), Gene that confers resistance to valine (ValR from *E. coli*), Enzyme: glucokinase (glk from *E. coli*), Polypeptide: 30 S ribosomal sununit protein S12 (rpsL from *E. coli*), Polypeptide: CynR DNA-binding transcriptional repressor (cynR from *E. coli*), Transporter: galactose:H+ symporter (galP from *E. coli*), aspartate aminotransferase (aspC from *E. coli*), Enzyme: alpha-ketoglutarate reductase (serA from *E. coli*), Enzyme: 6-phosphofructokinase I (pfkA from *E. coli*), Enzyme: phosphoenolpyruvate carboxylase (ppc from *E. coli*), Enzyme: succinate-semialdehyde dehydrogenase (NADP+) (gabD from *E. coli*), Enzyme: pyruvate kinase (pyk from *E. coli*), Enzyme: oxaloacetate 4-decarboxylase (OAD from *Leuconostoc mesenteroides*), Enzyme: trigger factor; a molecular chaperone involved in cell division (tig from *E. coli*), Transcription Unit (ptsHIcrr from *E. coli*), Enzyme: acetyl-CoA acetaldehyde dehydrogenase/alcohol dehydrogenase (adhE from *E. coli*), Enzyme: fattyacyl thioesterase I (tesA from *E. coli*), Enzyme: guanosine 3'-diphosphate 5'-triphosphate 3'-diphosphatase (spoT from *E. coli*), combination of genes encoding accABCD subunits (from *E. coli* and *Halomonas elongata*), pol (from *E. coli*), Enzyme: GDP pyrophosphokinase / GTP pyrophosphokinase (relA from *E. coli*), [Enzyme Name] (me from *E. coli*), Enzyme: citrate synthase (gltA from *E. coli*), Polypeptide: DNA gyrase, subunit A (gyrA from *E. coli*), Enzyme: multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate

aldolase and oxaloacetate decarboxylase (eda from *E. coli*), thiamin biosynthesis (thi from *E. coli*), Polypeptide: acetolactate synthase II (ilvG from *E. coli*), acetyl CoA carboxylase (accDACB from *E. coli*), Citrate synthase (ArCS from *Arthrobacter aurescens*), Acetyl-CoA carboxylase from Corynebacter glutamicum (CgACC from *Corynebacter glutamicum*), Polypeptide: ferrichrome / phage / antibiotic outer membrane porin FhuA (fhuA from *E. coli*), Transporter: phosphate:H+ symporter PitA (pitA from *E. coli*), Transporter: uracil:H+ symporter (uraA from *E. coli*), Enzyme: uracil phosphoribosyltransferase (upp from *E. coli*), Enzyme: acylphosphatase (yccX from *E. coli*), acetyl-CoA synthetase (acsA from *E. coli*), Polypeptide: restriction of methylated adenine (mrr from *E. coli*), Protein: TrpR transcriptional repressor (trpR from *E. coli*), Enzymes: glutamate 5-semialdehyde dehydrogenase / gamma-glutamyl kinase (proAB from *E. coli*), methylcytosine restriction system (mcrBC from *E. coli*), Protein: citrate lyase, citrate-ACP transferase component (citF from *E. coli*), Enzyme: thioesterase II (tesB from *E. coli*), Enzyme: DNA-specific endonuclease I (endA from *E. coli*), Enzyme: phosphate acetyltransferase (eutD from *E. coli*), Enzyme: propionate kinase (tdcD from *E. coli*), tRNA: tRNA glnV (supE from *E. coli*), Enzyme: DNA-binding, ATP-dependent protease La (lon from *E. coli*), Polypeptide: DNA strand exchange and recombination protein with protease and nuclease activity (recA from *E. coli*), Transcription Unit: restriction endonulease component of EcoKI restriction-modification system (hsdRMS from *E. coli*), Enzyme: restriction of DNA at 5-methylcytosine residues (mcrA from *E. coli*). In some embodiments the genetic modifications listed above are modified further with the genetic modules provided herein.

[00183] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the method of bioproduction of various chemicals which can be used to make various consumer products, including but not limited to those described herein.

[00184] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of 1,4-butanediol (1,4-BDO) . *See, e.g.,* U.S. Pub. No. 20110190513. In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of butanol. *See, e.g.,* U.S. App. No. 13/057,359. In some embodiments, the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of isobutanol. *See, e.g.,* U.S. App. No. 13/057,359.

[00185] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of 3-HP such and its aldehyde metabolites. *See, e.g.,* U.S. App. No. 13/062,917.

[00186] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of polyketide chemical products. *See, e.g.,* U.S. App. No. 13/575,581.

[00187] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of fatty acid methyl esters. *See e.g.,* U.S. Pub. No. 20110124063. In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of C4-C18 fatty acids. *See, e.g.,* U.S. App No. 61/682,127.

[00188] Genetic Modifications

[00189] Various methods to achieve such genetic modification in a host strain are known to one skilled in the art. Example of genetic modifications that can be used by the claimed invention include, but are not limited to, increasing expression of an endogenous genetic element; increasing expression of an exogenous genetic element; decreasing functionality of a repressor gene; increasing functionality of a repressor gene; increasing functionality of an activator gene; decreasing functionality of an activator gene; introducing a genetic change or element integrated in the host genome, introducing a heterologous genetic element permanently, by integration into the genome or transiently by transformation with plasmid; increasing copy number of a nucleic acid sequence encoding a polypeptide catalyzing an enzymatic conversion step; mutating a genetic element to provide a mutated protein to increase specific enzymatic activity; mutating a genetic element to provide a mutated protein to decrease specific enzymatic activity; over-expressing of a gene; reducing the expression of a gene; knocking out or deleting a gene; altering or modifying feedback inhibition; providing an enzyme variant comprising one or more of an impaired binding sites or active sites; increasing functionality of a siRNA or equivalent, decreasing functionality of a siRNA or equivalent, increasing functionality of an antisense molecule, decreasing functionality of an antisense molecule, addition of genetic modules such as RBS, '3 UTR elements to increase mRNA stability or translation; deletion of genetic modules such as RBS, '3 UTR elements to decrease mRNA stability or translation; addition or modification of genetic modules such as '5 UTR elements to increase transcription; deletion or modification of genetic modules such as '5 UTR and elements to increase transcription. In addition other genetic modules, provide herein, such a multicopy plasmids and various promoters can be used to further modify of the genetic modifications provide herein. Additionally, as known to those of ordinarily skill in the art compounds such as siRNA technology, anti-sense technology, and small molecule in inhibitors can be used to alter gene expression in the same manner as a genetic modification.

[00190] Screening methods, such as SCALE in combination with random mutagenesis may be practiced to provide genetic modifications that provide a benefit to increased production of 3-HP in a host cell. Examples of random mutagenesis can include insertions, deletions and substitutions of one or more nucleic acids in a nucleic acid of interest. In various embodiments, a genetic modification results in improved enzymatic specific activity and/or turnover number of an enzyme. Without being limited in any way, changes may be measured by one or more of the following: KM; Kcat, Kavidity, gene expression level, protein expression level, level of a product known to be produced by the enzyme, 3-HP tolerance, or by 3-HP production or by any other known means.

[00191] Host Cells

[00192] In some applications of the invention the host cell can be a gram-negative bacterium. In some applications of the invention the host cell can be from *Zymomonas, Escherichia, Pseudomonas, Alcaligenes, or Klebsiella*. In some applications of the invention the host cell can be *Escherichia coli, Cupriavidus necator, Oligotropha carboxidovorans*, or *Pseudomonas putida*. In some applications of the invention the host cell is one or more *E. coli* strains.

[00193] In some applications of the invention the host cell can be a gram-positive bacterium. In some applications of the invention the host cell can be from *Clostridium, Salmonella, Rhodococcus, Bacillus, Lactobacillus, Enterococcus, Paenibacillus, Arthrobacter, Corynebacterium,* or *Brevibacterium*. In some applications of the invention the host cell is *Bacillus licheniformis, Paenibacillus macerans, Rhodococcus erythropolis, Lactobacillus plantarum, Enterococcus faecium, Enterococcus gallinarium, Enterococcus faecalis,* or *Bacillus subtilis*. In some applications of the invention the host cell is *B. subtilis* strain.

[00194] In some applications of the invention the host cell can be yeast. In some applications of the invention the host cell can be from *Pichia, Candida, Hansenula or Saccharomyces*. In some applications of the invention the host cell is *Saccharomyces cerevisiae*. In some applications of the invention the host cell is *Saccharomyces pombe*.

[00195] In some applications of the invention the host cell can be an alga. In some applications of the invention the host cell can be a halophile. In some applications of the invention the host cell can be an alga. In some applications of the invention the host cell is a chemolithotrophic bacterium.

[00196] In some applications of the invention the host cell can be comprised of multiple host cell types. In some applications of the invention the host cell is comprised of one host cell type. In some applications of the invention the host cell is comprised of one more species or strain of a host cell type.

[00197] VIII. DOWNSTREAM CONSUMER PRODUCTS CHEMICALS

[00198] 3-HP purified according to the methods provided in this disclosure may be converted to various other products having industrial uses including, but not limited to, acrylamide, acrylic acid, esters of acrylic acid, 1,3-propanediol, and other chemicals, collectively referred to as "downstream chemical products" or "downstream products." In some instances, the conversion can be associated with a separation and/or purification process. These downstream chemical products can be useful for producing a variety of consumer products which are described in more detail below. The methods of the present invention can include steps to produce downstream products of 3-HP.

[00199] As a C3 building block, 3-HP can offer the potential for a variety of chemical conversions into commercially important intermediates, industrial end products, and consumer products. For example, 3-HP may be converted to acrylic acid, acrylates (*e.g.*, acrylic acid salts and esters), 1,3-propanediol, malonic acid, ethyl-3-hydroxypropionate, ethyl ethoxy propionate, propiolactone, acrylamide, or acrylonitrile.

[00200] Additionally, 3-HP may be oligomerized or polymerized to form poly(3-hydroxypropionate) homopolymers, or co-polymerized with one or more other monomers to form various co-polymers. Because 3-HP has a single stereoisomer, polymerization of 3-HP may not be complicated by the stereo-specificity of monomers during chain growth. This can be contrasted with (S)-2-hydroxypropanoic acid (also known as lactic acid), which has two (D, L) stereoisomers that should be considered during its polymerizations.

[00201] As will be further described, 3-HP can be converted into derivatives starting (i) substantially as the protonated form of 3-hydroxypropionic acid; (ii) substantially as the deprotonated form, 3-hydroxypropionate; or (iii) as mixtures of the protonated and deprotonated forms. Generally, the fraction of 3-HP present as the acid versus the salt can depend on the pH, the presence of other ionic species in solution, temperature (which can change the equilibrium constant relating the acid and salt forms), and, to some extent, pressure. Many chemical conversions may be carried out from either of the 3-HP forms, and overall process economics can likely dictate the form of 3-HP for downstream conversion.

[00202] Acrylic acid obtained from 3-HP purified by the methods described in this disclosure may be further converted to various polymers. For example, the free-radical polymerization of acrylic acid can take place by polymerization methods known to the skilled worker and can be carried out, for example, in an emulsion or suspension in aqueous solution or another solvent. Initiators, such as but not limited to organic peroxides, can be often added to aid in the polymerization. Among the classes of organic peroxides that may be used as initiators can be

diacyls, peroxydicarbonates, monoperoxycarbonates, peroxyketals, peroxyesters, dialkyls, and hydroperoxides. Another class of initiators can be azo initiators, which may be used for acrylate polymerization as well as co-polymerization with other monomers. U.S. Patent Nos. 5,470,928; 5,510,307; 6,709,919; and 7,678,869 teach various approaches to polymerization using a number of initiators, including organic peroxides, azo compounds, and other chemical types.

[00203] Accordingly, it can be further possible for co-monomers, such as crosslinkers, to be present during the polymerization. The free-radical polymerization of the acrylic acid obtained from dehydration of 3-HP, as produced herein, in at least partly neutralized form and in the presence of crosslinkers can be practiced in certain embodiments. This polymerization may result in hydrogels which can then be comminuted, ground and, where appropriate, surface-modified, by known techniques.

[00204] An important commercial use of polyacrylic acid can be for superabsorbent polymers. *See e.g.*, Modern Superabsorbent Polymer Technology, Buchholz and Graham (Editors), Wiley-VCH, 1997. Superabsorbent polymers can be used as absorbents for water and aqueous solutions for diapers, adult incontinence products, feminine hygiene products, and similar consumer products. In such consumer products, superabsorbent materials can replace traditional absorbent materials such as cloth, cotton, paper wadding, and cellulose fiber. Superabsorbent polymers can absorb, and retain under a slight mechanical pressure, up to 25 times or more their weight in liquid. The swollen gel can hold the liquid in a solid, rubbery state and can prevent the liquid from leaking. Superabsorbent polymer particles can be surface-modified to produce a shell structure with the shell being more highly cross-linked than the rest of the particle. This technique can improve the balance of absorption, absorption under load, and resistance to gel-blocking. It is recognized that superabsorbent polymers can have uses in fields other than consumer products, including agriculture, horticulture, and medicine.

[00205] Superabsorbent polymers can be prepared from acrylic acid (such as acrylic acid derived from 3-HP provided herein) and a crosslinker, by solution or suspension polymerization. Exemplary methods include those provided in U.S. Patent Nos. 5,145,906; 5,350,799; 5,342,899; 4,857,610; 4,985,518; 4,708, 997; 5,180,798; 4,666,983; 4,734,478; and 5,331,059.

[00206] Among consumer products, a diaper, a feminine hygiene product, and an adult incontinence product can be made with superabsorbent polymer that itself can be made substantially from acrylic acid converted from 3-HP made in accordance with the present invention.

[00207] Diapers and other personal hygiene products may be produced that incorporate superabsorbent polymers that can be made from acrylic acid that can be made from 3-HP which

can be produced and purified by the teachings of the present application. The following can provide general guidance for making a diaper that incorporates such superabsorbent polymer. The superabsorbent polymer first can be molded into an absorbent pad that may be vacuum formed, and in which other materials, such as a fibrous material (*e.g.*, wood pulp) can be added. The absorbent pad then can be assembled with sheet(s) of fabric, generally a nonwoven fabric (*e.g.*, that can be made from one or more of nylon, polyester, polyethylene, and polypropylene plastics) to form diapers.

[00208] More particularly, in one non-limiting process, multiple pressurized nozzles, located above a conveyer belt, can spray superabsorbent polymer particles (*e.g.*, about 400 micron size or larger), fibrous material, and/or a combination of these onto the conveyer belt at designated spaces/intervals. The conveyor belt can be perforated and under vacuum from below, so that the sprayed on materials can be pulled toward the belt surface to form a flat pad. In various embodiments, fibrous material can be applied first on the belt, followed by a mixture of fibrous material and the superabsorbent polymer particles, followed by fibrous material, so that the superabsorbent polymer can be concentrated in the middle of the pad. A leveling roller may be used toward the end of the belt path to yield pads of uniform thickness. Each pad thereafter may be further processed, such as to cut it to a proper shape for the diaper, or the pad may be in the form of a long roll sufficient for multiple diapers. Thereafter, the pad can be sandwiched between a top sheet and a bottom sheet of fabric (one generally being liquid pervious, the other liquid impervious), for example, on a conveyor belt, and these can be attached together, for example by gluing, heating or ultrasonic welding, and cut into diaper-sized units (if not previously so cut). Additional features may be provided, such as elastic components, strips of tape, etc., for fit and ease of wearing by a person.

[00209] The ratio of the fibrous material to polymer particles can affect performance characteristics. In some cases, this ratio can be between 75:25 and 90:10 (see *e.g.*, U.S. Patent No. 4,685,915). Other disposable absorbent articles may be constructed in a similar fashion, such as absorbent articles for adult incontinence, feminine hygiene (sanitary napkins), tampons, etc. (see, for example, U.S. Patent Nos. 5,009,653; 5,558,656; and 5,827,255.

[00210] Low molecular weight polyacrylic acid can have uses for water treatment, and as a flocculant and thickener for various applications including cosmetics and pharmaceutical preparations. For these applications, the polymer may be uncrosslinked or lightly cross-linked, depending on the specific application. The molecular weights can be typically from about 200 to about 1,000,000 g/mol. An example of the preparation of these low molecular weight

polyacrylic acid polymers is described in U.S. Patent Nos. 3,904,685; 4,301,266; 2,798,053; and 5,093,472.

[00211] Acrylic acid may be co-polymerized with one or more other monomers selected from the group consisting of: acrylamide, 2-acrylamido-2-methylpropanesulfonic acid, N,N-dimethylacrylamide, N-isopropylacrylamide, methacrylic acid, and methacrylamide, to name a few. The relative reactivity of the monomers can affect the microstructure and thus the physical properties of the polymer. Co-monomers may be derived from 3-HP, or otherwise provided, to produce co-polymers. *See e.g.*, Ullmann's Encyclopedia of Industrial Chemistry, Polyacrylamides and Poly(Acrylic Acids), WileyVCH Verlag GmbH, Wienham (2005).

[00212] Acrylic acid can in principle be copolymerized with almost any free-radically polymerizable monomers including styrene, butadiene, acrylonitrile, acrylic esters, maleic acid, maleic anhydride, vinyl chloride, acrylamide, itaconic acid, and so on. End-use applications typically dictate the co-polymer composition, which can influences properties. Acrylic acid also may have a number of optional substitutions and, after such substitutions, may be used as a monomer for polymerization, or co-polymerization reactions. As a general rule, acrylic acid (or one of its co-polymerization monomers) may be substituted by any substituent that does not interfere with the polymerization process, such as alkyl, alkoxy, aryl, heteroaryl, benzyl, vinyl, allyl, hydroxy, epoxy, amide, ethers, esters, ketones, maleimides, succinimides, sulfoxides, glycidyl, and silyl (see *e.g.*, U.S. Patent No. 7,678,869). The following paragraphs provide a few non-limiting examples of copolymerization applications.

[00213] Paints that comprise polymers and copolymers of acrylic acid and its esters are in wide use as industrial and consumer products. Aspects of the technology for making such paints can be found in *e.g.*, U.S. Patent Nos. 3,687,885 and 3,891,591. Generally, acrylic acid and its esters may form homopolymers or copolymers among themselves or with other monomers, such as amides, methacrylates, acrylonitrile, vinyl, styrene and butadiene. A desired mixture of homopolymers and/or copolymers, referred to in the paint industry as "vehicle" (or "binder") can be added to an aqueous solution and agitated sufficiently to form an aqueous dispersion that can include sub-micrometer sized polymer particles. The paint can cure by coalescence of these vehicle particles as the water and any other solvent evaporate. Other additives to the aqueous dispersion may include pigment, filler (*e.g.*, calcium carbonate, aluminum silicate), solvent (*e.g.*, acetone, benzol, alcohols, etc., although these are not found in certain no VOC paints), thickener, and additional additives depending on the conditions, applications, intended surfaces, etc. In many paints, the weight percent of the vehicle portion may range from about nine to about 26 percent, but for other paints the weight percent may vary beyond this range.

[00214] Acrylic-based polymers can be used for many coatings in addition to paints. For example, for paper coating latexes, acrylic acid can be used from 0.1-5.0%, along with styrene and butadiene, to enhance binding to the paper and modify rheology, freeze-thaw stability and shear stability. *See e.g.*, U.S. Patent Nos. 3,875,101 and 3,872,037. Acrylate-based polymers can be used in many inks, particularly UV curable printing inks. For water treatment, acrylamide and/or hydroxy ethyl acrylate can be co-polymerized with acrylic acid to produce low molecular-weight linear polymers. *See e.g.*, U.S. Patent Nos. 4,431,547 and 4,029,577. Co-polymers of acrylic acid with maleic acid or itaconic acid can also be produced for water-treatment applications, as described in U.S. Patent No. 5,135,677. Sodium acrylate (the sodium salt of glacial acrylic acid) can be co-polymerized with acrylamide (which may be derived from acrylic acid via amidation chemistry) to make an anionic co-polymer that can be used as a flocculant in water treatment.

[00215] For thickening agents, a variety of co-monomers can be used, such as those described in U.S. Patent Nos. 4,268,641 and 3,915,921. U.S. Patent No. 5,135,677 describes a number of co-monomers that can be used with acrylic acid to produce water-soluble polymers.

[00216] In some cases, conversion to downstream products may be made enzymatically. For example, 3-HP may be converted to 3-HP-CoA, which then may be converted into polymerized 3-HP with an enzyme having polyhydroxy acid synthase activity (EC 2.3.1.-). Also, 1,3-propanediol can be made using polypeptides having oxidoreductase activity or reductase activity (*e.g.* , enzymes in the EC 1.1.1.- class of enzymes). Alternatively, when creating 1,3-propanediol from 3-HP, a combination of (1) a polypeptide having aldehyde dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.34 class) and (2) a polypeptide having alcohol dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.32 class) can be used. Polypeptides having lipase activity may be used to form esters. Enzymatic reactions such as these may be conducted *in vitro*, such as using cell-free extracts, or i*n vivo*.

[00217] Thus, various embodiments described in this disclosure, such as methods of making a chemical, can include conversion steps to any downstream products of microbially produced 3-HP, including but not limited to those chemicals described herein and known in the art. For example, in some cases, 3-HP can be produced and can be converted to polymerized-3-HP (poly-3-HP) or acrylic acid. In some cases, 3-HP or acrylic acid can be used to produce polyacrylic acid (polymerized acrylic acid, in various forms), methyl acrylate, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP, malonic acid, 1,3-propanediol, ethyl acrylate, n-butyl acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, 2-ethylhexyl acrylate, and

acrylic acid or an acrylic acid ester to which an alkyl or aryl addition may be made, and/or to which halogens, aromatic amines or amides, and aromatic hydrocarbons may be added.

[00218] Reactions that form downstream compounds such as acrylates or acrylamides can be conducted in conjunction with use of suitable stabilizing agents or inhibiting agents reducing the likelihood of polymer formation. *See*, for example, U.S. Publication No. 2007/0219390. Stabilizing agents and/or inhibiting agents include, but are not limited to, *e.g.*, phenolic compounds (*e.g.*, dimethoxyphenol (DMP) or alkylated phenolic compounds such as di-tert-butyl phenol), quinones (*e.g.*, t-butyl hydroquinone or the monomethyl ether of hydroquinone (MEHQ)), and/or metallic copper or copper salts (*e.g.*, copper sulfate, copper chloride, or copper acetate). Inhibitors and/or stabilizers can be used individually or in combinations as will be known by those of skill in the art.

[00219] In some cases, the one or more downstream compounds can be recovered at a molar yield of up to about 100 percent, or a molar yield in the range from about 70 percent to about 90 percent, or a molar yield in the range from about 80 percent to about 100 percent, or a molar yield in the range from about 90 percent to about 100 percent. Such yields may be the result of single-pass (batch or continuous) or iterative separation and purification steps in a particular process.

[00220] The methods described in this disclosure can also be used to produce downstream compounds derived from 3-HP, such as but not limited to, polymerized-3-HP (poly-3-HP), acrylic acid, polyacrylic acid (polymerized acrylic acid, in various forms), copolymers of acrylic acid and acrylic esters, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP, malonic acid, and 1,3-propanediol. Also, among esters that are formed are methyl acrylate, ethyl acrylate, n-butyl acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, and 2-ethylhexyl acrylate. These and/or other acrylic acid and/or other acrylate esters may be combined, including with other compounds, to form various known acrylic acid-based polymers. Numerous approaches may be employed for such downstream conversions, generally falling into enzymatic, catalytic (chemical conversion process using a catalyst), thermal, and combinations thereof (including some wherein a desired pressure is applied to accelerate a reaction). For example, without being limiting, acrylic acid may be made from 3-HP via a dehydration reaction, methyl acrylate may be made from 3-HP via dehydration and esterification, the latter to add a methyl group (such as using methanol), acrylamide may be made from 3-HP via dehydration and amidation reactions, acrylonitrile may be made via a dehydration reaction and forming a nitrile moiety, propiolactone may be made from 3-HP via a ring-forming internal esterification reaction, ethyl-3-HP may be made from 3-HP via esterification with ethanol, malonic acid may be made

from 3-HP via an oxidation reaction, and 1,3-propanediol may be made from 3-HP via a reduction reaction. Additionally, it is appreciated that various derivatives of the derivatives of 3-HP and acrylic acid may be made, such as the various known polymers of acrylic acid and its derivatives. Production of such polymers can be considered within the scope of the present invention. Copolymers containing acrylic acid and/or esters have been widely used in the pharmaceutical formulation to achieve extended or sustained release of active ingredients, for example as coating material. Downstream compounds may also be converted to consumer products such as diapers, carpet, paint, and adhesives.

[00221] Another important product, acrylamide, has been used in a number of industrial applications. Acrylamide may be produced from 3-HP, for example, without being limiting, via an esterification-amidation-dehydration sequence. Refluxing an alcohol solution of 3-HP in the presence of an acid or Lewis acid catalyst described herein can lead to a 3-HP ester. Treatment of the 3-HP ester with either an ammonia gas or an ammonium ion could yield 3-HP amide. Finally, dehydration of the 3-HP amide with dehydration reagents described elsewhere in this disclosure could produce acrylamide. The steps mentioned herein may be rearranged to produce the same final product acrylamide. Polymerization of acrylamide can be achieved, for example, and without being limiting, by radical polymerization. Polyacrylamide polymers have been widely used as additives for treating municipal drinking water and waste water. In addition, they have found applications in gel electrophoresis, oil-drilling, papermaking, ore processing, and the manufacture of permanent press fabrics.

[00222] IX. EXPRESSION SYSTEMS GENERAL CONCEPTS

[00223] The following general concepts can be applicable to the embodiments of the invention described above.

[00224] Multicopy Plasmids

[00225] The researcher is faced with a myriad of genetic module options when designing a plasmid for expression of a heterologous protein in a host cell. How to optimize an expression plasmid system often depends on the downstream use of the expressed protein.

[00226] Different cloning vectors or plasmids are maintained at different copy numbers, dependent on the replicon of the plasmid. Most general cloning plasmids can carry a DNA insert up to about 15 kb in size.

[00227] Multicopy plasmids can be used for the expression of recombinant genes in *Escherichia coli*. Examples of include multicopy plasmids include high-copy, medium-copy and low-copy plasmids (**see *e.g.*, FIG. 8).** The high copy number plasmid is generally desired for maximum gene expression. However, the metabolic burden effects can result from multiple

plasmid copies could prove to be detrimental for maximum productivity in certain metabolic engineering applications by adding significant metabolic burden to the system.

[00228] The low-copy plasmids, for example, pBR322 is based on the original ColE1 replicon and thus has a copy number of about 15-20. The pACYC series of plasmids are based on the p15A replicon, which has a copy number of about 18-22, whereas pSC101 has even a lower copy number of about 5, and BACs are typically maintained at one copy per cell. Such low copy plasmids may be useful in metabolic engineering applications, particularly when one or more of the substrates used in the recombinant pathway are required for normal cellular metabolism and can be toxic to the cell at high levels.

[00229] However, the use of high-copy plasmids may be useful in enhanced cellular metabolism contexts. The mutant ColEl replicon, as found in the pUC series of plasmids produces a copy number of about 500-700 as a result of a point mutation within the RNAII regulatory molecule.

[00230] There are transcription and translation vectors. Transcription vectors are utilized when the DNA to be cloned has an ATG start codon and a prokaryotic ribosome-binding site. Translation vectors contain an efficient ribosome-binding site and, therefore, it is not necessary for the target DNA to contain one. This is particularly useful in cases where the initial portion of the gene may be cleaved in an effort to improve solubility. Another consideration when choosing a transcription or translation vector is the source of the DNA to be expressed. Prokaryotic genes usually have a ribosome-binding site that is compatible with the host *E. coli* translation machinery, whereas eukaryotic genes do not. Normal prokaryotic gene expression may be enhanced by use of an engineered promoter and ribosome-binding site.

[00231] Promoters

[00232] A promoter is a region of DNA that initiates transcription of a particular gene. In bacteria, transcription is initiated by the promoter being recognized by RNA polymerase and an associated sigma factor, which are often brought to the promoter site by an activator protein's binding to its own DNA binding site located proximal or distal (*e.g.*, near) to the promoter.

[00233] Promoter selection is an important factor when designing an expression plasmid system. A promoter is located upstream of the ribosome-binding site. Owing to the fact that many heterologous protein products are toxic to the cell, the promoter can be regulated so that the heterologous protein is expressed at the appropriate amount and time to reduce the burden on the cell host.

[00234] Historically, the most commonly used promoters have been the lactose (lac) and tryptophan (trp) promoters. These two promoters were combined to create the hybrid promoters

tac and trc that are also commonly used. Other common promoters are the phage lambda promoters, the phage T7 promoter (T7), and the alkaline phosphatase promoter (phoA).

[00235] Promoters can be constitutive and inducible. Constitutive promoters are active in all circumstances in the cell, while regulated or inducible promoters become active in response to specific stimuli. In addition, the strength of the promoter can also differ. A strong promoter has a high frequency of transcription and can generate a heterologous protein that is about 10-30% of the total cellular protein production (for examples see **FIG. 8)**. Chemically-inducible promoters that can be used in various aspects of the invention include, but are not limited to, promoters whose transcriptional activity is regulated by the presence or absence of alcohol, tetracycline, steroids, metal and other compounds. Physically-inducible promoters that can be used in various aspects of the invention include, but are not limited to, promoters whose transcriptional activity is regulated by the presence or absence of light and low or high temperatures.

[00236] In order to be an inducible promoter, the promoter should ideally initially be completely repressed to transcription. Although, most inducible promoters are known to have some leakage, these inducible promoters are also contemplated. Transcription is subsequently induced at any given time with the addition of an inducer into the desired host cell. Alternatively, an inducible promoter may be responsive to the lack of a substance, such as inorganic phosphate, such that the absence of inorganic phosphate will allow expression at a desired time in the host cell (for examples see **FIG. 8)**.

[00237] Ribosome Binding Sites

[00238] A Ribosome Binding Sites (RBS) is an RNA sequence upstream of the start codon that affects the rate at which a particular gene or open reading frame (ORF) is translated. A person of skill in the art can tailor an RBS site to a particular gene. Ribosome Binding Sites (RBSs) are typically short sequences, often less than 20 base pairs. Various aspects of RBS design are known to affect the rate at which the gene is translated in the cell. The RBS module can influence the translation rate of a gene largely by two known mechanisms. First, the rate at which ribosomes are recruited to the mRNA and initiate translation is dependent on the sequence of the RBS. Secondly, the sequence of the RBS can also affect the stability of the mRNA in the cell, which in turn affects the overall level of proteins. Through the use of genetic expression modules, the expression of desired genes, such as genes encoding enzymes in the biosynthetic pathway for 3-HP, can be tailored either at the transcriptional and translational level.

[00239] One can access the registry RBS collection as a starting point for designing an RBS <<http://partsregistry.org/Ribosome_Binding_Sites/Catalog>> ("Registry"). As of the date of application, the Registry has collections of RBSs that are recommended for general protein

expression in *E. coli* and other prokaryotic hosts. In addition, each family of RBSs has multiple members covering a range of translation initiation rates. There are also several consensus RBS sequence for *E. coli* that have been described. However, it is important to keep in mind the known RBS functions and mechanisms in a larger context. For example, in certain contexts the RBS can interact with upstream sequences, such as sequence that comprise the promoter or an upstream ORF. In other contexts, the RBS may interact with downstream sequences, for example, the ribosome enzyme binds jointly to the RBS and start codon at about the same time. These potential interactions should be considered in the overall RBS sequence design. The degree of secondary structure near the RBS can affect the translation initiation rate. This fact can be used to produce regulated translation initiation rates.

[00240] The Shine-Dalgarno portion of the RBS is critical to the strength of the RBS. The Shine-Dalgarno is found at the end of the 16s rRNA and is the portion that binds with the mRNA and includes the sequence 5´ - ACCUCC - 3´. RBSs will commonly include a portion of the Shine-Dalgarno sequence. One of the ribosomal proteins, S1, is known to bind to adenine bases upstream from the Shine-Dalgarno sequence. As a result, it is hypothesized that the RBS can be made stronger by adding more adenines in the sequence upstream of the RBS.

[00241] When considering the design of the spacing between the RBS and the start codon, it is important to think of the aligned spacing rather than just the absolute spacing. While the Shine-Dalgarno portion of the RBS is critical to the strength of the RBS, the sequence upstream of the Shine-Dalgarno sequence may also be important. Note that the promoter may add some bases onto the start of the mRNA that may affect the strength of the RBS by affecting S1 binding.

[00242] Computer programs that design RBS sequence to match protein coding sequences, desired upstream sequences including regulatory mRNA sequences, and account of secondary structure are known [*see, e.g.,* Salis, Mirsky, and Voight, Nature Biotechnology 27: 946-950, 2009] and were used to optimize RBSs for the ACCase subunit genes as described in (see **EXAMPLE 2**).

[00243] X. EXPORT OF 3-HYDROXYPROPIONIC ACID FROM CELLS

[00244] 3-HP is structurally and chemically similar to a number of amino acids (*e.g.*, serine, theronine, and to a lesser degree cysteine). These amino acids can be removed from cells by amino acid-specific flux proteins that belong to the major facilitator family (MF). *See, e.g.,* Kramer, R. (1994) Secretion of amino acids by bacteria · physiology and mechanism FEMS Microbiol. Rev. 13, 75-93; *see also e.g.*, Kramer, R. (1994) Systems and mechanisms of amino acid uptake and secretion in prokaryotes Arch. Microbiol. 162, 1-1. This protein family is part of a larger family of previously described and inferred drug extrusion translocases that also

include SMR (small multidrug resistance) family, RND (resistance nodulation cell division), ABC (ATP-b:inding cassette) family, and MATE (multidrug and toxic compound extrusion) family. *See, e.g.*, Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H. (2006) Effect of drug transporter genes on cysteine export and overproduction in *Escherichia coli*. Appl Environ Microbial. 2006 Jul;72(7):4735-42. Most of these proteins (all except the ABC family) can use the proton gradient or sodium gradient across the inner bacterial membrane to facilitate, *e.g.*, power translocation. Discussed herein, the proton gradient directed translocases are elucidated as increasing. Furthermore, modifying the effect of the proton gradient directed translocases is expected to preserve as much metabolic energy as possible. However, it can be appreciated that in some embodiments, ATP, sodium, or other metabolite gradient-directed translocases could also be used.

[00245] In some embodiments, the invention may be practiced in a range of microorganisms, such as those bacteria and yeast common use for industrial bioproduction of chemicals. Culture systems can comprise a population of a genetically modified microorganism according to an embodiment of the present invention placed in a media comprising suitable nutrients for growth.

[00246] In some embodiments, the genetically modified microorganisms according to the present invention may be cultured under aerobic, anaerobic, or microaerobic conditions. *See e.g.*, **FIG. 10**. Although some of these proteins have been shown to be fairly specific, members of this protein family have been shown to transport several different amino acids. There are more than 30 identified and putative transporters in the *E. coli* genome. However, the specificities of most of these proteins are unknown. In the MF family, the following specificities have been reported.

Serine	Threonine	Cysteine
rhtA	rhtA	eamA
rhtB	rhtC	eamB

[00247] This list does not necessarily reflect the full set of proteins involved in the transportation of these amino acids. Overexpression of 33 transporters have identified seven transporters that resulted in a significant increase of cysteine efflux (*see e.g.*, FIG. 11, adapted from Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H. (2006) Effect of drug transporter genes on cysteine export and overproduction in *Escherichia coli*. Appl Environ Microbial. 2006 Jul;72(7):4735-42).

[00248] The table below (Table 1) lists paralogs for the identified transport proteins listed in Biocyc. The first column shows the serine and threonine transports, the second column shows

the known cysteine transporters, and the third column shows other predicted transporters with operon information or expected functional information:

[00249] Table 1

rhtA, rhtB, rhtC, eamA, or eamB family	Bcrfamily	bcrfamily with unknown functions
yedA	emrB	garP (in operon with garRLK) likely importer
yijE	emrD	gudP(in operon with gudPX) likely importer
ytfF	emrY	hsrA (in operon with yieP) likely exporter
leuE	ydeD (known cysteine)	mdtD (in operon with mdtABC) likely exporter
rhtC	yfiK (known cysteine)	mdtG likely exporter
yahN		mdtl (next to tnaB - tryptophan transporter) likely exporter
		mdtM
		mhpT(3-(3-hydroxyphenyl)propionate transporter) likely exporter
		yajR
		ybjJ (next to riboflavin phosphates)
		ydhC (next to fatty acid synthesis gene) likely exporter
		ydjE
		ydjK(in an operon with putative threonine dehydrogenase ydjL)
		yfd (next to fatty acid

		synthesis gene) likely exporter
		yhjE
		yidE
		yjiJ

[00250] In a first evaluation, a subset of these proteins can be selected and tested for 3-HP export. As noted, the focus can concentrate on the proton gradient driven translocases, but in general sodium driven, ATP-driven, or other metabolite gradient driven translocases can be evaluated.

[00251] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the spirit of the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[00252] EXAMPLE 1: SALT INHIBITION STUDIES IN *E. COLI*

[00253] The activity of ACCase complex, a critical enzyme in the conversion of acetyl-CoA to malonyl-CoA, the immediate precursor for 3-HP, is severely inhibited by salt. Dose-dependent effects on ACCase activity was observed in the presence of NaCl, NH_4Cl, Na-3-HP , or NH_4-3-HP such that salt levels near 0.44M resulted in decreasing the activity of the ACCase enzyme by approximately 80%, while salts of 3-HP levels near 0.66M decreased the activity of the ACCase enzyme by approximately 80% relative to control (**FIG. 4**). Levels of greater than 0.66M (60 g/L) are expected to be present for commercially viable commercial production of 3-HP.

[00254] EXAMPLE 2: OTHER SALT-TOLERANT ENZYMES

[00255] Microorganisms comprising a genetic modifications to enzymes from slight , moderate, and/or extreme halophiles organisms are made. Homology with genes are determined by analysis with BLASTN version 2.0 provided through the NCBI website. Homology with proteins are determined by analysis with BLASTP version 2.2.2 provided through the NCBI website.

[00256] The enzymes from the following halophilic organisms are used: *Chromohalobacter salexigens, Flexistipes sinusarabici strain (MAS10T), Halobacterium sp. NRC-1*, *Haloarcula marismortui*, *Natronomonas pharaonis*, *Haloquadratum walsbyi*, *Haloferax volcanii*, *Halorubrum lacusprofundi*, *Halobacterium sp. R-1*, *Halomicrobium mukohataei*, *Halorhabdus utahensis*, *Halogeometricum borinquense*, *Haloterrigena turkmenica*, *Natronobacterium gregoryi SP2*, *Halalkalicoccus jeotgali*, *Natrialba magadii*, *Haloarcula hispanica*, *Halopiger xanaduensis*, *Halophilic archaeon DL31*, *Haloferax mediterranei*, *Halovivax ruber*, *Natronococcus gregoryi*, and *Natronococcus occultus*.

[00257] Moderate halophilic organisms in which homologous enzymes are derived are: eukaryotes such as crustaceans (*e.g.*, *Artemia salina*), insects (*e.g.*, *Ephydra hians*), certain plants from the genera *Salicornia spp*, algae (*e.g.*, *Dunaliella viridis*), fungi, and protozoa (*e.g.*, *Fabrea salina*), phototrophic organisms, such as planktonic and microbial mat-formers cyanobacteria as well as other anaerobic red and green sulphur bacteria from the genera *Ectothiorhodospira spp.*) and non-sulphur bacteria from the genera *Chromatium spp.*; gram-negative anaerobic bacteria, for example from the genera *Haloanaerobacter spp*. some of which are methanogenic, for example from the genera *Methanohalophilus* spp. and either aerobic or facultative such as species from the genera *Halomonas*, *Chromohalobacter*, *Salinovibrio*, *Pseudomonas* (*e.g.*, *Halomonase elongate*); gram-positive bacteria from genera such as *Halobacillus*, *Bacillus*, *Marinococcus*, etc. as well as some actinomycetes, *e.g.*, *Actinopolyspora halophila*.

[00258] Genomic and Proteomic Hallmarks of Halophilic Organisms

[00259] Proteins of microorganisms are genetically modified for salt tolerance such that they have low hydrophobicity, over-representation of acidic residues, especially Asp, under-representation of Cys, lower propensities for helix formation and higher propensities for coil structure.

[00260] Suitable salt-tolerant enzymes from salt-tolerant organisms are used. Salt-tolerant organisms (such as, *e.g.*, halophiles) include any living organism that are adapted to living in conditions of high salinity. Suitable salt-tolerant enzymes can include enzymes from salt-tolerant organism that are homologs of the following enzymes: Sucrose-6-phosphate hydrolase (*e.g.*, cscA from *E. coli*), glucose-6-phosphate isomerase (*e.g.*, pgi from *E. coli*), fructokinase (*e.g.*, cscK from *E. coli*), fructose-1,6-bisphosphatase (*e.g.*, yggF from *E. coli*), fructose 1,6-bisphosphatase (*e.g.*, ybhA from *E. coli*), fructose 1,6-bisphosphatase II (*e.g.*, glpX from *E. coli*), fructose-1,6-bisphosphatase monomer (*e.g.*, fbp from *E. coli*), 6-phosphofructokinase-1 monomer (*e.g.*, pfkA from *E. coli*), 6-phosphofructokinase-2 monomer (*e.g.*, pfkB from *E. coli*), fructose bisphosphate aldolase monomer (*e.g.*, fbaB from *E. coli*), fructose bisphosphate aldolase

monomer (*e.g.*, fbaA from *E. coli*), triose phosphate isomerase monomer (*e.g.*, tpiA), glyceraldehyde 3-phosphate dehydrogenase-A monomer (*e.g.*, gapA from *E. coli*), phosphoglycerate kinase (*e.g.*, pgk), 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (*e.g.*, gpmM from *E. coli*), 2,3-bisphosphoglycerate-dependent or tdcE (*e.g.*, from *E. coli*), phosphoglycerate mutase (*e.g.*, gpmA), enolase (*e.g.*, eno from *E. coli*), phosphoenolpyruvate carboxylase (*e.g.*, ppc from *E. coli*), malate dehydrogenase (*e.g.*, mdh), fumarase A (*e.g.*, fum from *E. coli*), fumarase B (*e.g.*, fumB), fumarase C (*e.g.*, fumC from *E. coli*), phosphoenolpyruvate synthetase (*e.g.*, ppsA from *E. coli*), pyruvate kinase I monomer (*e.g.*, pykF from *E. coli*), pyruvate kinase II monomer (*e.g.*, pykA from *E. coli*), fumarate reductase (*e.g.*, frdABCD from *E. coli*), lipoamide dehydrogenase (*e.g.*, lpd from *E. coli*), pyruvate dehydrogenase (*e.g.*, aceE from *E. coli*), pyruvate dehydrogenase (*e.g.*, aceF from *E. coli*), pyruvate formate-lyase (*e.g.*, pflB from *E. coli*), acetyl-CoA carboxylase (*e.g.*, accABCD from *E. coli*), malonyl CoA reductase (*e.g.*, mcr), 3HP dehydrogenase (*e.g.*, mmsB, NDSD, or ydfG), malonate semialdehyde reductase (*e.g.*, nemA, rutE from *E. coli*), or a combination thereof.

[00261] Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least and at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%,90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, or 70% overall amino acid or nucleotide identity to the above enzymes. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%,90% 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, or 70% amino acid or nucleotide to the essential protein function domains of the enzymes above. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%,90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, or 70% overall amino acid or nucleotide to the essential binding amino acids within an essential protein function domain of the enzymes above.

[00262] In accordance with a preferred embodiment of the invention, suitable salt-tolerant enzyme homologs are enzymes from one of the following organisms: *Halomonas elongata*, *Salinibacter rubu*r, or *Halobacterium* species (Archaea).

[00263] In accordance with a preferred embodiment of the present invention, there is provided a non-salt-tolerant organism that is genetically modified to make 3-HP, wherein the genetic modification includes a polynucleotide encoding an acetyl-CoA carboxylase from a salt-tolerant organism. In accordance with a preferred embodiment, the acetyl-CoA carboxylase subunits accA, accB, accC and accD is from *Halomonas elongata.*

[00264] EXAMPLE 3: ACCase FROM HALOPHILIC ORGANISM

[00265] Halophilic organisms, such as *Halomonas elongata*, are found in environments with high salt concentrations and, in general, have a salt internal concentration >2.5-3M. It is hypothesized that enzymes derived from any salt-tolerant species should be more resistant to enzyme inhibition by salts, such as 3-HP. Further, these enzymes that have greater salt tolerance should in turn have extended production under high salt conditions than enzymes with lower salt tolerance.

[00266] Accordingly, the genes encoding the accA, accB, accC, accD of *H. elongata* described in Table 1 were synthesized for expression in *E. coli* using codons optimized for this organism and supplied individually on pUC57 plasmids without promoters. Synthetic operons comprising the subunits were assembled using the Gibson assembly method.

Gene	Accession number	SEQ ID NO.
accA	YP_003898857.1	SEQ ID NO. 1, 2
accB	YP_003897250.1	SEQ ID NO. 3, 4
accC	YP_003897249.1	SEQ ID NO. 5, 6
accD	YP_003897309.1	SEQ ID NO. 7, 8

Table 1. Accession numbers for genes encoding ACCase subunits from *Halomonas elongate*.

[00267] Each gene was amplified by PCRs with *Pfu* Ultra II HS according to the manufacturer's instructions, and the PCR products were purified using the Zymo PCR Cleanup kit. Concentrations of products were measured using the Nanodrop spectophotometer. The Gibson Assembly kit (NEB) was used to construct plasmids expressing the ACCase subunit genes as directed by the manufacturer. The effect of NH_4-3-HP and NH_4Cl on *H. elongata* ACCase was tested and compared to the *E. coli* ACCase. As shown in **Fig. 4**, whereas the *E. coli* ACCase is significantly inhibited by the salts, the ACCase from the halophile is less affected by either NH_4-3-HP or by NH_4Cl. This result indicated that use of the *H. elongata* ACCase in 3-HP production strains would in beneficial in relieving 3-HP inhibition of the conversion of acetyl-CoA to malonyl-CoA, a critical step in the pathway.

[00268] EXAMPLE 4: RBS-OPTIMIZED GENES

[00269] Enzyme expression is regulated at transcriptional and translational levels in prokaryotes. Ribosome Binding Sites (RBS) are 15 nucleotide segments which are known to control the level of protein expression in microorganisms. To enhance *H. elongata* ACCase expression various customized RBS were constructed and optimized for *E. coli* translation expression. **Table 2** shows the RBS sequences used to increase expression of the individual subunits.

Table 2:

H	Modified RBS sequences preceeding ATG (underlined)

. *elongata* ACC expression plasmid	*He_accD*	*He_accA*	*He_accC*	*He_accB*
Parent 2-4	5′-GCGTAGTAAAGGA GGTAACATATG	5′-CAATTTATTTAAGGAGGACTCTTAAGATG	5′-GAAATTTCATACCACAGGCGAAGGAGGAAAAACCATG	5′-GGAAGAACAAGGGGTGTACATG
B2	Same as 2-4	Same as 2-4	Same as 2-4	5′-ggaagaattaaggggacaaggggaataATG
13A	5′-gcgtagtagccgggtgataaggagccgtaacATG	Same as 2-4	Same as 2-4	
14C	5′-gcgtagtagctgatataaaaggaggtaacggATG	Same as 2-4	Same as 2-4	Same as 2-4
15C	Same as 2-4	5′-caatttatttttgttcacccaaggagtattgctaATG	Same as 2-4	Same as 2-4
17C	Same as 2-4	5′-caatttatttaccgaaataaaa	Same as 2-4	Same as 2-4

		ggagggatgcg aATG		
3 5C	5′- gcgtagtagcc gggtgataagg agccgtaacATG	5′- caatttatttt tgttcacccaa ggagtattgct aATG	Same as 2-4	Same as 2-4
3 6C	5′- gcgtagtagcc gggtgataagg agccgtaacATG	5′- caatttattta ccgaaataaaa ggagggatgcg aATG	Same as 2-4	Same as 2-4
3 6C-8	5′- gcgtagtagcc gggtgataagg agccgtaacATG	5′- caatttattta ccgaaataaaa ggagggatgcg aATG	Same as 2-4	5′- ggaagaatta agggggacaa ggggggaataATG
7 2B	5′- gcgtagtagcc gggtgataagg agccgtaacATG	5′- caatttattta ccgaaataaaa ggagggatgcg aATG	5′- TCTTCCCACA ACACTGGCGG ACTCCATCATG	5′- GAAATTTCAT ACCACAGGCG AAGGAGGAAA AACCATG
1 05F	5′- gcgtagtagcc gggtgataagg agccgtaacATG	5′- caatttatttt tgttcacccaa ggagtattgct aATG	5′- TCTTCCCACA ACACTGGCGG ACTCCATCATG	5′- GAAATTTCAT ACCACAGGCG AAGGAGGAAA AACCATG

Table 2. RBS sequences used to enhance expression of *H. elongate* ACCase subunits.

[00270] The expression performance of the RBS-optimized *H. elongata* ACCases was evaluated by 3-HP production in a 96-well format, each in triplicate wells, and the averaged results shown in **Table 3.** Specific 3HP production is shown as g/L per OD_{600}. As may be seen in Table 3, enhancing the efficiency of the RBS in strains B2, 35C, and 72 B clearly resulted in increased malonyl-CoA production leading to increased 3-HP production. It is evident from

these results that combinations of enhanced RBS's before each of the individual genes *accA*, *accB*, *accC*, and *accD* may result in strains with even higher ACCase expression and activity.

H. elongata ACCase expression plasmid	3HP (g/l.OD)
Parent 2-4	0.06
B2	0.81
13A	0.01
14C	0.54
15C	0.14
17C	0.08
35C	0.68
36C	0.31
36C-8	0.31
72B	0.57
105F	0.19

Table 3. Improvement in 3-HP production by RBS-optimized expression of *H. elongata* ACCase subunits.

[00271] EXAMPLE 5: COORDINATED EXPRESSION BY SUBUNIT FUSIONS

[00272] In nature ACCase subunit genes from prokaryotes such as *E. coli* and *H. elongata* have been shown to have a quaternary structure: $accA_2$:$accD_2$:$accB_4$:$accC_2$. However, the intrinsic levels of the ACCase subunit genes are too low for optimal production. Therefore, for optimal production it is ideal to have overexpression to be coordinated in a similar manner.

[00273] Expression of the genes encoding each ACCase subunit is regulated at transcriptional and translational levels. Coordinated overexpression of the ACCase subunit genes, *accA*, *accB*, *accC*, *accD* should give better ACCase activity. Examples of fusions of *accC-B* proteins exist in bacteria. It is hypothesized that coordinated overexpression may be achieved by fusion of subunit genes should ensures equimolar expression of the subunit genes at the optimal time.

[00274] The following ACCase subunit gene fusion were constructed and the constructs overexpressed in *E. coli*: (A) Control ABCD, (B) fusion of *accC-B* (SEQ ID NOs. 9, 10) subunit genes as seen in bacteria, (C) fusion of *accD-A* subunit genes using a flexible 15-amino acid linker (Linker sequence LSGGGGSGGGGSGGGGSGGGGSAAA; SEQ ID NOs. 11, 12) as depicted in **FIG. 5**.

[00275] The performance of the ACC fusions were tested for their ACCase activity and for various 3-HP production metrics in **Table 4**. ACCase activity was determined in cell lysates

using an assay for malonyl-CoA production as described in Kroeger, Zarzycki, and Fuchs, Analytical Biochem. 411:100-105, 2011, incorporated herein by reference in its entirety. Production of 3-HP was determined in cells co-transformed with a plasmid bearing the genes encoding the malonyl-CoA reductase from *S. tokadaii* and *E. coli ydfG* providing a 3-HP dehydrogenase to complete the metabolic pathway from malonyl-CoA to 3HP. These results show that the strain with the fused *accDA* genes had higher average specific productivity of 3-HP compared to the parental strain in which the overexpressed ACCase is not fused. **Fig. 6** shows that the benefit of the *accDA* fusion were also manifested in 3-HP production in fermentors with environmental controls of nutrient feed, pH, aeration, and temperature.

Strain designation	Plasmid	Avg specific prodn rate (g/gDCW.h) at TS+6	Avg specific prodn rate (g/gDCW.hr) at TS+20	ACCase specific activity at TS+6 (U/mg)
BX3_783	Parent (unfused ACCase)	0.160	0.146	0.057
BX3_829	No ACC	0.069	0.062	0.000
BX3_837	EC ACC DA fusion	0.209	0.201	0.054

Table 4: ACC Fusions and ACCase activity

[00276] EXAMPLE 6 3-HP EXPORTER

[00277] Growth inhibition has been demonstrated for E. coli strains grown in the presence of 3-HP at levels as low as 20 g/L. To produce high titers of 3-HP the production host is required to balance production with overcoming inhibition. A known chemical exporter from *E. coli* that has been previously characterized for homoserine transport, *rhtA*, was evaluated for increased production of 3-HP. A mutant version of the exporter, *rhtA*(P2S) (SEQ ID NOs. 30 and 31) was synthesized behind the PtpiA promoter and inserted into the pTRC-PyibD-MCR plasmid behind a terminator using the Gibson Assembly kit (NEB) according to manufacturer's instructions. The effects of overexpression of *rhtA* were evaluated in 1L fermentation compared to the control plasmid without *rhtA*. As shown in **FIG 7**, overexpression of *rhtA* resulted in a significant improvement in 3HP titer compared to the control production strain. Construction of plasmids expressing another putative transporter, ydcO (SEQ ID NOs. 32 and 33) is carried out in the same manner.

[00278] EXAMPLE 7 BICARBONATE IMPORTER

[00279] Increased import of bicarbonate to increase availability of bicarbonate for the ACCase reaction will increase production of malonyl-CoA and hence products derived metabolically from malonyl-CoA, such as 3-HP. The gene encoding the bicA bicarbonate transporter (SEQ ID NO. 13) of Synechococcus sp. was synthesized using codons optimized for expression in *E. coli*

(SEQ ID NO. 14) and expressed using the *E. coli* tal promoter in a strain co-transformed with plasmids encoding ACCase and MCR functions. Production of 3-HP by this strain is compared to that achieved by a control strain without overexpressed bicA.

[00280] EXAMPLE 8: MONOFUNCTIONAL MCR

[00281] One of the steps in the biosynthesis of 3-HP involves the conversion of malonyl-CoA (MCA) to malonate semialdehyde (MSA) and the conversion of malonate semialdehyde (MSA) to 3-HP. In order to enhance cellular bioproduction of 3-HP in a host cell, a genetically modified organism having novel enzymes and/or combinations of enzymes to catalyze the MCA to MSA reaction are made.

[00282] Organisms that comprise novel enzyme compositions or that co-express a combinations of enzymes to catalyze the conversion of malonyl-CoA to 3-HP are made. For example, malonyl-CoA is converted to malonate semialdehyde by a monofunctional malonyl-CoA reductase that catalyzes the malonyl-CoA conversion, but does not catalyze the malonate semialdehyde conversion.

[00283] The organism is genetically modified to comprise and/or express a monofunctional malonyl-CoA reductase from *Sulfolobus tokodaii* (stMCR) (SEQ ID NOs. 15 and 16) or a functional homolog of stMCR or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity. Other organisms are produced to comprise a genetic modification that include a bi-functional malonyl-CoA reductase comprising two protein fragments with at least one fragment that has malonyl-CoA reductase activity and the other fragment has malonate semialdehyde dehydrogenase activity that is derived from *Chloroflexus aurantiacus* (caMCR).

[00284] EXAMPLE 9: MCR-DEHYDROGENASE ENZYMES FOR CONVERSION OF 3-HP IONS

[00285] Following the conversion of the malonyl-CoA to malonate semialdehyde, the malonate semialdehyde is converted to 3-HP through either or both of two alternative pathways. Malonate semialdehyde may exist in at least three states; the keto form, the enol form, and hydrate form.

[00286] A genetically modified organism comprising and/or expressing a 3-hydroxy acid dehydrogenase enzyme (*e.g., ydfG*; SEQ ID NOs. 21 and 22), a 3-hydroxyisobutyrate dehydrogenase enzyme (*e.g., Pseudomonas aeruginosa mmsB*; SEQ ID NOs. 23 and 24), and/or NAD+-dependent serine dehydrogenase (*e.g., Pseudomonas NDSD*; SEQ ID NOs. 25 and 26) are made.

[00287] Genetically modified organisms are made that also comprise and/or express an N-ethylmaleimide reductase (*e.g., nemA*; SEQ ID NOs. 17 and 18), and/or a malonic semialdehyde

reductase (*e.g., rutE*, SEQ ID NOs. 19 and 20) from *E. coli*, with or without the previous aforementioned genes.

[00288] EXAMPLE 10: GENETICALLY MODIFIED ORGANISMS AND/OR MODIFIED/FUSED ENZYMES

[00289] The genetically modified organisms are made to comprise and/or express a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; and one or more genes encoding one or more of the following enzymes: *ydfG*, *mmsB*, *NDSD*, *rutE*, and *nemA* or a functional homolog or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00290] A genetically modified organism capable of making 3-HP is also created. The genetically modified organism comprises and/or expresses a polynucleotide encoding a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP; and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form or the malonate semialdehyde hydrated form to 3-HP.

[00291] Also made are monofunctional malonyl-CoA reductase enzymes fused to a dehydrogenase enzyme that is either: (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible. Also made are monofunctional malonyl-CoA reductase enzymes fused to a dehydrogenase enzyme that is NADPH-dependent.

[00292] Also made are genetically modified organisms containing and/or expressing 3-HP dehydrogenase enzymes that are largely NADH-dependent, *e.g.*, *mmsB* and/or *NDSD*. Also made are genetically modified organism containing and/or expressing malonate reductase enzymes that are flavin-dependent, *e.g.*, *rutE* and/or *nemA*. Also made are genetically modified organism containing and/or expressing 3-HP dehydrogenase enzymes that are less susceptible 3-HP inhibition at high concentrations, including *e.g.*, *ydfG* and/or *NDSD*. Also made are genetically modified organism containing and/or expressing 3-HP dehydrogenase or malonate semialdehyde dehydrogenase enzymes that catalyze a reaction pathway to 3-HP that is substantially irreversible, *e.g.*, *rutE* and/or *nemA*.

[00293] Monofunctional malonyl-CoA reductase enzyme are also fused to one or more dehydrogenase enzymes. For example, malonyl-CoA reductase is fused with malonate

semialdehyde dehydrogenase to create a multi-domain protein (*e.g.*, two domain protein) and having the MCR and dehydrogenase domains adjacent in the sequence.

[00294] A first monofunctional malonyl-CoA reductase enzyme is fused to a first dehydrogenase enzyme of one type and second monofunctional malonyl-CoA reductase enzyme is fused to a dehydrogenase enzyme of a different type than the first dehydrogenase enzyme.

[00295] In certain fusions, the malonyl-CoA reductase from *S. tokadaii* is fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme includes one of the following the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA – rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00296] In other fusions, the malonyl-CoA reductase from *C. aggregans* is fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, or *nemA* (or some combination thereof). The fused enzyme includes one of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr* - nemA, mcr - ydfG - mmsB, mcr - *ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA - rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00297] In other fusions, the malonyl-CoA reductase from *O. trichoides* is fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, or *nemA* (or some combination thereof). The fused enzyme may include one of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, or *mcr - nemA - rutE*, functional homologs, or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00298] EXAMPLE 11: ENHANCED MUTATED MONOFUNCTIONAL MCR FOR BIOPRODUCTION

[00299] Microorganisms comprising a genetic modification having a mutated form of *stMCR* that have enhanced activity at about 0 °C to about 10°C, at about 8°C to about 15°C, at about 12°C to about 21°C, at 20°C to about 44°C, at about 30°C to about 37°C, at about 32°C to about 38°C, at about 35°C to about 42°C, at about 40°C to about 50°C, at about 50°C to about 60°C, and/or at about 59°C to less than equal to about 72°C, are made.

[00300] Other mutations are also created. The carboxylase domains of the malonyl-CoA reductase derived from *Chloroflexus aggregans* and/or *Oscillochloris trichoides* are enhanced by mutations in the carboxylase binding domain to provide increased 3-HP production over the natural occurring enzyme. Additional mutation are also made. For example, mutations where the carboxylase activity of the malonyl-CoA reductase derived from *Chloroflexus aurantiacus* is enhanced, are made.

[00301] Other fusions include microorganisms comprising a genetic modification that include carboxylase domains of the malonyl-CoA reductase derived from *C. aggregans* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). Any of the enhanced mutated MCR are also fused one of the following configurations including *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA* - ydfG, *mcr - nemA - mmsB*, mcr - *nemA - NDSD*, *mcr - nemA – rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00302] EXAMPLE 12: TEMPERATURE SENSITIVE MALONYL-CoA REDUCTASE

[00303] In this example, a malonyl-CoA reductase (MCR) gene is mutated to enhance its activity at lower temperatures or enhance its activity within a specific temperature range. Any MCR can be used, including, an MCR gene derived from *Sulfolobus tokodaii.*

[00304] Mutation is achieved by site directed mutagenesis and/or random mutagenesis. Subsequent screen methods are used to isolate lower temperature enhanced MCRs and/or specific temperature enhanced MCRs. Screening methods can include any methods to measure any of the substrates and/or products of MCR, *e.g.*, malonate semialdehyde, malonyl-CoA, CoA, NADP+, NADPH, Mg^{2+}, and/or H+. The substrates and/or products can be labeled, *e.g.*, fluorescence or radioactive.

[00305] By repeating this procedure, MCRs with further enhanced activity at lower temperatures and/or MCRs with enhanced activity within a specific temperature range, can be

isolated. For example, a mutated MCR having enhanced activity at or about less than 0°C to at or about at 37°C; at or about at 20°C to at or about at 44°C; at or about at 30°C to at or about at 37°C; at or about at 32°C to at or about at 38°C, can be isolated.

[00306] EXAMPLE 13: THE GROWTH PHASE

[00307] A genetically modified organism is used in a controlled multi-phase production process which includes an initiation and/or completion of one or more phases of the production process. For example, the bioproduction process includes one or more of: (1) a growth phase; (2) an induction phase; and (3) a production phase.

[00308] During the growth phase, the genetically modified organism is replicated while a biocatalyst is built up. During the induction phase, key enzymes are expressed, which are used in production. During the production phase, the genetically modified organism produces the desired chemical product.

[00309] During the entire process, the initiation and/or completion of the growth, induction and/or production phases are controlled. Because in some cases, the growth phase is dependent on the presence of a critical external reactant that will initiate growth, the initiation and completion of the growth phase is controlled by the addition and amount of the initiating reactant added to the reaction vessel. For example, the reactant may be phosphate, which is needed for replication of genetically modified organisms, such as, *E. coli* cells. In some cases, the growth phase is initiated by the addition of phosphate to a reaction vessel (together with a carbon source such as sugar and a genetically modified organism such as *E. coli* cell), and the duration of the growth phase is controlled by the amount of phosphate added to the system.

[00310] EXAMPLE 14: THE INDUCTION PHASE

[00311] The induction phase can be controlled by genetic modifications additional genetic modifications. The key enzymes triggered during this phase are engineered using promoters that are sensitive to (*e.g.*, activated by or deactivated by) the depletion of the initiating reactant. As a result, once the initiating reactant is depleted, the growth phase ends, and the additional enzymes are activated and the induction phase begins.

[00312] The induction phase can be controlled by genes that encode for enzymes in the biosynthetic pathway for the product by using promoters that are activated by for example, phosphate depletion. When desiring to produce 3-HP, *E. coli* can be genetically modified by manipulating one or more of the following genes: mcr, mmsB, ydfG, rutE, nemA and NDSD; genes that encode individual or fused subunits of ACCase, such as accA, accB, accC, accD, accDA fusion, and accCB fusion, and the promoters may include one or more of the promoters

that direct expression of the following *E. coli* genes: amn, tktB, xasA, yibD, ytfK, pstS, phoH, phnC, or other phosphate-regulated genes.

[00313] EXAMPLE 15: THE PRODUCTION PHASE

[00314] The production phase may also be controlled by genetic modifications. For example, the organism is engineered to include mutated forms of enzymes for initiating production of the chemical product. These initiation enzymes facilitate initiation of production either by: (1) becoming active and serving a key function in the production pathway; and/or (2) becoming inactive and thereby turning off a branch pathway or other competitive pathway that prevents or limits the production pathway leading to the chemical product, for example, 3-HP.

[00315] For example, the initiation enzymes are mutated to form temperature sensitive variants of the enzymes that are either activated by or deactivated at certain temperatures. As a result, the production phase is initiated by changing the changing the temperature within the reaction vessel. For example, the production phase is controlled by genetically modifying the organism with a heterologous nucleotide sequence encoding for one or more of the following temperature sensitive enzymes: $fabI^{ts}$ (SEQ ID NO. 27), $fabB^{ts}$ (SEQ ID NO.28) and $fabD^{ts}$ (SEQ ID NO. 29). These enzymes are deactivated or shut-off at the desired temperature for production of the chemical product. Once the reaction vessel temperature is changed, the temperature sensitive enzymes are deactivated and the production pathway of the chemical product is ramped up.

[00316] EXAMPLE 16: CHEMICAL PRODUCTION

[00317] In this example, a genetically modified organism is produced, that is capable of converting a renewable carbon source to a chemical product. The genetically modified organism requires inorganic phosphate for growth and comprises at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system. The at least one heterologous gene converts a carbon source to a chemical product and is not required for the genetically modified organism to replicate. The genetically modified organism also comprises a gene encoding a temperature-sensitive enzyme.

[00318] In this example, a culture system comprising a carbon source in an aqueous medium and the genetically modified organism is created. The culture system is maintained under conditions that allow the genetically modified microorganism to replicate. A sufficient level of inorganic phosphate within said culture system is also maintained. However, once the inorganic phosphate is allowed to be depleted, the expression of the gene regulated by a promoter sensitive to inorganic phosphate levels is triggered. The temperature of the culture system is also altered thereby activating or deactivating said temperature-sensitive enzyme and initiating the production of the desired chemical product.

[00319] EXAMPLE 17: PRODUCING 3-HP

[00320] A genetically modified organism capable of converting a renewable carbon source to 3-HP is produced. The genetically modified organism comprises at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system. The specific gene regulated by the phosphate sensitive promoter is selected from the group consisting of mcr, mmsB, ydfG, rutE, nemA, NDSD, accA, accB, accC, accD, accDA fusion, and accCB fusion. Also included in the genetically modified organism is a gene encoding a temperature-sensitive enzyme selected from the group consisting of fabI, fabB and fabD.

[00321] The genetically modified organism is combined with a carbon source in an aqueous medium comprising phosphate. This initiates the growth phase during which the genetically modified microorganism replicates. The level of inorganic phosphate is maintained until the desired level of cell growth is achieved. By allowing the inorganic phosphate to become depleted, initiates an induction phase which begins the expression of said gene regulated by a promoter sensitive to inorganic phosphate levels. Additionally, changing the temperature of the culture system, activates or deactivates said temperature-sensitive enzyme and initiating a growth phase during which said genetically modified microorganism produces 3-HP.

[00322] EXAMPLE 18: FERMENTATION CONDITIONS

[00323] The growth phase can last between about 10 to about 40 hours, or about 15 to about 35 hours, or about 20 to about 30 hours. The induction phase can last between about 1 to about 6 hours, about 1 to about 5 hours, or about 2 to about 4 hours. The production phase can last from and from about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 hours depending on the amount of chemical product that is desired.

[00324] The growth phase and induction phase are conducted at a temperature of about 25°C to about 35°C, about 28°C to about 32°C, or about 30 °C. The production phase is conducted at a temperature of about 35°C to about 45°C, about 35°C to about 40°C, or about 36°C to about 38 °C. The production phase temperature is typically higher than the induction phase temperature, and the increase in temperature that initiates the production phase occurs over a period of about 1 to about 5 hours, about 1 to about 3 hours, about 2 hours, or about 1 hour.

[00325] Depending on the host cell fermentation may be performed under aerobic, microaerobic, or anaerobic conditions, with or without agitation.

[00326] Suitable pH ranges for fermentation depend on the multiple factors such as the host cell. In some applications of the invention fermentation can occur between various pH ranges for example, pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, or pH 9.0 to pH 10.0.

[00327] EXAMPLE 19: pH VALUES ON 3-HP INHIBITION

[00328] At pH 7.0, growth occurs up to about 30gr/L3-HP. However, at lower pHs growth in 3-HP becomes more inhibitory. As a result several pH and 3-HP values were screened in this example and compared to controls in order to possibly give greater sensitivity within these assays. In order to do this part of the evaluation, buffered media were evaluated for their overall effect, and then selected for use to evaluate the effect of different pH values on 3-HP inhibition on a microorganism (with or without the genetic modification introducing one or more nucleic acids encoding the proteins described herein). *See* FIG. 12.

[00329] As a carboxylic acid group without a strong electronegative group nearby, 3-HP has a typical pKa ~4.5. Therefore, diffusion across the membrane should be limited at pHs much above this pH. Exporters are used for one or more fermentations that occur at pH above about 4.5.

[00330] EXAMPLE 20: STRUCTURAL CONSIDERATIONS

[00331] MF family proteins typically exhibit common transmembrane spanning structures, although there are varying reports of whether these protein work as monomers, dimer, or trimers. In the case of rhtA, multiple sequence alignments appear to show 10 transmembrane stretches (FIGs. 13A-D). Pileup sequence comparisons for directing protein evolution experiments were conducted (FIGs. 14A-K). Thus, site-saturated mutagenesis for perturbing functional aspects of the protein at specific spots without altering essential residues, are performed. Random mutagenesis are also performed to yield additional mutations for increasing function.

[00332] EXAMPLE 21: EFFLUX PROTEINS

[00333] Accessory protein(s) known to work with these efflux proteins, are increased (*e.g.*, increased performance, expression and/or levels). For example, some accessory proteins include, but are not limited to, out membrane proteins belonging to the Tol family (such as talC) and Omp family (such as oprM).

[00334] Cloning of potential efflux protein genes

[00335] The following genes were PCR amplified from genomic *E.coli* K12 DNA using a phosphorylated 5' containing forward primer and reverse primer containing both SnaBI and SphI sites:

rhtA	emaA	cusA
rht8	ema8	Bcr
rhtC	emrKY	

[00336] Two backbones were prepared by PCR amplification. The first contained a Ptac promoter with ribosome binding site using a pKKll3 vector as template. The second backbone

contains Psh and Ptrc promoters and a ribosome binding site at one end and a SphI site on the opposing end. The template for this PCR was a pJ284:29990 vector produced by the gene synthesis services of DNA2.0.

[00337] For the pKK223 backbone, inserts and backbone were gel purified and blunt end ligated together. Colonies were screened, and plasmids showing positive insertions were cultured and mini prepped. Finally the insets were check for proper orientation and will be sent for sequencing.

[00338] For pJ284 backbones, inserts and backbone pieces were digested with SphI, gel purified, and semi-directly ligated together. Colonies were screened, and plasmids showing positive insertions were cultured and miniprepped. Finally the insets were check for proper orientation and will be sent for sequencing.

[00339] Plasmids of each efflux protein are transformed into BW25113 strains, as well as their corresponding Kieo collection strain containing the Knockout of these genes. In other words, efflux proteins able to transport 3-HP are indentified.

[00340] Strains carrying the plasmids, and control strain (wild type and Keio deletion strains) are cultured in M9 media at several (2 to 3) of 3-HP and pH (2 to 3 levels). OD is checked at 600nm to evaluate growth (using IPTG were appropriate). Cultures showing growth are plated and isolated.

[00341] EXAMPLE 22: MUTAGENESIS FOR INCREASED TOLERANCE TO 3-HP

[00342] Efflux proteins showing the most initial tolerance for 3-HP are subject to random mutagenesis and/or site-directed enzyme evolution to increase functionality for increased tolerance to 3-HP. Random mutagenesis are conducted by epPCR (error prone) and site specific mutagenesis is performed by site-saturation mutagenesis.

[00343] Efficient screening is accomplished by plating transformed cultures on minimal media plates prepared at increasing 3-HP levels and various pH values.

[00344] The best growing colonies are cultured and mutations are sequenced to determine the set of mutations conferring better fitness to 3-HP tolerance. The plasmids carrying gene conferring increased fitness are carried forward to repeated screen to develop greater tolerance in strains, and multiple combinations of the isolated mutations are combined.

[00345] Efflux proteins are also evaluated *in vitro*. In such a case, a simple membrane extraction protocol is used where by bacterial membranes are isolated and extruded into vesicles. These vesicles are used to collect 3-HP under the right conditions in an assay similar to that disclosed in Rayman MK, Lo TC, Sanwal BD. Transport of succinate in Escherichia coli. II.

Characteristics of uptake and energy coupling with transport in membrane preparations. J. Biol. Chem. 1972 Oct 10;247(19):6332-9.

[00346] EXAMPLE 23: DOWNSTREAM CHEMICAL PRODUCTS AND CONSUMER PRODUCTS

[00347] 3-HP purified according to the methods provided in this disclosure are converted to various other products having industrial uses including, but not limited to, acrylic acid, acrylates (*e.g.*, acrylic acid salts and esters), 1,3-propanediol, malonic acid, ethyl-3-hydroxypropionate, ethyl ethoxy propionate, propiolactone, acrylamide, or acrylonitrile, and other chemicals, collectively referred to as "downstream chemical products" or "downstream products." For example, 3-HP is produced and converted to polymerized-3-HP (poly-3-HP) or acrylic acid. 3-HP or acrylic acid are also used to produce polyacrylic acid (polymerized acrylic acid, in various forms), methyl acrylate, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP, malonic acid, 1,3-propanediol, ethyl acrylate, n-butyl acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, 2-ethylhexyl acrylate, and acrylic acid or an acrylic acid ester to which an alkyl or aryl addition are made, and/or to which halogens, aromatic amines or amides, and aromatic hydrocarbons are optionally added. In some instances the conversion is associated with the separation and/or purification steps.

[00348] The downstream chemical products are useful for producing a variety of consumer products. The methods of the present invention include steps to produce downstream products of 3-HP.

[00349] Numerous approaches are employed for such downstream conversions, generally falling into enzymatic, catalytic (chemical conversion process using a catalyst), thermal, and combinations thereof (including some wherein a desired pressure is applied to accelerate a reaction). For example, without being limiting, acrylic acid is made from 3-HP via a dehydration reaction, methyl acrylate is made from 3-HP via dehydration and esterification, the latter to add a methyl group (such as using methanol), acrylamide is made from 3-HP via dehydration and amidation reactions, acrylonitrile is made via a dehydration reaction and forming a nitrile moiety, propiolactone is made from 3-HP via a ring-forming internal esterification reaction, ethyl-3-HP is made from 3-HP via esterification with ethanol, malonic acid is made from 3-HP via an oxidation reaction, and 1,3-propanediol is made from 3-HP via a reduction reaction. Various derivatives of the derivatives of 3-HP and acrylic acid are also made, such as the various known polymers of acrylic acid and its derivatives. Additionally, copolymers containing acrylic acid and/or esters have been widely used in the pharmaceutical formulation to achieve extended or sustained release of active ingredients, for example as

coating material. Downstream compounds may also be converted to consumer products such as diapers, carpet, paint, and adhesives.

[00350] 3-HP is oligomerized or polymerized to form poly(3-hydroxypropionate) homopolymers, or co-polymerized with one or more other monomers to form various co-polymers. Because 3-HP has a single stereoisomer, polymerization of 3-HP is not complicated by the stereo-specificity of monomers during chain growth.

[00351] 3-HP is converted into derivatives starting (i) substantially as the protonated form of 3-hydroxypropionic acid; (ii) substantially as the deprotonated form, 3-hydroxypropionate; or (iii) as mixtures of the protonated and deprotonated forms. Generally, the fraction of 3-HP present as the acid versus the salt will depend on the pH, the presence of other ionic species in solution, temperature (which changes the equilibrium constant relating the acid and salt forms), and, to some extent, pressure. Chemical conversions are carried out from either of the 3-HP forms.

[00352] Acrylic acid obtained from 3-HP purified by the methods described in this disclosure can be further converted to various polymers. For example, the free-radical polymerization of acrylic acid takes place by polymerization methods known to the skilled worker and is carried out, for example, in an emulsion or suspension in aqueous solution or another solvent. Initiators, such as but not limited to organic peroxides, are optionally added to aid in the polymerization. Among the classes of organic peroxides that are used as initiators are diacyls, peroxydicarbonates, monoperoxycarbonates, peroxyketals, peroxyesters, dialkyls, and hydroperoxides. Another class of initiators is azo initiators, which are used for acrylate polymerization as well as co-polymerization with other monomers.

[00353] Co-monomers, such as crosslinkers, are also optionally present during the polymerization. The free-radical polymerization of the acrylic acid obtained from the dehydration of 3-HP, as produced herein, in at least partly neutralized form and in the presence of crosslinkers is also prepared. This polymerization may, in some case, result in hydrogels which are then comminuted, ground and, where appropriate, surface-modified.

[00354] Superabsorbent polymers are prepared from acrylic acid (such as acrylic acid derived from 3-HP provided herein) and a crosslinker, by solution or suspension polymerization. This superabsorbent polymer is used in the manufacture of products requiring absorbents for water and aqueous solutions, such as for diapers, adult incontinence products, feminine hygiene products, and similar consumer products. The superabsorbent polymer particles are also surface-modified to produce a shell structure with the shell being more highly cross-linked than the rest of the particle. This technique improves the balance of absorption, absorption under load, and

resistance to gel-blocking. Superabsorbent polymers are also adapted for use in agriculture, horticulture, and medicine.

[00355] When making diapers and other personal hygiene products, the superabsorbent polymer first is molded into an absorbent pad that are vacuum formed, and in which other materials, such as a fibrous material (*e.g.*, wood pulp) are added. The absorbent pad then is assembled with sheet(s) of fabric, generally a nonwoven fabric (*e.g.*, made from one or more of nylon, polyester, polyethylene, and polypropylene plastics) to form diapers.

[00356] Multiple pressurized nozzles, located above a conveyer belt, spray superabsorbent polymer particles (*e.g.*, about 400 micron size or larger), fibrous material, and/or a combination of these onto the conveyer belt at designated spaces/intervals. The conveyor belt is perforated and under vacuum from below, so that the sprayed on materials are pulled toward the belt surface to form a flat pad. In various embodiments, fibrous material is applied first on the belt, followed by a mixture of fibrous material and the superabsorbent polymer particles, followed by fibrous material, so that the superabsorbent polymer is concentrated in the middle of the pad. A leveling roller is used toward the end of the belt path to yield pads of uniform thickness. Each pad thereafter are further processed, such as to cut it to a proper shape for the diaper, or the pad is in the form of a long roll sufficient for multiple diapers. Thereafter, the pad is sandwiched between a top sheet and a bottom sheet of fabric (one generally being liquid pervious, the other liquid impervious), for example, on a conveyor belt, and these are attached together, for example by gluing, heating or ultrasonic welding, and cut into diaper-sized units (if not previously so cut). Additional features are optionally provided, such as elastic components, strips of tape, etc., for fit and ease of wearing by a person.

[00357] Other disposable absorbent articles are constructed in a similar fashion, such as absorbent articles for adult incontinence, feminine hygiene (sanitary napkins), tampons, etc.

[00358] Low molecular weight polyacrylic acid made by the methods described herein, are used for water treatment, for example, as a flocculant and thickener for various applications including cosmetics and pharmaceutical preparations. For these applications, the polymer is uncrosslinked or lightly cross-linked. The molecular weights are typically from about 200 to about 1,000,000 g/mol.

[00359] Acrylic acid is also co-polymerized with one or more other monomers selected from the group consisting of acrylamide, 2-acrylamido-2-methylpropanesulfonic acid, N,N-dimethylacrylamide, N-isopropylacrylamide, methacrylic acid, and methacrylamide. Co-monomers are also derived from 3-HP, or otherwise provided, to produce co-polymers.

[00360] Acrylic acid is also copolymerized with any free-radically polymerizable monomers including, but not limited to, styrene, butadiene, acrylonitrile, acrylic esters, maleic acid, maleic anhydride, vinyl chloride, acrylamide, and itaconic acid. Acrylic acid (or one of its co-polymerization monomers) are also substituted by any substituent that does not interfere with the polymerization process, such as alkyl, alkoxy, aryl, heteroaryl, benzyl, vinyl, allyl, hydroxy, epoxy, amide, ethers, esters, ketones, maleimides, succinimides, sulfoxides, glycidyl and silyl.

[00361] Acrylic acid and its esters are also formed into paints. A desired mixture of homopolymers and/or copolymers, referred to in the paint industry as "vehicle" (or "binder") are added to an aqueous solution and agitated sufficiently to form an aqueous dispersion that includes sub-micrometer sized polymer particles. The paint cures by coalescence of these vehicle particles as the water and any other solvent evaporate. Other additives to the aqueous dispersion include, but are not limited to, pigment, filler (*e.g.*, calcium carbonate, aluminum silicate), solvent (*e.g.*, acetone, benzol, alcohols, etc., although these are not found in certain no VOC paints), thickener, and additional additives depending on the conditions, applications, intended surfaces, etc.

[00362] Acrylic-based polymers are used for many coatings in addition to paints. For example, for paper coating latexes, acrylic acid is used from 0.1-5.0%, along with styrene and butadiene, to enhance binding to the paper and modify rheology, freeze-thaw stability and shear stability. Acrylate-based polymers also are used in many inks, particularly UV curable printing inks. For water treatment, acrylamide and/or hydroxy ethyl acrylate are commonly co-polymerized with acrylic acid to produce low molecular-weight linear polymers. Co-polymers of acrylic acid with maleic acid or itaconic acid are also produced for water-treatment applications. Sodium acrylate (the sodium salt of glacial acrylic acid) are also co-polymerized with acrylamide (which may be derived from acrylic acid via amidation chemistry) to make an anionic co-polymer that is used as a flocculant in water treatment.

[00363] For thickening agents, a variety of co-monomers are used.

[00364] Conversion to downstream products are also made enzymatically. For example, 3-HP are converted to 3-HP-CoA, which then is converted into polymerized 3-HP with an enzyme having polyhydroxy acid synthase activity (EC 2.3.1.-). Also, 1,3-propanediol is made using polypeptides having oxidoreductase activity or reductase activity (*e.g.* , enzymes in the EC 1.1.1.- class of enzymes). Alternatively, when creating 1,3-propanediol from 3-HP, a combination of (1) a polypeptide having aldehyde dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.34 class) and (2) a polypeptide having alcohol dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.32 class) are used. Polypeptides having lipase activity are used to form esters.

Enzymatic reactions such as these are conducted *in vitro*, such as using cell-free extracts, or i*n vivo*.

[00365] Reactions that form downstream compounds such as acrylates or acrylamides are conducted in conjunction with use of suitable stabilizing agents or inhibiting agents reducing the likelihood of polymer formation. Stabilizing agents and/or inhibiting agents including, but not limited to, *e.g.*, phenolic compounds (*e.g.*, dimethoxyphenol (DMP) or alkylated phenolic compounds such as di-tert-butyl phenol), quinones (*e.g.*, t-butyl hydroquinone or the monomethyl ether of hydroquinone (MEHQ)), and/or metallic copper or copper salts (*e.g.*, copper sulfate, copper chloride, or copper acetate), are used. Inhibitors and/or stabilizers are also used individually or in combinations.

[00366] The one or more downstream compounds are also recovered at a molar yield of up to about 100 percent, or a molar yield in the range from about 70 percent to about 90 percent, or a molar yield in the range from about 80 percent to about 100 percent, or a molar yield in the range from about 90 percent to about 100 percent. Such yields are the result of single-pass (batch or continuous) or iterative separation and purification steps in a particular process.

[00367] In any embodiment herein, a microorganism can comprise at least 10, at least 15, at least 20, at least 25, at least 30, about 10, about 15, about 20, about 25, or about 30 contiguous polynucleotides or amino acids of any one of SEQ ID NOs: 1-100. In any embodiment herein, a microorganism can comprise a polynucleotide or polypeptide having at least: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%; or about: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology to a polynucleotide or polypeptide of any one of SEQ ID NOS; 1-100. In any embodiment herein, a microorganism can comprise a polynucleotide coding for an MCR having at least about: 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology to caMCR, stMCR, oaMCR and/or otMCR. In any embodiment herein, a microorganism can comprise a polynucleotide coding for ACCase or an ACCase and/or any subunit thereof having at least about: 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology to an ACCase or any subunit any subunit thereof. In any embodiment herein, the genetically modified microorganism can have at least one disrupted gene selected from the group consisting of: araD, araB, lacZ, rhaD, rhaB, hsdR, ldhA, pflB, mgsA, poxB, pta-ack, fabI, fabB, fabF, fabD, aldA, aldB, puuC, and any combination thereof. In any embodiment herein, the genetically modified microorganism can comprise an exogenous polynucleotide encoding a malonyl-CoA reductase. In any embodiment herein, the malonyl-CoA reductase can be monofunctional or bifunctional (e.g., monofunctional reduces malonyl-

CoA to malonate semialdehyde, and bifunctional reduces malonyl-CoA to 3-HP. In any embodiment herein, the genetically modified microorganism herein can comprise an acetyl-CoA carboxylase or one or more subunits thereof and/or a polynucleotide encoding these.

[00368] Acrylamide is also produced from 3-HP, for example, via an esterification-amidation-dehydration sequence. Refluxing an alcohol solution of 3-HP in the presence of an acid or Lewis acid catalyst described herein leads to an 3-HP ester. Treatment of the 3-HP ester with either an ammonia gas or an ammonium ion yields 3-HP amide. Finally, dehydration of the 3-HP amide with dehydration reagents described produces acrylamide. The steps mentioned herein are optionally rearranged to produce the same final product acrylamide. Polymerization of acrylamide is also achieved, for example, by radical polymerization. Polyacrylamide polymers are used as additives for treating municipal drinking water and waste water, in addition to applications in gel electrophoresis, oil-drilling, papermaking, ore processing, and the manufacture of permanent press fabrics.

SEQUENCE LISTING

SEQ ID NO. 1: ACCA PROTEIN, HALOMONAS ELONGATA

MNPNYLDFEQPIAELQAKIEELRMVGNDSQVNLSDEIGRLEEKSRKLTES
IFKDLSAWQVSQLSRHPQRPYTLDYLEHVFTDFDELHGDRRFADDAAIVG
GVARLDDKPVMVIGHQKGRDVHEKVRRNFGMPRPEGYRKACRLMEMAERF
HMPVLTFIDTPGAYPGIDAEERGQSEAIAYNLGVMSRLKTPIISTVVGEG
GSGGALAIGVCDELAMLQYSTYSVISPEGCASILWKSADKASEAAQAMGI
TAERLKELGFVDTLIPEPLGGAHRQPSATAERIKTALLESLDRLETMETD
ALLERRYERLMSYGAPV

SEQ ID NO. 2: ACCA NUCLEIC ACID, HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGAATCCGAACTATCTGGACTTTGAACAACCGATCGCTGAACTGCAAGCCAAAATCGAA
GAACTGCGTATGGTGGGCAACGACTCACAGGTGAACCTGTCTGATGAAATTGGCCGTCTG
GAAGAAAAAAGTCGCAAACTGACCGAATCCATCTTTAAAGACCTGTCAGCGTGGCAAGTT
AGCCAACTGTCTCGTCATCCGCAACGCCCGTATACCCTGGATTACCTGGAACATGTCTTT
ACGGATTTCGACGAACTGCACGGTGACCGTCGCTTTGCAGATGACGCGGCCATTGTTGGC
GGTGTCGCTCGTCTGGATGACAAACCGGTCATGGTGATCGGCCATCAGAAAGGTCGTGAT
GTGCACGAAAAAGTTCGTCGCAACTTCGGCATGCCGCGCCCGGAAGGTTATCGTAAAGCG
TGCCGCCTGATGGAAATGGCCGAACGCTTTCACATGCCGGTGCTGACCTTCATTGATACG
CCGGGCGCATATCCGGGTATCGACGCTGAAGAACGTGGCCAAAGCGAAGCGATTGCCTAC
AATCTGGGTGTTATGTCGCGCCTGAAAACCCCGATTATCAGCACGGTGGTTGGCGAAGGC
GGTTCTGGCGGTGCACTGGCTATCGGTGTCTGCGATGAACTGGCGATGCTGCAATATAGT
ACCTACTCCGTGATTTCACCGGAAGGCTGTGCCTCGATCCTGTGGAAAAGCGCAGATAAA
GCTTCTGAAGCAGCTCAAGCGATGGGCATTACCGCCGAACGTCTGAAAGAACTGGGTTTC
GTTGACACGCTGATCCCGGAACCGCTGGGCGGTGCACATCGTCAGCCGAGTGCGACCGCC
GAACGCATTAAAACGGCCCTGCTGGAAAGCCTGGATCGCCTGGAAACGATGGAAACGGAT
GCCCTGCTGGAACGCCGCTATGAACGCCTGATGTCTTACGGTGCCCCGGTCTGA

SEQ ID NO. 3: ACCB PROTEIN, HALOMONAS ELONGATA

MDIRKVKKLIELLEESNISEIEIQEGEESVRISRHPNGTEHPQPAAPAWP
ATAAAPAPQPAAAPVESPAEVDEGPAYQGQPIVSPMVGTFYRAPAPGAKA
FVELGQSVKKGETVCIVEAMKMMNQIEADRDGVVEAILVEDGEPVEFEQP
MVVIS

SEQ ID NO. 4: ACCB NUCLEIC ACID HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGGACATCCGCAAAGTGAAAAAACTGATCGAACTGCTGGAAGAAAGTAACATCTCTGAA
ATTGAAATCCAAGAAGGCGAAGAAAGCGTGCGTATTAGTCGCCATCCGAACGGCACCGAA
CACCCGCAGCCGGCGGCACCGGCATGGCCGGCCACGGCAGCTGCGCCGGCGCCGCAACCG
GCCGCAGCTCCGGTGGAAAGCCCGGCAGAAGTTGATGAAGGCCCGGCTTATCAGGGTCAA
CCGATCGTTTCTCCGATGGTCGGCACCTTTTACCGTGCGCCGGCACCGGGTGCAAAAGCT
TTCGTCGAACTGGGCCAGAGCGTTAAAAAAGGTGAAACGGTCTGCATTGTGGAAGCCATG
AAAATGATGAATCAAATCGAAGCCGATCGCGACGGTGTGGTTGAAGCAATCCTGGTGGAA
GATGGTGAACCGGTGGAATTTGAACAGCCGATGGTGGTGATTAGTTAA

SEQ ID NO. 5: ACCC PROTEIN, HALOMONAS ELONGATA

MLDKVLIANRGEIALRILRACKELGIRTVAVHSKADRELMHVRLADEAVC
IGPASSAQSYLNIPALISAAEVTDTSAIHPGYGFLSENADFAEQVERSGF
TFIGPSAETIRLMGDKVSAINAMKEAGVPTVPGSNGPLGDDEGEILATAR
RIGYPVIIKAAAGGGGRGMRVVHAEGHLLSAVNVTRTEAHSSFGDGTVYM
EKFLENPRHVEVQVLADGQGNAIHLYDRDCSLQRRHQKVLEEAPAPGLDQ
QAREQVFKACRDACVKIGYRGAGTFEFLYENGEFFFIEMNTRVQVEHPVT
EMVTGVDIVREQLRIASGLPLSIRQEDVELSGHAFECRINAEDSRTFMPS
PGRVTLYHPPGGLGVRMDSHVYTGYTVPPHYDSLIGKLITWGDDRETALI
RMRNALDELLVEGIKTNTDLHKDLVRDGYFQQGGVNIHYLEKKLGL

SEQ ID NO. 6: ACCC NUCLEIC ACID, HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGCTGGACAAAGTGCTGATTGCGAATCGTGGCGAAATTGCGCTGCGTATCCTGCGTGCG
TGTAAAGAACTGGGTATCCGTACCGTCGCTGTTCATTCAAAAGCGGACCGTGAACTGATG
CACGTCCGCCTGGCCGATGAAGCAGTGTGCATTGGTCCGGCTAGCTCTGCGCAGTCGTAT
CTGAACATTCCGGCACTGATCAGTGCGGCCGAAGTGACCGACACGTCCGCGATCCATCCG
GGCTACGGTTTCCTGAGCGAAAATGCCGATTTTGCAGAACAAGTCGAACGTTCAGGTTTC
ACCTTTATTGGCCCGTCGGCCGAAACGATCCGCCTGATGGGTGATAAAGTTAGTGCTATT
AACGCGATGAAAGAAGCAGGCGTGCCGACCGTTCCGGGTTCCAATGGTCCGCTGGGTGAT
GACGAAGGTGAAATTCTGGCCACCGCACGTCGCATCGGCTATCCGGTTATTATCAAAGCA
GCTGCGGGCGGTGGCGGTCGTGGTATGCGTGTGGTTCATGCTGAAGGCCACCTGCTGAGC
GCGGTCAACGTGACCCGTACGGAAGCGCATAGTTCCTTCGGCGATGGCACCGTTTATATG

GAAAAATTTCTGGAAAACCCGCGTCACGTTGAAGTCCAGGTGCTGGCCGATGGCCAGGGT
AATGCAATTCATCTGTACGATCGCGACTGCTCTCTGCAACGTCGCCACCAAAAAGTGCTG
GAAGAAGCTCCGGCACCGGGTCTGGACCAGCAAGCACGTGAACAGGTTTTTAAAGCCTGC
CGCGATGCATGTGTCAAAATTGGTTATCGTGGCGCGGGCACCTTCGAATTTCTGTACGAA
AACGGCGAATTTTTCTTTATCGAAATGAATACGCGCGTTCAGGTCGAACATCCGGTGACC
GAAATGGTCACGGGTGTGGATATTGTTCGTGAACAGCTGCGTATCGCATCAGGTCTGCCG
CTGTCGATTCGCCAAGAAGACGTTGAACTGAGCGGTCATGCCTTCGAATGTCGTATCAAT
GCAGAAGATAGCCGCACCTTTATGCCGTCTCCGGGTCGTGTCACGCTGTATCACCCGCCG
GGCGGTCTGGGTGTCCGTATGGACAGCCATGTGTATACCGGCTACACGGTTCCGCCGCAC
TACGATTCTCTGATTGGTAAACTGATCACCTGGGGCGATGACCGTGAAACGGCTCTGATT
CGTATGCGCAACGCCCTGGATGAACTGCTGGTTGAAGGCATCAAAACCAATACGGATCTG
CACAAAGACCTGGTTCGCGATGGCTACTTTCAGCAAGGCGGTGTCAACATTCACTACCTG
GAAAAAAAACTGGGTCTGTAA

SEQ ID NO. 7: ACCD PROTEIN, HALOMONAS ELONGATA

MSWLDKIVPSVGRIQRKERRTSVPDGLWRKCPKCESVLYLPELEKHHNVC
PKCDHHLRLTARKRLDWFLDKEGREEIAADLEPVDRLKFRDSKKYKDRLS
AAQKATGEKDGLVAMRGTLEGLPVVAVAFEFTFMGGSMGAVVGEKFVRAA
TQALDEGVPLVCFSASGGARMQEALFSLMQMAKTSAALEKLKQAGVPYIS
VLTDPVFGGVSASLAMLGDLNIAEPNALIGFAGPRVIEQTVREQLPEGFQ
RSEFLLEHGAVDMIVHRQQIRERLGGVLRKLTHQPASGPAVVENDEPDLV
DAAEQAEPQPEAPEAVETSESEAPTEKGVEADSEETDESPRSGDNR

SEQ ID NO. 8: ACCD NUCLEIC ACID, HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGTCTTGGTTAGATAAAATCGTCCCGTCAGTGGGTCGCATTCAACGCAAAGAACGTCGC
ACCTCGGTCCCGGATGGTCTGTGGCGTAAATGCCCGAAATGTGAATCAGTTCTGTATCTG
CCGGAACTGGAAAAACATCACAACGTCTGCCCGAAATGTGATCATCACCTGCGTCTGACC
GCGCGTAAACGCCTGGACTGGTTCCTGGATAAAGAAGGCCGCGAAGAAATTGCGGCCGAC
CTGGAACCGGTGGATCGTCTGAAATTTCGCGACAGCAAAAAATACAAAGATCGTCTGAGC
GCGGCGCAGAAAGCAACCGGTGAAAAAGACGGTCTGGTGGCCATGCGTGGCACGCTGGAA
GGTCTGCCGGTGGTTGCAGTTGCTTTTGAATTTACCTTTATGGGCGGTAGCATGGGCGCA
GTCGTGGGTGAAAAATTCGTTCGTGCGGCCACGCAGGCTCTGGATGAAGGTGTGCCGCTG
GTTTGCTTCAGCGCATCTGGCGGTGCCCGCATGCAGGAAGCACTGTTTAGTCTGATGCAA

ATGGCTAAAACCTCCGCAGCTCTGGAAAAACTGAAACAGGCGGGCGTGCCGTATATTTCT
GTTCTGACGGACCCGGTCTTCGGCGGTGTGAGTGCGTCCCTGGCCATGCTGGGTGATCTG
AACATTGCAGAACCGAATGCTCTGATCGGCTTTGCGGGTCCGCGTGTCATCGAACAGACC
GTGCGCGAACAACTGCCGGAAGGCTTCCAGCGTTCAGAATTTCTGCTGGAACATGGTGCC
GTTGATATGATTGTCCACCGTCAGCAAATCCGTGAACGCCTGGGCGGTGTGCTGCGCAAA
CTGACGCATCAACCGGCATCGGGTCCGGCCGTTGTCGAAAATGATGAACCGGACCTGGTC
GATGCGGCCGAACAGGCAGAACCGCAACCGGAAGCACCGGAAGCTGTTGAAACCTCAGAA
TCGGAAGCACCGACGGAAAAAGGCGTGGAAGCAGACTCGGAAGAAACGGATGAATCACCG
CGCTCAGGCGACAACCGCTAA

SEQ ID NO. 9: FUSION ACCCB PROTEIN, E. COLI

MLDKIVIANRGEIALRILRACKELGIKTVAVHSSADRDLKHVLLADETVC
IGPAPSVKSYLNIPAIISAAEITGAVAIHPGYGFLSENANFAEQVERSGF
IFIGPKAETIRLMGDKVSAIAAMKKAGVPCVPGSDGPLGDDMDKNRAIAK
RIGYPVIIKASGGGGGRGMRVVRGDAELAQSISMTRAEAKAAFSNDMVYM
EKYLENPRHVEIQVLADGQGNAIYLAERDCSMQRRHQKVVEEAPAPGITP
ELRRYIGERCAKACVDIGYRGAGTFEFLFENGEFYFIEMNTRIQVEHPVT
EMITGVDLIKEQLRIAAGQPLSIKQEEVHVRGHAVECRINAEDPNTFLPS
PGKITRFHAPGGFGVRWESHIYAGYTVPPYYDSMIGKLICYGENRDVAIA
RMKNALQELIIDGIKTNVDLQIRIMNDENFQHGGTNIHYLEKKLGLQEKD
IRKIKKLIELVEESGISELEISEGEESVRISRAAPAASFPVMQQAYAAPM
MQQPAQSNAAAPATVPSMEAPAAAEISGHIVRSPMVGTFYRTPSPDAKAF
IEVGQKVNVGDTLCIVEAMKMMNQIEADKSGTVKAILVESGQPVEFDEPL
VVIE

SEQ ID NO. 10: FUSION ACCCB NUCLEIC ACID, E. COLI

ATGCTGGACAAGATCGTCATCGCCAACCGCGGCGAAATCGCCCTGCGCATCTTGCGCGCG
TGTAAAGAGCTGGGCATTAAGACTGTTGCCGTGCATTCCAGCGCAGACCGCGACCTGAAG
CATGTTCTGCTGGCCGACGAAACGGTTTGCATCGGTCCGGCACCGAGCGTGAAAAGCTAT
CTGAACATCCCGGCCATCATCTCTGCGGCAGAGATCACCGGTGCAGTGGCGATTCATCCG
GGCTACGGTTTCCTGAGCGAGAACGCTAACTTTGCTGAACAAGTGGAGCGTAGCGGTTTC
ATCTTCATTGGCCCTAAGGCGGAGACGATTCGCCTGATGGGCGACAAAGTGAGCGCCATT
GCAGCGATGAAAAAGGCCGGTGTGCCGTGTGTTCCGGGCAGCGATGGTCCGCTGGGTGAC
GATATGGACAAGAACCGTGCCATCGCTAAACGTATTGGCTACCCGGTCATTATCAAAGCC

TCTGGTGGTGGCGGTGGCCGTGGTATGCGTGTCGTCCGTGGTGATGCGGAACTGGCGCAA
AGCATCAGCATGACCCGTGCGGAAGCCAAAGCGGCGTTCTCTAACGATATGGTGTATATG
GAGAAGTATCTGGAGAATCCGCGCCACGTTGAAATCCAAGTTCTGGCGGATGGTCAGGGC
AATGCGATCTACTTGGCAGAACGTGATTGCTCCATGCAACGCCGTCATCAGAAGGTGGTG
GAAGAGGCACCGGCTCCGGGTATTACGCCGGAACTGCGTCGCTACATCGGTGAGCGCTGT
GCGAAAGCGTGTGTGGACATTGGTTACCGTGGTGCGGGTACGTTTGAGTTCCTGTTCGAA
AATGGTGAGTTTTACTTCATTGAAATGAATACCCGCATCCAGGTTGAGCACCCGGTGACC
GAGATGATTACTGGCGTTGATCTGATCAAAGAGCAACTGCGCATTGCGGCTGGTCAGCCG
CTGTCGATCAAGCAAGAAGAGGTGCACGTTCGTGGTCACGCGGTCGAGTGCCGTATCAAT
GCGGAGGACCCGAATACCTTTCTGCCGAGCCCTGGTAAGATCACTCGTTTTCACGCGCCA
GGTGGTTTTGGCGTTCGTTGGGAGTCTCACATCTACGCCGGTTACACCGTGCCGCCGTAC
TATGACAGCATGATTGGTAAACTGATCTGCTATGGCGAAAATCGTGATGTCGCGATCGCC
CGCATGAAAAACGCGCTGCAAGAGCTGATCATTGATGGCATTAAGACCAATGTGGATTTG
CAGATCCGCATTATGAACGACGAGAATTTCCAGCACGGCGGTACGAACATTCACTACCTG
GAAAAGAAACTGGGCCTGCAAGAGAAAGACATCCGCAAGATCAAGAAGCTGATCGAACTG
GTGGAAGAGTCTGGCATCAGCGAGCTGGAGATCAGCGAAGGTGAAGAGAGCGTCCGTATT
TCCCGTGCGGCACCGGCAGCGAGCTTTCCGGTTATGCAGCAAGCATACGCCGCTCCGATG
ATGCAACAGCCGGCACAGAGCAACGCCGCTGCACCGGCGACCGTTCCAAGCATGGAGGCA
CCGGCAGCGGCCGAGATTTCGGGTCATATCGTGCGTAGCCCGATGGTGGGCACCTTCTAT
CGCACGCCGTCGCCGGACGCAAAAGCCTTCATCGAAGTCGGCCAGAAGGTCAATGTCGGC
GACACGCTGTGTATCGTTGAGGCAATGAAAATGATGAACCAGATTGAAGCGGATAAGAGC
GGTACTGTTAAAGCGATCCTGGTGGAATCCGGCCAGCCTGTTGAGTTCGATGAACCGCTG
GTTGTGATCGAGTAA

SEQ ID NO. 11: FUSION ACCDA PROTEIN, E. COLI

MSWIERIKSNITPTRKASIPEGVWTKCDSCGQVLYRAELERNLEVCPKCD
HHMRMTARNRLHSLLDEGSLVELGSELEPKDVLKFRDSKKYKDRLASAQK
ETGEKDALVVMKGTLYGMPVVAAAFEFAFMGGSMGSVVGARFVRAVEQAL
EDNCPLICFSASGGARMQEALMSLMQMAKTSAALAKMQERGLPYISVLTD
PTMGGVSASFAMLGDLNIAEPKALIGFAGPRVIEQTVREKLPPGFQRSEF
LIEKGAIDMIVRRPEMRLKLASILAKLMNLPAPNPEAPREGVVVPPVPDQ
EPEALSGGGGSGGGGSGGGGSGGGGSAAASLNFLDFEQPIAELEAKIDSL
TAVSRQDEKLDINIDEEVHRLREKSVELTRKIFADLGAWQIAQLARHPQR

PYTLDYVRLAFDEFDELAGDRAYADDKAIVGGIARLDGRPVMIIGHQKGR

ETKEKIRRNFGMPAPEGYRKALRLMQMAERFKMPIITFIDTPGAYPGVGA

EERGQSEAIARNLREMSRLGVPVVCTVIGEGGSGGALAIGVGDKVNMLQY

STYSVISPEGCASILWKSADKAPLAAEAMGIIAPRLKELKLIDSIIPEPL

GGAHRNPEAMAASLKAQLLADLADLDVLSTEDLKNRRYQRLMSYGYA

SEQ ID NO. 12: FUSION ACCDA NUCLEIC ACID; E. COLI

ATGAGCTGGATCGAGCGCATCAAGAGCAACATCACCCCGACCCGCAAGGCGAGCATCCCT

GAAGGCGTCTGGACCAAATGCGATAGCTGCGGTCAGGTTTTGTATCGTGCGGAGCTGGAG

CGTAACCTGGAAGTGTGCCCGAAATGCGACCATCACATGCGTATGACCGCTCGTAATCGT

CTGCATAGCCTGCTGGATGAGGGCAGCCTGGTCGAGCTGGGTAGCGAACTGGAACCGAAA

GATGTTCTGAAATTCCGTGATTCCAAGAAGTATAAGGATCGTTTGGCATCTGCACAAAAA

GAAACCGGTGAGAAGGACGCACTGGTTGTTATGAAAGGCACCCTGTATGGTATGCCGGTT

GTTGCTGCGGCGTTCGAGTTTGCGTTTATGGGTGGCAGCATGGGTTCCGTGGTGGGCGCA

CGCTTTGTGCGTGCCGTGGAGCAGGCGCTGGAGGATAACTGTCCTCTGATTTGTTTCAGC

GCGAGCGGTGGTGCGCGTATGCAAGAGGCCCTGATGAGCCTGATGCAGATGGCAAAAACC

TCGGCAGCCCTGGCGAAGATGCAAGAACGCGGCCTGCCGTACATTTCCGTCCTGACCGAC

CCTACGATGGGCGGTGTCAGCGCCAGCTTTGCGATGCTGGGTGATTTGAACATCGCAGAG

CCGAAGGCTCTGATTGGTTTTGCTGGTCCGCGTGTTATTGAACAGACGGTTCGCGAAAAG

TTGCCGCCTGGTTTCCAGCGCAGCGAGTTCCTGATTGAGAAAGGTGCCATCGACATGATC

GTTCGCCGTCCAGAAATGCGTCTGAAACTGGCGAGCATTCTGGCGAAATTGATGAATCTG

CCGGCTCCGAATCCTGAAGCACCGCGTGAGGGTGTCGTGGTTCCGCCGGTCCCGGACCAA

GAGCCGGAGGCTCTGAGCGGCGGAGGTGGCTCTGGTGGAGGCGGTTCAGGAGGCGGTGGC

AGTGGTGGCGGCGGATCTGCGGCAGCTTCTCTGAACTTCCTGGACTTCGAGCAGCCGATC

GCCGAACTGGAGGCGAAGATTGACAGCCTGACCGCGGTTAGCCGTCAAGATGAGAAACTG

GACATTAACATCGACGAAGAGGTCCACCGTTTGCGTGAGAAGTCTGTTGAACTGACTCGC

AAAATCTTTGCTGATTTGGGCGCATGGCAGATTGCCCAGTTGGCTCGCCACCCACAACGC

CCATATACCCTGGACTACGTGCGCCTGGCGTTTGACGAGTTCGACGAACTGGCAGGCGAC

CGCGCCTATGCGGACGATAAAGCAATTGTCGGCGGTATTGCTCGTTTGGATGGCCGTCCG

GTGATGATTATCGGCCATCAAAAAGGCCGCGAGACAAAGGAAAAGATTCGTCGTAATTTC

GGAATGCCGGCACCGGAGGGCTACCGCAAGGCCCTGCGTCTGATGCAAATGGCCGAACGC

TTTAAGATGCCGATTATCACGTTCATTGATACGCCTGGTGCGTACCCAGGCGTTGGTGCG

GAAGAGCGTGGTCAGAGCGAGGCCATCGCACGTAACCTGCGTGAGATGTCTCGTCTGGGT

GTGCCGGTCGTTTGCACCGTGATTGGCGAGGGCGGTAGCGGTGGTGCGTTGGCGATCGGT
GTCGGTGATAAGGTCAACATGCTGCAATACAGCACGTACAGCGTCATTAGCCCGGAAGGT
TGCGCTTCCATTCTGTGGAAGAGCGCGGATAAAGCACCATTGGCAGCGGAAGCGATGGGT
ATCATCGCACCGCGTCTGAAAGAACTGAAGTTGATTGATTCTATCATCCCGGAACCGCTG
GGCGGTGCTCACCGTAATCCGGAGGCGATGGCAGCCAGCCTGAAGGCCCAGCTGCTGGCG
GACCTGGCGGATCTGGACGTGCTGAGCACGGAGGATCTGAAAAACCGTCGCTATCAGCGC
TTGATGAGCTATGGCTACGCTTAA

SEQ ID NO. 13: BICA PROTEIN, SYNECHOCOCCUS SP.

MQITNKIHFRNIRGDIFGGLTAAVIALPMALAFGVASGAGAEAGLWGAVL
VGFFAALFGGTPTLISEPTGPMTVVMTAVIAHFTASAATPEEGLAIAFTV
VMMAGVFQIIFGSLKLGKYVTMMPYTVISGFMSGIGIILVILQLAPFLGQ
ASPGGGVIGTLQNLPTLLSNIQPGETALALGTVAIIWFMPEKFKKVIPPQ
LVALVLGTVIAFFVFPPEVSDLRRIGEIRAGFPELVRPSFSPVEFQRMIL
DAAVLGMLGCIDALLTSVVADSLTRTEHNSNKELIGQGLGNLFSGLFGGI
AGAGATMGTVVNIQSGGRTALSGLVRAFVLLVVILGAASLTATIPLAVLA
GIAFKVGVDIIDWSFLKRAHEISPKGALIMYGVILLTVLVDLIVAVGVGV
FVANVLTIERMSNLQSEKVQTVSDADDNIRLTTTEKRWLDEGQGRVLLFQ
LSGPMIFGVAKAIAREHNAMGDCDALVFDIGEVPHMGVTASLALENAIEE
ALDKERQVYIVGAAGQTRRRLEKLKLFKRVPPDKCLMSREEALKNAVLGI
YPHLADGVTAPSSEMG

SEQ ID NO. 14: BICA NUCLEIC ACID, SYNECHOCOCCUS SP.

ATGCAGATTACCAACAAGATCCATTTCCGTAACATTCGTGGCGACATTTTTGGTGGCCTG
ACCGCTGCTGTGATTGCGCTGCCGATGGCACTGGCTTTTGGTGTGGCAAGTGGTGCAGGT
GCAGAAGCAGGTCTGTGGGGTGCAGTTCTGGTGGGCTTTTTCGCAGCACTGTTCGGTGGT
ACGCCGACCCTGATTTCAGAACCGACGGGCCCGATGACCGTGGTTATGACGGCCGTGATC
GCACATTTTACCGCATCGGCAGCTACGCCGGAAGAAGGCCTGGCTATTGCGTTCACCGTC
GTGATGATGGCCGGTGTTTTTCAGATTATCTTCGGCAGCCTGAAACTGGGCAAGTATGTT
ACCATGATGCCGTACACGGTCATCAGTGGTTTTATGTCCGGTATTGGCATTATCCTGGTG
ATCCTGCAGCTGGCACCGTTCCTGGGTCAAGCCAGTCCGGGCGGTGGCGTTATTGGCACC
CTGCAGAACCTGCCGACGCTGCTGTCCAATATCCAACCGGGTGAAACCGCCCTGGCACTG
GGTACGGTCGCGATTATCTGGTTCATGCCGGAAAAGTTCAAGAAGGTTATCCCGCCGCAG
CTGGTTGCGCTGGTTCTGGGCACCGTCATCGCGTTTTTCGTGTTTCCGCCGGAAGTTAGC

GATCTGCGTCGCATTGGCGAAATCCGTGCAGGTTTCCCGGAACTGGTGCGTCCGAGCTTT
TCTCCGGTTGAATTTCAGCGCATGATTCTGGATGCGGCCGTGCTGGGCATGCTGGGTTGC
ATCGATGCGCTGCTGACCAGCGTTGTCGCCGACTCTCTGACGCGTACCGAACATAACAGC
AATAAAGAACTGATTGGTCAGGGCCTGGGTAACCTGTTTTCTGGCCTGTTCGGTGGTATT
GCTGGTGCAGGTGCAACGATGGGCACCGTGGTTAATATCCAAAGTGGTGGCCGTACCGCA
CTGTCCGGTCTGGTGCGTGCTTTTGTTCTGCTGGTCGTGATTCTGGGTGCAGCTTCTCTG
ACGGCAACCATTCCGCTGGCTGTGCTGGCAGGCATCGCCTTTAAAGTGGGTGTTGATATT
ATCGACTGGTCATTCCTGAAACGCGCCCACGAAATCTCGCCGAAGGGCGCACTGATTATG
TATGGTGTGATCCTGCTGACCGTCCTGGTGGATCTGATTGTTGCGGTCGGCGTGGGTGTT
TTTGTCGCCAACGTTCTGACCATCGAACGTATGTCAAATCTGCAGTCGGAAAAAGTCCAA
ACCGTGAGCGATGCGGATGACAACATTCGCCTGACCACGACCGAAAAGCGTTGGCTGGAC
GAGGGTCAGGGTCGTGTGCTGCTGTTTCAACTGTCTGGCCCGATGATTTTCGGTGTTGCA
AAAGCTATCGCGCGTGAACATAACGCAATGGGTGATTGCGACGCTCTGGTGTTTGATATT
GGCGAAGTCCCGCACATGGGTGTGACCGCAAGTCTGGCTCTGGAAAATGCGATTGAAGAA
GCCCTGGACAAAGAACGCCAGGTTTACATCGTCGGTGCAGCAGGTCAAACCCGTCGCCGT
CTGGAAAAACTGAAGCTGTTTAAACGCGTGCCGCCGGATAAGTGTCTGATGTCCCGTGAA
GAAGCACTGAAGAATGCTGTTCTGGGTATCTATCCGCATCTGGCTGACGGTGTTACGGCT
CCGAGTTCCGAAATGGGCTAA

SEQ ID NO. 15: MCR DNA ORGANISM NAME: SULFOLOBUS TOKODAII

ATGTCTCGTC GCACCCTGAA AGCGGCTATC CTGGGCGCCA CCGGCCTGGT TGGTATCGAA
60

TATGTCCGTA TGCTGTCAAA TCATCCGTAT ATCAAACCGG CGTATCTGGC CGGCAAAGGT
120

TCAGTTGGCA AACCGTACGG TGAAGTGGTT CGTTGGCAGA CCGTTGGCCA AGTCCCGAAA
180

GAAATCGCCG ATATGGAAAT TAAACCGACG GACCCGAAAC TGATGGATGA CGTGGATATT
240

ATCTTTTCGC CGCTGCCGCA GGGTGCGGCC GGTCCGGTTG AAGAACAATT TGCAAAAGAA
300

GGCTTCCCGG TCATCAGCAA CTCTCCGGAT CATCGTTTCG ATCCGGACGT CCCGCTGCTG
360

GTGCCGGAAC TGAATCCGCA CACCATTAGT CTGATCGATG AACAGCGCAA ACGTCGCGAA
420

TGGAAAGGTT TTATTGTTAC CACGCCGCTG TGCACGGCAC AAGGTGCAGC TATCCCGCTG
480

GGTGCTATCT TCAAAGATTA CAAAATGGAC GGCGCGTTCA TTACCACGAT CCAGAGTCTG
540

TCCGGTGCAG GTTACCCGGG TATCCCGTCT CTGGATGTCG TGGACAACAT TCTGCCGCTG
600

GGCGATGGTT ATGACGCGAA AACCATTAAA GAAATCTTCC GTATTCTGTC AGAAGTTAAA
660

CGCAATGTCG ATGAACCGAA ACTGGAAGAC GTTTCGCTGG CGGCCACCAC GCATCGTATC
720

GCCACCATTC ATGGCCACTA TGAAGTGCTG TACGTTAGTT TTAAAGAAGA AACCGCAGCT
780

GAAAAAGTGA AAGAAACGCT GGAAAACTTC CGCGGTGAAC CGCAGGATCT GAAACTGCCG
840

ACCGCACCGT CCAAACCGAT TATCGTCATG AATGAAGATA CGCGTCCGCA AGTGTACTTT
900

GATCGCTGGG CTGGCGACAT TCCGGGTATG AGCGTTGTCG TGGGCCGTCT GAAACAGGTG
960

AACAAACGTA TGATCCGCCT GGTGTCTCTG ATTCACAATA CCGTTCGCGG TGCGGCGGGC
1020

GGTGGCATCC TGGCTGCTGA ACTGCTGGTT GAAAAAGGTT ACATTGAAAA A 1071

<212> TYPE : DNA

<211> LENGTH : 1071

SEQUENCENAME : SULFOLOBUS TOKODAII MCR DNA

SEQ ID NO. 16: MCR PROTEIN ORGANISM NAME : SULFOLOBUS TOKODAII

MSRRTLKAAI LGATGLVGIE YVRMLSNHPY IKPAYLAGKG SVGKPYGEVV RWQTVGQVPK
60

EIADMEIKPT DPKLMDDVDI IFSPLPQGAA GPVEEQFAKE GFPVISNSPD HRFDPDVPLL 120

VPELNPHTIS LIDEQRKRRE WKGFIVTTPL CTAQGAAIPL GAIFKDYKMD GAFITTIQSL 180

SGAGYPGIPS LDVVDNILPL GDGYDAKTIK EIFRILSEVK RNVDEPKLED VSLAATTHRI 240

ATIHGHYEVL YVSFKEETAA EKVKETLENF RGEPQDLKLP TAPSKPIIVM NEDTRPQVYF 300

DRWAGDIPGM SVVVGRLKQV NKRMIRLVSL IHNTVRGAAG GGILAAELLV EKGYIEK 357

<212> TYPE : PRT

<211> LENGTH : 357

SEQUENCENAME : SULFOLOBUS TOKODAII MCR PROTEIN

SEQ ID NO. 17 : NEMA DNA ORGANISM NAME : ESCHERICHIA COLI

ATGTCATCTG AAAAACTGTA TTCCCCACTG AAAGTGGGCG CGATCACGGC GGCAAACCGT

60

ATTTTTATGG CACCGCTGAC GCGTCTGCGC AGTATTGAAC CGGGTGACAT TCCTACCCCG

120

TTGATGGCGG AATACTATCG CCAACGTGCC AGTGCCGGTT TGATTATTAG TGAAGCCACG

180

CAAATTTCTG CCCAGGCAAA AGGATATGCA GGTGCGCCTG GCATCCATAG TCCGGAGCAA

240

ATTGCCGCAT GGAAAAAAAT CACCGCTGGC GTTCATGCTG AAAATGGTCA TATGGCCGTG

300

CAGCTGTGGC ACACCGGACG CATTTCTCAC GCCAGCCTGC AACCTGGCGG TCAGGCACCG

360

GTAGCGCCTT CAGCACTTAG CGCGGGAACA CGTACTTCTC TGCGCGATGA AAATGGTCAG

420

GCGATCCGTG TTGAAACATC CATGCCGCGT GCGCTTGAAC TGGAAGAGAT TCCAGGTATC

480

GTCAATGATT TCCGTCAGGC CATTGCTAAC GCGCGTGAAG CCGGTTTTGA TCTGGTAGAG

540

CTCCACTCTG CTCACGGTTA TTTGCTGCAT CAGTTCCTTT CTCCTTCTTC AAACCATCGT

600

ACCGATCAGT ACGGCGGCAG CGTGGAAAAT CGCGCACGTT TGGTACTGGA AGTGGTCGAT

660

GCCGGGATTG AAGAATGGGG TGCCGATCGC ATTGGCATTC GCGTTTCACC AATCGGTACT

720

TTCCAGAACA CAGATAACGG CCCGAATGAA GAAGCCGATG CACTGTATCT GATTGAACAA

780

CTGGGTAAAC GCGGCATTGC TTATCTGCAT ATGTCAGAAC CAGATTGGGC GGGGGGTGAA
840

CCGTATACTG ATGCGTTCCG CGAAAAAGTA CGCGCCCGTT TCCACGGTCC GATTATCGGC
900

GCAGGTGCAT ACACAGTAGA AAAAGCTGAA ACGCTGATCG GCAAAGGGTT AATTGATGCG
960

GTGGCATTTG GTCGTGACTG GATTGCGAAC CCGGATCTGG TCGCCCGCTT GCAGCGCAAA
1020

GCTGAGCTTA ACCCACAGCG TGCCGAAAGT TTCTACGGTG GCGGCGCGGA AGGCTATACC
1080

GATTACCCGA CGTTGTAA 1098

<212> TYPE : DNA

<211> LENGTH : 1098

SEQUENCENAME : ESCHERICHIA COLI NEMA DNA

SEQ ID NO. 18: NEMA PROTEIN ORGANISM NAME: ESCHERICHIA COLI

MSSEKLYSPL KVGAITAANR IFMAPLTRLR SIEPGDIPTP LMAEYYRQRA SAGLIISEAT 60

QISAQAKGYA GAPGIHSPEQ IAAWKKITAG VHAENGHMAV QLWHTGRISH ASLQPGGQAP
120

VAPSALSAGT RTSLRDENGQ AIRVETSMPR ALELEEIPGI VNDFRQAIAN AREAGFDLVE 180

LHSAHGYLLH QFLSPSSNHR TDQYGGSVEN RARLVLEVVD AGIEEWGADR IGIRVSPIGT
240

FQNTDNGPNE EADALYLIEQ LGKRGIAYLH MSEPDWAGGE PYTDAFREKV RARFHGPIIG
300

AGAYTVEKAE TLIGKGLIDA VAFGRDWIAN PDLVARLQRK AELNPQRAES FYGGGAEGYT
360

DYPTL 365

<212> TYPE : PRT

<211> LENGTH : 365

SEQUENCENAME : ESCHERICHIA COLI NEMA PROTEIN

SEQ ID NO. 19: RUTE DNA ORGANISM NAME: ESCHERICHIA COLI

ATGAACGAAG CCGTTAGCCC AGGTGCGCTT AGCACCCTGT TCACCGATGC CCGCACTCAC
60

AACGGCTGGC GGGAGACACC CGTCAGCGAT GAGACGTTAC GGGAGATTTA TGCCCTGATG
120

AAATGGGGGC CGACATCAGC TAACTGTTCT CCGGCACGGA TCGTGTTTAC CCGCACGGCA
180

GAAGGAAAAG AACGTCTGCG CCCGGCACTT TCCAGCGGCA ATCTGCAAAA AACCCTGACC
240

GCGCCCGTCA CCGCTATCGT CGCCTGGGAC AGTGAATTTT ATGAACGGTT ACCACTACTG
300

TTTCCCCACG GTGATGCCCG CAGTTGGTTT ACCTCCAGCC CACAACTTGC CGAAGAAACA
360

GCGTTTCGCA ACAGTTCCAT GCAGGCGGCC TATCTGATCG TCGCCTGCCG GGCGCTGGGA
420

CTGGATACCG GCCCGATGTC GGGCTTTGAC CGTCAACACG TGGACGACGC CTTTTTTACG
480

GGCAGCACGC TGAAGAGCAA TCTGCTGATT AATATCGGCT ATGGCGATAG CAGCAAGCTT
540

TATGCGCGCC TGCCACGTCT GTCCTTTGAA GAAGCCTGCG GGCTGTTGTA A 591

<212> TYPE : DNA

<211> LENGTH : 591

SEQUENCENAME : ESCHERICHIA COLI RUTE DNA

SEQ ID NO. 20 RUTE PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MNEAVSPGAL STLFTDARTH NGWRETPVSD ETLREIYALM KWGPTSANCS PARIVFTRTA
60

EGKERLRPAL SSGNLQKTLT APVTAIVAWD SEFYERLPLL FPHGDARSWF TSSPQLAEET 120

AFRNSSMQAA YLIVACRALG LDTGPMSGFD RQHVDDAFFT GSTLKSNLLI NIGYGDSSKL
180

YARLPRLSFE EACGLL 196

<212> TYPE : PRT

<211> LENGTH : 196

SEQUENCENAME : ESCHERICHIA COLI RUTE PROTEIN

SEQ ID NO. 21: YDFG DNA ORGANISM NAME : ESCHERICHIA COLI

ATGGTCGTTT TAGTAACTGG AGCAACGGCA GGTTTTGGTG AATGCATTAC TCGTCGTTTT
60

ATTCAACAAG GGCATAAAGT TATCGCCACT GGCCGTCGCC AGGAACGGTT GCAGGAGTTA
120

AAAGACGAAC TGGGAGATAA TCTGTATATC GCCCAACTGG ACGTTCGCAA CCGCGCCGCT
180

ATTGAAGAGA TGCTGGCATC GCTTCCTGCC GAGTGGTGCA ATATTGATAT CCTGGTAAAT
240

AATGCCGGCC TGGCGTTGGG CATGGAGCCT GCGCATAAAG CCAGCGTTGA AGACTGGGAA
300

ACGATGATTG ATACCAACAA CAAAGGCCTG GTATATATGA CGCGCGCCGT CTTACCGGGT
360

ATGGTTGAAC GTAATCATGG TCATATTATT AACATTGGCT CAACGGCAGG TAGCTGGCCG
420

TATGCCGGTG GTAACGTTTA CGGTGCGACG AAAGCGTTTG TTCGTCAGTT TAGCCTGAAT
480

CTGCGTACGG ATCTGCATGG TACGGCGGTG CGCGTCACCG ACATCGAACC GGGTCTGGTG
540

GGTGGTACCG AGTTTTCCAA TGTCCGCTTT AAAGGCGATG ACGGTAAAGC AGAAAAAACC
600

TATCAAAATA CCGTTGCATT GACGCCAGAA GATGTCAGCG AAGCCGTCTG GTGGGTGTCA
660

ACGCTGCCTG CTCACGTCAA TATCAATACC CTGGAAATGA TGCCGGTTAC CCAAAGCTAT
720

GCCGGACTGA ATGTCCACCG TCAGTAA 747

<212> TYPE : DNA

<211> LENGTH : 747

SEQUENCENAME : ESCHERICHIA COLI YDFG DNA

SEQ ID NO. 22: YDFG PROTEIN ORGANISM NAME: ESCHERICHIA COLI

MVVLVTGATA GFGECITRRF IQQGHKVIAT GRRQERLQEL KDELGDNLYI AQLDVRNRAA
60

IEEMLASLPA EWCNIDILVN NAGLALGMEP AHKASVEDWE TMIDTNNKGL VYMTRAVLPG
120

MVERNHGHII NIGSTAGSWP YAGGNVYGAT KAFVRQFSLN LRTDLHGTAV RVTDIEPGLV
180

GGTEFSNVRF KGDDGKAEKT YQNTVALTPE DVSEAVWWVS TLPAHVNINT LEMMPVTQSY
240

AGLNVHRQ 248

<212> TYPE : PRT

<211> LENGTH : 248

SEQUENCENAME : ESCHERICHIA COLI YDFG PROTEIN

SEQ ID NO. 23: MMSB DNA ORGANISM NAME : P. AERUGINOSA

ATGGCCGACA TTGCGTTTCT GGGTCTGGGC AATATGGGCG GTCCGATGGC CGCGAACCTG
60

CTGAAAGCCG GCCACCGTGT GAATGTGTTC GACCTGCAAC CAAAAGCGGT CCTGGGCTTG
120

GTTGAGCAAG GCGCGCAGGG CGCAGACTCT GCTCTGCAAT GTTGTGAGGG TGCGGAGGTC
180

GTGATTTCTA TGCTGCCAGC AGGCCAGCAT GTGGAAAGCC TGTACCTGGG CGATGATGGT
240

CTGCTGGCAC GCGTGGCGGG CAAGCCTTTG CTGATTGACT GTAGCACCAT CGCACCGGAA
300

ACGGCGCGTA AGGTGGCGGA GGCAGCCGCA GCAAAGGGCC TGACGCTGCT GGATGCCCCG
360

GTTTCGGGCG GTGTCGGTGG TGCCCGTGCA GGTACGCTGT CGTTTATCGT GGGTGGTCCG
420

GCGGAGGGTT TTGCGCGTGC GCGTCCGGTT CTGGAGAATA TGGGTCGCAA CATTTTCCAC
480

GCGGGTGATC ACGGCGCTGG TCAGGTGGCG AAAATCTGTA ACAACATGCT GCTGGGTATC
540

TTGATGGCGG GCACCGCCGA AGCCTTGGCG CTGGGCGTCA AAAACGGTCT GGACCCGGCA
600

GTGCTGTCCG AAGTGATGAA ACAGAGCAGC GGTGGTAACT GGGCGCTGAA TCTGTACAAT
660

CCGTGGCCGG GTGTGATGCC GCAGGCCCCA GCCTCTAATG GCTACGCAGG CGGCTTCCAA
720

GTGCGCCTGA TGAACAAAGA CCTGGGCCTG GCGCTGGCGA ATGCGCAAGC GGTCCAAGCG
780

AGCACCCCGC TGGGCGCACT GGCCCGTAAC CTGTTTAGCC TGCACGCTCA AGCCGACGCC
840

GAGCACGAAG GTCTGGACTT CAGCTCTATT CAAAAACTGT ATCGCGGTAA GGATTAG
897

<212> TYPE : DNA

<211> LENGTH : 897

SEQUENCE NAME : P. AERUGINOSA MMSB DNA

SEQ ID NO. 24: MMSB PROTEIN ORGANISM NAME: P. AERUGINOSA

MADIAFLGLG NMGGPMAANL LKAGHRVNVF DLQPKAVLGL VEQGAQGADS ALQCCEGAEV
60

VISMLPAGQH VESLYLGDDG LLARVAGKPL LIDCSTIAPE TARKVAEAAA AKGLTLLDAP 120

VSGGVGGARA GTLSFIVGGP AEGFARARPV LENMGRNIFH AGDHGAGQVA KICNNMLLGI
180

LMAGTAEALA LGVKNGLDPA VLSEVMKQSS GGNWALNLYN PWPGVMPQAP ASNGYAGGFQ
240

VRLMNKDLGL ALANAQAVQA STPLGALARN LFSLHAQADA EHEGLDFSSI QKLYRGKD
298

<212> TYPE : PRT

<211> LENGTH : 298

SEQUENCENAME : P. AERUGINOSA MMSB PROTEIN

SEQ ID NO. 25: NDSD DNA ORGANISMNAME : PSEUDOMONAS AERUGINOSA

ATGGGCAAAC AGATCGCCTT CATCGGCCTG GGCCATATGG GCGCACCTAT GGCCACCAAC
60

CTGCTGAAGG CCGGCTACCT GCTGAATGTG TTCGACCTGG TGCAGAGCGC CGTGGATGGT
120

TTAGTGGCCG CAGGTGCAAG TGCAGCACGC AGTGCACGCG ATGCCGTTCA GGGTGCCGAC
180

GTGGTGATCA GCATGCTGCC TGCCAGCCAA CACGTGGAGG GTCTGTACCT GGACGACGAT
240

GGTCTGCTGG CCCACATTGC CCCTGGCACC TTAGTGCTGG AGTGCAGCAC AATCGCCCCG
300

ACCAGTGCAC GCAAGATTCA TGCAGCAGCC CGCGAGCGTG GTCTGGCAAT GCTGGACGCA
360

CCGGTTAGCG GTGGTACAGC AGGTGCCGCA GCAGGCACCC TGACCTTCAT GGTGGGCGGT
420

GACGCCGAAG CCCTGGAAAA AGCACGCCCG CTGTTTGAGG CAATGGGCCG TAACATCTTC
480

CATGCCGGCC CTGATGGCGC AGGTCAGGTG GCCAAAGTGT GCAATAACCA GCTGCTGGCA
540

GTGCTGATGA TCGGTACCGC CGAGGCAATG GCACTGGGCG TGGCAAACGG CTTAGAGGCC
600

AAGGTGCTGG CAGAAATCAT GCGCCGTAGT AGCGGCGGTA ACTGGGCCCT GGAGGTGTAC
660

AACCCGTGGC CTGGCGTGAT GGAGAATGCA CCGGCCAGTC GTGACTACAG CGGCGGTTTC
720

ATGGCACAGC TGATGGCCAA GGACCTGGGC TTAGCCCAAG AGGCAGCCCA AGCCAGCGCC
780

AGTAGTACCC CGATGGGCAG CTTAGCCCTG AGTCTGTACC GCTTACTGCT GAAGCAGGGC
840

TACGCCGAAC GCGACTTCAG CGTGGTGCAG AAGCTGTTCG ACCCGACCCA AGGCCAGTAA
900

<212> TYPE : DNA

<211> LENGTH : 900

SEQUENCENAME : P. AERUGINOSA NDSD DNA

SEQ ID NO. 26: NDSD PROTEIN PSEUDOMONAS AERUGINOSA

MGKQIAFIGL GHMGAPMATN LLKAGYLLNV FDLVQSAVDG LVAAGASAAR SARDAVQGAD
60

VVISMLPASQ HVEGLYLDDD GLLAHIAPGT LVLECSTIAP TSARKIHAAA RERGLAMLDA 120

PVSGGTAGAA AGTLTFMVGG DAEALEKARP LFEAMGRNIF HAGPDGAGQV AKVCNNQLLA

180

VLMIGTAEAM ALGVANGLEA KVLAEIMRRS SGGNWALEVY NPWPGVMENA PASRDYSGGF

240

MAQLMAKDLG LAQEAAQASA SSTPMGSLAL SLYRLLLKQG YAERDFSVVQ KLFDPTQGQ

299

<212> TYPE : PRT

<211> LENGTH : 299

SEQUENCENAME : P. AERUGINOSA NDSD PROTEIN

SEQ ID NO. 27: FABI(TS) PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MGFLSGKRIL VTGVASKLSI AYGIAQAMHR EGAELAFTYQ NDKLKGRVEE FAAQLGSDIV 60

LQCDVAEDAS IDTMFAELGK VWPKFDGFVH SIGFAPGDQL DGDYVNAVTR EGFKIAHDIS

120

SYSFVAMAKA CRSMLNPGSA LLTLSYLGAE RAIPNYNVMG LAKASLEANV RYMANAMGPE

180

GVRVNAISAG PIRTLAASGI KDFRKMLAHC EAVTPIRRTV TIEDVGNSAA FLCSDLSAGI 240

FGEVVHVDGG FSIAAMNELE LK 262

<212> TYPE : PRT

<211> LENGTH : 262

SEQUENCENAME : ESCHERICHIA COLI FABI(TS) PROTEIN

SEQ ID NO. 28: FABB(TS) PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MKRAVITGLG IVSSIGNNQQ EVLASLREGR SGITFSQELK DSGMRSHVWG NVKLDTTGLI

60

DRKVVRFMSD ASIYAFLSME QAIADAGLSP EAYQNNPRVG LIAGSGGGSP RFQVFGADAM

120

RGPRGLKAVG PYVVTKAMAS GVSACLATPF KIHGVNYSIS SACATSAHCI GNAVEQIQLG

180

KQDIVFAGGG EELCWEMACE FDAMGALSTK YNDTPEKASR TYDAHRDGFV IAGGGGMVVV

240

EELEHALARG AHIYAEIVGY GATSDGADMV APSGEGAVRC MKMAMHGVDT PIDYLNSHGT

300

STPVGDVKEL AAIREVFGDK SPAISATKVM TGHSLGAAGV QEAIYSLLML EHGFIAPSIN 360

IEELDEQAAG LNIVTETTDR ELTTVMSNSF GFGGTNATLV MRKLKD 406

<212> TYPE : PRT

<211> LENGTH : 406

SEQUENCENAME : ESCHERICHIA COLI FABB(TS) PROTEIN

SEQ ID NO. 29: FABD(TS) PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MTQFAFVFPG QGSQTVGMLA DMAASYPIVE ETFAEASAAL GYDLWALTQQ GPAEELNKTW

60

QTQPALLTAS VALYRVWQQQ GGKAPAMMAG HSLGEYSALV CAGVIDFADA VRLVEMRGKF

120

MQEAVPEGTG AMAAIIGLDD ASIAKACEEA AEGQVVSPVN FNSPGQVVIA GHKEAVERAG

180

AACKAAGAKR ALPLPVSVPS HCALMKPAAD KLAVELAKIT FNAPTVPVVN NVDVKCETNG

240

DAIRDALVRQ LYNPVQQTKS VEYMAAQGVE HLYEVGPGKV LTGLTKRIVD TLTASALNEP

300

SAMAAALEL 309

<212> TYPE : PRT

<211> LENGTH : 309

SEQUENCENAME : ESCHERICHIA COLI FABD(TS) PROTEIN

SEQ ID NO. 30: RHTA DNA ORGANISM NAME : ESCHERICHIA COLI

ATGAGCGGTT CATTACGTAA AATGCCGGTC TGGTTACCAA TAGTCATATT GCTCGTTGCC

60

ATGGCGTCTA TTCAGGGTGG AGCCTCGTTA GCTAAGTCAC TTTTTCCTCT GGTGGGCGCA

120

CCGGGTGTCA CTGCGCTGCG TCTGGCATTA GGAACGCTGA TCCTCATCGC GTTCTTTAAG

180

CCATGGCGAC TGCGCTTTGC CAAAGAGCAA CGGTTACCGC TGTTGTTTTA CGGCGTTTCG

240

CTGGGTGGGA TGAATTATCT TTTTTATCTT TCTATTCAGA CAGTACCGCT GGGTATTGCG

300

GTGGCGCTGG AGTTCACCGG ACCACTGGCG GTGGCGCTGT TCTCTTCTCG TCGCCCGGTA
360

GATTTCGTCT GGGTTGTGCT GGCGGTTCTT GGTCTGTGGT TCCTGCTACC GCTGGGGCAA
420

GACGTTTCCC ATGTCGATTT AACCGGCTGT GCGCTGGCAC TGGGGGCCGG GGCTTGTTGG
480

GCTATTTACA TTTTAAGTGG GCAACGCGCA GGAGCGGAAC ATGGCCCTGC GACGGTGGCA
540

ATTGGTTCGT TGATTGCAGC GTTAATTTTC GTGCCAATTG GAGCGCTTCA GGCTGGTGAA
600

GCACTCTGGC ACTGGTCGGT TATTCCATTG GGTCTGGCTG TCGCTATTCT CTCGACCGCT
660

CTGCCTTATT CGCTGGAAAT GATTGCCCTC ACCCGTTTGC CAACACGGAC ATTTGGTACG
720

CTGATGAGCA TGGAACCGGC GCTGGCTGCC GTTTCCGGGA TGATTTTCCT CGGAGAAACA
780

CTGACACCCA TACAGCTACT GGCGCTCGGC GCTATCATCG CCGCTTCAAT GGGGTCTACG
840

CTGACAGTAC GCAAAGAGAG CAAAATAAAA GAATTAGACA TTAATTAA 888

<212> TYPE : DNA

<211> LENGTH : 888

SEQUENCENAME : ESCHERICHIA COLI RHTA DNA

SEQ ID NO. 31: RHTA PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MSGSLRKMPV WLPIVILLVA MASIQGGASL AKSLFPLVGA PGVTALRLAL GTLILIAFFK 60

PWRLRFAKEQ RLPLLFYGVS LGGMNYLFYL SIQTVPLGIA VALEFTGPLA VALFSSRRPV 120

DFVWVVLAVL GLWFLLPLGQ DVSHVDLTGC ALALGAGACW AIYILSGQRA GAEHGPATVA
180

IGSLIAALIF VPIGALQAGE ALWHWSVIPL GLAVAILSTA LPYSLEMIAL TRLPTRTFGT 240

LMSMEPALAA VSGMIFLGET LTPIQLLALG AIIAASMGST LTVRKESKIK ELDIN 295

<212> TYPE : PRT

<211> LENGTH : 295

SEQUENCENAME : ESCHERICHIA COLI RHTA PROTEIN

SEQ ID NO. 32: YDCO DNA ORGANISM NAME : ESCHERICHIA COLI

ATGCGACTCC TCCGGCAAAA CGGAAGTTTA TCACTTGTGC GTTATAACGG ACAAATGCTA
60

CGGTGCCTGT ACGCTATAAC GCACGAGGTG ACTATGCGTC TGTTTTCTAT TCCTCCACCC
120

ACGCTACTGG CGGGGTTTCT GGCGGTATTA ATTGGCTACG CCAGTTCAGC GGCAATAATC
180

TGGCAAGCAG CGATTGTCGC CGGAGCCACC ACTGCACAAA TCTCTGGCTG GATGACGGCG
240

CTGGGGCTGG CAATGGGCGT CAGTACGCTG ACTCTGACAT TATGGTATCG CGTACCTGTT
300

CTCACCGCAT GGTCAACGCC TGGCGCGGCT TTGTTGGTCA CCGGATTGCA GGGACTAACA
360

CTTAACGAAG CCATCGGCGT TTTTATTGTC ACCAACGCGC TAATAGTCCT CTGCGGCATA
420

ACGGGACTCT TTGCTCGTCT GATGCGCATT ATTCCGCACT CGCTTGCGGC GGCAATGCTT
480

GCCGGGATTT TATTACGCTT TGGTTTACAG GCGTTTGCCA GTCTGGACGG TCAATTTACG
540

TTGTGTGGAA GTATGTTGCT GGTATGGCTG GCAACCAAGG CCGTTGCGCC GCGCTATGCG
600

GTAATTGCCG CGATGATTAT TGGGATCGTG ATCGTCATCG CGCAAGGTGA CGTTGTCACA
660

ACTGATGTTG TCTTTAAACC CGTTCTCCCC ACTTATATTA CCCCTGATTT TTCGTTTGCT
720

CACAGCCTGA GCGTTGCACT CCCCCTTTTT CTGGTGACGA TGGCATCGCA AAACGCACCG
780

GGTATCGCAG CAATGAAAGC AGCTGGATAT TCGGCTCCTG TTTCGCCATT AATTGTATTT
840

ACTGGATTGC TGGCACTGGT TTTTTCCCCT TTCGGCGTTT ATTCCGTCGG TATTGCGGCA
900

ATCACCGCGG CTATTTGCCA AAGCCCGGAA GCGCATCCGG ATAAAGATCA ACGTTGGCTG
960

GCCGCTGCCG TTGCAGGCAT TTTCTATTTG CTCGCAGGTC TGTTTGGTAG TGCCATTACC
1020

GGGATGATGG CTGCCCTGCC CGTAAGTTGG ATCCAGATGC TGGCAGGTCT GGCGCTGTTA
1080

AGTACCATCG GCGGCAGTTT GTATCAGGCG CTGCATAATG AGCGTGAGCG AGACGCGGCG
1140

GTGGTGGCAT TTCTGGTAAC GGCAAGTGGA TTGACGCTGG TCGGGATTGG TTCTGCGTTT
1200

TGGGGATTAA TTGCCGGAGG CGTTTGTTAC GTGGTGTTGA ATTTAATCGC TGACAGAAAC
1260

CGATATTGA 1269

<212> TYPE : DNA

<211> LENGTH : 1269

SEQUENCENAME : ESCHERICHIA COLI YDCO DNA

SEQ ID NO. 33: YDCO PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MRLLRQNGSL SLVRYNGQML RCLYAITHEV TMRLFSIPPP TLLAGFLAVL IGYASSAAII 60

WQAAIVAGAT TAQISGWMTA LGLAMGVSTL TLTLWYRVPV LTAWSTPGAA LLVTGLQGLT
120

LNEAIGVFIV TNALIVLCGI TGLFARLMRI IPHSLAAAML AGILLRFGLQ AFASLDGQFT 180

LCGSMLLVWL ATKAVAPRYA VIAAMIIGIV IVIAQGDVVT TDVVFKPVLP TYITPDFSFA 240

HSLSVALPLF LVTMASQNAP GIAAMKAAGY SAPVSPLIVF TGLLALVFSP FGVYSVGIAA 300

ITAAICQSPE AHPDKDQRWL AAAVAGIFYL LAGLFGSAIT GMMAALPVSW IQMLAGLALL
360

STIGGSLYQA LHNERERDAA VVAFLVTASG LTLVGIGSAF WGLIAGGVCY VVLNLIADRN 420

RY 422

<212> TYPE : PRT

<211> LENGTH : 422

SEQUENCENAME : ESCHERICHIA COLI YDCO PROTEIN

SEQ ID NO. 34: ACRD ORGANISM NAME : ESCHERICHIA COLI

MANFFIDRPI FAWVLAILLC LTGTLAIFSL PVEQYPDLAP PNVRVTANYP GASAQTLENT VTQVIEQNMT GLDNLMYMSS QSSGTGQASV TLSFKAGTDP DEAVQQVQNQ LQSAMRKLPQ AVQNQGVTVR KTGDTNILTI AFVSTDGSMD KQDIADYVAS NIQDPLSRVN GVGDIDAYGS QYSMRIWLDP AKLNSFQMTA KDVTDAIESQ NAQIAVGQLG GTPSVDKQAL NATINAQSLL QTPEQFRDIT LRVNQDGSEV RLGDVATVEM GAEKYDYLSR FNGKPASGLG VKLASGANEM ATAELVLNRL DELAQYFPHG LEYKVAYETT SFVKASIEDV VKTLLEAIAL VFLVMYLFLQ NFRATLIPTI AVPVVLMGTF SVLYAFGYSV NTLTMFAMVL AIGLLVDDAI VVVENVERIM SEEGLTPREA TRKSMGQIQG ALVGIAMVLS AVFVPMAFFG GTTGAIYRQF SITIVAAMVL SVLVAMILTP ALCATLLKPL KKGEHHGQKG FFAWFNQMFN RNAERYEKGV AKILHRSLRW IVIYVLLLGG MVFLFLRLPT SFLPLEDRGM FTTSVQLPSG STQQQTLKVV EQIEKYYFTH EKDNIMSVFA TVGSGPGGNG QNVARMFIRL KDWSERDSKT GTSFAIIERA TKAFNQIKEA RVIASSPPAI SGLGSSAGFD MELQDHAGAG HDALMAARNQ LLALAAENPE LTRVRHNGLD DSPQLQIDID QRKAQALGVA IDDINDTLQT AWGSSYVNDF MDRGRVKKVY VQAAAPYRML PDDINLWYVR NKDGGMVPFS AFATSRWETG SPRLERYNGY SAVEIVGEAA PGVSTGTAMD IMESLVKQLP NGFGLEWTAM SYQERLSGAQ APALYAISLL VVFLCLAALY ESWSVPFSVM LVVPLGVIGA LLATWMRGLE NDVYFQVGLL TVIGLSAKNA ILIVEFANEM NQKGHDLFEA TLHACRQRLR PILMTSLAFI FGVLPMATST GAGSGGQHAV GTGVMGGMIS ATILAIYFVP LFFVLVRRRF PLKPRPE

SEQ ID NO. 35: ATTA ORGANISM NAME : ESCHERICHIA COLI

MAHPPLLHLQ DITLSLGGNP LLDGAGFAVG RGERLCLVGR NGSGKSTLLK IAAGVIQPDS GSVFVQPGAS LRYLPQEPDL SAYATTADYV VGQIGDPDMA WRATPLLDAL GLTGRESTQN LSGGEGRRCA IAGVLAAAPD VLLLDEPTNH LDMPTIEWLE RELLSLGAMV IISHDRRLLS TLSRSVVWLD RGVTRRLDEG FGRFEAWREE VLEQEERDAH KLDRKIAREE DWMRYGVTAR RKRNVRRVRE LADLRTARKE AIRAPGTLTL NTQLRPHRKL VAVAEDISKA WGEKQVVRHL DLRILRGDRL GIVGANGAGK TTLLRMLTGL DQPDSGTISL GPSLNMVTLD QQRRTLNPER TLADTLTEGG GDMVQVGTEK RHVVGYMKDF LFRPEQARTP VSALSGGERG RLMLACALAK PSNLLVLDEP TNDLDLETLD ILQDMLASCE GTVLLVSHDR DFLDRVATSV LATEGDGNWI EYAGGYSDML AQRHQKPLTT ASVVENEPTK PKETTAARGP TKKLSYKDQF ALDNLPKEME KLEAQAANCV KNWQIQIYME KTPRSLRNFR LIYRSSKQSW QNLKNAGWNW K

SEQ ID NO. 36: BCR ORGANISM NAME : ESCHERICHIA COLI

MTTRQHSSFA IVFILGLLAM LMPLSIDMYL PALPVISAQF GVPAGSTQMT LSTYILGFAL GQLIYGPMAD SFGRKPVVLG GTLVFAAAAV ACALANTIDQ LIVMRFFHGL AAAAASVVIN

ALMRDIYPKE EFSRMMSFVM LVTTIAPLMA PIVGGWVLVW LSWHYIFWIL ALAAILASAM IFFLIKETLP PERRQPFHIR TTIGNFAALF RHKRVLSYML ASGFSFAGMF SFLSAGPFVY IEINHVAPEN FGYYFALNIV FLFVMTIFNS RFVRRIGALN MFRSGLWIQF IMAAWMVISA LLGLGFWSLV VGVAAFVGCV SMVSSNAMAV ILDEFPHMAG TASSLAGTFR FGIGAIVGAL LSLATFNSAW PMIWSIAFCA TSSILFCLYA SRPKKR

SEQ ID NO. 37: CUSA ORGANISM NAME : ESCHERICHIA COLI

MIEWIIRRSV ANRFLVLMGA LFLSIWGTWT IINTPVDALP DLSDVQVIIK TSYPGQAPQI VENQVTYPLT TTMLSVPGAK TVRGFSQFGD SYVYVIFEDG TDPYWARSRV LEYLNQVQGK LPAGVSAELG PDATGVGWIY EYALVDRSGK HDLADLRSLQ DWFLKYELKT IPDVAEVASV GGVVKEYQVV IDPQRLAQYG ISLAEVKSAL DASNQEAGGS SIELAEAEYM VRASGYLQTL DDFNHIVLKA SENGVPVYLR DVAKVQIGPE MRRGIAELNG EGEVAGGVVI LRSGKNAREV IAAVKDKLET LKSSLPEGVE IVTTYDRSQL IDRAIDNLSG KLLEEFIVVA VVCALFLWHV RSALVAIISL PLGLCIAFIV MHFQGLNANI MSLGGIAIAV GAMVDAAIVM IENAHKRLEE WQHQHPDATL DNKTRWQVIT DASVEVGPAL FISLLIITLS FIPIFTLEGQ EGRLFGPLAF TKTYAMAGAA LLAIVVIPIL MGYWIRGKIP PESSNPLNRF LIRVYHPLLL KVLHWPKTTL LVAALSVLTV LWPLNKVGGE FLPQINEGDL LYMPSTLPGI SAAEAASMLQ KTDKLIMSVP EVARVFGKTG KAETATDSAP LEMVETTIQL KPQEQWRPGM TMDKIIEELD NTVRLPGLAN LWVPPIRNRI DMLSTGIKSP IGIKVSGTVL ADIDAMAEQI EEVARTVPGV ASALAERLEG GRYINVEINR EKAARYGMTV ADVQLFVTSA VGGAMVGETV EGIARYPINL RYPQSWRDSP QALRQLPILT PMKQQITLAD VADIKVSTGP SMLKTENARP TSWIYIDARD RDMVSVVHDL QKAIAEKVQL KPGTSVAFSG QFELLERANH KLKLMVPMTL MIIFVLLYLA FRRVGEALLI ISSVPFALVG GIWLLWWMGF HLSVATGTGF IALAGVAAEF GVVMLMYLRH AIEAVPSLNN PQTFSEQKLD EALYHGAVLR VRPKAMTVAV IIAGLLPILW GTGAGSEVMS RIAAPMIGGM ITAPLLSLFI IPAAYKLMWL HRHRVRK

SEQ ID NO. 38: CYNX ORGANISM NAME: ESCHERICHIA COLI

MLLVLVLIGL NMRPLLTSVG PLLPQLRQAS GMSFSVAALL TALPVVTMGG LALAGSWLHQ HVSERRSVAI SLLLIAVGAL MRELYPQSAL LLSSALLGGV GIGIIQAVMP SVIKRRFQQR TPLVMGLWSA ALMGGGGLGA AITPWLVQHS ETWYQTLAWW ALPAVVALFA WWWQSAREVA SSHKTTTTPV RVVFTPRAWT LGVYFGLING GYASLIAWLP AFYIEIGASA QYSGSLLALM TLGQAAGALL MPAMARHQDR RKLLMLALVL QLVGFCGFIW LPMQLPVLWA MVCGLGLGGA FPLCLLLALD HSVQPAIAGK LVAFMQGIGF IIAGLAPWFS GVLRSISGNY LMDWAFHALC VVGLMIITLR FAPVRFPQLW VKEA

SEQ ID NO. 39: DEDA ORGANISM NAME : ESCHERICHIA COLI

MDLIYFLIDF ILHIDVHLAE LVAEYGVWVY AILFLILFCE TGLVVTPFLP GDSLLFVAGA LASLETNDLN VHMMVVLMLI AAIVGDAVNY TIGRLFGEKL FSNPNSKIFR RSYLDKTHQF YEKHGGKTII LARFVPIVRT FAPFVAGMGH MSYRHFAAYN VIGALLWVLL FTYAGYFFGT IPMVQDNLKL LIVGIIVVSI LPGVIEIIRH KRAAARAAK

SEQ ID NO. 40: EAMA ORGANISM NAME : ESCHERICHIA COLI

MSRKDGVLAL LVVVVWGLNF VVIKVGLHNM PPLMLAGLRF MLVAFPAIFF VARPKVPLNL LLGYGLTISF AQFAFLFCAI NFGMPAGLAS LVLQAQAFFT IMLGAFTFGE RLHGKQLAGI ALAIFGVLVL IEDSLNGQHV AMLGFMLTLA AAFSWACGNI FNKKIMSHST RPAVMSLVIW SALIPIIPFF VASLILDGSA TMIHSLVTID MTTILSLMYL AFVATIVGYG IWGTLLGRYE TWRVAPLSLL VPVVGLASAA LLLDERLTGL QFLGAVLIMT GLYINVFGLR WRKAVKVGS

SEQ ID NO. 41: EAMB ORGANISM NAME : ESCHERICHIA COLI

MTPTLLSAFW TYTLITAMTP GPNNILALSS ATSHGFRQST RVLAGMSLGF LIVMLLCAGI SFSLAVIDPA AVHLLSWAGA AYIVWLAWKI ATSPTKEDGL QAKPISFWAS FALQFVNVKI ILYGVTALST FVLPQTQALS WVVGVSVLLA MIGTFGNVCW ALAGHLFQRL FRQYGRQLNI VLALLLVYCA VRIFY

SEQ ID NO. 42: EMRB ORGANISM NAME : ESCHERICHIA COLI

MQQQKPLEGA QLVIMTIALS LATFMQVLDS TIANVAIPTI AGNLGSSLSQ GTWVITSFGV ANAISIPLTG WLAKRVGEVK LFLWSTIAFA IASWACGVSS SLNMLIFFRV IQGIVAGPLI PLSQSLLLNN YPPAKRSIAL ALWSMTVIVA PICGPILGGY ISDNYHWGWI FFINVPIGVA VVLMTLQTLR GRETRTERRR IDAVGLALLV IGIGSLQIML DRGKELDWFS SQEIIILTVV AVVAICFLIV WELTDDNPIV DLSLFKSRNF TIGCLCISLA YMLYFGAIVL LPQLLQEVYG YTATWAGLAS APVGIIPVIL SPIIGRFAHK LDMRRLVTFS FIMYAVCFYW RAYTFEPGMD FGASAWPQFI QGFAVACFFM PLTTITLSGL PPERLAAASS LSNFTRTLAG SIGTSITTTM WTNRESMHHA QLTESVNPFN PNAQAMYSQL EGLGMTQQQA SGWIAQQITN QGLIISANEI FWMSAGIFLV LLGLVWFAKP PFGAGGGGGG AH

SEQ ID NO. 43: EMRD ORGANISM NAME : ESCHERICHIA COLI

MKRQRNVNLL LMLVLLVAVG QMAQTIYIPA IADMARDLNV REGAVQSVMG AYLLTYGVSQ LFYGPISDRV GRRPVILVGM SIFMLATLVA VTTSSLTVLI AASAMQGMGT GVGGVMARTL PRDLYERTQL RHANSLLNMG ILVSPLLAPL IGGLLDTMWN WRACYLFLLV LCAGVTFSMA RWMPETRPVD APRTRLLTSY KTLFGNSGFN CYLLMLIGGL AGIAAFEACS GVLMGAVLGL SSMTVSILFI LPIPAAFFGA WFAGRPNKRF STLMWQSVIC CLLAGLLMWI PDWFGVMNVW

TLLVPAALFF FGAGMLFPLA TSGAMEPFPF LAGTAGALVG GLQNIGSGVL ASLSAMLPQT GQGSLGLLMT LMGLLIVLCW LPLATRMSHQ GQPV

SEQ ID NO. 44: EMRKY ORGANISM NAME : ESCHERICHIA COLI

MAITKSTPAP LTGGTLWCVT IALSLATFMQ MLDSTISNVA IPTISGFLGA STDEGTWVIT SFGVANAIAI PVTGRLAQRI GELRLFLLSV TFFSLSSLMC SLSTNLDVLI FFRVVQGLMA GPLIPLSQSL LLRNYPPEKR TFALALWSMT VIIAPICGPI LGGYICDNFS WGWIFLINVP MGIIVLTLCL TLLKGRETET SPVKMNLPGL TLLVLGVGGL QIMLDKGRDL DWFNSSTIII LTVVSVISLI SLVIWESTSE NPILDLSLFK SRNFTIGIVS ITCAYLFYSG AIVLMPQLLQ ETMGYNAIWA GLAYAPIGIM PLLISPLIGR YGNKIDMRLL VTFSFLMYAV CYYWRSVTFM PTIDFTGIIL PQFFQGFAVA CFFLPLTTIS FSGLPDNKFA NASSMSNFFR TLSGSVGTSL TMTLWGRRES LHHSQLTATI DQFNPVFNSS SQIMDKYYGS LSGVLNEINN EITQQSLSIS ANEIFRMAAI AFILLTVLVW FAKPPFTAKG VG

SEQ ID NO. 45: EMRY ORGANISM NAME : ESCHERICHIA COLI

MAITKSTPAP LTGGTLWCVT IALSLATFMQ MLDSTISNVA IPTISGFLGA STDEGTWVIT SFGVANAIAI PVTGRLAQRI GELRLFLLSV TFFSLSSLMC SLSTNLDVLI FFRVVQGLMA GPLIPLSQSL LLRNYPPEKR TFALALWSMT VIIAPICGPI LGGYICDNFS WGWIFLINVP MGIIVLTLCL TLLKGRETET SPVKMNLPGL TLLVLGVGGL QIMLDKGRDL DWFNSSTIII LTVVSVISLI SLVIWESTSE NPILDLSLFK SRNFTIGIVS ITCAYLFYSG AIVLMPQLLQ ETMGYNAIWA GLAYAPIGIM PLLISPLIGR YGNKIDMRLL VTFSFLMYAV CYYWRSVTFM PTIDFTGIIL PQFFQGFAVA CFFLPLTTIS FSGLPDNKFA NASSMSNFFR TLSGSVGTSL TMTLWGRRES LHHSQLTATI DQFNPVFNSS SQIMDKYYGS LSGVLNEINN EITQQSLSIS ANEIFRMAAI AFILLTVLVW FAKPPFTAKG VG

SEQ ID NO. 46: GARP ORGANISM NAME : ESCHERICHIA COLI

MILDTVDEKK KGVHTRYLIL LIIFIVTAVN YADRATLSIA GTEVAKELQL SAVSMGYIFS AFGWAYLLMQ IPGGWLLDKF GSKKVYTYSL FFWSLFTFLQ GFVDMFPLAW AGISMFFMRF MLGFSEAPSF PANARIVAAW FPTKERGTAS AIFNSAQYFS LALFSPLLGW LTFAWGWEHV FTVMGVIGFV LTALWIKLIH NPTDHPRMSA EELKFISENG AVVDMDHKKP GSAAASGPKL HYIKQLLSNR MMLGVFFGQY FINTITWFFL TWFPIYLVQE KGMSILKVGL VASIPALCGF AGGVLGGVFS DYLIKRGLSL TLARKLPIVL GMLLASTIIL CNYTNNTTLV VMLMALAFFG KGFGALGWPV ISDTAPKEIV GLCGGVFNVF GNVASIVTPL VIGYLVSELH SFNAALVFVG CSALMAMVCY LFVVGDIKRM ELQK

SEQ ID NO. 47: GUDP ORGANISM NAME: ESCHERICHIA COLI

MSSLSQAASS VEKRTNARYW IVVMLFIVTS FNYGDRATLS IAGSEMAKDI GLDPVGMGYV FSAFSWAYVI GQIPGGWLLD RFGSKRVYFW SIFIWSMFTL LQGFVDIFSG FGIIVALFTL RFLVGLAEAP SFPGNSRIVA AWFPAQERGT AVSIFNSAQY FATVIFAPIM GWLTHEVGWS HVFFFMGGLG IVISFIWLKV IHEPNQHPGV NKKELEYIAA GGALINMDQQ NTKVKVPFSV KWGQIKQLLG SRMMIGVYIG QYCINALTYF FITWFPVYLV QARGMSILKA GFVASVPAVC GFIGGVLGGI ISDWLMRRTG SLNIARKTPI VMGMLLSMVM VFCNYVNVEW MIIGFMALAF FGKGIGALGW AVMADTAPKE ISGLSGGLFN MFGNISGIVT PIAIGYIVGT TGSFNGALIY VGVHALIAVL SYLVLVGDIK RIELKPVAGQ

SEQ ID NO. 48: HSRA ORGANISM NAME: ESCHERICHIA COLI

MSDKKKRSMA GLPWIAAMAF FMQALDATIL NTALPAIAHS LNRSPLAMQS AIISYTLTVA MLIPVSGWLA DRFGTRRIFT LAVSLFTLGS LACALSNSLP QLVVFRVIQG IGGAMMMPVA RLALLRAYPR NELLPVLNFV AMPGLVGPIL GPVLGGVLVT WATWHWIFLI NIPIGIAGLL YARKHMPNFT TARRRFDITG FLLFGLSLVL FSSGIELFGE KIVASWIALT VIVTSIGLLL LYILHARRTP NPLISLDLFK TRTFSIGIVG NIATRLGTGC VPFLMPLMLQ VGFGYQAFIA GCMMAPTALG SIIAKSMVTQ VLRRLGYRHT LVGITVIIGL MIAQFSLQSP AMAIWMLILP LFILGMAMST QFTAMNTITL ADLTDDNASS GNSVLAVTQQ LSISLGVAVS AAVLRVYEGM EGTTTVEQFH YTFITMGIIT VASAAMFMLL KTTDGNNLIK RQRKSKPNRV PSESE

SEQ ID NO. 49: LEUE ORGANISM NAME : ESCHERICHIA COLI

MFAEYGVLNY WTYLVGAIFI VLVPGPNTLF VLKNSVSSGM KGGYLAACGV FIGDAVLMFL AWAGVATLIK TTPILFNIVR YLGAFYLLYL GSKILYATLK GKNSEAKSDE PQYGAIFKRA LILSLTNPKA ILFYVSFFVQ FIDVNAPHTG ISFFILAATL ELVSFCYLSF LIISGAFVTQ YIRTKKKLAK VGNSLIGLMF VGFAARLATL QS

SEQ ID NO. 50: MDLB ORGANISM NAME : ESCHERICHIA COLI

MRSFSQLWPT LKRLLAYGSP WRKPLGIAVL MMWVAAAAEV SGPLLISYFI DNMVAKNNLP LKVVAGLAAA YVGLQLFAAG LHYAQSLLFN RAAVGVVQQL RTDVMDAALR QPLSEFDTQP VGQVISRVTN DTEVIRDLYV TVVATVLRSA ALVGAMLVAM FSLDWRMALV AIMIFPVVLV VMVIYQRYST PIVRRVRAYL ADINDGFNEI INGMSVIQQF RQQARFGERM GEASRSHYMA RMQTLRLDGF LLRPLLSLFS SLILCGLLML FGFSASGTIE VGVLYAFISY LGRLNEPLIE LTTQQAMLQQ AVVAGERVFE LMDGPRQQYG NDDRPLQSGT IEVDNVSFAY RDDNLVLKNI NLSVPSRNFV ALVGHTGSGK STLASLLMGY YPLTEGEIRL DGRPLSSLSH SALRQGVAMV QQDPVVLADT FLANVTLGRD ISEERVWQAL ETVQLAELAR SMSDGIYTPL GEQGNNLSVG

QKQLLALARV LVETPQILIL DEATASIDSG TEQAIQHALA AVREHTTLVV IAHRLSTIVD ADTILVLHRG QAVEQGTHQQ LLAAQGRYWQ MYQLQLAGEE LAASVREEES LSA

SEQ ID NO. 51: MDTD ORGANISM NAME : ESCHERICHIA COLI

MTDLPDSTRW QLWIVAFGFF MQSLDTTIVN TALPSMAQSL GESPLHMHMV IVSYVLTVAV MLPASGWLAD KVGVRNIFFT AIVLFTLGSL FCALSGTLNE LLLARALQGV GGAMMVPVGR LTVMKIVPRE QYMAAMTFVT LPGQVGPLLG PALGGLLVEY ASWHWIFLIN IPVGIIGAIA TLLLMPNYTM QTRRFDLSGF LLLAVGMAVL TLALDGSKGT GLSPLTIAGL VAVGVVALVL YLLHARNNNR ALFSLKLFRT RTFSLGLAGS FAGRIGSGML PFMTPVFLQI GLGFSPFHAG LMMIPMVLGS MGMKRIVVQV VNRFGYRRVL VATTLGLSLV TLLFMTTALL GWYYVLPFVL FLQGMVNSTR FSSMNTLTLK DLPDNLASSG NSLLSMIMQL SMSIGVTIAG LLLGLFGSQH VSVDSGTTQT VFMYTWLSMA LIIALPAFIF ARVPNDTHQN VAISRRKRSA Q

SEQ ID NO. 52: MDTG ORGANISM NAME : ESCHERICHIA COLI

MSPCENDTPI NWKRNLIVAW LGCFLTGAAF SLVMPFLPLY VEQLGVTGHS ALNMWSGIVF SITFLFSAIA SPFWGGLADR KGRKLMLLRS ALGMGIVMVL MGLAQNIWQF LILRALLGLL GGFVPNANAL IATQVPRNKS GWALGTLSTG GVSGALLGPM AGGLLADSYG LRPVFFITAS VLILCFFVTL FCIREKFQPV SKKEMLHMRE VVTSLKNPKL VLSLFVTTLI IQVATGSIAP ILTLYVRELA GNVSNVAFIS GMIASVPGVA ALLSAPRLGK LGDRIGPEKI LITALIFSVL LLIPMSYVQT PLQLGILRFL LGAADGALLP AVQTLLVYNS SNQIAGRIFS YNQSFRDIGN VTGPLMGAAI SANYGFRAVF LVTAGVVLFN AVYSWNSLRR RRIPQVSN

SEQ ID NO. 53: MDTL ORGANISM NAME : ESCHERICHIA COLI

MSRFLICSFA LVLLYPAGID MYLVGLPRIA ADLNASEAQL HIAFSVYLAG MAAAMLFAGK VADRSGRKPV AIPGAALFII ASVFCSLAET STLFLAGRFL QGLGAGCCYV VAFAILRDTL DDRRRAKVLS LLNGITCIIP VLAPVLGHLI MLKFPWQSLF WAMAMMGIAV LMLSLFILKE TRPAAPAASD KPRENSESLL NRFFLSRVVI TTLSVSVILT FVNTSPVLLM EIMGFERGEY ATIMALTAGV SMTVSFSTPF ALGIFKPRTL MITSQVLFLA AGITLAVSPS HAVSLFGITL ICAGFSVGFG VAMSQALGPF SLRAGVASST LGIAQVCGSS LWIWLAAVVG IGAWNMLIGI LIACSIVSLL LIMFVAPGRP VAAHEEIHHH A

SEQ ID NO. 54: MDTM ORGANISM NAME : ESCHERICHIA COLI

MPRFFTRHAA TLFFPMALIL YDFAAYLSTD LIQPGIINVV RDFNADVSLA PAAVSLYLAG GMALQWLLGP LSDRIGRRPV LITGALIFTL ACAATMFTTS MTQFLIARAI QGTSICFIAT VGYVTVQEAF GQTKGIKLMA IITSIVLIAP IIGPLSGAAL MHFMHWKVLF AIIAVMGFIS FVGLLLAMPE TVKRGAVPFS AKSVLRDFRN VFCNRLFLFG AATISLSYIP MMSWVAVSPV

ILIDAGSLTT SQFAWTQVPV FGAVIVANAI VARFVKDPTE PRFIWRAVPI QLVGLSLLIV GNLLSPHVWL WSVLGTSLYA FGIGLIFPTL FRFTLFSNKL PKGTVSASLN MVILMVMSVS VEIGRWLWFN GGRLPFHLLA VVAGVIVVFT LAGLLNRVRQ HQAAELVEEQ

SEQ ID NO. 55: MHPT ORGANISM NAME : ESCHERICHIA COLI

MSTRTPSSSS SRLMLTIGLC FLVALMEGLD LQAAGIAAGG IAQAFALDKM QMGWIFSAGI LGLLPGALVG GMLADRYGRK RILIGSVALF GLFSLATAIA WDFPSLVFAR LMTGVGLGAA LPNLIALTSE AAGPRFRGTA VSLMYCGVPI GAALAATLGF AGANLAWQTV FWVGGVVPLI LVPLLMRWLP ESAVFAGEKQ SAPPLRALFA PETATATLLL WLCYFFTLLV VYMLINWLPL LLVEQGFQPS QAAGVMFALQ MGAASGTLML GALMDKLRPV TMSLLIYSGM LASLLALGTV SSFNGMLLAG FVAGLFATGG QSVLYALAPL FYSSQIRATG VGTAVAVGRL GAMSGPLLAG KMLALGTGTV GVMAASAPGI LVAGLAVFIL MSRRSRIQPC ADA

SEQ ID NO. 56: RHTA ORGANISM NAME : ESCHERICHIA COLI

MPGSLRKMPV WLPIVILLVA MASIQGGASL AKSLFPLVGA PGVTALRLAL GTLILIAFFK PWRLRFAKEQ RLPLLFYGVS LGGMNYLFYL SIQTVPLGIA VALEFTGPLA VALFSSRRPV DFVWVVLAVL GLWFLLPLGQ DVSHVDLTGC ALALGAGACW AIYILSGQRA GAEHGPATVA IGSLIAALIF VPIGALQAGE ALWHWSVIPL GLAVAILSTA LPYSLEMIAL TRLPTRTFGT LMSMEPALAA VSGMIFLGET LTPIQLLALG AIIAASMGST LTVRKESKIK ELDIN

SEQ ID NO. 57: RHTB ORGANISM NAME : ESCHERICHIA COLI

MTLEWWFAYL LTSIILSLSP GSGAINTMTT SLNHGYRGAV ASIAGLQTGL AIHIVLVGVG LGTLFSRSVI AFEVLKWAGA AYLIWLGIQQ WRAAGAIDLK SLASTQSRRH LFQRAVFVNL TNPKSIVFLA ALFPQFIMPQ QPQLMQYIVL GVTTIVVDII VMIGYATLAQ RIALWIKGPK QMKALNKIFG SLFMLVGALL ASARHA

SEQ ID NO. 58: RHTC ORGANISM NAME : ESCHERICHIA COLI

MLMLFLTVAM VHIVALMSPG PDFFFVSQTA VSRSRKEAMM GVLGITCGVM VWAGIALLGL HLIIEKMAWL HTLIMVGGGL YLCWMGYQML RGALKKEAVS APAPQVELAK SGRSFLKGLL TNLANPKAII YFGSVFSLFV GDNVGTTARW GIFALIIVET LAWFTVVASL FALPQMRRGY QRLAKWIDGF AGALFAGFGI HLIISR

SEQ ID NO. 59: YAHN ORGANISM NAME : ESCHERICHIA COLI

MMQLVHLFMD EITMDPLHAV YLTVGLFVIT FFNPGANLFV VVQTSLASGR RAGVLTGLGV ALGDAFYSGL GLFGLATLIT QCEEIFSLIR IVGGAYLLWF AWCSMRRQST PQMSTLQQPI SAPWYVFFRR GLITDLSNPQ TVLFFISIFS VTLNAETPTW ARLMAWAGIV LASIIWRVFL SQAFSLPAVR RAYGRMQRVA SRVIGAIIGV FALRLIYEGV TQR

SEQ ID NO. 60: YAJR ORGANISM NAME : ESCHERICHIA COLI

MNDYKMTPGE RRATWGLGTV FSLRMLGMFM VLPVLTTYGM ALQGASEALI GIAIGIYGLT QAVFQIPFGL LSDRIGRKPL IVGGLAVFAA GSVIAALSDS IWGIILGRAL QGSGAIAAAV MALLSDLTRE QNRTKAMAFI GVSFGITFAI AMVLGPIITH KLGLHALFWM IAILATTGIA LTIWVVPNSS THVLNRESGM VKGSFSKVLA EPRLLKLNFG IMCLHILLMS TFVALPGQLA DAGFPAAEHW KVYLATMLIA FGSVVPFIIY AEVKRKMKQV FVFCVGLIVV AEIVLWNAQT QFWQLVVGVQ LFFVAFNLME ALLPSLISKE SPAGYKGTAM GVYSTSQFLG VAIGGSLGGW INGMFDGQGV FLAGAMLAAV WLTVASTMKE PPYVSSLRIE IPANIAANEA LKVRLLETEG IKEVLIAEEE HSAYVKIDSK VTNRFEIEQA IRQA

SEQ ID NO. 61: YBBP ORGANISM NAME: ESCHERICHIA COLI

MIARWFWREW RSPSLLIVWL ALSLAVACVL ALGNISDRME KGLSQQSREF MAGDRALRSS REVPQAWLEE AQKRGLKVGK QLTFATMTFA GDTPQLANVK AVDDIYPMYG DLQTNPPGLK PQAGSVLLAP RLMALLNLKT GDTIDVGDAT LRIAGEVIQE PDSGFNPFQM APRLMMNLAD VDKTGAVQPG SRVTWRYKFG GNENQLDGYE KWLLPQLKPE QRWYGLEQDE GALGRSMERS QQFLLLSALL TLLLAVAAVA VAMNHYCRSR YDLVAILKTL GAGRAQLRKL IVGQWLMVLT LSAVTGGAIG LLFENVLMVL LKPVLPAALP PASLWPWLWA LGTMTVISLL VGLRPYRLLL ATQPLRVLRN DVVANVWPLK FYLPIVSVVV VLLLAGLMGG SMLLWAVLAG AVVLALLCGV LGWMLLNVLR RMTLKSLPLR LAVSRLLRQP WSTLSQLSAF SLSFMLLALL LVLRGDLLDR WQQQLPPESP NYFLINIATE QVAPLKAFLA EHQIVPESFY PVVRARLTAI NDKPTEGNED EALNRELNLT WQNTRPDHNP IVAGNWPPKA DEVSMEEGLA KRLNVALGDT VTFMGDTQEF RAKVTSLRKV DWESLRPNFY FIFPEGALDG QPQSWLTSFR WENGNGMLTQ LNRQFPTISL LDIGAILKQV GQVLEQVSRA LEVMVVLVTA CGMLLLLAQV QVGMRQRHQE LVVWRTLGAG KKLLRTTLWC EFAMLGFVSG LVAAIGAETA LAVLQAKVFD FPWEPDWRLW IVLPCSGALL LSLFGGWLGA RLVKGKALFR QFAG

SEQ ID NO.62: YBIF (RHTA) ORGANISM NAME : ESCHERICHIA COLI

MPGSLRKMPV WLPIVILLVA MASIQGGASL AKSLFPLVGA PGVTALRLAL GTLILIAFFK PWRLRFAKEQ RLPLLFYGVS LGGMNYLFYL SIQTVPLGIA VALEFTGPLA VALFSSRRPV DFVWVVLAVL GLWFLLPLGQ DVSHVDLTGC ALALGAGACW AIYILSGQRA GAEHGPATVA IGSLIAALIF VPIGALQAGE ALWHWSVIPL GLAVAILSTA LPYSLEMIAL TRLPTRTFGT LMSMEPALAA VSGMIFLGET LTPIQLLALG AIIAASMGST LTVRKESKIK ELDIN

SEQ ID NO.63: YBJJ ORGANISM NAME : ESCHERICHIA COLI

MTVNSSRNAL KRRTWALFMF FFLPGLLMAS WATRTPAIRD ILSVSIAEMG GVLFGLSIGS MSGILCSAWL VKRFGTRNVI LVTMSCALIG MMILSLALWL TSPLLFAVGL GVFGASFGSA EVAINVEGAA VEREMNKTVL PMMHGFYSLG TLAGAGVGMA LTAFGVPATV HILLAALVGI APIYIAIQAI PDGTGKNAAD GTQHGEKGVP FYRDIQLLLI GVVVLAMAFA EGSANDWLPL LMVDGHGFSP TSGSLIYAGF TLGMTVGRFT GGWFIDRYSR VAVVRASALM GALGIGLIIF VDSAWVAGVS VVLWGLGASL GFPLTISAAS DTGPDAPTRV SVVATTGYLA FLVGPPLLGY LGEHYGLRSA MLVVLALVIL AAIVAKAVAK PDTKTQTAME NS

SEQ ID NO. 64: YCAP ORGANISM NAME: ESCHERICHIA COLI

MKAFDLHRMA FDKVPFDFLG EVALRSLYTF VLVFLFLKMT GRRGVRQMSL FEVLIILTLG SAAGDVAFYD DVPMVPVLIV FITLALLYRL VMWLMAHSEK LEDLLEGKPV VIIEDGELAW SKLNNSNMTE FEFFMELRLR GVEQLGQVRL AILETNGQIS VYFFEDDKVK PGLLILPSDC TQRYKVVPES ADYACIRCSE IIHMKAGEKQ LCPRCANPEW TKASRAKRVT

SEQ ID NO. 65: YDCO ORGANISM NAME : ESCHERICHIA COLI

MRLFSIPPPT LLAGFLAVLI GYASSAAIIW QAAIVAGATT AQISGWMTAL GLAMGVSTLT LTLWYRVPVL TAWSTPGAAL LVTGLQGLTL NEAIGVFIVT NALIVLCGIT GLFARLMRII PHSLAAAMLA GILLRFGLQA FASLDGQFTL CGSMLLVWLA TKAVAPRYAV IAAMIIGIVI VIAQGDVVTT DVVFKPVLPT YITPDFSFAH SLSVALPLFL VTMASQNAPG IAAMKAAGYS APVSPLIVFT GLLALVFSPF GVYSVGIAAI TAAICQSPEA HPDKDQRWLA AAVAGIFYLL AGLFGSAITG MMAALPVSWI QMLAGLALLS TIGGSLYQAL HNERERDAAV VAFLVTASGL TLVGIGSAFW GLIAGGVCYV VLNLIADRNRY

SEQ ID NO. 66: YDDG ORGANISM NAME : ESCHERICHIA COLI

MTRQKATLIG LIAIVLWSTM VGLIRGVSEG LGPVGGAAAI YSLSGLLLIF TVGFPRIRQI PKGYLLAGSL LFVSYEICLA LSLGYAATHH QAIEVGMVNY LWPSLTILFA ILFNGQKTNW LIVPGLLLAL VGVCWVLGGD NGLHYDEIIN NITTSPLSYF LAFIGAFIWA AYCTVTNKYA RGFNGITVFV LLTGASLWVY YFLTPQPEMI FSTPVMIKLI SAAFTLGFAY AAWNVGILHG NVTIMAVGSY FTPVLSSALA AVLLSAPLSF SFWQGALMVC GGSLLCWLAT RRG

SEQ ID NO. 67: YDED (EAMA) ORGANISM NAME : ESCHERICHIA COLI

MSRKDGVLAL LVVVVWGLNF VVIKVGLHNM PPLMLAGLRF MLVAFPAIFF VARPKVPLNL LLGYGLTISF AQFAFLFCAI NFGMPAGLAS LVLQAQAFFT IMLGAFTFGE RLHGKQLAGI ALAIFGVLVL IEDSLNGQHV AMLGFMLTLA AAFSWACGNI FNKKIMSHST RPAVMSLVIW SALIPIIPFF VASLILDGSA TMIHSLVTID MTTILSLMYL AFVATIVGYG IWGTLLGRYE TWRVAPLSLL VPVVGLASAA LLLDERLTGL QFLGAVLIMT GLYINVFGLR WRKAVKVGS

SEQ ID NO. 68: YDGE (MDTI) ORGANISM NAME : ESCHERICHIA COLI

MAQFEWVHAA WLALAIVLEI VANVFLKFSD GFRRKIFGLL SLAAVLAAFS ALSQAVKGID LSVAYALWGG FGIAATLAAG WILFGQRLNR KGWIGLVLLL AGMIMVKLA

SEQ ID NO. 69: YDHC ORGANISM NAME : ESCHERICHIA COLI

MQPGKRFLVW LAGLSVLGFL ATDMYLPAFA AIQADLQTPA SAVSASLSLF LAGFAAAQLL WGPLSDRYGR KPVLLIGLTI FALGSLGMLW VENAATLLVL RFVQAVGVCA AAVIWQALVT DYYPSQKVNR IFAAIMPLVG LSPALAPLLG SWLLVHFSWQ AIFATLFAIT VVLILPIFWL KPTTKARNNS QDGLTFTDLL RSKTYRGNVL IYAACSASFF AWLTGSPFIL SEMGYSPAVI GLSYVPQTIA FLIGGYGCRA ALQKWQGKQL LPWLLVLFAV SVIATWAAGF ISHVSLVEIL IPFCVMAIAN GAIYPIVVAQ ALRPFPHATG RAAALQNTLQ LGLCFLASLV VSWLISISTP LLTTTSVMLS TVVLVALGYM MQRCEEVGCQ NHGNAEVAHS ESH

SEQ ID NO. 70: YDHP ORGANISM NAME : ESCHERICHIA COLI

MKINYPLLAL AIGAFGIGTT EFSPMGLLPV IARGVDVSIP AAGMLISAYA VGVMVGAPLM TLLLSHRARR SALIFLMAIF TLGNVLSAIA PDYMTLMLSR ILTSLNHGAF FGLGSVVAAS VVPKHKQASA VATMFMGLTL ANIGGVPAAT WLGETIGWRM SFLATAGLGV ISMVSLFFSL PKGGAGARPE VKKELAVLMR PQVLSALLTT VLGAGAMFTL YTYISPVLQS ITHATPVFVT AMLVLIGVGF SIGNYLGGKL ADRSVNGTLK GFLLLLMVIM LAIPFLARNE FGAAISMVVW GAATFAVVPP LQMRVMRVAS EAPGLSSSVN IGAFNLGNAL GAAAGGAVIS AGLGYSFVPV MGAIVAGLAL LLVFMSARKQ PETVCVANS

SEQ ID NO. 71: YDIN ORGANISM NAME: ESCHERICHIA COLI

MSQNKAFSTP FILAVLCIYF SYFLHGISVI TLAQNMSSLA EKFSTDNAGI AYLISGIGLG RLISILFFGV ISDKFGRRAV ILMAVIMYLL FFFGIPACPN LTLAYGLAVC VGIANSALDT GGYPALMECF PKASGSAVIL VKAMVSFGQM FYPMLVSYML LNNIWYGYGL IIPGILFVLI TLMLLKSKFP SQLVDASVTN ELPQMNSKPL VWLEGVSSVL FGVAAFSTFY VIVVWMPKYA MAFAGMSEAE ALKTISYYSM GSLVCVFIFA ALLKKMVRPI WANVFNSALA TITAAIIYLY PSPLVCNAGA FVIGFSAAGG ILQLGVSVMS EFFPKSKAKV TSIYMMMGGL ANFVIPLITG YLSNIGLQYI IVLDFTFALL ALITAIIVFI RYYRVFIIPE NDVRFGERKF CTRLNTIKHR G

SEQ ID NO. 72: YDIM ORGANISM NAME: ESCHERICHIA COLI

MKNPYFPTAL GLYFNYLVHG MGVLLMSLNM ASLETLWQTN AAGVSIVISS LGIGRLSVLL FAGLLSDRFG RRPFIMLGMC CYMAFFFGIL QTNNIIIAYV FGFLAGMANS FLDAGTYPSL MEAFPRSPGT ANILIKAFVS SGQFLLPLII SLLVWAELWF GWSFMIAAGI MFINALFLYR

CTFPPHPGRR LPVIKKTTSS TEHRCSIIDL ASYTLYGYIS MATFYLVSQW LAQYGQFVAG MSYTMSIKLL SIYTVGSLLC VFITAPLIRN TVRPTTLLML YTFISFIALF TVCLHPTFYV VIIFAFVIGF TSAGGVVQIG LTLMAERFPY AKGKATGIYY SAGSIATFTI PLITAHLSQR SIADIMWFDT AIAAIGFLLA LFIGLRSRKK TRHHSLKENV APGG

SEQ ID NO. 73: YDJE ORGANISM NAME : ESCHERICHIA COLI

MEQYDQIGAR LDRLPLARFH YRIFGIISFS LLLTGFLSYS GNVVLAKLVS NGWSNNFLNA AFTSALMFGY FIGSLTGGFI GDYFGRRRAF RINLLIVGIA ATGAAFVPDM YWLIFFRFLM GTGMGALIMV GYASFTEFIP ATVRGKWSAR LSFVGNWSPM LSAAIGVVVI AFFSWRIMFL LGGIGILLAW FLSGKYFIES PRWLAGKGQI AGAECQLREV EQQIEREKSI RLPPLTSYQS NSKVKVIKGT FWLLFKGEML RRTLVAITVL IAMNISLYTI TVWIPTIFVN SGIDVDKSIL MTAVIMIGAP VGIFIAALII DHFPRRLFGS TLLIIIAVLG YIYSIQTTEW AILIYGLVMI FFLYMYVCFA SAVYIPELWP THLRLRGSGF VNAVGRIVAV FTPYGVAALL THYGSITVFM VLGVMLLLCA LVLSIFGIET RKVSLEEISE VN

SEQ ID NO. 74: YDJF ORGANISM NAME: ESCHERICHIA COLI

MAAKDRIQAI KQMVANDKKV TVSNLSGIFQ VTEETIRRDL EKLEDEGFLT RTYGGAVLNT AMLTENIHFY KRASSFYEEK QLIARKALPF IDNKTTMAAD SSSTVMELLK LLQDRSGLTL LTNSAEAIHV LAQSEIKVVS TGGELNKNTL SLQGRITKEI IRRYHVDIMV MSCKGLDINS GALDSNEAEA EIKKTMIRQA TEVALLVDHS KFDRKAFVQL ADFSHINYII TDKSPGAEWI AFCKDNNIQL VW

SEQ ID NO. 75: YDJI ORGANISM NAME : ESCHERICHIA COLI

MLADIRYWEN DATNKHYAIA HFNVWNAEML MGVIDAAEEA KSPVIISFGT GFVGNTSFED FSHMMVSMAQ KATVPVITHW DHGRSMEIIH NAWTHGMNSL MRDASAFDFE ENIRLTKEAV DFFHPLGIPV EAELGHVGNE TVYEEALAGY HYTDPDQAAE FVERTGCDSL AVAIGNQHGV YTSEPQLNFE VVKRVRDAVS VPLVLHGASG ISDADIKTAI SLGIAKINIH TELCQAAMVA VKENQDQPFL HLEREVRKAV KERALEKIKL FGSDGKAE

SEQ ID NO. 76: YDJK ORGANISM NAME : ESCHERICHIA COLI

MEQITKPHCG ARLDRLPDCR WHSSMFAIVA FGLLVCWSNA VGGLILAQLK ALGWTDNSTT ATFSAITTAG MFLGALVGGI IGDKTGRRNA FILYEAIHIA SMVVGAFSPN MDFLIACRFV MGVGLGALLV TLFAGFTEYM PGRNRGTWSS RVSFIGNWSY PLCSLIAMGL TPLISAEWNW RVQLLIPAIL SLIATALAWR YFPESPRWLE SRGRYQEAEK VMRSIEEGVI RQTGKPLPPV VIADDGKAPQ AVPYSALLTG VLLKRVILGS CVLIAMNVVQ YTLINWLPTI FMTQGINLKD SIVLNTMSMF GAPFGIFIAM LVMDKIPRKT MGVGLLILIA VLGYIYSLQT SMLLITLIGF

FLITFVYMYV CYASAVYVPE IWPTEAKLRG SGLANAVGRI SGIAAPYAVA VLLSSYGVTG VFILLGAVSI IVAIAIATIG IETKGVSVES LSIDAVANK

SEQ ID NO. 77: YEAN ORGANISM NAME : ESCHERICHIA COLI

MTCSTSLSGK NRIVLIAGIL MIATTLRVTF TGAAPLLDTI RSAYSLTTAQ TGLLTTLPLL AFALISPLAA PVARRFGMER SLFAALLLIC AGIAIRSLPS PYLLFGGTAV IGGGIALGNV LLPGLIKRDF PHSVARLTGA YSLTMGAAAA LGSAMVVPLA LNGFGWQGAL LMLMCFPLLA LFLWLPQWRS QQHANLSTSR ALHTRGIWRS PLAWQVTLFL GINSLVYYVI IGWLPAILIS HGYSEAQAGS LHGLLQLATA APGLLIPLFL HHVKDQRGIA AFVALMCAVG AVGLCFMPAH AITWTLLFGF GSGATMILGL TFIGLRASSA HQAAALSGMA QSVGYLLAAC GPPLMGKIHD ANGNWSVPLM GVAILSLLMA IFGLCAGRDK EIR

SEQ ID NO. 78: YEAS (LEUE) ORGANISM NAME : ESCHERICHIA COLI

MFAEYGVLNY WTYLVGAIFI VLVPGPNTLF VLKNSVSSGM KGGYLAACGV FIGDAVLMFL AWAGVATLIK TTPILFNIVR YLGAFYLLYL GSKILYATLK GKNSEAKSDE PQYGAIFKRA LILSLTNPKA ILFYVSFFVQ FIDVNAPHTG ISFFILAATL ELVSFCYLSF LIISGAFVTQ YIRTKKKLAK VGNSLIGLMF VGFAARLATL QS

SEQ ID NO. 79: YEBS ORGANISM NAME : ESCHERICHIA COLI

MALNTPQITP TKKITVRAIG EELPRGDYQR CPQCDMLFSL PEINSHQSAY CPRCQAKIRD GRDWSLTRLA AMAFTMLLLM PFAWGEPLLH IWLLGIRIDA NVMQGIWQMT KQGDAITGSM VFFCVIGAPL ILVTSIAYLW FGNRLGMNLR PVLLMLERLK EWVMLDIYLV GIGVASIKVQ DYAHIQAGVG LFSFVALVIL TTVTLSHLNV EELWERFYPQ RPATRRDEKL RVCLGCHFTG YPDQRGRCPR CHIPLRLRRR HSLQKCWAAL LASIVLLLPA NLLPISIIYL NGGRQEDTIL SGIMSLASSN IAVAGIVFIA SILVPFTKVI VMFTLLLSIH FKCQQGLRTR ILLLRMVTWI GRWSMLDLFV ISLTMSLINR DQILAFTMGP AAFYFGAAVI LTILAVEWLD SRLLWDAHES GNARFDD

SEQ ID NO. 80: YEDA ORGANISM NAME : ESCHERICHIA COLI

MRFRQLLPLF GALFALYIIW GSTYFVIRIG VESWPPLMMA GVRFLAAGIL LLAFLLLRGH KLPPLRPLLN AALIGLLLLA VGNGMVTVAE HQNVPSGIAA VVVATVPLFT LCFSRLFGIK TRKLEWVGIA IGLAGIIMLN SGGNLSGNPW GAILILIGSI SWAFGSVYGS RITLPVGMMA GAIEMLAAGV VLMIASMIAG EKLTALPSLS GFLAVGYLAL FGSIIAINAY MYLIRNVSPA LATSYAYVNP VVAVLLGTGL GGETLSKIEW LALGVIVFAV VLVTLGKYLF PAKPVVAPVI QDASSE

SEQ ID NO. 81: YEEO ORGANISM NAME : ESCHERICHIA COLI

MLRHILTAKN LLSNPIFKFP NCLPFLSTVC CICRQFVGEN LCSFADSPSL FEMWFHFLQL RSALNISSAL RQVVHGTRWH AKRKSYKVLF WREITPLAVP IFMENACVLL MGVLSTFLVS WLGKDAMAGV GLADSFNMVI MAFFAAIDLG TTVVVAFSLG KRDRRRARVA TRQSLVIMTL FAVLLATLIH HFGEQIIDFV AGDATTEVKA LALTYLELTV LSYPAAAITL IGSGALRGAG NTKIPLLING SLNILNIIIS GILIYGLFSW PGLGFVGAGL GLTISRYIGA VAILWVLAIG FNPALRISLK SYFKPLNFSI IWEVMGIGIP ASVESVLFTS GRLLTQMFVA GMGTSVIAGN FIAFSIAALI NLPGSALGSA STIITGRRLG VGQIAQAEIQ LRHVFWLSTL GLTAIAWLTA PFAGVMASFY TQDPQVKHVV VILIWLNALF MPIWSASWVL PAGFKGARDA RYAMWVSMLS MWGCRVVVGY VLGIMLGWGV VGVWMGMFAD WAVRAVLFYW RMVTGRWLWK YPRPEPQKCE KKPVVSE

SEQ ID NO. 82: YEGH ORGANISM NAME: ESCHERICHIA COLI

MEWIADPSIW AGLITLIVIE LVLGIDNLVF IAILAEKLPP KQRDRARVTG LLLAMLMRLL LLASISWLVT LTQPLFSFRS FTFSARDLIM LFGGFFLLFK ATMELNERLE GKDSNNPTQR KGAKFWGVVT QIVVLDAIFS LDSVITAVGM VDHLLVMMAA VVIAISLMLM ASKPLTQFVN SHPTIVILCL SFLLMIGFSL VAEGFGFVIP KGYLYAAIGF SVMIEALNQL AIFNRRRFLS ANQTLRQRTT EAVMRLLSGQ KEDAELDAET ASMLVDHGNQ QIFNPQERRM IERVLNLNQR TVSSIMTSRH DIEHIDLNAP EEEIRQLLER NQHTRLVVTD GDDAEDLLGV VHVIDLLQQS LRGEPLNLRV LIRQPLVFPE TLPLLPALEQ FRNARTHFAF VVDEFGSVEG IVTLSDVTET IAGNLPNEVE EIDARHDIQK NADGSWTANG HMPLEDLVQY VPLPLDEKRE YHTIAGLLME YLQRIPKPGE EVQVGDYLLK TLQVESHRVQ KVQIIPLRKD GEMEYEV

SEQ ID NO. 83: YGGA (ARGO) ORGANISM NAME: ESCHERICHIA COLI

MFSYYFQGLA LGAAMILPLG PQNAFVMNQG IRRQYHIMIA LLCAISDLVL ICAGIFGGSA LLMQSPWLLA LVTWGGVAFL LWYGFGAFKT AMSSNIELAS AEVMKQGRWK IIATMLAVTW LNPHVYLDTF VVLGSLGGQL DVEPKRWFAL GTISASFLWF FGLALLAAWL APRLRTAKAQ RIINLVVGCV MWFIALQLAR DGIAHAQALF S

SEQ ID NO. 84: YFCJ ORGANISM NAME : ESCHERICHIA COLI

MTAVSQTETR SSANFSLFRI AFAVFLTYMT VGLPLPVIPL FVHHELGYGN TMVGIAVGIQ FLATVLTRGY AGRLADQYGA KRSALQGMLA CGLAGGALLL AAILPVSAPF KFALLVVGRL ILGFGESQLL TGALTWGLGI VGPKHSGKVM SWNGMAIYGA LAVGAPLGLL IHSHYGFAAL AITTMVLPVL AWACNGTVRK VPALAGERPS LWSVVGLIWK PGLGLALQGV GFAVIGTFVS LYFASKGWAM AGFTLTAFGG AFVVMRVMFG WMPDRFGGVK VAIVSLLVET VGLLLLWQAP

GAWVALAGAA LTGAGCSLIF PALGVEVVKR VPSQVRGTAL GGYAAFQDIA LGVSGPLAGM LATTFGYSSV FLAGAISAVL GIIVTILSFR RG

SEQ ID NO. 85: YFIK (EAMB) ORGANISM NAME: ESCHERICHIA COLI

MTPTLLSAFW TYTLITAMTP GPNNILALSS ATSHGFRQST RVLAGMSLGF LIVMLLCAGI SFSLAVIDPA AVHLLSWAGA AYIVWLAWKI ATSPTKEDGL QAKPISFWAS FALQFVNVKI ILYGVTALST FVLPQTQALS WVVGVSVLLA MIGTFGNVCW ALAGHLFQRL FRQYGRQLNI VLALLLVYCA VRIFY

SEQ ID NO. 86: YHJE ORGANISM NAME: ESCHERICHIA COLI

MQATATTLDH EQEYTPINSR NKVLVASLIG TAIEFFDFYI YATAAVIVFP HIFFPQGDPT AATLQSLATF AIAFVARPIG SAVFGHFGDR VGRKATLVAS LLTMGISTVV IGLLPGYATI GIFAPLLLAL ARFGQGLGLG GEWGGAALLA TENAPPRKRA LYGSFPQLGA PIGFFFANGT FLLLSWLLTD EQFMSWGWRV PFIFSAVLVI IGLYVRVSLH ESPVFEKVAK AKKQVKIPLG TLLTKHVRVT VLGTFIMLAT YTLFYIMTVY SMTFSTAAAP VGLGLPRNEV LWMLMMAVIG FGVMVPVAGL LADAFGRRKS MVIITTLIIL FALFAFNPLL GSGNPILVFA FLLLGLSLMG LTFGPMGALL PELFPTEVRY TGASFSYNVA SILGASVAPY IAAWLQTNYG LGAVGLYLAA MAGLTLIALL LTHETRHQSL

SEQ ID NO. 87:YIDE ORGANISM NAME: ESCHERICHIA COLI

MSDIALTVSI LALVAVVGLF IGNVKFRGIG LGIGGVLFGG IIVGHFVSQA GMTLSSDMLH VIQEFGLILF VYTIGIQVGP GFFASLRVSG LRLNLFAVLI VIIGGLVTAI LHKLFDIPLP VVLGIFSGAV TNTPALGAGQ QILRDLGTPM EMVDQMGMSY AMAYPFGICG ILFTMWMLRV IFRVNVETEA QQHESSRTNG GALIKTINIR VENPNLHDLA IKDVPILNGD KIICSRLKRE ETLKVPSPDT IIQLGDLLHL VGQPADLHNA QLVIGQEVDT SLSTKGTDLR VERVVVTNEN VLGKRIRDLH FKERYDVVIS RLNRAGVELV ASGDISLQFG DILNLVGRPS AIDAVANVLG NAQQKLQQVQ MLPVFIGIGL GVLLGSIPVF VPGFPAALKL GLAGGPLIMA LILGRIGSIG KLYWFMPPSA NLALRELGIV LFLSVVGLKS GGDFVNTLVN GEGLSWIGYG ALITAVPLIT VGILARMLAK MNYLTMCGML AGSMTDPPAL AFANNLHPTS GAAALSYATV YPLVMFLRII TPQLLAVLFW SIG

SEQ ID NO. 88: YIGK (RHTB) ORGANISM NAME: ESCHERICHIA COLI

MTLEWWFAYL LTSIILSLSP GSGAINTMTT SLNHGYRGAV ASIAGLQTGL AIHIVLVGVG LGTLFSRSVI AFEVLKWAGA AYLIWLGIQQ WRAAGAIDLK SLASTQSRRH LFQRAVFVNL TNPKSIVFLA ALFPQFIMPQ QPQLMQYIVL GVTTIVVDII VMIGYATLAQ RIALWIKGPK QMKALNKIFG SLFMLVGALL ASARHA

SEQ ID NO. 89:YIGJ (RHTC) ORGANISM NAME : ESCHERICHIA COLI

MLMLFLTVAM VHIVALMSPG PDFFFVSQTA VSRSRKEAMM GVLGITCGVM VWAGIALLGL HLIIEKMAWL HTLIMVGGGL YLCWMGYQML RGALKKEAVS APAPQVELAK SGRSFLKGLL TNLANPKAII YFGSVFSLFV GDNVGTTARW GIFALIIVET LAWFTVVASL FALPQMRRGY QRLAKWIDGF AGALFAGFGI HLIISR

SEQ ID NO. 90: YIJE ORGANISM NAME : ESCHERICHIA COLI

MSAAGKSNPL AISGLVVLTL IWSYSWIFMK QVTSYIGAFD FTALRCIFGA LVLFIVLLLR GRGMRPTPFK YTLAIALLQT CGMVGLAQWA LVSGGAGKVA ILSYTMPFWV VIFAALFLGE RLRRGQYFAI LIAAFGLFLV LQPWQLDFSS MKSAMLAILS GVSWGASAIV AKRLYARHPR VDLLSLTSWQ MLYAALVMSV VALLVPQREI DWQPTVFWAL AYSAILATAL AWSLWLFVLK NLPASIASLS TLAVPVCGVL FSWWLLGENP GAVEGSGIVL IVLALALVSR KKKEAVSVKRI

SEQ ID NO. 91: YJII (KPTA) ORGANISM NAME : ESCHERICHIA COLI

MAKYNEKELA DTSKFLSFVL RHKPEAIGIV LDREGWADID KLILCAQKAG KRLTRALLDT VVATSDKKRF SYSSDGRCIR AVQGHSTSQV AISFAEKTPP QFLYHGTASR FLDEIKKQGL IAGERHYVHL SADEATARKV GARHGSPVIL TVKAQEMAKR GLPFWQAENG VWLTSTVAVE FLEW

SEQ ID NO. 92: YJIJ ORGANISM NAME : ESCHERICHIA COLI

MPSSTHPVER FSFSTALFGM LVLTLGMGLG RFLYTPMLPV MMAEGSFSFS QLSWIASGNY AGYLAGSLLF SFGAFHQPSR LRPFLLASAL ASGLLILAMA WLPPFILVLL IRVLAGVASA GMLIFGSTLI MQHTRHPFVL AALFSGVGIG IALGNEYVLA GLHFDLSSQT LWQGAGALSG MMLIALTLLM PSKKHAITPM PLAKTEQQIM SWWLLAILYG LAGFGYIIVA TYLPLMAKDA GSPLLTAHLW TLVGLSIVPG CFGWLWAAKR WGALPCLTAN LLVQAICVLL TLASDSPLLL IISSLGFGGT FMGTTSLVMT IARQLSVPGN LNLLGFVTLI YGIGQILGPA LTSMLSNGTS ALASATLCGA AALFIAALIS TVQLFKLQVV TS

SEQ ID NO. 93: YJIO (MDTM) ORGANISM NAME : ESCHERICHIA COLI

MPRFFTRHAA TLFFPMALIL YDFAAYLSTD LIQPGIINVV RDFNADVSLA PAAVSLYLAG GMALQWLLGP LSDRIGRRPV LITGALIFTL ACAATMFTTS MTQFLIARAI QGTSICFIAT VGYVTVQEAF GQTKGIKLMA IITSIVLIAP IIGPLSGAAL MHFMHWKVLF AIIAVMGFIS FVGLLLAMPE TVKRGAVPFS AKSVLRDFRN VFCNRLFLFG AATISLSYIP MMSWVAVSPV ILIDAGSLTT SQFAWTQVPV FGAVIVANAI VARFVKDPTE PRFIWRAVPI QLVGLSLLIV

GNLLSPHVWL WSVLGTSLYA FGIGLIFPTL FRFTLFSNKL PKGTVSASLN MVILMVMSVS VEIGRWLWFN GGRLPFHLLA VVAGVIVVFT LAGLLNRVRQ HQAAELVEEQ

SEQ ID NO. 94: YKGH ORGANISM NAME : ESCHERICHIA COLI

MREQIKQDID LIEILFYLKK KIRVILFIMA ICMAMVLLFL YINKDNIKVI YSLKINQTTP GILVSCDSNN NFACQTTMTE DVIQRITTFF HTSPDVKNRE IRLEWSGDKR ALPTAEEEIS RVQASIIKWY ASEYHNGRQV LDEIQTPSAI NSELYTKMIY LTRNWSLYPN GDGCVTISSP EIKNKYPAAI CLALGFFLSI VISVMFCLVK KMVDEYQQNS GQ

SEQ ID NO. 95: YPJD ORGANISM NAME : ESCHERICHIA COLI

MQRLEQRSPD AILLLFLIAQ TVDITMPVFA LLALVAYSVS LALIVPGLLQ KNGGWRRMAI ISAVIALVCH AIALEARILP DGDSGQNLSL LNVGSLVSLM ICTVMTIVAS RNRGWLLLPI VYAFALINLA LATFMPNEYI THLEATPGML VHIGLSLFSY ATLIIAALYA LQLAWIDYQL KNKKLAFNQE MPPLMSIERK MFHITQIGVV LLTLTLCTGL FYMHNLFSME NIDKAVLSIV AWFVYIVLLW GHYHEGWRGR RVVWFNVAGA VILTLAYFGS RIVQQLIS

SEQ ID NO. 96: YTFF ORGANISM NAME : ESCHERICHIA COLI

MPVMISGVLY ALLAGLMWGL IFVGPLIVPE YPAMLQSMGR YLALGLIALP IAWLGRVRLR QLARRDWLTA LMLTMMGNLI YYFCLASAIQ RTGAPVSTMI IGTLPVVIPV FANLLYSQRD GKLAWGKLAP ALICIGIGLA CVNIAELNHG LPDFDWARYT SGIVLALVSV VCWAWYALRN ARWLRENPDK HPMMWATAQA LVTLPVSLIG YLVACYWLNT QTPDFSLPFG PRPLVFISLM VAIAVLCSWV GALCWNVASQ LLPTVILGPL IVFETLAGLL YTFLLRQQMP PLMTLSGIAL LVIGVVIAVR AKPEKPLTES VSES

SEQ ID NO. 97: YTFL ORGANISM NAME: ESCHERICHIA COLI

MLNSILVILC LIAVSAFFSM SEISLAASRK IKLKLLADEG NINAQRVLNM QENPGMFFTV VQIGLNAVAI LGGIVGDAAF SPAFHSLFSR YMSAELSEQL SFILSFSLVT GMFILFADLT PKRIGMIAPE AVALRIINPM RFCLYVCTPL VWFFNGLANI IFRIFKLPMV RKDDITSDDI YAVVEAGALA GVLRKQEHEL IENVFELESR TVPSSMTPRE NVIWFDLHED EQSLKNKVAE HPHSKFLVCN EDIDHIIGYV DSKDLLNRVL ANQSLALNSG VQIRNTLIVP DTLTLSEALE SFKTAGEDFA VIMNEYALVV GIITLNDVMT TLMGDLVGQG LEEQIVARDE NSWLIDGGTP IDDVMRVLDI DEFPQSGNYE TIGGFMMFML RKIPKRTDSV KFAGYKFEVV DIDNYRIDQL LVTRIDSKAT ALSPKLPDAK DKEESVA

SEQ ID NO. 98: CHLOROFLEXUS AGGREGANS (CGMCR)

<400> 785

MET SER VAL THR GLY ARG LEU ALA GLY LYS ILE ALA LEU ILE THR GLY

1 5 10 15

GLY ALA GLY ASN ILE GLY SER GLU MET THR ARG ARG PHE LEU ALA GLU

20 25 30

GLY ALA THR VAL ILE ILE SER GLY ARG ASN SER ALA LYS LEU ALA ALA

35 40 45

LEU ALA GLU ARG LEU ARG SER GLU ALA GLY VAL PRO ALA LYS ARG ILE

50 55 60

ASP LEU GLU VAL MET ASP GLY SER ASP PRO ALA ALA VAL ARG ALA GLY

65 70 75 80

VAL ALA ALA ILE ILE GLY ARG HIS GLY HIS ILE ASP ILE LEU VAL ASN

85 90 95

ASN ALA GLY SER THR GLY ALA GLN ARG ARG LEU ALA GLU ILE PRO LEU

100 105 110

ASN GLU THR ASP ARG ASP LEU ASP ASP GLU GLU ALA LEU SER THR SER

115 120 125

VAL ALA ASN LEU LEU GLY MET ALA TRP HIS LEU MET ARG ILE LEU SER

130 135 140

PRO HIS MET PRO PRO GLY SER ALA ILE ILE ASN ILE SER THR ILE PHE

145 150 155 160

SER ARG ALA GLU TYR TYR GLY ARG ILE PRO TYR VAL VAL PRO LYS ALA

165 170 175

ALA LEU ASN THR LEU THR GLN ILE ALA ALA ARG GLU LEU GLY ILE ARG

180 185 190

GLY ILE ARG VAL ASN THR ILE PHE PRO GLY PRO ILE GLU SER GLU ARG

195 200 205

ILE GLN THR VAL PHE GLN ARG MET ASP GLN LEU LYS GLY ARG PRO GLU

210 215 220

GLY ASP THR ALA SER GLN PHE LEU ALA THR MET ARG LEU TYR ARG ALA

225 230 235 240

ASN ASP GLN GLY GLN LEU GLU ARG ARG PHE PRO THR ILE CYS ASP VAL

245 250 255

ALA ASP ALA ALA VAL PHE LEU ALA SER ASP GLU ALA ALA ALA LEU THR

260 265 270

GLY GLU THR ILE GLU VAL THR HIS GLY MET GLU LEU PRO THR SER SER

275 280 285

GLU THR SER LEU LEU ALA ARG THR ASP LEU ARG THR ILE ASP ALA ASN

290 295 300

GLY ARG THR THR LEU ILE CYS ALA GLY ASP GLN ILE GLU GLU VAL MET

305 310 315 320

ALA LEU THR GLY MET LEU ARG THR CYS GLY SER GLU VAL ILE ILE GLY

325 330 335

PHE ARG SER GLU ALA ALA LEU ALA GLN PHE GLU GLN ALA ILE GLY GLU

340 345 350

SER ARG ARG LEU ALA GLY GLU SER PHE ILE PRO PRO ILE ALA LEU PRO

355 360 365

ILE ASP LEU ARG ASN PRO SER THR ILE ASP ALA LEU PHE ASP TRP ALA

370 375 380

GLY GLU ASN THR GLY GLY ILE HIS ALA ALA VAL ILE LEU PRO ALA SER

385 390 395 400

GLY ARG GLU PRO ALA THR GLN VAL ILE ASP ILE ASP ASP ALA HIS VAL

405 410 415

GLN ALA PHE LEU ASN ASP GLU ILE VAL GLY SER ILE ILE ILE ALA SER

420 425 430

ARG LEU ALA ARG TYR TRP GLN ALA GLN ARG ILE ALA PRO GLY ALA ARG

435 440 445

ALA ARG GLU PRO ARG VAL ILE PHE LEU SER ASN GLY ALA SER THR ALA

450 455 460

GLY ASN PRO TYR GLY ARG ILE GLN SER ALA ALA ILE GLU GLN LEU ILE

465 470 475 480

ARG VAL TRP ARG HIS GLU ALA ALA LEU ASP TYR GLU ARG ALA THR ALA

485 490 495

ALA GLY GLU ARG VAL LEU PRO ALA VAL TRP ALA SER GLN ILE VAL ARG

500 505 510

PHE ALA ASN ARG SER LEU GLU GLY LEU GLU PHE ALA CYS ALA TRP THR

515 520 525

ALA GLN LEU LEU HIS SER GLN ARG ARG ILE ASN GLU ILE THR LEU THR

530 535 540

ILE PRO ALA ASP ILE SER ALA THR THR GLY ALA ARG SER ALA SER VAL

545 550 555 560

GLY TRP ALA GLU SER LEU ILE GLY LEU HIS LEU GLY LYS VAL ALA LEU

565 570 575

ILE THR GLY GLY SER ALA GLY ILE GLY GLY GLN ILE GLY ARG LEU LEU

580 585 590

ALA LEU SER GLY ALA ARG VAL MET LEU ALA ALA ARG ASP PRO HIS LYS

595 600 605

LEU GLU GLN ILE GLN ALA THR ILE ARG ALA GLU LEU ALA GLU VAL GLY

610 615 620

TYR THR ASP VAL GLU GLU ARG VAL GLN ILE ALA PRO GLY CYS ASP VAL

625 630 635 640

SER SER GLU GLU GLN LEU VAL ASP LEU VAL GLU ARG THR LEU ALA ALA

645 650 655

PHE GLY THR VAL ASP TYR LEU ILE ASN ASN ALA GLY ILE ALA GLY VAL

660 665 670

GLU GLU MET VAL ILE ASP MET PRO VAL GLU GLY TRP ARG ASN THR LEU

675 680 685

TYR ALA ASN LEU ILE SER ASN TYR SER LEU MET ARG LYS LEU ALA PRO

690 695 700

LEU MET LYS LYS GLN GLY SER GLY TYR VAL LEU ASN VAL SER SER TYR

705 710 715 720

PHE GLY GLY GLU LYS ASP ALA ALA ILE PRO TYR PRO ASN ARG ALA ASP

725 730 735

TYR ALA VAL SER LYS ALA GLY GLN ARG ALA MET ALA GLU VAL PHE ALA

740 745 750

ARG PHE LEU GLY PRO GLU ILE GLN ILE ASN ALA ILE ALA PRO GLY PRO

755 760 765

VAL GLU GLY ASP ARG LEU ARG GLY THR GLY GLU ARG PRO GLY LEU PHE

770 775 780

ALA ARG ARG ALA ARG LEU ILE LEU GLU ASN LYS ARG LEU ASN GLU LEU

785 790 795 800

HIS ALA ALA LEU ILE THR ALA ALA ARG THR ASP ASN ARG PRO MET ARG

805 810 815

GLU LEU VAL GLU LEU LEU LEU PRO ASN ASP VAL ALA ALA LEU ALA GLN

820 825 830

HIS PRO ALA ALA PRO ASP VAL LEU ARG THR LEU ALA LYS ARG PHE GLN

835 840 845

SER GLU GLY ASP PRO ALA ALA SER SER SER SER PHE LEU LEU ASN ARG

850 855 860

SER ILE ALA ALA LYS LEU LEU ALA ARG LEU ILE ASN GLY GLY TYR ASP

865 870 875 880

LEU PRO ALA ASP ILE PHE ALA ASN LEU ALA VAL PRO PRO ASP PRO PHE

885 890 895

PHE THR ARG ALA GLN ILE ASP ARG GLU ALA ARG LYS VAL ARG ASP GLY

900 905 910

ILE MET GLY MET LEU TYR LEU GLN ARG MET PRO THR GLU PHE ASP VAL

915 920 925

ALA MET ALA THR VAL TYR TYR LEU ALA ASP ARG ASN VAL SER GLY GLU

930 935 940

THR PHE HIS PRO SER GLY GLY LEU ARG TYR GLU ARG THR PRO THR GLY

945 950 955 960

GLY GLU LEU PHE GLY LEU PRO ALA PRO GLU ARG LEU ALA GLU LEU VAL

965 970 975

GLY SER THR VAL TYR LEU ILE GLY GLU HIS LEU THR GLU HIS LEU ASN

980 985 990

LEU LEU ALA ARG ALA TYR LEU GLU ARG TYR GLY ALA ARG GLN VAL VAL

995 1000 1005

MET ILE VAL GLU THR GLU ALA GLY ALA GLU LYS MET ARG HIS LEU

1010 1015 1020

LEU HIS ASP HIS VAL GLU ALA GLY ARG LEU PRO ILE ILE VAL ALA

1025 1030 1035

GLY ASP GLN ILE GLU ALA ALA ILE ASP GLN ALA ILE ALA ASN TYR

1040 1045 1050

GLY ARG PRO GLY PRO VAL VAL CYS THR PRO PHE ARG PRO LEU PRO

1055 1060 1065

SER ALA PRO LEU VAL GLY ARG LYS ASP SER ASP TRP SER THR VAL

1070 1075 1080

LEU SER GLU ALA GLU PHE ALA GLU LEU CYS GLU HIS GLN LEU THR

1085 1090 1095

HIS HIS PHE ARG VAL ALA ARG LYS ILE ALA LEU SER ASP GLY ALA

1100 1105 1110

SER LEU ALA LEU VAL THR PRO GLU THR THR ALA THR SER SER THR

1115 1120 1125

GLU GLN PHE ALA LEU ALA ASN PHE VAL LYS THR THR LEU HIS ALA

1130 1135 1140

PHE THR ALA THR ILE GLY VAL GLU SER GLU ARG THR ALA GLN ARG

1145 1150 1155

ILE LEU ILE ASN GLN VAL ASP LEU THR ARG ARG ALA ARG ALA GLU

1160 1165 1170

GLU PRO ARG ASP PRO ARG GLU ARG GLN GLN GLU LEU GLU ARG PHE

1175 1180 1185

ILE GLU ALA VAL LEU LEU VAL THR ALA PRO LEU PRO PRO GLU ALA

1190 1195 1200

ASP THR ARG TYR ALA GLY ARG ILE HIS ARG GLY ARG ALA ILE THR VAL

1205 1210 1215

SEQ ID NO. 99: MCR OSCILLOCHLORIS TRICHOIDES (OTMCR)

MFMTRLNDKIALITGGAGTIGEVITRRYLEEGATVVMAGRNRDKLDRYRERLITEFHALP

ERVMVVRMDGSSNAEVRMGIAAVVAHFGRIDILVNNAGSAGARQRLPAIPLLRSELQADE

TETLADSIGNLIGITWNLIRAAAPFMPAGSSVINISTIFARTDYYGRIPYVVPKAALHAL

TLAAATELGERGIRVNQINPGPIDSDRIRTVFRRMDELKGVPEQSTADGFFQMMRLRRPN

AEGDLVKGFPKTLDVANVAVFLGSAESAALSGETLDVTHGMAVPTESRTTLTSRPGLRAV

DGSGHTTLICVGDQIEEAAALTGVLRACGAEVVIGFRSRAAIARFDHLIERGRHLPSQEH

VAPVLLYLNPTEPESIDQALRWMATNLDLPTSVIILPAQRQPLPPSVVRASDEEVAYFLR
DELSGMIVLASRLARFWQQATLAPGNAPIQPRVLFMTNPDDGQGNLYAEILRAGVEQLCR
VWRHESQLDYTRLAQMDAHPPHIRPVWANQLVRFANNEQENLEYCCAWVAKILLSERTIE
ELNLYLPRQIGSTTGSRQPSFGWAENLIGLHLGKTALITGGSAGIGSQIARLLALSGARV
MLCARDERKLIQMRDMIIAELTEVGYNQVESRVQICAGCDVGEEEQLEIAVQRTLDLFGH
VDYLINNAGIAGAEEMVLDLPLEAWQRTLRTNLISNYSLIRKLAPQMKSRGSGYILNVSS
YFGGEKYAAIPYPNRADYAVSKAGQRALGEALARLLGPEVQINAMAPGPVEGERLRGSGD
RPGLFLRRGRLILENKRLNDLHATLIAAERETQVGMRDLLARLLHNDVCALIDDPAAPTH
LRALAERIWEQSDPNSYARAFFMNANIATKLLARLFNADQIDAQTFHTSQPNLPPEPFFA
RTQIDREARRVRDGVMSMLYLQRMPTEFDVALATVYYLNDRSVSGETFHPSGGLRHERTP
TGAELYGSPAPQRLASLAGSTVYLIGESMAAHLEALARAYIERYAATRVVLICATPAGVE
RFSHHLADHLASGALAILSAEEGIEAALSEALRRFGPPGPVVSTPFQPLPSQPLIGRNDS
DWSTVLDVAGFSAMCEQQLTHHFRVTRKLSLVAGVSLVLVTPETDSHSSTEQFALANFVK
TTLHAFTATVGVECERTAHRILVNQVDLGRQARAEEPRSPAEQAQEMERFIDAIMLTTAP
IPAEEDNRYTGRIYRGRAITV

SEQ ID NO. 100: CHLOROFLEXUS AURANTIACUS (CAMCR)

MSGTGRLAGKIALITGGAGNIGSELTRRFLAEGATVIISGRNRAKLTALAERMQAEAGVP
AKRIDLEVMDGSDPVAVRAGIEAIVARHGQIDILVNNAGSAGAQRRLAEIPLTEAELGPG
AEETLHASIANLLGMGWHLMRIAAPHMPVGSAVINVSTIFSRAEYYGRIPYVTPKAALNA
LSQLAARELGARGIRVNTIFPGPIESDRIRTVFQRMDQLKGRPEGDTAHHFLNTMRLCRA
NDQGALERRFPSVGDVADAAVFLASAESAALSGETIEVTHGMELPACSETSLLARTDLRT
IDASGRTTLICAGDQIEEVMALTGMLRTCGSEVIIGFRSAAALAQFEQAVNESRRLAGAD
FTPPIALPLDPRDPATIDAVFDWGAGENTGGIHAAVILPATSHEPAPCVIEVDDERVLNF
LADEITGTIVIASRLARYWQSQRLTPGARARGPRVIFLSNGADQNGNVYGRIQSAAIGQL
IRVWRHEAELDYQRASAAGDHVLPPVWANQIVRFANRSLEGLEFACAWTAQLLHSQRHIN
EITLNIPANISATTGARSASVGWAESLIGLHLGKVALITGGSAGIGGQIGRLLALSGARV
MLAARDRHKLEQMQAMIQSELAEVGYTDVEDRVHIAPGCDVSSEAQLADLVERTLSAFGT
VDYLINNAGIAGVEEMVIDMPVEGWRHTLFANLISNYSLMRKLAPLMKKQGSGYILNVSS
YFGGEKDAAIPYPNRADYAVSKAGQRAMAEVFARFLGPEIQINAIAPGPVEGDRLRGTGE
RPGLFARRARLILENKRLNELHAALIAAARTDERSMHELVELLLPNDVAALEQNPAAPTA
LRELARRFRSEGDPAASSSSALLNRSIAAKLLARLHNGGYVLPADIFANLPNPPDPFFTR
AQIDREARKVRDGIMGMLYLQRMPTEFDVAMATVYYLADRNVSGETFHPSGGLRYERTPT

GGELFGLPSPERLAELVGSTVYLIGEHLTEHLNLLARAYLERYGARQVVMIVETETGAET
MRRLLHDHVEAGRLMTIVAGDQIEAAIDQAITRYGRPGPVVCTPFRPLPTVPLVGRKDSD
WSTVLSEAEFAELCEHQLTHHFRVARKIALSDGASLALVTPETTATSTTEQFALANFIKT
TLHAFTATIGVESERTAQRILINQVDLTRRARAEEPRDPHERQQELERFIEAVLLVTAPL
PPEADTRYAGRIHRGRAITV

We claim:

1. A genetically modified organism capable of producing a chemical product of interest, comprising:

(a) at least one nucleic acid encoding for a monofunctional malonyl-CoA reductase capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde, and

(b) at least one nucleic acid encoding for an enzyme selected from the group consisting of: ydfG, mmsB, NDSD, rutE, nemA, and combinations thereof.

2. The genetically modified organism of any claim, wherein the at least one nucleic acid of (b) encodes for an enzyme capable of catalyzing the conversion of malonate semialdehyde to 3-hydroxypropionic acid (3-HP).

3. The genetically modified organism of any claim, wherein the malonyl-CoA reductase exhibits a reduced capability of converting malonate semialdehyde to 3-HP.

4. The genetically modified organism of any claim, wherein said malonyl-CoA reductase is from or is derived from *Sulfolobus tokodaii.*

5. The genetically modified organism of any claim, wherein the at least one nucleic acid of (b) encodes for ydfG, mmsB, or NDSD, and wherein the ydfG, mmsB, or NDSD is capable of catalyzing the conversion of the malonate semialdehyde keto form to 3-hydroxypropionic acid (3-HP).

6. The genetically modified organism of any claim, wherein the at least one nucleic acid of (b) encodes for ydfG and is from or is derived from *E. coli.*

7. The genetically modified organism of any claim, wherein the at least one nucleic acid of (b) encodes for mmsB and NDSD and is from or is derived from *Pseudomonas.*

8. The genetically modified organism of any claim, wherein the at least one nucleic acid of (b) encodes for nemA or rutE, wherein the nemA or rutE is capable of catalyzing the conversion of malonate semialdehyde enol form to 3-hydroxypropionic acid (3-HP).

9. The genetically modified organism of any claim 1, wherein the at least one nucleic acid of (b) encodes for nemA and rutE and is from or is derived from *E. coli.*

10. The genetically modified organism of any claim, wherein the at least one nucleic acid of (b) encodes for an enzyme capable of catalyzing the conversion of malonate semialdehyde hydrated form to 3-hydroxypropionic acid (3-HP).

11. A genetically modified organism capable of producing a chemical product of interest, comprising:

(a) at least one nucleic acid encoding for a monofunctional malonyl-CoA reductase capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde,

(b) at least one nucleic acid encoding for an enzyme capable of converting malonate semialdehyde keto form to 3-hydroxypropionic acid (3-HP); and

(c) at least one nucleic acid sequence encoding for an enzyme capable of converting the malonate semialdehyde enol form and/or the malonate semialdehyde hydrated form into 3-HP.

12. A genetically modified organism capable of producing a chemical product of interest, comprising at least one nucleic acid encoding for a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme,

wherein the fused enzyme exhibits at least one characteristic selected from the group consisting of: (a) primarily not NADPH-dependent, (b) primarily NADH-dependent, (c) primarily flavin-dependent, (d) less susceptible to 3-HP inhibition at high concentration, and/or (e) catalyzes a reaction pathway to 3-HP that is substantially irreversible.

13. A genetically modified organism capable of producing a chemical product of interest, comprising at least one nucleic acid encoding for a monofunctional malonyl-CoA reductase enzyme fused to at least one dehydrogenase enzyme.

14. The genetically modified organism of any claim, wherein the at least one dehydrogenase enzyme is selected from the group consisting of: ydfG, mmsB, NDSD, rutE, and nemA.

15. The genetically modified organism of any claim, comprising:

(a) at least one nucleic acid encoding for a first monofunctional malonyl-CoA reductase enzyme fused to a first dehydrogenase enzyme, and

(b) at least one nucleic acid encoding for a second monofunctional malonyl-CoA reductase enzyme fused to a second dehydrogenase enzyme,

wherein said first and second dehydrogenase enzymes are capable of converting different forms of malonate semialdehyde to 3-hydroxypropionic acid (3-HP).

16. The genetically modified organism of any claim, wherein the first dehydrogenase enzyme is capable of converting malonate semialdehyde enol form to 3-hydroxypropionic acid (3-HP), and

wherein said second dehydrogenase enzyme is capable of converting malonate semialdehyde keto form to 3-HP.

17. The genetically modified organism of any claim, wherein the first monofunctional malonyl-CoA reductase enzyme is identical to the second monofunctional malonyl-CoA reductase enzyme.

18. The genetically modified organism of any claim, wherein the first monofunctional malonyl-CoA reductase enzyme is different from the second monofunctional malonyl-CoA reductase enzyme.

19. The genetically modified organism of any claim, wherein the configuration of the monofunctional malonyl-CoA reductase enzyme fused to one or more dehydrogenase enzymes is selected from the group consisting of: mcr-ydfG, mcr-mmsB, mcr-NDSD, mcr-rutE, mcr-nemA, mcr-ydfG-mmsB, mcr-ydfG-NDSD, mcr-ydfG-rutE, mcr-ydfG-nemA, mcr-mmsB-ydfG, mcr-mmsB-NDSD, mcr-mmsB-rutE, mcr-mmsB-nemA, mcr-NDSD-ydfG, mcr-NDSD-mmsB, mcr-NDSD-rutE, mcr-NDSD-nemA, mcr-rutE-ydfG, mcr-rutE-mmsB, mcr-rutE-NDSD, mcr-rutE-nemA, mcr-nemA-ydfG, mcr-nemA-mmsB, mcr-nemA-NDSD, and mcr-nemA-rutE.

20. A method of producing 3-hydroxypropionic acid (3-HP) using the genetically modified organism of any claim, wherein the organism synthesizes 3-HP by:

(a) converting a carbon source to malonyl-CoA,

(b) converting malonyl-CoA to malonate semialdehyde, and

(c) converting malonate semialdehyde to 3-HP.

21. A method of producing a product, comprising forming the product with the genetically a modified organism of any claim.

22. A product made by the genetically modified organism of any claim.

23. A product made from the method of any claim.

24. The product of any claim, wherein the product is selected from the group consisting of: acetyl-CoA, malonyl-CoA, malonate semialdehyde, 3-HP, acrylic acid, 1,3 propanediol, malonic acid, ethyl 3-HP, propiolactone, acrylonitrile, acrylamide, methyl acrylate, a polymer, a superabsorbent polymer, polyacrylic acid, a consumer product, and any combination thereof.

25. The method of any claim, wherein said product comprises at least one product selected from the group consisting of: 3-hydroxypropionic acid (3-HP), a derivative of 3-HP, 1,4-butanediol, butanol, isobutanol, a polyketide chemical product, a C4-C18 fatty acid chain, and any combination thereof.

26. The method of any claim, wherein the at least one product is converted to acrylic acid, acrylates, 1,3-propanediol, malonic acid, ethyl-3-hydroxypropionate, ethyl ethoxy propionate, propiolactone, acrylamide, acrylonitrile, or any combination thereof.

27. The method of any claim, wherein the at least one product is oligomerized or polymerized into polyacrylic acid, oligomerized or polymerized: methyl acrylate, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP (ethyl 3-hydroxypropionic acid), ethyl acrylate, n-butyl

acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, 2-ethylhexyl acrylate, an acrylic acid ester, and combinations thereof.

28. The method of any claim, wherein the at least one product comprises acrylic acid ester, wherein an alkyl, aryl, halogens, aromatic amines or amides, and/or aromatic hydrocarbons, are added to the acrylic acid ester.

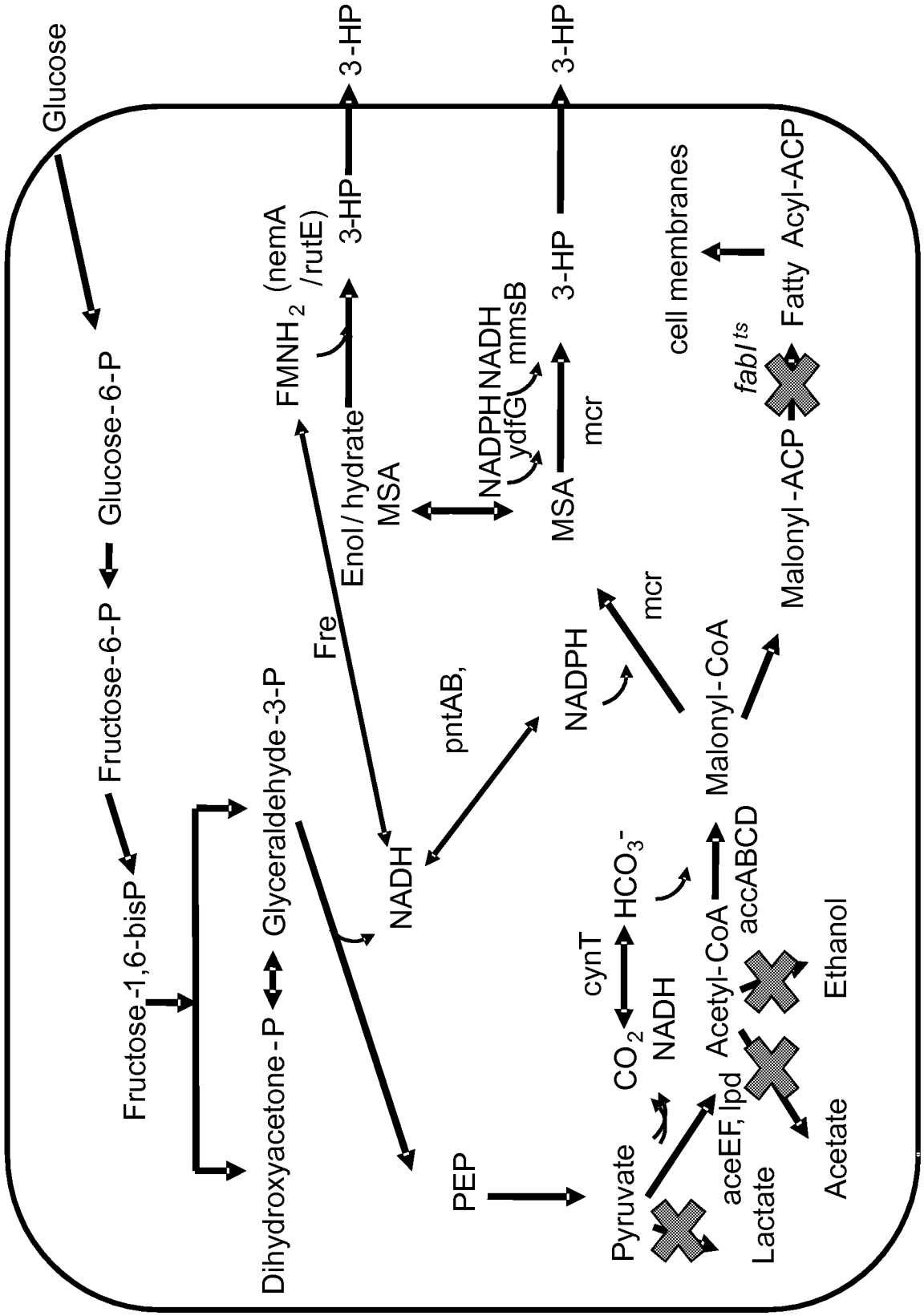

Glucose
Glucose-6-P
Fructose-6-P
Fructose-1,6-bisP
Dihydroxyacetone-P
Glyceraldehyde-3-P
PEP
Pyruvate
Lactate
Acetate
Ethanol
Acetyl-CoA
Malonyl-CoA
Malonyl-ACP
Fatty Acyl-ACP
cell membranes
3-HP
3-HP
3-HP
3-HP
FMNH2
(nemA /rutE)
Fre
Enol/hydrate
MSA
MSA
NADPH
NADH
ydfG
mmsB
mcr
mcr
NADPH
NADH
pntAB,
NADH
cynT
CO2
HCO3-
NADH
aceEF, lpd
accABCD
fabIts

FIG. 1

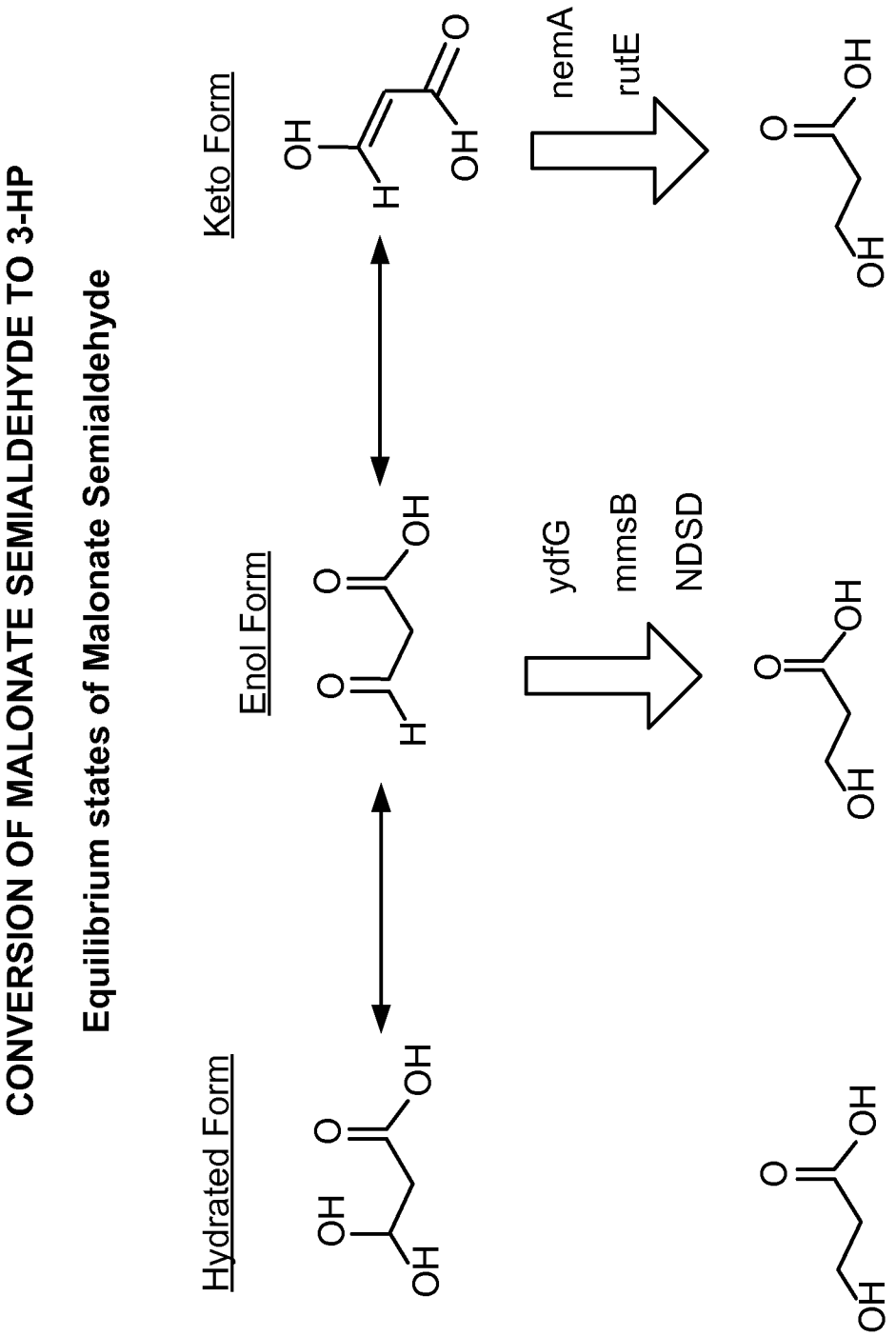

CONVERSION OF MALONATE SEMIALDEHYDE TO 3-HP
Equilibrium states of Malonate Semialdehyde
Keto Form
Enol Form
Hydrated Form
nemA
rutE
ydfG
mmsB
NDSD

FIG. 2

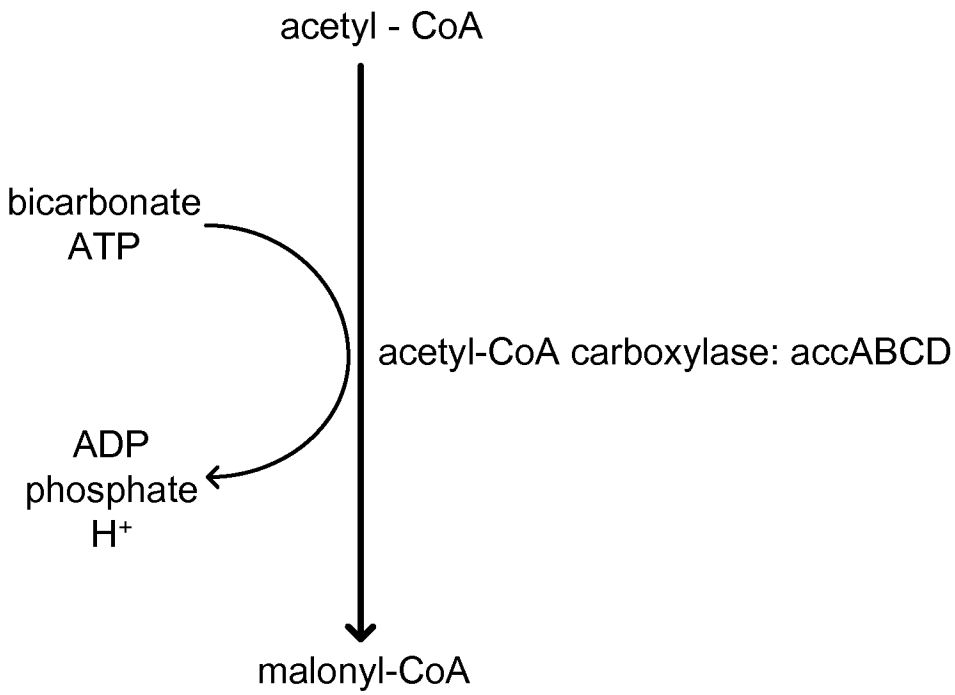

acetyl - CoA
bicarbonate
ATP
acetyl-CoA carboxylase: accABCD
ADP
phosphate
H+
malonyl-CoA

FIG. 3

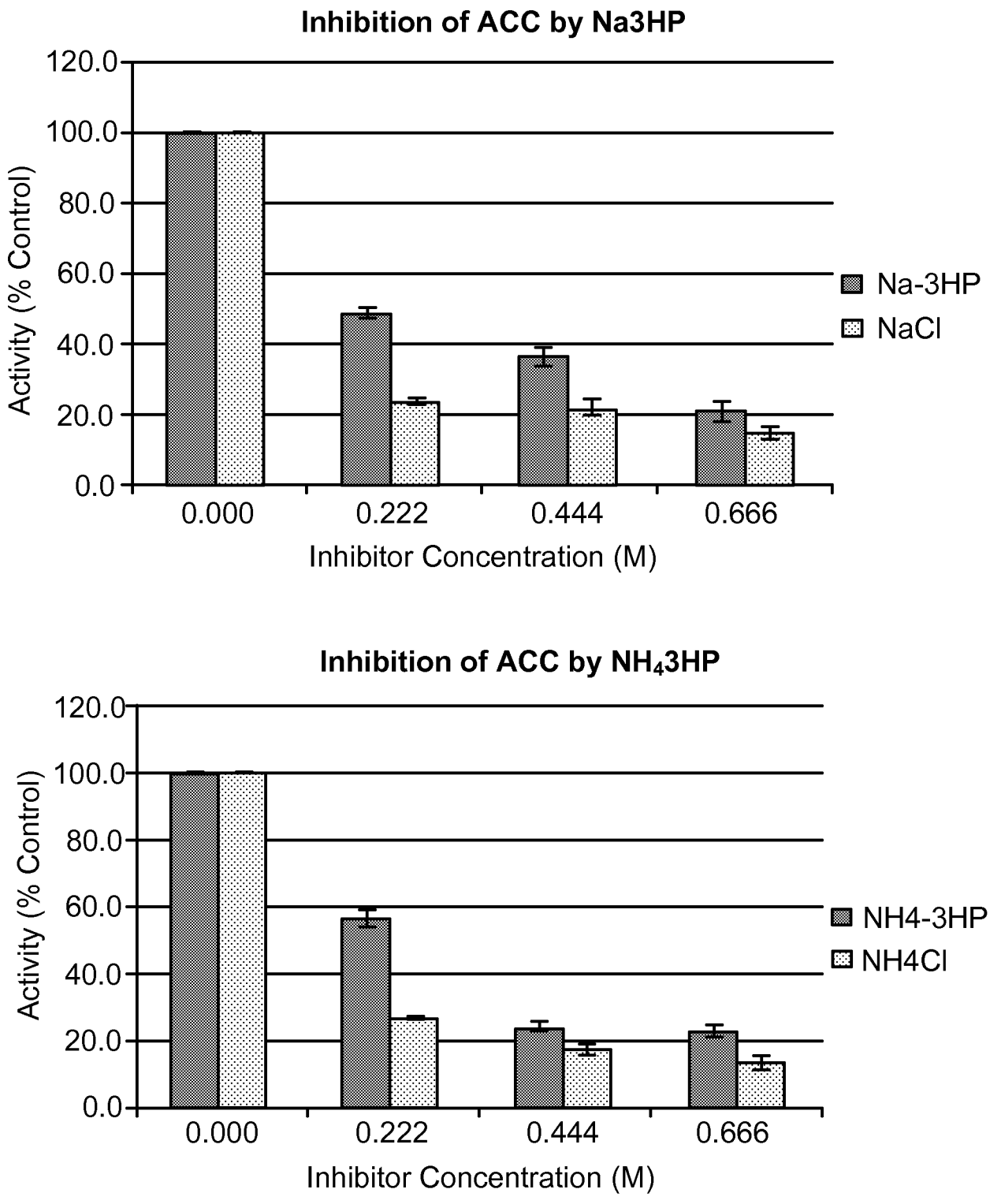

Inhibition of ACC by Na3HP
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Activity (% Control)
0.000
0.222
0.444
0.666
Inhibitor Concentration (M)
Na-3HP
NaCl
Inhibition of ACC by NH4 3HP
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Activity (% Control)
0.000
0.222
0.444
0.666
Inhibitor Concentration (M)
NH4-3HP
NH4Cl

FIG. 4

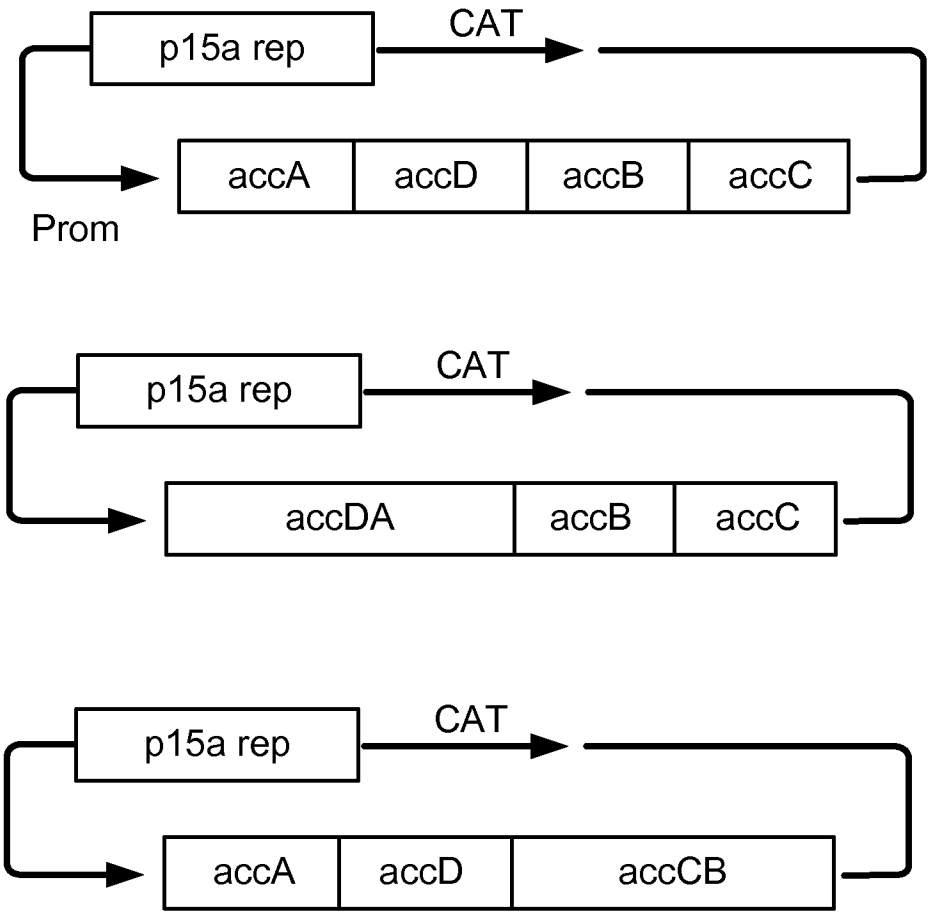

p15a rep
CAT
accA
accD
accB
accC
Prom
p15a rep
CAT
accDA
accB
accC
p15a rep
CAT
accA
accD
accCB

FIG. 5

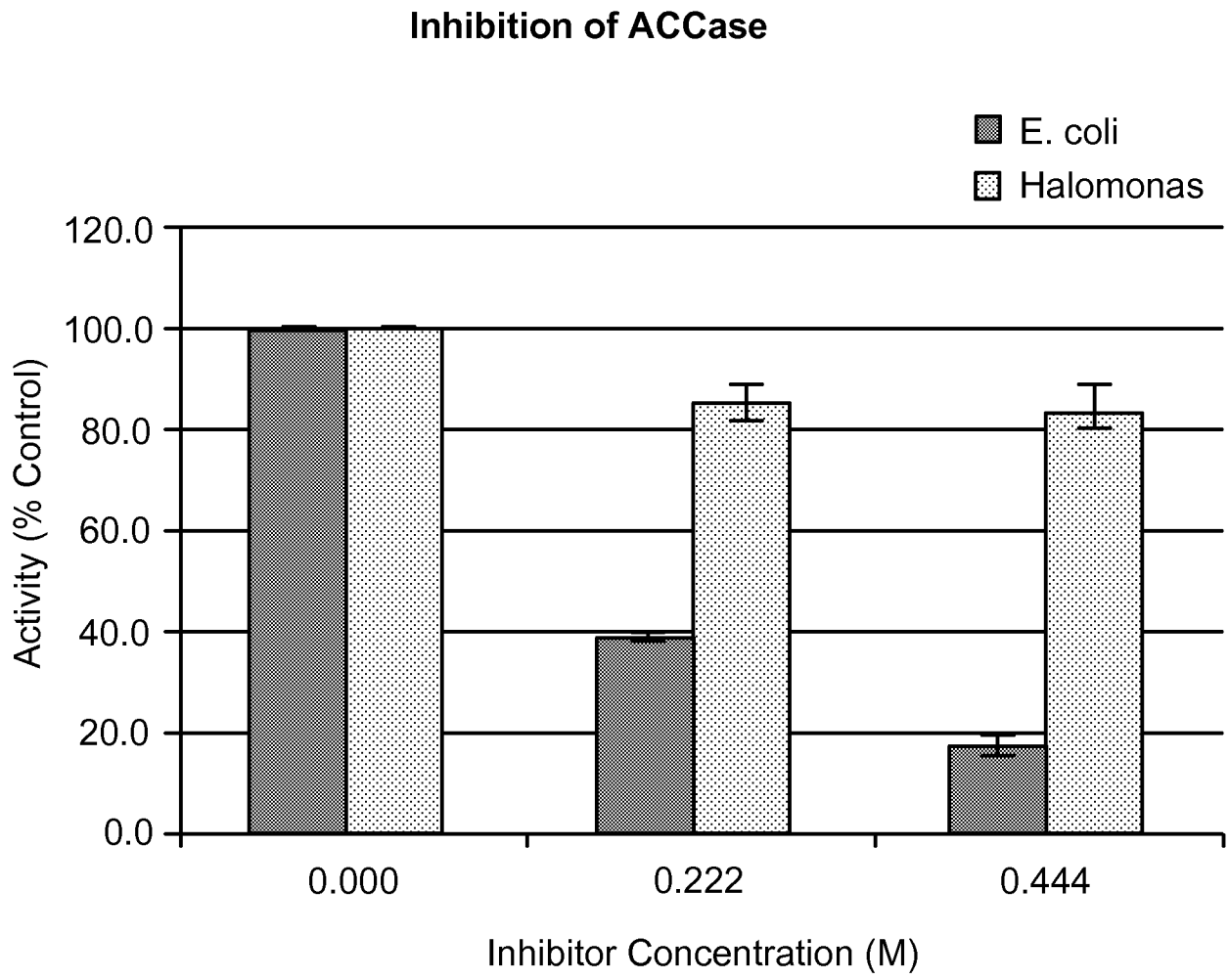

Inhibition of ACCase
E. coli
Halomonas
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Activity (% Control)
0.000
0.222
0.444
Inhibitor Concentration (M)

FIG. 6

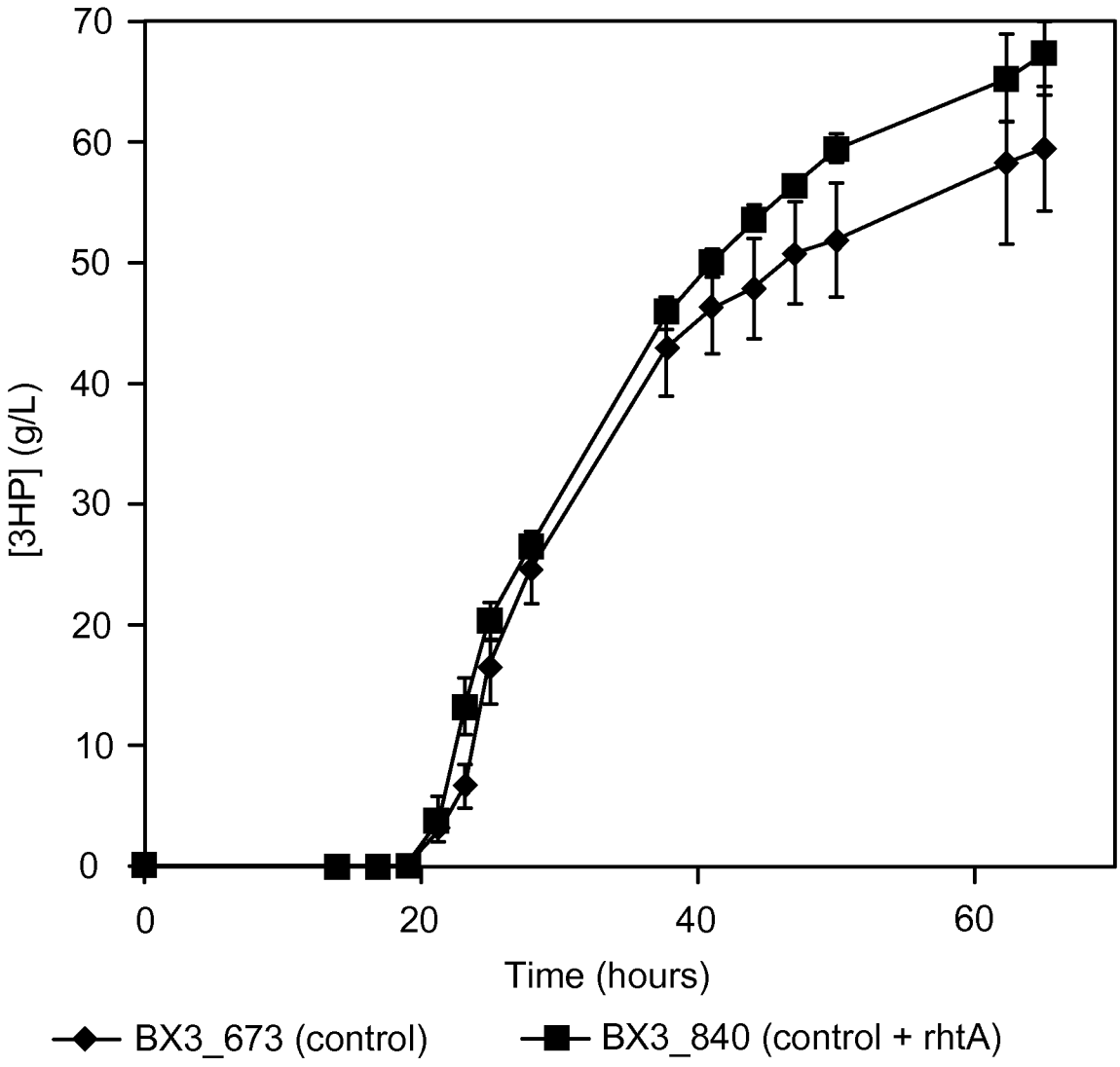

70
60
50
40
30
20
10
0
[3HP] (g/L)
0
20
40
60
Time (hours)
BX3_673 (control)
BX3_840 (control + rhtA)

FIG. 7

Promoters:
- Regulated-
  - Phosphate
  - PstsIH
  - PyibD
  - $ProD_{plot}$
  - $ProX_{plot}$
  - IPTG
  - PT7
  - Ptrc
  - Plac
  - temperature
  - Lambda repressor
- Constitutive:
  - $PT5_{-orl}$
  - $PT5_{-FWA-1}$
  - $PT5_{-to\ ATL}$
  - PlpId
  - PrpiA
  - Pscp1-4 (ProA-D)
  - Pkan
  - PgapA
  - Library Designs

RRS
- Many

Transcriptional terminators
- rrnOT1T2
- tonB
- soxR
- BiobrickTT

Plasmids
- Origins: (copy number at 30C)
  - pBBR1 (15-20)
    - pRMT1-4, pRM1-4
  - pSC101 (5)
    - pSC101
    - pSC101ts(RepA-A56V) (pKD46,pRed/p708)
  - p15 (10-12)
    - pACYC, pJ251
  - pMB1* (20-30, >200 at 37°C)
    - pUC, pCR4/Blunt, pJ244
  - pMB1/ColE1 (15-20)
    - pET, pTrc, pBR322
  - CluDF13(20-40, ~50 at 37°C)
    - pCDF-Duet
  - RSF1030(>100?)
    - pRSF-Duet

Plasmid Selection:
- Antibiotic
  - Amp/Carb
  - Kan
  - Cam
  - Tet
  - Strp
  - Bsd
  - Zeo
- Nonantibiotic
  - ccdAB
  - gapA
  - CitSyn

Other Modules
- Recombination Sites
  - FRT
  - loxP

FIG. 8

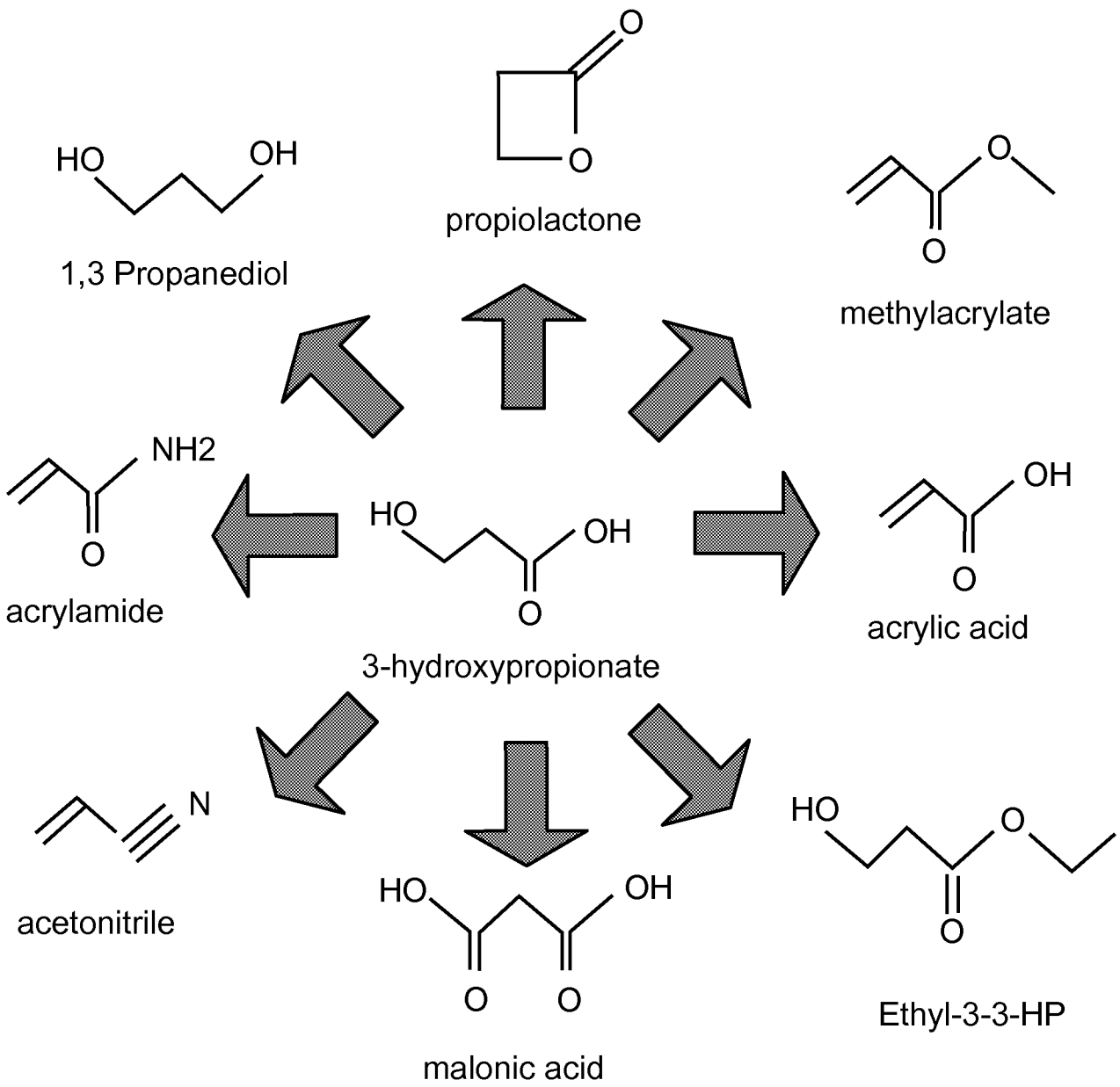

HO
OH
1,3 Propanediol
O
O
propiolactone
O
O
methylacrylate
NH2
O
acrylamide
HO
OH
O
3-hydroxypropionate
OH
O
acrylic acid
N
acetonitrile
HO
OH
O
O
malonic acid
HO
O
O
Ethyl-3-3-HP

FIG. 9

Examples: Enzymatic reaction of: transport of homoserine (threonine and homoserine exporter) (Biocyc)

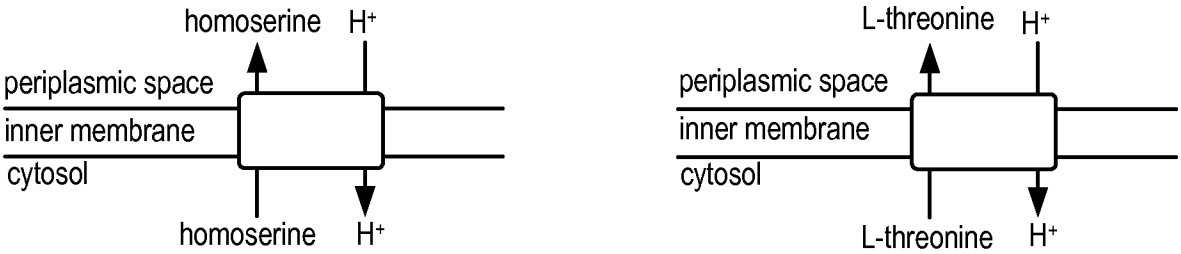
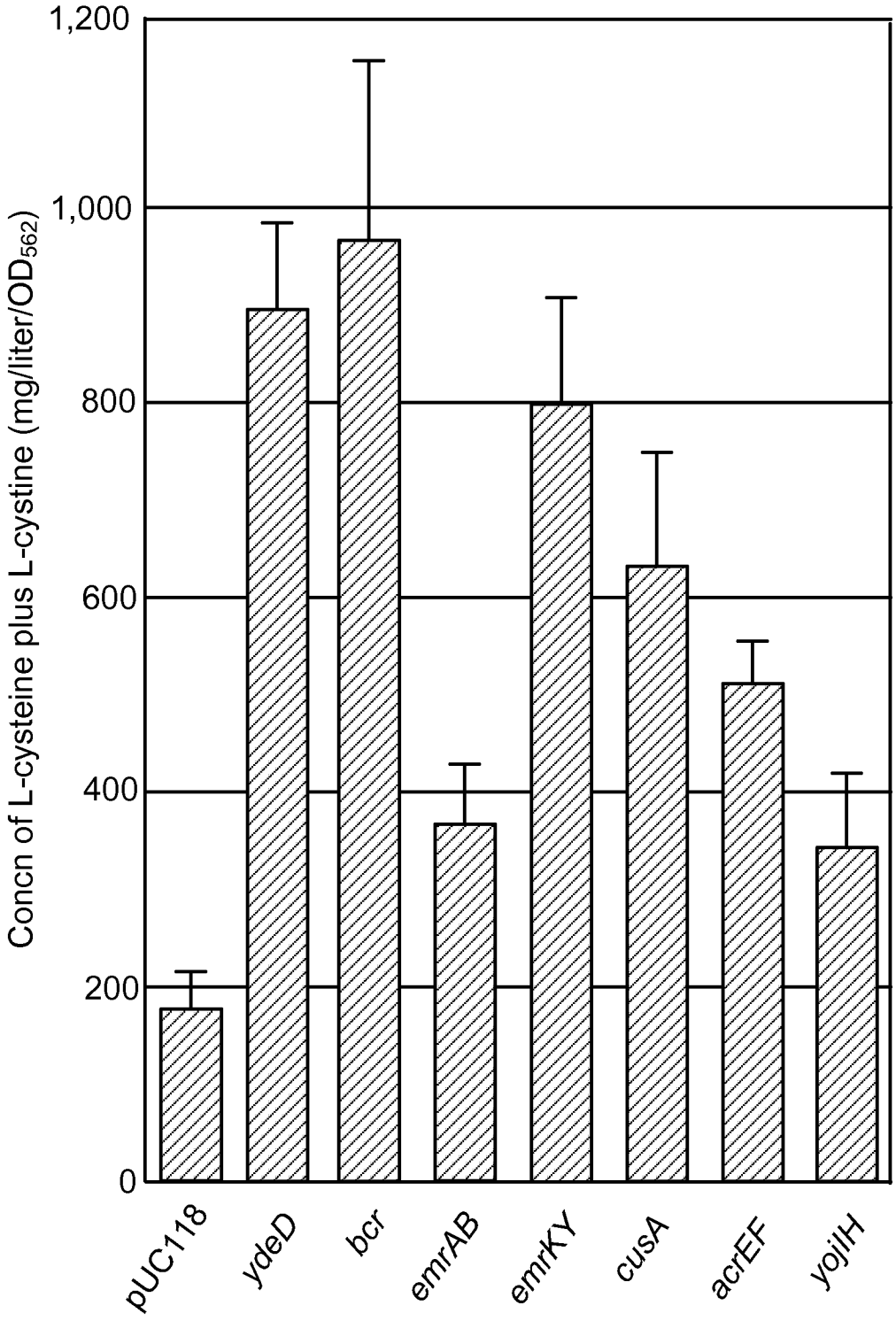


FIG. 10



Production of L-cysteine pluse L-cystine by *E. coli* strain JM39Δ*tnaA* with pACYC-M256l carrying the altered *cysE* gene and plasmids carrying drug transporter genes after 48 h of cultivation. Detailed procedures for culture are described in the text. The concentration of L-cysteine plus L-cystine was determined by a microbioassay.

FIG. 11

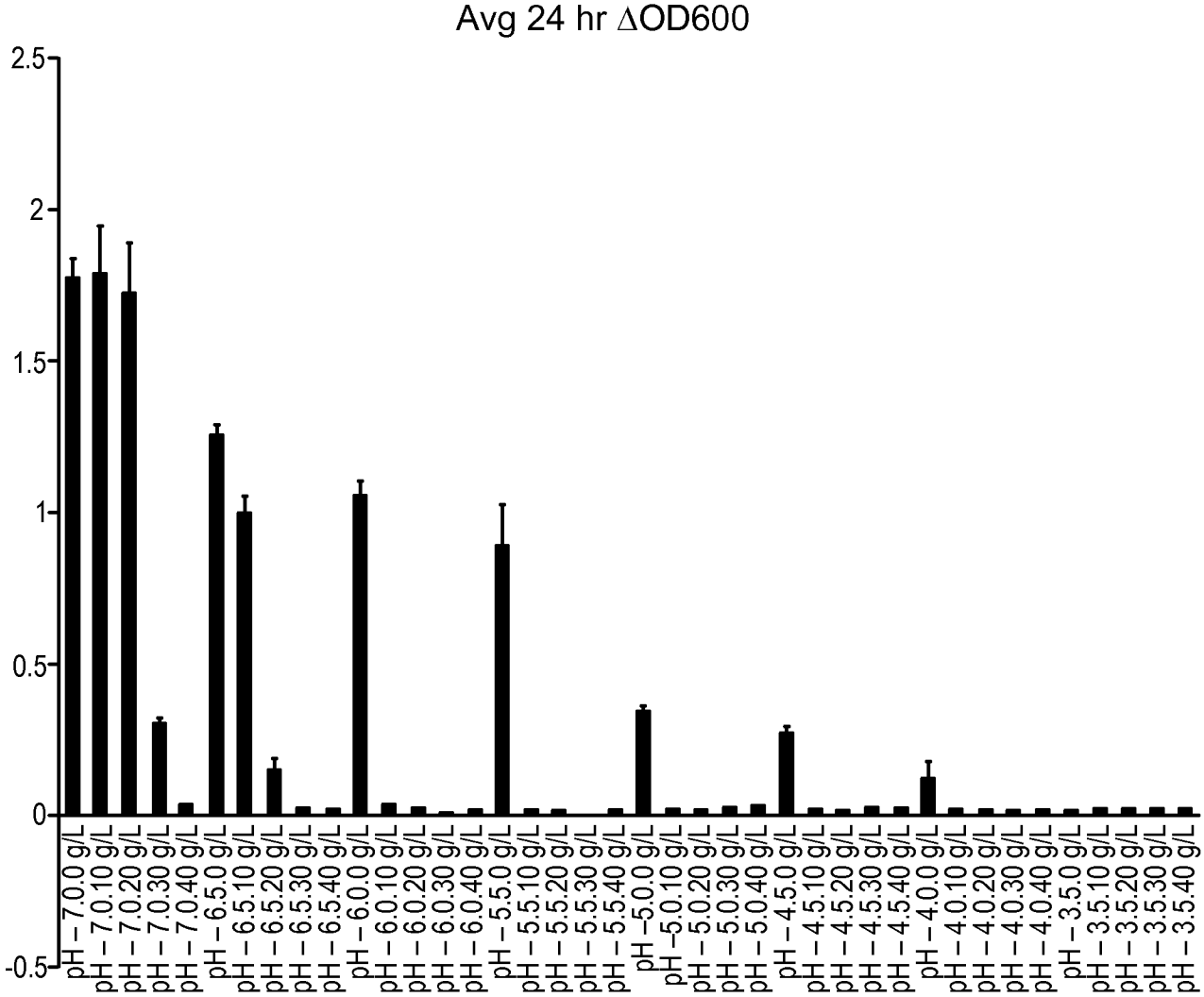

Avg 24 hr ΔOD600
2.5
2
1.5
1
0.5
0
-0.5
pH – 7.0.0 g/L
pH – 7.0.10 g/L
pH – 7.0.20 g/L
pH – 7.0.30 g/L
pH – 7.0.40 g/L
pH – 6.5.0 g/L
pH – 6.5.10 g/L
pH – 6.5.20 g/L
pH – 6.5.30 g/L
pH – 6.5.40 g/L
pH – 6.0.0 g/L
pH – 6.0.10 g/L
pH – 6.0.20 g/L
pH – 6.0.30 g/L
pH – 6.0.40 g/L
pH – 5.5.0 g/L
pH – 5.5.10 g/L
pH – 5.5.20 g/L
pH – 5.5.30 g/L
pH – 5.5.40 g/L
pH – 5.0.0 g/L
pH – 5.0.10 g/L
pH – 5.0.20 g/L
pH – 5.0.30 g/L
pH – 5.0.40 g/L
pH – 4.5.0 g/L
pH – 4.5.10 g/L
pH – 4.5.20 g/L
pH – 4.5.30 g/L
pH – 4.5.40 g/L
pH – 4.0.0 g/L
pH – 4.0.10 g/L
pH – 4.0.20 g/L
pH – 4.0.30 g/L
pH – 4.0.40 g/L
pH – 3.5.0 g/L
pH – 3.5.10 g/L
pH – 3.5.20 g/L
pH – 3.5.30 g/L
pH – 3.5.40 g/L

FIG. 12

```
Protein: rhtA
Conservation 0
Iteration 1 SP= 374273.00 AvSp= 19,736 Std= 13898 AvSid= 0.733 Nseq= 12 Len= 301 Naa=3503 Ngaps= 109
*         1 -      1. gi|26246789|ref|NP_752829.1|
*         13 -     2. gi|156934692|ref|YP_001438608.1|
*         25 -     5. gi|170023846|ref|YP_001720351.1|
*         37 -     12. gi|158369726|ref|YP_001477715.1|
*         49 -     4. gi|162418170|ref|YP_001606257.1|
*         61 -     6. gi|227325920|ref|ZP_03829944.1|
*         73 -     9. gi|253687975|ref|YP_003017165.1|
*         85 -     10. gi|50121689|ref|YP_050856.1|
*         97 -     8. gi|227115303|ref|ZP_03828959.1|
*         109 -    7. gi|253990387|ref|YP_003041743.1|
*         121 -    3. gi|238749896|ref|ZP_04611400.1|
*         133 -    11. gi|238753380|ref|ZP_04614743.1|
gi|26246789|ref
000055555555555888888999MASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIAFFKPWRLRFA66QRLPLLFYGVSLGGMNYLFYL
SIQTVP
                    ttttttttttttttttttttt      ttttttttttttttttttttttttttt          ttttttttttttttttt    tt
gi|156934692|re----
6666666666888888999MASIQSGASLAKSLFPVVGAPGVTALRLALGTLILAVVFKPWRLRFT66QRMPLLIYGLALGAMNYLFYLSIRTVP
                                    ttttttttttttttttttttt      ttttttttttttttttttt
gi|170023846|re-
9223336666666888888999MISIQGALAKSLFPLVGAQGITSLRLGIGTLILFVIFKPWRMRFAA6SRLPLLIYGVALGGMNFLFYMSLKTVP
                                    ttttttttttttttttttttt      ttttttttttttttttttt
gi|157369726|re
9922677788888888888888999MVSIQTGASLAKSLFPLVGAEGITTLRLSIGTLILFIIFRPWRMRFAA6SRLPLFIYGLTLGAMNYLFYLSLRTLP
                   ttttttttttttttttttttt    ttttttttttttttttttttttttttt          ttttttttttttttttttt    tt
gi|162418170|re-
8755556666666888888999MISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFVIFKPWRMRFAA65RLPLLIYGVALGGMNFLFYMSLKTVP
                                    ttttttttttttttttttt        ttttttttttttttttttt
```

FIG. 13A

```
gi|237325920|re --
886667777777888888999MLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCHFKPWRMRP977NRLPLLVYGTTLGGMNFLFYLSLQTVP
                     ttttttttttttttttttttt     ttttttttttttttttttttttt          ttttttttttttttttttt
gi|253687975|re --
886667777777888888999MLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCVIFKPWRMRF977NRLPLLVYGITLGGMNFLFYLSLQTVP
                     ttttttttttttttttttttt     ttttttttttttttttttttttt          ttttttttttttttttttt
gi|50121689|ref --
886667777777888888999MLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCVIFKPWRMRF977NRLPLLVYGITLGGMNFLFYLSLQTVP
                     ttttttttttttttttttttt     ttttttttttttttttttttttt          ttttttttttttttttttt
gi|227115303|re --
886667777777888888999MLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCLIFKPWRMRF977NRLPLLVYGMTLGGMNFLFYLSLQTVP
                     ttttttttttttttttttttt     ttttttttttttttttttttttt          ttttttttttttttttttt
gi|25399037|re
992233334444444888888999MASIQSGASLAKTLFPVIGAPGVTALRLSLGTHIILFLIFKPWRLHF666SMMPLLTYGIALGAMNYTFYMALT
TIP
                                          ttttttttttttttttttt          ttttttttttttttttttt    tt
gi|238749896|re ----------
999MISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFIIFKPWRMRFAA6SRLPLLIYGVTLGGMNFLFYLSLKTVP
                                  ttttttttttttttttttttttttt          ttttttttttttttttttttt
gi|238753380|re ----------
MVSIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFVIFKPWKMRFAA6SRLALLIYGLALGGMNFLFYLSKTVP
                                    ttttttttttttttttttt          ttttttttttttttttttt
Avrg Consist  886656666666666888888999999999999999999999999999999999999999999999999269999999999999999999999999

gi|26246789|ref
LGIAVALEFTGPLAVALFSSRRPVDFVWVVLAVLGLWFLLPLGQDVSHVDLTGCALALGAGACWAIYILSGQRAGAEHGPATVAIGSLIAALIF
VPIGAL
          ttttttttttttttttttt     ttttttttttttttttttttt     ttttttttttttttttttt          ttttttttttttttttttt
gi|156934692|re
LGIAVALEFTGPLAVALFGSRRPVDFLWVALAVLGLWFLLPLGQDVTHVDPLGAACALGAGVCWAIYIIAGQKAGAEHGPATVALGSLIAAIVF
VPIGAA
          ttttttttttttttttttt     ttttttttttttttttttttt     ttttttttttttttttttt          ttttttttttttttttttt
```

FIG. 13B

```
gi|170023846|re
LGIAVALEFTGPLAVAMLSSRRAVDFIWVGLAVILGLWFLLPLGHNIGTIDLFGAACALGAGACWAIYIISGQKAGGDHGPATVAVGSLIAAL
IVFCPIGVA
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|157369726|re
LGIAVALEFTGPLAVAMFSSRRPIDFLWVALAIAGLWFLLPLGHDMGSIDPIGAACALGAGACWAVYIICGQKAGGDHGPGPGTVAVGSL
IAAVVFCPIGAW
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|162418170|re
LGIAVALEFTGPLAVAMFSSRRAVDFIWVGLAILGLWFLLPLGHNIGTIDLFGAACALGAGACWAIYIISGQKAGGDHGPGTVAVGSLIAAL
IFCPIGVA
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|227325920|re
LGIAVALEFTGPLAVAMLASRRPIDFLWVFLAVAGLWFLLPLGHNIGNVDLTGAACAVGAGACWALYILFGQKAGANHGPGTVAIGSCIA
ALIFCPIGAY
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|253687975|re
LGIAVALEFTGPLAVAMLASRRPIDFLWVFLAVAGLWFLLPLGHNIGNVDLVGAACAVGAGACWALYILFGQKAGSNHGPGTVAIGSCIA
ALIFCPIGAY
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|50121689|ref
LGIAVALEFTGPLAVAMLASRRPIDFLWVFLAVAGLWFLLPLGHNIGNVDLTGAACAVGAGACWALYILFGQKAGANHGPGTVAVGSCIA
ALIFCPIGVY
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|227115303|ref
LGIAVALEFTGPLAVAMLASRRPIDFLWVFLAVAGLWFLLPLGHNIGNVDLTGAACAVGAGACWALYILFGQKAGANHGPGTVAVGSCIA
ALIFCPIGAY
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
gi|253990387|ref
LGIAVALEFTGPLAVAMFSSRRPIDFLWVALVIAGLAFLLPIGNNIDRLDPVGIIYALSAGVCWALYIIFGQRAGAGYGAATVSIGSFIAALIFF
PVGLI
        ttttttttttttttttttt  ttttttttttttttttttt  ttttttttttttttttttt
```

FIG. 13C

gi|238749896|re
LGIAVALEFTGPLALAMLSSRRPVDFIWVGLALGLWFLLPLGNNIGSIDLGAACALGAGVCWIYIVSGQKAGGDHGPGTVAIGSLIAALVFC
PIGVA
ttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttt
gi|238753380|re
LGIAVALEFTGPLAVAMFSSRRPIDFLWVGLAILGLTFLLPLGQSVERIDLFGAACALGAGACWAIYILSGQKAGGDHGAGTVAIGSLIAALV
FCPIGVA
ttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttt
Avrg Consist 99999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999

gi|26246289|refQAG-
EALWHWSVIPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGMIFLGETLTPIQLLALGAIIAASMGSTLTVRKESKIKELD55
ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|156934692|re AAG-
EALWHWSVLPAGIGIAILSTALPYSLEMTALTRLPTRTFGTLMSMEPALAAFTGMIFLGETLTLVQWLALLSHIASVGSTLTLRRETQIK7
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|170023846|re YNG-
MALFDPAILPIALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGLVFLNEHLTLVQWLALASIISASVGASLTIKPKPQLDQVT-
tttt ttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|157369726|re
QTG99999NVDILPIALAVAILSTALPYSLEHALPKIPARTFGTLMSLEPALAALSGMLFLNEHLTTVQWLALAAIIAASMGATLTIKPKPQIEKLS-
ttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|162418170|re YNG-
MALFDPAILPIALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGLVFLNEHLTLVQWLALASIISASVGASLTIKPKPQLDQVT-
tttt ttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|227325920|re
Y999STLFSLSILPLGIAVAIMSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGILFLGEHLSFIQWMALIFHIASIGATLTIK88777766644
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|253687975|re
Y999STLFSLSILPLGVAVAVLSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGILFLGEHLSFIQWMALIFHIASIGATLTIK99999988844
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt

FIG. 13D

gi|50121689|ref
Y999STLFSFSILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGILFLGEHLAFIQWMALMFHIASIGATLTIK98888888855
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|227115303|re
Y999STLFSFSILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGILFLGEHLSFIQWMALMFHIASIGATLTIK88777766644
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|253990387|re
Q6609999DMSIMPLALAIAILSTAFPYTLEMIALTRLPAQTFGTLMSLEPALGALSGIVFLNHHLTTAQWLALFCHFASIGSTSTIK66666666626
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|238749896|re YNG-
LALFDPAILPVALAVAILSTALPYSLEMVALTRLPTRTFGTLMSLEPALAAISGLIFLNEHLTLIQWLALVSHIASIGATLTIRP9777777777
tttt ttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|238753380|re YNG-
MALFSPAILPVALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGLIFLNEHLSLVQWLALASIICASIGATLTIR88888888888
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
Avrg Consist 99989999999999999999999999999999999999999999999999999999999999999999999999999999999888888777756

gi|50121689|ref
Y999STLFSFSILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGILFLGEHLAFIQWMALMFHIASIGATLTIK9888888885555
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt
gi|227115303|re
Y999STLFSFSILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGILFLGEHLSFIQWMALMFHIASIGATLTIK8877776664444
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt

gi|253990387|re
Q6609999DMSIMPLALAIAILSTAFPYTLEMIALTRLPAQTFGTLMSLEPALGALSGIVFLNHHLTTAQWLALFCHFASIGSTSTIK6666666626626
t ttttttttttttttttttttt ttttttttttttttttttt ttttttttttttttttttt

FIG. 13E

```
gi|238749896|re  YNG-
LALFDPAILPVALAVAILSTALPYSLEMVALTRLPTRTFGTLMSLEPALAAISGLIFLNEHLTLIQWLALVSHIASIGATLTIRP9777777777
                 tttt  ttttttttttttttttttttt      ttttttttttttttttttttt    ttttttttttttttttttttt
gi|238753380|re  YNG-
MALFSPAILPVALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGLIFLNEHLSLVQWLALASIICASIGATLTIR88888888888
                   t  ttttttttttttttttttttttt      ttttttttttttttttttttt    ttttttttttttttttttttt
Avrg Consist  99989999999999999999999999999999999999999999999999999999999999999999999999999888888
77756

gi|26246289|ref -
gi|156934692|re 7
gi|170023846|re -
gi|157369726|re -
gi|162418170|re -
gi|227325920|re 3
gi|253687975|re 3
gi|50121689|ref 3
gi|227115303|re 3
gi|253990387|re 6
gi|238749896|re-
gi|238753380|re-

Avrg Consist  7
```

FIG. 13F

```
CLUSTAL2.0.11 multiple sequence alignment

gi|148548707|ref|YP_001268809, ----------------------------------------
gi|26988963|ref|NP_744388.1|   ----------------------------------------
gi|167032871|ref|YP_001668102. ----------------------------------------
gi|170722598|ref|YP_001750286. ----------------------------------------
gi|104782554|ref|YP_609052.1| ----------------------------------------
gi|229589563|ref|YP_002871682. ----------------------------------------
gi|77460125|ref|YP_349632.1|  ----------------------------------------
gi|70731497|ref|YP_261238.1|  ----------------------------------------
gi|213971379|ref|ZP_03399494.1 ----------------------------------------
gi|237799531|ref|ZP_04587992.1 ----------------------------------------
gi|71737736|ref|YP_275301.1|  MIYKVFSFPSAPEVFRHDRSQRASTVNFKITEIARFVITYIEFKAKIAQ 50
gi|257484608|ref|ZP_05638649.1 ----------------------------------------
gi|66046446|ref|YP_236287.1|  ----------------------------------------
gi|15596557|ref|NP_250051.1|  ----------------------------------------
gi|49085542|gb|AAT51286.1|    ----------------------------------------
gi|254239729|ref|ZP_04933051.1 ----------------------------------------
gi|152988797|ref|YP_001349377. ----------------------------------------
gi|44662925|gb|AAS47543.1|    ----------------------------------------
gi|92112331|ref|YP_572259.1|  ----------------------------------------
gi|227325920|ref|ZP_03829944.1| ----------------------------------------
gi|227115303|ref|ZP_03828959.1 ----------------------------------------
gi|25368797|ref|YP_003017165 ----------------------------------------
gi|50121689|ref|YP_050856.1|  ----------------------------------------
gi|255053893|ref|ZP_05306142.1 ----------------------------------------
gi|251790084|ref|YP_003004805. ----------------------------------------
gi|258631979|ref|ZP_05724793.1 ----------------------------------------
gi|242239813|ref|YP_002987994. ----------------------------------------
gi|123443052|ref|YP_001007026. ----------------------------------------
gi|238788823|ref|ZP_04632614.1 ----------------------------------------
```

FIG. 14A

gi|170023846|ref|YP_001720351. ----------
gi|22125575|ref|NP_668998.1| ----------
gi|162418170|ref|YP_001606257. ----------
gi|238793410|gb|ZP_04637035.1 ----------
gi|238761036|ref|ZP_04622013.1 ----------
gi|238798464|ref|ZP_04641944.1 ----------
gi|238784064|ref|ZP_04628079.1 ----------
gi|238749896|ref|ZP_04611400.1 ----------
gi|238757260|ref|ZP_04618447.1 ----------
gi|238753380|ref|ZP_04614743.1 ----------
gi|157369726|ref|YP_001477715. ----------
gi|258636050|ref|ZP_05728810.1 ----------
gi|188534331|ref|YP_001908128. ----------
gi|152969399|ref|YP_001334508. ----------
gi|206577533|ref|YP_002239546. ----------
gi|195940762|ref|ZP_03086144.1 ----------
gi|209907267|ref|ZP_03281751.1 ----------
gi|146310958|ref|YP_001176032. ----------
gi|227332340|ref|ZP_03835996.1 ----------
gi|237730791|ref|ZP_04561272.1 ----------
gi|16764194|ref|NP_459809.1| ----------
gi|198245156|ref|YP_002214796. ----------
gi|200389664|ref|ZP_03216275.1 ----------
gi|62179398|ref|YP_215815.1| ----------
gi|16759750|ref|NP_455367.1 ----------
gi|213022641|ref|ZP_03337088.1 ----------
gi|213163835|ref|ZP_03349545.1. ----------
gi|161504001|ref|YP_001571113. ----------
gi|157146546|ref|YP_001453865. ----------
gi|440181|gb|AAA21854.1| ----------
gi|30062297|ref|NP_836468.1| ----------
gi|218704191|ref|YP_002411710. ----------

FIG. 14B

```
gi|26246789|ref|NP_752829.1|  ----------
gi|170684270|ref|YP_001742917.  ----------
gi|15800565|ref|NP_286577.1|  ----------
gi|170020831|ref|YP_001725785.  ----------
gi|215485900|ref|YP_002328331.  ----------
gi|227884223|ref|ZP_04002028.1  ----------
gi|157158672|ref|YP_001462008.  ----------
gi|170769078|ref|ZP_02903531.1  ----------
gi|218548334|ref|YP_002382125.  ----------
gi|156934692|ref|YP_001438608.  ----------
gi|253990387|ref|YP_003041743.  ----------
gi|37525482|ref|NP_928826.1|  ----------
gi|183598233|ref|ZP_02959726.1  ----------
gi|223990759|ref|ZP_03639931.1  ----------
gi|212712149|ref|ZP_03320277.1  ----------
gi|212708023|ref|ZP_03316151.1  ----------
gi|197284529|ref|YP_002150401.  ----------
gi|237807801|ref|YP_002892241.  ----------
gi|238898963|ref|YP_002924645.  ----------
gi|146307537|ref|YP_001188002  ----------
gi|108759302|ref|YP_628438.1|  ----------

gi|148548707|ref|YP_001268809  ----------MNT----------QPRSL 8
gi|26988963|ref|NP_744388.1|  ----------MNT----------QPRSL 8
gi|167032871|ref|YP_001668102.  ----------MNT----------QPRSL 8
gi|170722598|ref|YP_001750286  ----------MNT----------QPRSM 8
gi|104782554|ref|YP_609052.1|  ----------MNT----------APRSL 8
gi|229589563|ref|YP_002871682.  ----------MNT----------SPRSL 8
gi|77460125|ref|YP_349632.1|  ----------MND----------QPRSL 8
gi|70731497|ref|YP_261238.1|  ----------
gi|213971379|ref|ZP_03399494.1  ----------MSD----------KPHTL 8
```

FIG. 14C

gi|237799531|ref|ZP_04587992.1| ----------MPY----------KSNNL 8
gi|71737736|ref|YP_275301.1| YIAHASIVAHHVKLSVPGSHPGYITKMSE----------KPHTL84
gi|257484608|ref|ZP_05638649.1 ----------
gi|66046446|ref|YP_236287.1| ----------MSE----------KSRNL 8
gi|15596557|ref|NP_250051.1| ----------MTS----------TSRGL 8
gi|49085542|gb|AAT51286.1| ----------MTS----------TSRGL 8
gi|254239729|ref|ZP_04933051.1 ----------MTS----------TSRGL 8
gi|152988797|ref|YP_001349677. ----------MTS----------TSRGL 8
gi|44662925|gb|AA547543.1| ----------MTT----------SPRGL 8
gi|92112331|ref|YP_572259.1 ----------MPQKQGGVMSV----------AVREF 16
gi|227325920|ref|ZP_03829944.1 ----------MTLF----------I-IIR 7
gi|227115303|ref|ZP_03828959.1 ----------MTLF----------N-IIR 7
gi|253687975|ref|YP_003017165. ----------MTLF----------S-IIR 7
gi|50121689|ref|YP_050856.1| ----------MTLF----------S-IIR 7
gi|255053893|ref|ZP_05306142.1 ----------MTLF----------S-IIR 7
gi|251790084|ref|YP_003004805. ----------MTLI----------PALK 8
gi|258631979|ref|ZP_05724793.1 ----------MTLI----------SALK 8
gi|242239813|ref|YP_002987994. ----------MTLY----------SSIK 8
gi|123443052|ref|YP_001007026. ----------MSL-SP----------PSKII 9
gi|238788823|ref|ZP_04632614.1 ----------
gi|170023846|ref|YP_001720351. ----------MSL-SP----------SSKN 9
gi|22125575|ref|NP_668998.1| ----------MSL-SP----------SSKN 9
gi|162418170|ref|YP_001606257. ----------MSL-SP----------SSKN 7
gi|238793410|ref|ZP_04637035.1 ----------MSL-SP----------SSKII 9
gi|238761036|ref|ZP_04622013.1 ----------
gi|238798464|ref|ZP_04641944.1 ----------
gi|238784064|ref|ZP_04628079.1 ----------
gi|238749896|ref|ZP_04611400.1 ----------
gi|238757260|ref|ZP_04618447.1 ----------
gi|238753380|ref|ZP_04614743.1 ----------
gi|157369726|ref|YP_001477715. ----------MSS-SA----------TAKA 9

FIG. 14D

```
gi|258636050|ref|ZP_05728810.1 ----------MSAPLT----------AKKL 10
gi|188534331|ref|YP_001908128. ----------MSS-LQ----------VKN- 8
gi|152969399|ref|YP_001334508. ----------MPG-----------PSRK 7
gi|206577533|ref|YP_002239546. ----------MTG-----------PSRK 7
gi|195940762|ref|ZP_03086144.1 ----------MTG-----------LSRK 7
gi|209907267|ref|ZP_03281751.1 ----------
gi|146310958|ref|YP_001176032. ----------MPG-----------LSRK 7
gi|227332340|ref|ZP_03835996.1 ----------MPG-----------SLRK 7
gi|237730791|ref|ZP_04561272.1 ----------MPG-----------LKRK 7
gi|16764194|ref|NP_459809.1|   ----------MPG-----------STRK 7
gi|198245156|ref|YP_002214796. ----------MPG-----------STRK 7
gi|200389664|ref|ZP_03216275.1 ----------MPG-----------STRK 7
gi|62179398|ref|YP_215815.1|   ----------MPG-----------STRK 7
gi|16759750|ref|NP_455367.1|   ----------MPG-----------STRK 7
gi|213022641|ref|ZP_03337088.1 ----------
gi|213163835|ref|ZP_03349545.1 ----------MPG-----------STRK 7
gi|161504001|ref|YP_001571113. ----------MPG-----------STRK 7
gi|157146546|ref|YP_001453865. ----------MPG-----------SSRK 7
gi|440181|gb|AAA21854.1|       ----------MPG-----------SLRK 7
gi|30062297|ref|NP_836468.1|   ----------MPG-----------SLRK 7
gi|218704191|ref|YP_002411710. ----------MPG-----------SLRK 7
gi|262467891ref|NP_752829.1|   ----------MGERMPG-------SLRK 11
gi|170684270|ref|YP_001742917. ----------MPG-----------SLRK 7
gi|15800565|ref|NP_286577.1|   ----------MPG-----------SLRK 7
gi|170020831|ref|YP_001725785. ----------MPG-----------SLRK 7
gi|215485900|ref|YP_002328331. ----------MPG-----------SLRK 7
gi|227884223|ref|ZP_04002028.1 ----------
gi|157158672|ref|YP_001462008. ----------MPG-----------SLRK 7
gi|170769078|ref|ZP_02903531.1 ----------MPG-----------SLRK 7
gi|218548334|ref|YP_002382125. ----------MPG-----------SKRK 7
gi|156934692|ref|YP_001438608. ----------MPT-----------LPRK 7
```

FIG. 14E

```
gi|253990387|ref|YP_003041743.  ----------MSS-SK----------LQRN 9
gi|37525482|ref|NP_928826.1|    ----------MSP-SK----------LQKS 9
gi|183598233|ref|ZP_02959726.1  ----------MS-AD----------TKKS 8
gi|223990759|ref|ZP_03639931.1  ----------MS-AD----------TKKS 8
gi|212712149|ref|ZP_03320277.1  ----------MS-AD----------TKKS 8
gi|212708023|ref|ZP_03316151.1  ----------MS-AD----------TKKS 8
gi|197284529|ref|YP_002150401.  ----------MLS-AK----------HSAK 9
gi|237807801|ref|YP_002892241.  ----------MSSSSD----------R 7
gi|238898963|ref|YP_002924645.  ----------MQKEVEAILNHIKRITQLRV 20
gi|146307537|ref|YP_001188002.  ----------MPR 3
gi|108759302|ref|YP_638438.1    ----------MSR----------VLI KP8
gi|148548707|ref|YP_001268809.  AATLFPIGLLLIAMASIQSGASLAKSMFPIIGAQGTTTLRLIFASIIMLL 58
gi|26988963|ref|NP_744388.1|    AATLFPIGLLLIAMASIQSGASLAKSMFPIVGAQGTTTLRLIFASIIMLL 58
gi|167032871|ref|YP_001668102.  AATLFPIGLLLIAMASIQSGASLAKSMFPIIGAQGTTTLRLIFASIIMLL 58
gi|170722598|ref|YP_001750286.  ATTLFPVGLLLIAMASIQSGASLAKSMFPIIGAQGTTTLRLIFASIIMLL 58
gi|104782554|ref|YP_609052.1|   AATLFPIGLLLIAMASIQSGASLAKSMFPIVGAQGTTALRLIFASIIMLL 58
gi|229589563|ref|YP_002871682.  ASTLFPVGLLLIAMASIQSGASLAKSMFPIVGAQGTTALRLIFASVIMLL 58
gi|77460125|ref|YP_349632.1|    ASTLFPVGLLLIAMASIQSGASLAKSMFPIVGAQGTTTLRLIFASVIMLV 58
gi|70731497|ref|YP_261238.1|    -----MGLLLIAMISIQSGASLAKSLFPVVGAQGTTTLRLVFASVIMLL 44
gi|213971379|ref|ZP_03399494.1  ASALFPVGLLLIAMISIQSGASLAKSLFPVVGAQGTTTLRLVFASLILLV 58
gi|237799531|ref|ZP_04587992.1  LSALFPVGLLLIAMASIQSGASLAKSMFPIVGAQGTTALRLIFASVIMLLV 58
gi|71737736|ref|YP_275301.1|    ASALFPLGLLMIAMISIQSGASLAKSLFPVVGAQGTTTLRLVFASLILIA 134
gi|257484608|ref|ZP_05638649.1  -----MIAMISIQSGASLAKSLFPVVGAQGTTTLRLVFASLIMIA 40
gi|66046446|ref|YP_236287.1|    ASALFPLGLLMIAMVSIQSGASLAKSLFPVVGAQGTTTLRLVFASLILVA 58
gi|15596557|ref|NP_250051.1|    AGTLLPVGLLLIAMASIQSGASLAKSMFPLVGAQGTTALRLFFAALILLL 58
gi|49085542|gb|AAT51286.1|      AGTLLPVGLLLIAMASIQSGASLAKSMFPLVGAQGTTALRLFFAALILLL 58
gi|254239729|ref|ZP_04933051.1| AGTLLPVGLLLIAMASIQSGASLAKSMFPLVGAQGTTALRLFFAALILLL 58
gi|152988797|ref|YP_001349377.  AGTLLPVGLLLIAMASIQSGASLAKSMFPLVGAQGTTALRLFFAALILLL 58
gi|44662925|gb|AAS47543.1|      AAMLLPVGLLLVAMASIQSGASLAKSLFPLVGAQGATALRLLFAALILSV 58
gi|92112331|ref|YP_572259.1|    VARVWPVAMLIVAMCSIQIGASIAKTLFPVIGATGTTSIRLILAAGLLLI 66
gi|227325920|ref|ZP_03829944.1  SPSFLPVLLIIISMLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCL 57
```

FIG. 14F

```
gi|227115303|ref|ZP_03828959.1  SPSFLPVLLIIISMLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCL 57
gi|253687975|ref|YP_003017165.  SSSFVPVLLIIISMLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCV 57
gi|50121689|ref|YP_0050856.1|  SSSFVPVLLIIISMLSIQSGAALAKSLFPLIGATGVTALRLGIGTIILCI 57
gi|255053893|ref|ZP_05306142.1  LPSFIPVLLIIISMLSIQSGAALAKNLFPLIGATGVTALRLGIGTIILCI 57
gi|251790084|ref|YP_003004805.  NSVLLPVLLIVTAMLSIQTGAAMAKMVFPLIGASGVTALRLAIGSLILLM 58
gi|258631979|ref|ZP_05724793.1  NSVLLPVLLLIAMLSIQTGAALAKTVFPLIGASGMTVLRLAIGSLILLI 58
gi|242239813|ref|YP_002987994  TSVLIPVLMIVIAMISLQVGAALAKTLFPTIGASGMTALRLAIGTLILLV 58
gi|123443052|ref|YP_001007026.  SLPLLPIFLLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFI 59
gi|238788823|ref|ZP_04632614.1  ----------MISIQSGASLAKSLFPVVGAQGITSLRLGIGILILFV 37
gi|170023846|ref|YP_001720351.  NLPLLPILLLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 59
gi|22125575|ref|NP_668998.1| SLPLLPILLLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 59
gi|162418170|ref|YP_001606257.  SLPLLPILLLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 57
gi|238793410|ref|ZP_04637035.1  SLPLVPILLLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 59
gi|238761036|ref|ZP_04622013.1  ----------MIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFI 40
gi|238798464|ref|ZP_04641944.1  ---MLPIFLLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 47
gi|238784064|ref|ZP_04628079.1  ----------MLVIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 42
gi|238749896|ref|ZP_04611400.1  ----------MIAMISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFI 40
gi|238757260|ref|ZP_04618447.1  ---MLPILLLVIAMISIQNGASLAKSLFPLVGAQGITSLRLGIGTLILFI 47
gi|238753380|ref|ZP_04614743.1  ----------MVISIQSGASLAKSLFPLVGAQGITSLRLGIGTLILFV 37
gi|157369726|ref|YP_001477715.  SSTLVPVCLLIIAMVSIQTGASLAKSLFPLVGAEGITTLRLSIGTLILFI 59
gi|258636050|ref|ZP_05728810.1  TPVVFPIAVLLIAMLSLQGGASLAKSLFPTVGAPGITALRLGIGTLILCA 60
gi|188534331|ref|YP_001908128.  -TVWQPVALLLIAMTSIQGGAALAKTLFPTVGAAGITALRLGLATLILCI 57
gi|152969399|ref|YP_001334508.  AAAWLPILVILVAMTSIQSGASLAKSLFPVVGAPGITALRLALGTLILVV 57
gi|206577533|ref|YP_002239546.  AAAWLPILVILIAMTSIQSGASLAKSLFPLVGAPGVTALRLALGTLILVV 57
gi|195940762|ref|ZP_03086144.1  SSVWMPVAVILIAMMSIQSGASLAKSLFPLVGAPGVTALRIALGTLILVV 57
gi|209907267|ref|ZP_03281751.1  ----MPVAVILIAMMSIQSGASLAKSLFPIVGAPGVTALRIALGTLILVV 46
gi|146310958|ref|YP_001176032.  APVWLPIVVILIAMLSIQSGASLAKSLFPIIGAPGVTALRIALGTLILVI 57
gi|227332340|ref|ZP_03835996.1  APVWLPILVLLIAMSSIQSGASLAKSLFPLIGAPGVTALRIALGTLILIA 57
gi|227730791|ref|ZP_04561272.1  APVWLPILVLLIAMSSIQSGASLAKSLFPLIGAPGVTALRIALGTLILIA 57
gi|16764194|ref|NP_459809.1| LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|198245156|ref|YP_002214796.  LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
```

FIG. 14G

```
gi|200389664|ref|ZP_03216275.1  LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|62179398|ref|YP_215815.1|    LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|16759750|ref|NP_455367.1|    LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|213022641|ref|ZP_03337088.1  ---------MLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 41
gi|213163835|ref|ZP_03349545.1  LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|161504001|ref|YP_001571113.  LPVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|157146546|ref|YP_001453865.  -PVWLPILVLLIAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 56
gi|440181|gb|AAA21854.1|        MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|30062297|ref|NP_836468.1|    MPVWLPIVILLVAMASIQGGASLAKSLFPLVSAPGVTALRLALGTLILIA 57
gi|218704191|ref|YP_002411710.  MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLLLIA 57
gi|26246789|ref|NP_752829.1|    MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 61
gi|170684270|ref|YP_001742917.  MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|15800565|ref|NP_286577.1|    MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|170020831|ref|YP_001725785.  MPIWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|215485900|ref|YP_002328331.  MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|227884223|ref|ZP_04002028.1  MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 50
gi|157158672|ref|NP_001462008.  MPVWLPIIILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|170769078|ref|ZP_02903531.1  MPVWLPIVILLVAMASIQGGASLAKSLFPLVGAPGVTALRLALGTMILIT 57
gi|218548334|ref|YP_002382125.  LPVWLPILILLVAMSSIQSGASLAKSLFPLVGAPGVTALRLALGTLILIA 57
gi|156934692|ref|YP_001438608.  TPLWLPVIVLIVAMASIQSGASLAKSLFPVVGAPGVTALRLALGTLILAV 57
gi|253990387|ref|YP_003041743.  AFPLIPVILLLFSMASIQSGASLAKTLFPVIGAPGVTALRLSLGTIILFL 59
gi|37525482|ref|NP_928826.1|    AFPLIPVILLLLAMTSIQGGASLAKTLFPVIGAPGVTALRLGLGTLILFL 59
gi|183598233|ref|ZP_02959726.1  AFPLLPVGLLLLAMLSIQSGASLAKTLFPVIGAPGVTGLRLLLATIILFV 58
gi|223990759|ref|ZP_03639931.1  AFPLLPIGLLLLAMTSIQSGASLAKTLFPVIGAPGVTGLRLLLATIILFV 58
gi|212712149|ref|ZP_03320277.1  AFPLLPIGLLLLAMASIQSGASLAKTLFPLIGAPGVTGLRLLLATIILFV 58
gi|212708023|ref|ZP_03316151.1  AFPLLPIGLLLLAMASIQSGASLAKTLFPLIGAPGVTGLRLLLATIILFV 58
gi|197284529|ref|YP_002150401.  LQVFYPVLLLLLLSMLSIQSGASLAKSLFPVIGAPAVTALRLFLGTLILFF 59
gi|237807801|ref|YP_002892241.  SSVLLPVFLIIIAMISVQSGASMAKMLFPLVGAEGATSLRLMIGAIALAL 57
gi|238898963|ref|YP_002924645.  SKFVLPIGALLIAMISLQSGASLAKGLFPLVGAPGVTSLRLGIGAFILLF 70
gi|146307537|ref|YP_001188002.  LALLAPIGLLLVAMISIQSGASLAKSLFPLVGAEGTTALRLVLGATILSL 53
gi|108759302|ref|YP_628438.1|   GPVTVAVLTLLVAMASIQTGASLAKQVFPVLGAAGATALRVFFAALILAA 58
```

FIG. 14H

gi|148548707|ref|YP_001268809. ILRPWRVRMTANTLRNVVIYGMALGGMNFLFYMALQTVPIGIAVALEFTG 108
gi|26988963|ref|NP_744388.1| ILRPWRVRMTANTLRNVVIYGMALGGMNFLFYMALQTVPIGIAVALEFTG 108
gi|167032871|ref|YP_001668102. ILRPWRVRMTANTLRNVVIYGMALGGMNFLFYMALQTLPIGIAVALEFTG 108
gi|170722598|ref|YP_001750286. ILRPWRVRMTAQTLRNVIIYGMALGGMNFLFYMALQTTPLGIAVALEFTG 108
gi|104782554|ref|YP_609052.1| LLRPWRAIILDINILRSIIIYGMALGGMNFLFYMSLRTVPLGIAVALEFTG 108
gi|229589563|ref|YP_002871682. LLRPWRAIILDINILRSIIIYGMALGGMNFLFYMSLRTVPLGIAVALEFTG 108
gi|77400125|ref|YP_349632.1 LLKPWRAKLTAKSLRTVIVYGMALGGMNFLFYMSLRTVPLGIAVALEFTG 108
gi|70731497|ref|YP_261238.1 LLRPWRAKFTTNTLRTVIIYGMALGGMNFLFYMSLRSVPLGIAVALEFTG 94
gi|213971379|ref|ZP_03399494.1 ILRPWRARLTARSLRTVVIYGIALGSMNFLFYMSLQTVPLGIAVALEFTG 108
gi|232799531|ref|ZP_04587992.1 ILRPWRASLTAKSLRTVVIYGIALGCMNFLFYMSLQTVPLGIAVALEFHR 108
gi|71737736|ref|YP_275301.1 ILRPWRANLTAKSLRTVVIYGIALGSMNLLFYMSLQTVPLGIAVALEFTG 184
gi|257484608|ref|ZP_05638649.1 ILRPWRANLTAKSLRTVVIYGIALGSMNLLFYMSLQTVPLGIAVALEFTG 90
gi|66046446|ref|YP_236287.1| VLRPWRANLTAKSLRTVVIYGVALGGMNLLFYMSLQTVPLGIAVALEFTG 108
gi|15596557|ref|YP_250051.1| LLRPWRKRLSLQSLRSVVVYGIALGGMNLLFYLSVRTVPLGIAVALEFTG 108
gi|49085542|gb|AAT51286.1| LLRPWRKRLSLQSLRSVVVYGIALGGMNLLFYLSVRTVPLGIAVALEFTG 108
gi|254239729|ref|ZP_04933051.| LLRPWRKRLSLQSLRSVVVYGIALGGMNLLFYLSVRTVPLGIAVALEFTG 108
gi|152988797|ref|YP_001349377. FLRPWRKRLSLQSLRSVVVYGIALGGMNLLFYLSVRTVPLGIAVALEFTG 108
gi|44662925|gb|AAS47543.1| LLRPWRLRFNLNSLRSVVIYGIALGGMNLMFYLSVRTVPLGVAVALEFTG 108
gi|92112331|ref|YP_572259.1| VMRPWRQRFSRRAWKSTMIYGVALGVMNLLFYQALQTVPLGIAVALEFTG 116
gi|227325920|ref|ZP_03829944.1 IFKPWRMRFS-SNRLPLLVYGITLGGMNFLFYLSLQTVPLGIAVALEFTG 106
gi|227115303|ref|ZP_03828959.1 IFKPWRMRFS-SNRLPLLVYGMTLGGMNFLFYLSLQTVPLGIAVALEFTG 106
gi|253687975|ref|YP_003017165 IFKPWRMRFS-SNRLPLLVYGITLGGMNFLFYLSLQTVPLGIAVALEFTG 106
gi|50121689|ref|YP_050856.1| IFKPWRMRFS-SNRLPLLVYGITLGGMNFLFYLSLQTVPLGIAVALEFTG 106
gi|255053893|ref|ZP_05306142.1 IFKPWRMRFS-SNRLPLLIYGITLGGMNFLFYLSLQTVPLGIAVALEFTG 106
gi|251790084|ref|YP_003004805. VFRPWRLRFG-SERLPLLVYGVILGGMNYLFYLALRTVPLGIAVALEFTG 107
gi|258631979|ref|ZP_05724793.1 AFKPWRLRAG-SNRLPLLIYGITLGGMNYLFYLAVRTVPLGIAVALEFTG 107
gi|242239813|ref|YP_002987994. IFKPWRMHLG-SNRWPLLVYGITLGGMNYLFYLALRTVPLGVVVALEFTG 107
gi|123443052|ref|YP_001007026. IFKPWRMRFEAGSRLPLLVYGVTLGGMNFLFYLSLKTVPLGIAVALEFTG 109
gi|238788823|ref|ZP_04632614.1 IFKPWRMRFEAGSRLPLLIYGVTLGGMNFLFYLSLKTVPLGIAVALEFTG 87
gi|170023846|ref|YP_001720351. IFKPWRMRFAAGSRLPLLIYGVALGGMNFLFYMSLKTVPLGIAVALEFTG 109
gi|221255575|ref|NP_668998.1| IFKPWRMRFAAGSRLPLLIYGVALGGMNFLFYMSLKTVPLGIAVALEFTG 109
gi|162418170|ref|YP_001606257. IFKPWRMRFAAGSRLPLLIYGVALGGMNFLFYMSLKTVPLGIAVALEFTG 109

FIG. 14I

gi|238761036|ref|ZP_04622013.1 IFKPWRMRFEAGSRLPLLIYGITLGGMNFLFYLSLKTVPLGIAVALEFTG 90
gi|238798464|ref|ZP_04641944.1 IFKPWRMRFAAGSRLPLLVYGVTLGGMNFLFYLSLKTVPLGIAVALEFTG 97
gi|238784064|ref|ZP_04628079.1 IFKPWRMRFEAGNRLPLLVYGVTLGGMNFLFYLNLKTVPLGIAVALEFTG 92
gi|238749896|ref|ZP_04611400.1 IFKPWRMRFAAGSRLPLLIYGVTLGGMNFLFYLSLKTVPLGIAVALEFTG 90
gi|238757260|ref|ZP_04618447.1 IFKPWRMRFAAGSRLPLLVYGVALGGMNFLFYLSLKTVPLGIAVALEFTG 97
gi|238753380|ref|ZP_04614743.1 IFKPWKMRFAAGSRLALLIYGVALGGMNFLFYLSLKTVPLGIAVALEFTG 87
gi|157369726|ref|YP_001477715. IFRPWRMRFAAGSRLPLFIYGLTLGAMNYLFYLSLRTLPLGIAVALEFTG 109
gi|268636050|ref|ZP_05728810.1 IFKPWRLRFTREQRLPLLMYGLALGGMNYLFYLSIRTVPLGVAVGLEFTG 110
gi|188534331|ref|YP_001908128. IFKPWRLBFSRQQIVPLIYGLALGGMNYAFYMSIRTVPLGIAVALEFTG 107
gi|152969399|ref|YP_001334508. VFKPWRLRFSPAQRVPLLLYGLALGAMNYLFYLSLQRIPLGVAVALEFTG 107
gi|206577533|ref|YP_002239546. VFKPWRLRFSPAQRVPLLLYGLALGAMNYLFYLSLQRIPLGVAVALEFTG 107
gi|195940762|ref|ZP_03086144.1 IFKPWRLRFKKEQRLPLLFYGLALGEMNYMFYLSIQTIPLGIAVALEFTG 107
gi|209907267|ref|ZP_03281751.1 IFKPWRLRFKKEQRLPLLYYGLSLGAMNYMFYLSIQTIPLGIAVALEFTG 96
gi|146310958|ref|YP_001176032. IFKPWRLRFKKEQRLPLLFYGLSLGAMNYLFYLSIQTVPLGVAVALEFTG 107
gi|227332340|ref|ZP_03835996.1 FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|237730791|ref|ZP_04561272.1 FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|16764194|ref|NP_459809.1| FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|198245156|ref|YP_002214796. FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|200389664|ref|ZP_03216375.1 FFKPWRLHFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|62179398|ref|YP_215815.1| FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|16759750|ref|NP_455367.1| FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|213022641|ref|ZP_03337088.1 FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 91
gi|213163835|ref|ZP_03349545.1 FFKPWRLRFAKEQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|161504001|ref|YP_001571113. FFKPWRLRFAKAQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|157146546|ref|YP_001453865. FFKPWRLRFAREQRLPLLFYGLSLGGMNYLFYLSIQTVPLGIAVALEFTG 106
gi|440181|gb|AAA21854.1| FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|30062297|ref|NP_836468.1| FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|21870419|ref|YP_002411710. FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|26246789|ref|NP_752829.1| FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 111
gi|170684270|ref|YP_001742917. FFKPWRLRFAKEQRLLLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|15800565|ref|NP_286577.1| FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107

FIG. 14J

```
gi|170020831|ref|YP_001725785.  FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|215485900|ref|YP_002328331.  FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|227884223|ref|ZP_04002028.1  FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 100
gi|157158672|ref|YP_001462008.  FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|170769078|ref|ZP_02903531.1  FFKPWRLRFAKEQRLPLLFYGVSLGGMNYLFYLSIQTVPLGIAVALEFTG 107
gi|218548334|ref|YP_002382125.  FFKPWRLRFAKEQRLPLLFYGLSLGAMNYLFYLAIQTVPLGIAVALEFTG 107
gi|156934692|ref|YP_001438608.  VFKPWRLRFTRSQRMPLLIYGLALGAMNYLFYLSIRTVPLGIAVALEFTG 107
gi|253990387|ref|YP_003041743.  IFKPWRLHFRRDSMMPLLIYGIALGAMNYTFYMALTTIPLGIAVALEFTG 109
gi|37525482|ref|NP_928826.1|    IFKPWRLRFSRKSIMPLLIYGIALGAMNYTFYMALTTIPLGIAVALEFTG 109
gi|183598233|ref|ZP_02959726.1  IFKPWRLKFRRNSLIPLLTYGLSLGTMNYLFYLSLERIPLGIAVALEFTG 108
gi|223990759|ref|ZP_03639931.1  IFKPWRLKFRKNSLVPLLAYGLSLGTMNYLFYLSLERIPLGIAVALEFTG 108
gi|212712149|ref|ZP_03320277.1  IFKPWRLNFRKSSLVPLLAYGLSLGTMNYLFYLALERIPLGIAVALEFTG 108
gi|212708023|ref|ZP_03316151.1  IFKPWRLNFRKSSLVPLLAYGLSLGTMNYLFYLALERIPLGIAVALEFTG 108
gi|197284529|ref|YP_002150401.  IFKPWRLSFSRSAIMPLFLYGLSLGAMNYLFYLALERIPLGIAVALEFTG 109
gi|237807801|ref|YP_002892241.  FYKPWRTRLVPGNIGPLLIYGVVLGGMNYLFYQSIRTLPLGVAVAFEFTG 107
gi|238898963|ref|YP_002924645.  IFKPWRIRFPRGDRMALFLYAFALGGMNFLFYFSLKKLPLGVAVALEFTG 120
gi|146307537|ref|YP_001188002.  VMQPWRTRLNLAAYRSLLAYGLALGGMNLLFYMSLQSIPLGIAVALEFTG 103
gi|108759302|ref|YP_628438.1|   VFRPWRQRLTRKDLRAVAIYGAALGGMNLTFYLALERIPLGIAVAIEFTG 108

gi|148548707|ref|YP_001268809.  PLAVAIFSSRRPIDFLWIALAIAGLLLLLPAG-HSGQALDPVGAAYALGA 157
gi|26988963|ref|YP_744388.1|    PLAVAIFSSRRAIDFLWIALAIAGLLLLLPAG-HSGQALDPVGAAYALGA 157
gi|167032871|ref|YP_001668102.  PLAVAIFSSRRPIDFLWIALAIAGLLLLLPAG-QSGQILDPLGAAYALGA 157
gi|170722598|ref|YP_001750286.  PLAVALFASRRAIDFLWIALAVSGLLLLIPVG-HGGQPLDLTGAAYALGA 157
gi|104782554|ref|YP_609052.1|   PLTVALLASRRALDFVWIVLAVIGLLLLIPVG-QAGGGLDPVGAAYALGA 157
gi|229589563|ref|YP_002871682.  PLAVAIYASRRAVDFLWIVLAIIGLLLLIPMG-EASSGIDLLGAGYALGA 157
gi|77460125|ref|YP_349632.1|    PLAVAIYASRRAIDFLWIALAAIGLLLLIPTG-ATTSGIDLVGAGYALGA 157
gi|70731497|ref|YP_261238.1|    PLAVAIYASRKAIDFLWIALAIVGLLLLIPTG-ATETAIDLVGAGYALGA 143
gi|213971379|ref|ZP_03399494.1  PLAVALLSSRKPIDFLWVTMAVIGLLLLIPLR-HSDAAIDLAGAGYALGA 157
gi|237799531|ref|ZP_04587992.1  PMAVALLSSRKPIDFLWVTLAVIGLLLLIPLG-NSDAAVDLVGAGYALGA 157
gi|71737736|ref|YP_275301.1|    PLAVALLSSRKPIDFLWVSLAVIGLLLLIPLG-ASNEAIDLLGAAYALGA 233
gi|257484608|ref|ZP_05638649.1  PLAVALLSSRKPIDFLWVSLAVIGLLLLIPLG-ASNEAIDLLGAAYALGA 139
gi|66046446|ref|YP_236287.1|    PLAVALLSSRKPIDFLWVTLAVIGLLLLIPTG-TSSAAIDLLGAGYALGA 157
```

FIG. 14K

```
gi|15596557|ref|NP_250051.1|   PLLVAILSSRRLLDFLWIGLAVFGIWLLLPLG-NSAEPLDLVGAAVALGA 157
gi|49085542|gb|AAT51286.1|   PLLVAILSSRRLLDFLWIGLAVFGIWLLLPLG-NSAEPLDLVGAAYALGA 157
gi|254239729|ref|ZP_04933051.1   PLLVAILSSRRLLDFLWIGLAVFGIWLLLPLG-NSAEPLDLVGAAYALGA 157
gi|152988797|ref|YP_001349377.   PLLVAILSSRRLLDFLWIGLAVFGIWLLLPLG-DSAEPLDLVGAAYALGA 157
gi|44662925|gb|AAS47543.1|   PLLVAILSSRRLLDFLWIGLAVFGLWLLLPLG-RSAEPVDPVGATYALSA 157
gi|92112331|ref|YP_572259.1|   PLAVAILSSRRWLDLLWVAFAVLGLACLLLLGDESNGAVDPYGAACALGA 166
gi|227325920|ref|ZP_03829944.1   PLAVAMLASRRPIDFLWVFLAVAGLWFLLPLG-HNIGNVDLTGAACAVGA 155
gi|227115303|ref|ZP_03828959.1   PLAVAMLASRRPIDFLWVFLAVAGLWFLLPLG-HNIGNVDLTGAACAVGA 155
gi|253687975|ref|YP_003017165.   PLAVAMLASRRPIDFLWVFLAVAGLWFLLPLG-HNIGNVDLVGAACAVGA 155
gi|50121689|ref|YP_050856.1|   PLAVAMLASRRPIDFLWVFLAVAGLWFLLPLG-HNIGNVDLTGAACAVGA 155
gi|255053893|ref|ZP_05306142.1   PLAVAMLASRRPIDFLWVFLAVAGLWFLLPLG-HNIGNVDLVGAAYAIGA 155
gi|251790084|ref|YP_003004805.   PLAVAILASRRLLDFGWIVLAAAGLCLLLPLG-QHINNVDFVGAAYAVGA 156
gi|258631979|ref|ZP_05724793.1   PLAVAILASRRVLDFVWIALAAAGLWLLLPLG-QHVNNVDLAGAAFAVGA 156
gi|242239813|ref|YP_002987994.   PLAVAVLSSRRLLDFLWIALAASGLWLLLPLG-GGTGDLDPLGILFAIGA 156
gi|123443052|ref|YP_001007026.   PLAVAVLSSRRPVDFIWVGLAILGLWFLLPLG-SSIGSIDLFGAACALGA 158
gi|238788823|ref|ZP_04632614.1   PLAVAMLASRRPVDFIWVGLAILGLWFLLPLG-NSIGTIDLFGAACALGA 136
gi|170023846|ref|YP_001720351.   PLAVAMFSSRRAVDFIWVGLAILGLWFLLPLG-HNIGTIDLFGAACALGA 158
gi|221255575|ref|NP_668998.1|   PLAVAMFSSRRAVDFIWVGLAILGLWFLLPLG-HNIGTIDLFGAACALGA 158
gi|162418170|ref|YP_001606257.   PLAVAMFSSRRAVDFIWVGLAILGLWFLLPLG-HNIGTIDLFGAACALGA 156
gi|238793410|ref|YP_04637035.1   PLAVAMFSSRRPVDFIWVGLAVLGLWFLLPLG-HNIGTIDLFGAACALGA 158
gi|238761036|ref|ZP_04622013.1   PLAVAMLSSRRPVDFIWVGLAILGLWFLLPLG-NSIGTIDLFGAACALGA 139
gi|238798464|ref|ZP_04641944.1   PLAVAMLSSRRPVDFIWVGLAVLGLWFLLPLG-HNVGTIDPFGAACALGA 146
gi|238784064|ref|ZP_04628079.1   PLAVAMLSSRRPVDFIWVGLAVLGLWFLLPLG-HNVGTIDLFGAACALGA 141
gi|238749896|ref|ZP_04611400.1   PLAVAMLSSRRPVDFIWVGLAVLGLWFLLPLG-NNIGSIDPFGAACALGA 139
gi|238757260|ref|ZP_04618447.1   PLAVAMFSSRRAVDFIWVGLAILGLWFLLPLG-HSVGTIDLFGAACALGA 146
gi|238753380|ref|ZP_04614743.1   PLAVAMFSSRRPIDFLWVGLAILGLTFLLPLG-QSVERIDLFGAACALGA 136
gi|157369726|ref|YP_001477715.   PLAVAMFSSRRPIDFLWVALAIAGLWFLLPLG-HDMGSIDIGAACALGA 158
gi|258636050|ref|ZP_05728810.1   PLTLALLGSRRPLDFLWVLLAVVGLWFLLPIG-TGISHIDPLGALLAVGA 159
gi|188534331|ref|YP_001908128.   PLMLALVGSRRALDFIWIALAVTGLLFLLPVG-HDISSVDPVGALLALTA 156
gi|152969399|ref|YP_001334508.   PLAVALFGSRRPLDFVWVALAILGLWYLLPLF-QNVAQVDLTGALFALGA 156
gi|206577533|ref|YP_002239546.   PLAVALLGSRRPLDFVWVALAILGLWYLLPLG-QNVAQVDLTGALFALGA 156
gi|195940762|ref|ZP_03086144.1   PLAVALFSSRRPVDFIWVILAVLGLWFLLPLG-QSVSQVDLTGAALALGA 156
```

FIG. 14L

```
gi|209907267|ref|ZP_03281751.1  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QSVSQIDLTGAALALGA 145
gi|146310958|ref|YP_001176032.  PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QNISHVDLTGAALALGA 156
gi|227332340|ref|ZP_03835996.1  PLAVALFSSRRPVDFIWVALAVLGLWFLLPLG-QDVSHVDLTGAALALGA 156
gi|237730791|ref|ZP_04561272.1  PLAVALFSSRRPVDFIWVALAVLGLWFLLPLG-QDVSHVDLTGAALALGA 156
gi|16764194|ref|NP_459809.1|    PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 156
gi|198245156|ref|YP_002214796.  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 156
gi|200389664|ref|ZP_03216275.1  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 156
gi|62179398|ref|YP_215815.1|    PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 156
gi|16759750|ref|NP_455367.1|    PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 156
gi|213022264|ref|YP_03337088.1  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 140
gi|213163835|ref|ZP_03349545.1  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDMSHVDLTGAALALGA 156
gi|161504001|ref|YP_001571113.  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDVSHVDLTGAALALGA 156
gi|157146546|ref|YP_001453865.  PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QGVSHVDLTGAALALGA 155
gi|440181|gb|AAA21854.1|        PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|30062297|ref|NP_836468.1|    PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|218704191|ref|YP_002411710.  PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|26246789|ref|NP_752829.1|    PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 160
gi|170684270|ref|YP_001742917.      PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|15800565|ref|NP_286577.1|    PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|170020831|ref|YP_001725785.      PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|215485900|ref|YP_002328331.      PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|227884223|ref|ZP_04002028.1      PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 149
gi|157158672|ref|YP_001462008.      PLAVALFSSRRPVDFVWVVLAVLGLWFLLPLG-QDVSHVDLTGCALALGA 156
gi|170769078|ref|ZP_02903531.1      PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLN-QDVSHVDLTGCALALGA 156
gi|218548334|ref|YP_002382125.      PLAVALFSSRRPVDFIWVVLAVLGLWFLLPLG-QDVSHVDLKGAAMALGA 156
gi|156934692|ref|YP_001438608.      PLAVALFGSRRPVDFLWVALAVLGLWFLLPLG-QDVTHVDPLGAACALGA 156
gi|253990387|ref|YP_003041743.      PLAVAMFSSRRPIDFLWVALVIAGLAFLLPKG-NNIDRLDPVGHYALSA 158
gi|37525482|ref|NP_928826.1|    PLAVAMFSSRRPIDFLWVALVIAGLAFLLPIGNNNINSLDPIGHYALSA 159
gi|183598233|ref|ZP_02959726.1      PLAVAMFSSRRPIDFLWIALVIVGLLFLLPIG-DDINGLDLVGALYALGA 157
gi|223990759|ref|ZP_03639931.1      PLAVAMFSSRRPIDFLWIALVIVGLLFLLPIG-DNIDSIDTLGALYALGA 157
gi|212712149|ref|ZP_03320277.1      PLAVAMFSSRRPIDFLWIGLVITGLLFLLPIG-DNIDSLDLLGVAYALGA 157
gi|212708023|ref|ZP_03316151.1      PLAVAMFSSRRPIDFLWIGLVITGLLFLLPIG-DEINAIDPLGAAYALGA 157
```

FIG. 14M

```
gi|197284529|ref|YP_002150401.  PLAVAMFSSRRAIDFLWIILVIAGLGLLLPIG-DNIHGLDPLGILYALGA 158
gi|237807801|ref|YP_002892241.  PLAVAIFSSRRAVDFLWIVLAMSGLGLLLPIG-HSMSDIDIPGALYALAA 156
gi|238898963|ref|YP_002924645.  PLAVVMFSSRRIVDFVWLGFAILGLWLLLPLH-QKTGGVDLFSAACALAA 169
gi|146307537|ref|YP_001188002.  PLGLALLSSRRLLDFVWVALAVFGLWLLLPSG-LAQTQLDPLGMALALAA 152
gi|108759302|ref|YP_628438.1|   PLALAIFSTRRALDFVWALLAVAGILLILPLG-ETSRSLDWMGVFWALVA 157

gi|148548707|ref|YP_001268809.    GVCWALYILFGQRAG-AEHGIQSAALGVVVAALFVAPIGIAHAG-SALLT 205
gi|26988963|ref|NP_744388.1|   GVCWALYILFGQRAG-AEHGIQSAALGVVVAALFVAPIGIAHAG-SALLT 205
gi|167032871|ref|YP_001668102.    GVCWALYILFGQRAG-AEHGIQSAALGVVVAALFVAPIGIAHAG-SALLT 205
gi|170722598|ref|YP_001750286.    GVCWALYILFGQRAG-AEHGIQSAALGVVVAAIFVAPIGIVHAG-TALLT 205
gi|104782554|ref|YP_609052.1|    GVCWALYILYGQRAG-AEHGIQTAALGVVIAALFVAPIGIVHAG-SALLT 205
gi|229589563|ref|YP_002871682.    GVCWALYILYGQKAG-NDNGVQTAALGVMIAALFVAPIGIVHAG-SALLT 205
gi|77460125|ref|YP_349632.1|   GFCWALYILFGQKAG-ADNGVTIAALGVMIAALFVAPIGIVHAG-SALLT 205
gi|70731497|ref|YP_261238.1|   GVCWALYILFGQKAG-AENGIQIAALGVMIAALFVAPIGIVHAG-AALLT 191
gi|213971379|ref|ZP_03399494.1    GVCWAAYIVFGQKAG-ADNGVQTAALGVMIAAIFIAPIGVVHAG-AALLD 205
gi|237799531|ref|ZP_04587992.1    GVCWAAYIVFGQKAG-ADNGVQTAALGVMIAALFIGPIGVIHAG-SALLD 205
gi|71737736|ref|YP_275301.1|   GVCWAAYTVFGHKAG-ADNGVQTAALGVMIAALFIGPVGVIHAG-SALFD 281
gi|257484608|ref|ZP_05638649.1    GVCWAAYIVFGHKAG-ADNGVQTAALGVMIAALFIGPVGVIHAG-SALFD 187
gi|66046446|ref|YP_236287.1|   GVCWAAYIVFGHKAG-ADNGVQTAALGVVIAALFIGPIGVIHAG-SALLD 205
gi|15596557|ref|NP_250051.1|   GVCWALYILFGQRAG-ADHGAQGAALGVLVAAILVVPIGVAHAG-ADLLD 205
gi|49085542|gb|NAAT51286.1|    GVCWALYILFGQRAG-ADHGAQGAALGVLVAAILVVPIGVAHAG-ADLLD 205
gi|254239729|ref|ZP_04933051.1    GVCWALYILFGQRAG-ADHGAQGAALGVLVAAILVVPIGVAHAG-ADLLD 205
gi|152988797|ref|YP_001349377.    GVCWALYILFGQRAG-ADHGAQGAALGVLVAAILVVPIGVAHAG-ADLLD 205
gi|44662925|gb|AAS47543.1|    GVCWALYILFGQRAG-ADYGTQGAALGVLVAAFCVVPVGIVHAG-ADLLD 205
gi|92112331|ref|YP_572259.1|   GVCWALYILFGQKAG-SVNGAQSATLGITIAAVVIAPVGLIDVG-ADLLD 214
gi|227325920|ref|ZP_03829944.1    GACWALYILFGQKAG-ANHGPGTVAIGSCIAALIFCPIGAYYA-ESTLFS 203
gi|227115303|ref|ZP_03828959.1    GACWALYILFGQKAG-ANHGPGTVAIGSCIAALIFCPIGAYYA-ESTLFS 203
gi|253687975|ref|YP_003017165.    GACWALYILFGQKAG-SNHGPGTVAIGSCIAALIFCPIGAYYA-ESTLFS 203
gi|50121689|ref|YP_050856.1|   GACWALYILFGQKAG-ANHGPGTVAVGSCIAALIFCPIGVYYA-ESTLFS 203
gi|255053893|ref|ZP_05306142.1    GACWALYILFGQKAG-ANHGPGTVAIGSCIAALIFCPIGAYYA-ESTLFS 203
gi|251790084|ref|YP_003004805.    GACWAGYILFGQKAG-ANHGAGTVALGSLIAALVYCPVGLLFSDVNTLFS 205
```

FIG. 14N

```
gi|258631979|ref|ZP_05724793.1 GACWAGYILFGQKAG-ASHGASTVAMGSLIAALVYCPIGLLFSDISSLLS 205
gi|242239813|ref|YP_002987994. GACWAGYILFGKKAG-ARHGAGTVAMGSLIAALVYCPIGAFFS-SSALFS 204
gi|123443052|ref|YP_001007026. GACWALYIISGQKAG-GDHGPGTVAVGSLIAALVFCPIGVAYNG-LALFD 206
gi|238788823|ref|ZP_04632614.1 GACWALYIVSGQKAG-GDHGPGTVAVGSLIAALVFCPIGVAYNG-LALFA 184
gi|170023846|ref|YP_001720351. GACWAIYIISGQKAG-GDHGPATVAVGSLIAALIFCPIGVAYNG-MALFD 206
gi|22125575|ref|NP_668998.1| GACWAIYIISGQKAG-GDHGPATVAVGSLIAALIFCPIGVAYNG-MALFD 206
gi|162418170|ref|YP_001606257. GACWAIYIISGQKAG-GDHGPATVAVGSLIAALIFCPIGVAYNG-MALFD 204
gi|238793410|ref|ZP_04637035.1 GACWALYIISGQKAG-GDHGPGTVAVGSLIAALVFCPIGVAYNG-LALFD 206
gi|238761036|ref|ZP_04622013.1 GVCWALYIVSGQKAG-GDHGPGTVAIGSLIAAIIFCPIGVAYNG-MALFS 187
gi|238798464|ref|ZP_04641944.1 GACWALYIISGQKAG-GDHGPGTVAVGSLIAALVFCPIGVAYNG-MALFD 194
gi|238784064|ref|ZP_04628079.1 GACWALYIISGQKAG-GDHGAGTVAIGSLIAALVFCPIGVAYNG-MALFD 189
gi|238749894|ref|ZP_04611400.1 GVCWAIYIVSGQKAG-GDHGPGTVAIGSLIAALVFCPIGVAYNG-LALFD 187
gi|238757260|ref|ZP_04618447.1 GACWAIYIVSGQKAG-GDHGPGTVAIGSLIAALIFCPIGVVYNG-MALFD 194
gi|238753380|ref|ZP_04614743.1 GACWAIYILSGQKAG-GDHGATVAIGSLIAALVFCPIGVAYNG-MALFS 184
gi|157369726|ref|YP_001477715. GACWAVYIICGQKAG-GDHGPTVAVGSLIAAVVPCPIGAWQTG-SALLN 206
gi|258636050|ref|ZP_05728810.1 GACWAVYILAGQRAG-AEHGPATVAMGSLVASIIFVPLGLTFAH-SGIWH 207
gi|188534331|ref|YP_001908128. GGCWAIYILAGQRAG-SQHGPTVAAGTLIGALVFVPLGLVYAN-PGIWT 204
gi|152969399|ref|YP_001334508. GACWAVYILTGQRAG-EEHGPATVAMGSLIASLVFVPLGMAQAT-DTLWQ 204
gi|206577533|ref|YP_002239546. GACWAVYILTGQRAG-EEHGPATVAMGSLIASLVFVPLGMAQAT-DTLWQ 204
gi|195940762|ref|ZP_03086144.1 GACWAVYILTGQRAG-EEHGPATVALGSLIAAVIFVPIGMAQAT-DSIWQ 204
gi|209907267|ref|ZP_03281751.1 GACWAIYILSGQRAG-EEHGPATVALGSLIAAHFVPLGMAQAT-ESIWQ 193
gi|146310958|ref|YP_001176032. GACWAVYILTGQRAG-EEHGPATVALGSLIAAHFVPLGMAQAT-ESIWQ 204
gi|227332340|ref|ZP_03835996.1 GACWAIYILTGQRAG-AEHGPATVAVGSLIAALVFVPIGALQAG-DALWH 204
gi|237730791|ref|ZP_04561272.1 GACWAIYILTGQRAG-AEHGPATVAVGSFIAALVFVPIGALQAG-DALWH 204
gi|167641941|ref|NP_459809.1| GACWAVYILTGQRAG-AEHGPATVAVGSLIAAIIFVPIGAVQAG-DALWH 204
gi|198245156|ref|YP_002214796. GACWAVYILTGQRAG-AEHGPATVAVGSLIAAIIFVPIGAVQAG-DALWH 204
gi|200389664|ref|ZP_03216275.1 GACWAVYILTGQRAG-AEHGPATVAVGSLIAAIIFVPIGAVQAG-DALWH 204
gi|62179398|ref|YP_215815.1| GACWAVYILTGQRAG-AEHGPATVAVGSLIAAIIFVPIGAVQAG-DALWH 204
gi|167597501|ref|NP_455367.1| GACWAVYILTGQRAG-AEHGPATVAVGSLITAIIFVPIGAVQAG-DALWH 204
gi|213022641|ref|ZP_03337088.1 GACWAVYILTGQRAG-AEHGPATVAVGSLITAIIFVPIGAVQAG-DALWH 188
gi|213163835|ref|ZP_03349545.1 GACWAVYILTGQRAG-AEHGPATVAVGSLITAIIFVPIGAVQAG-DALWH 204
gi|161504001|ref|YP_001571113. GACWAVYILTGQRAG-AEHGPATVAVGSLIAAIIFVPIGAVQAG-DALWH 204
```

FIG. 14O

```
gi|157146546|ref|YP_001453865.   GACWAIYILTGQRAG-EEHGPATVAVGSLIAAIVFVPVGALQAG-EALWH 203
gi|440181|gb|AAA21854.1|         GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|30062297|ref|NP_836468.1|     GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|218704191|ref|YP_002411710.   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|26246789|ref|NP_752829.1|     GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 208
gi|170684270|ref|YP_001742917.   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGVLQAG-EALWH 204
gi|158005651|ref|NP_286577.1|    GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|170020831|ref|YP_001725785.   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|215485900|ref|YP_002328331.   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|227884223|ref|ZP_04002028.1   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 197
gi|157158672|ref|YP_001462008.   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|170769078|ref|ZP_02903531.1   GACWAIYILSGQRAG-AEHGPATVAIGSLIAALIFVPIGALQAG-EALWH 204
gi|218548334|ref|YP_002382125.   GACWAIYILTGQRAG-AEHGPATVAIGSLIAAMVFVPIGAMQAG-EALWH 204
gi|156934692|ref|YP_001438608.   GVCWAIYIIAGQKAG-AEHGPATVALGSLIAAIVFVPIGAAAAG-EALWH 204
gi|253990387|ref|YP_003041743.   GVCWALYIIAGQRAG-AGYGAATVSIGSFIAALIFFPVGLIQTGPELLFD 207
gi|37525482|ref|NP_928826.1|     GVCWALYIIFGQRAG-AGHGAATVSIGSFIAAMIFFPVGLIQTGPELLFD 208
gi|183598233|ref|ZP_02959726.1   GVCWGIYIIFGQRAG-AGYGAATVAIGSFISTLIFFPIGLMQTGAEVMFD 206
gi|223990759|ref|ZP_03639931.1   GVCWGLYIIFGQRAG-AGYGAATVAIGSFISAMIFFPIGLMQTGVEVMFD 206
gi|212712149|ref|ZP_03320277.1   GVCWAIYIIFGQRAG-AGYGAATVAVGSLISALIFFPIGLMQTGVEAMFD 206
gi|212708023|ref|ZP_03316151.1   GVCWGIYIIFGKRAG-AGYGAATVAVGSLISALIFFPIGLMQTGVEAMFD 206
gi|197284529|ref|YP_002150401.   GVGWALYIVFGQRAG-KGYGAATVSIGSLIAAFIFFPIGMLQSSPELVFS 207
gi|237807801|ref|YP_002892241.   GGCWALYIIFGKKSG-MKNGPTTVALGVGIATVCFAPIGVWQNG-MALLS 204
gi|238898963|ref|YP_002924645.   GACWALYIIFGQKTG-VHHGPGAVSVGSLIAACLFCPIGIIDNG-MALCS 217
gi|146307537|ref|YP_001188002.   GLCWALYIVFGQKAG-AAHGRQTVALGTWVAALLVLPIGLWQAG-GSLFS 200
gi|108759302|ref|YP_628438.1|    ATCWALYILFGQRAGGTVHGGTAASLGMCVAALLVMPPGVAIIAG-MKLLD 206

gi|148548707|ref|YP_001268809.   PAVIPMALAVAILSTALPYSLEMVALTRIPARTFGTLMSIEPAFGALSGL 255
gi|26988963|ref|NP_744388.1|     PAVIPMAFAVAILSTALPYSLEMVALTRIPARTFGTLMSIEPAFGALSGL 255
gi|167032871|ref|YP_001668102.   PAVIPMALAVAVLSTALPYSLEMVALTRIPARTFGTLMSIEPAFGALSGL 255
gi|170722598|ref|YP_001750286.   PALIPLALGVAILSTALPYSLEMVALTRMPARTFGTLMSIEPAIGALSGL 255
gi|104782554|ref|YP_609052.1|    PALLPVALGVAILSTALPYSLEMVALTRMPARTFGTLMSIEPAFGALSGL 255
gi|229589563|ref|YP_002871682.   PALIPVAIGVAILSTALPYTLEMVALTRLPARTFGTLMSIEPAFGAVSGL 255
```

FIG. 14P

gi|77460125|ref|YP_349632.1| PALIPIAIGVAILSTALPYTLEMVALTRMPARTFGTLMSIEPAFGALSGL 255
gi|70731497|ref|YP_261238.1| PSLIPVALGVAILSTALPYSLEMVALTRMPARTFGTLMSIEPAFGALSGL 241
gi|213971379|ref|ZP_03399494.1 ISLIPAAIGVAILSTALPYSLEMVALTRMSARTFGTLASLEPVFAALSGL 255
gi|237799531|ref|ZP_04587992.1 ISLIPAALGVAILSTALPYSLEMIALTRMSARTFGTLASLEPVFAALSGL 255
gi|71737736|ref|YP_275301.1| ISLVPAALGVAILSTALPYSLEMIALTRMSARTFGTLASLEPVFAALSGL 331
gi|257484608|ref|ZP_05638649.1 ISLVPAALGVAILSTALPYSLEMIALTRMSARTFGTLASLEPVFAALSGL 237
gi|66046446|ref|YP_236287.1| ISLIPAALGVAILSTALPYSLEMIALTRMSARTFGTLASLEPVFAALSGL 255
gi|15596557|ref|NP_250051.1| PALIPLALGVAVLSTALPYTLEMVALTRLPARTFGTLMSIEPAFGALSGL 255
gi|49085542|gb|AAT51286.1| PALIPLALGVAVLSTALPYTLEMVALTRLPARTFGTLMSIEPAFGALSGL 255
gi|254239729|ref|ZP_04933051.1 PALIPLALGVAVLSTALPYTLEMVALTRLPARTFGTLMSIEPAFGALSGL 255
gi|152988797|ref|YP_001349377. PALIPLALGVAVLSTALPYTLEMVALTRLPARTFGTLMSIEPAFGALSGL 255
gi|44662925|gb|AAS47543.1| PVLIPVALEVAILSTALPYSLEMVALTRLPAITFGTLMSVEPAFGALSGL 255
gi|92112331|ref|YP_572299.1| PEVLPLAFAVAVLSTALPYTLEMVALTRLPTQTFGTLMSLEPAIGALSGL 264
gi|227325920|ref|ZP_03829944.1 LSILPLGIAVAIMSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGI 253
gi|227115303|ref|ZP_03828959.1 LSILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGI 253
gi|253687975|ref|YP_003017165. LSILPLGVAVAVLSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGI 253
gi|50121689|ref|YP_050856.1| FSILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGI 253
gi|255053893|ref|ZP_05306142.1 LTILPLGIAVAILSTALPYSLEMVALTRLPARTFSTLMSMEPAIAALSGI 253
gi|251790084|ref|YP_003004805. LDVLPIGIAVALLSTALPYTLEMLALTRLPTRTFSTLMSLEPAVAAMSGI 255
gi|258631979|ref|ZP_05724793.1 MSILPIGIAVAVLSTALPYILEMMALTRLPTRTFSTLMSLEPAVAALSGI 255
gi|242239813|ref|YP_002987994. SAVLPVGIAVALLSTALPYSLEMLALTRLPTRTFSTLMSLEPAIAAMSGI 254
gi|123443052|ref|YP_001007026. PAILPVALAVAILSTALPYSLEMVALTRLPTRTFGTLMSLEPALAAISGL 256
gi|238788823|ref|ZP_04632614.1 PAILPVALAVAILSTALPYSLEMVALTRLPTRTFGTLMSLEPALAAISGL 234
gi|170023846|ref|YP_001720351. PAILPVALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGL 256
gi|22125575|ref|NP_668998.1| PAILPVALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGL 256
gi|162418170|ref|YP_001606257. PAILPIALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGL 254
gi|238793410|ref|ZP_04637035 1. PAILPVALAVAILSTALPYSLEMVALTRLPTRTFGTLMSLEPALAAISGL 256
gi|238761036|ref|ZP_04622013.1 PAILPVALAVAILSTALPYSLEMVALTRLPTRTFGTLMSLEPALAAVSGL 237
gi|238798464|ref|ZP_04641944.1 PAILPIALAVAILSTALPYSLEMVALTNLPTRTFGTLMSLEPALAAISGL 244
gi|238749896|ref|ZP_04611400.1 PAILPIALAVAILSTALPYSLEMVALTNLPTRTFGTLMSLEPALAAISGL 237
gi|238757260|ref|ZP_04618447.1 PAILPIALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAVSGL 244
gi|238753380|ref|ZP_04614743.1 PAILPVALAVAILSTALPYSLEMVALTRLPARTFGTLMSLEPALAAISGL 234

FIG. 14Q

```
gi|157369726|ref|YP_001477715.  VDILPIALAVAILSTALPYSLEMHALPKIPARTFGTLMSLEPALAALSGM 256
gi|258636050|ref|ZP_05728810.1  WEILPLALVIALLSSAIPYSLEMMALTRLPARIFGTLMSLEPAMAALSGM 257
gi|188534331|ref|YP_001980812 8.   LSLIPIGLAIAVMSSALPYSLEMMALTRLPARTFGTLMSLEPAMAAISGM 254
gi|152969399|ref|YP_001334508.  WSLLPLGLGIAILSTALPYSLEMMALTRLPTRTFGTLMSLEPALAALSGM 254
gi|206577533|ref|YP_002239546.  WSLLPMGLGIAILSTALPYSLEMMALTRLPTRTFGTLMSLEPALAALSGM 254
gi|195940762|ref|ZP_03086144.1  WSILPVGLAVAILSTALPYSLEMIALTRLPTRIFGTLMSMEPALAAISGM 254
gi|209907267|ref|ZP_03281751.1  WSVMPIGLAVAILSTALPYSLEMIALTRLPTRIFGTLMSMEPALAAISGM 243
gi|146310958|ref|YP_001176032.  WSVLPIGLAVAVLSTALPYSLEMIALTRLPTRIFGTLMSMEPALAAMSGM 254
gi|227332340|ref|ZP_03835996.1  WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|237730791|ref|ZP_04561272.1  WSILPLGLAIAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|16764194|ref|NP_459809.1|    WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|198245156|ref|YP_002214796.  WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPAL------ 248
gi|200389664|ref|ZP_03216275.1  WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|62179398|ref|YP_215815.1|    WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|16759750|ref|NP_455367.1|    WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPVLAAISGM 254
gi|213022641|ref|ZP_03337088.1|    WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPVLAAISGM 238
gi|213163835|ref|ZP_03349545.1|  WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|161504001|ref|ZP_001571113.  WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|157146546|ref|YP_001453865.  WSILPLGLAVAVLSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 253
gi|440181|gb|AAA21854.1|        WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|30062297|ref|NP_836468.1|    WSIVPLGLAVVILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|218704191|ref|YP_002411710.  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|26246789|ref|NP_752829.1|    WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 258
gi|170684270|ref|YP_001742917.  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|15800565|ref|NP_286577.1|    WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|170020831|ref|YP_001725785.  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|215485900|ref|YP_002328331.  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|227884223|ref|ZP_04002028.1  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 247
gi|157158672|ref|YP_001462008.  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPALAAVSGM 254
gi|170769078|ref|ZP_02903531.1  WSIVPLGLAVAILSTALPYSLEMIALTRLPTRTFGTLMSMEPAMAAISGM 254
gi|218548334|ref|YP_002382125.  WSVPLGLAVAILSTALPYSLEMIALTRLPTRIFGTLMSMEPALAAISGM 254
gi|156934692|ref|YP_001438608.  WSVLPAGIGIAILSTALPYSLEMTALTRLPTRIFGTLMSMEPALAAFTGM 254
```

FIG. 14R

```
gi|253990387|ref|YP_003041743.   MSIMPLALAIAILSTAFPYTLEMIALTRLPAQTFGTLMSLEPALGALSGI 257
gi|37525482|ref|NP_928826.1|     MSILPLALAIAILSTAFPYTLEMIALTRLPAQTFGTLMSLEPALGALSGI 258
gi|183598233|ref|ZP_02959726.1    PAILPLALAVAVLSTAFPYTLEMIALIRLPAKTFGTLMSLEPCMGAFLGI 256
gi|223990759|ref|ZP_03639931.1    PSILPIALALAILSTAFPYTLEMIALTRLPARTFGTLMSLEPCMGAFLGI 256
gi|221712149|ref|ZP_03320277.1    PAILPLALAVAILSTAFPYTLEMIALTKLPAKTFGTLMSLEPCMGAFLGI 256
gi|221708023|ref|ZP_03316151.1    PAILPIALAVAILSTAFPYTLEMIALTKLPAKTFGTLMSLEPCMGAFLGI 256
gi|197284529|ref|YP_002150401.    WSILPIGLAIAILSTAFPYTLEMIALTRLPAKTFGTLMSLEPCMGAFLGI 257
gi|237807801|ref|YP_002892241.    PDVLPLALGVGILSTALPFSLEMMALTRIPARTFGTLMSLEPAVGALSGM 254
gi|238898963|ref|YP_002924645.    SEVLPPFIAIAILSSVVPFSLEMVALTRLPARTFGTLMSLEPAFGALSGV 267
gi|237807801|ref|YP_002892241.    PDVLPLALGVGILSTALPFSLEMMALTRIPARTFGTLMSLEPAVGALSGM 250
gi|108759302|ref|YP_628438.1|     VSLLPLHLGVAVLSSALPYSLEMYALKALPTRTFGILMSLEPALAAVSGL 256

gi|148548707|ref|YP_001268809.    LFLGEVLTLTQWLAILAIITASVGTTLSMR-KAPSPAIAAD---- 295
gi|26988963|ref|NP_744388.1|     LFLGEVLTLTQWLAILAIITASVGTTLSMR-KAPSPAIAAD---- 295
gi|167032871|ref|YP_001668102.    LFLGEVLTVTQWLAILAIITASVGTTLSMR-NTSKPAIAAD---- 295
gi|170722598|ref|YP_001750286.    LFLGELLSVTQWLAILAIIAASVGATLSMR-RDAKPPVAAD---- 295
gi|104782554|ref|YP_609052.1|     LFLGEQLILWQWLAILAIIATASIGATLSMK-RDAKPPVAAD---- 295
gi|229589563|ref|YP_002871682.    LFLHEIILSIAQWLAIICIILASVGATVIMK-SESKPLVPAD---- 295
gi|77460125|ref|YP_349632.1|     LFLQEYLSLSQWMAILCIILASVGATMTMG-NIAKPAIAAD---- 295
gi|70731497|ref|YP_261238.1|     LFLHEYLSLAQWTAIACIILASVGATMTMR-REAKPIVAVD---- 281
gi|213971379|ref|ZP_03399494.1    VFLHENLSLTQWLAIGAIIFASIGATLSSA-NAKPQLVPAD---- 295
gi|227799531|ref|ZP_04587992.1    IFLHEDLSLTQWLAIGAIIFASIGATLSSA-NAKPQLVPAD---- 295
gi|71737736|ref|YP_275301.1|     VFLHESLSLTQWLAIGAIIFASIGATLGSA-NIKPQLVPAD---- 371
gi|257484608|ref|ZP_05638649.1    VFLHESLSLTQWLAIGAIIFASIGATLGSA-NIKPQLVPAD---- 277
gi|66046446|ref|YP_236287.1|     MFLHESLSLTQWLAIGAIIFASIGATLGSA-NSKPQLVPAD---- 295
gi|15596557|ref|NP_250051.1|     LFLGERLSPTQWLAIGAIILASVGATTLSVKSRGAAPALPAKD--- 297
gi|49085542|gb|AAT51286.1|       LFLGERLSPTQWLAIGAIILASVGATTLSVKSRGAAPALPAKDG-- 298
gi|254239729|ref|ZP_04933051.1    LFLGERLNPTQWLAIGAIILASVGTTLSVKSRVAAPALPAKD--- 297
gi|152988797|ref|YP_001349377.    LFLGERLSPTQWLAIGAIILASVGTTLSVKSRGPAPALPAKD--- 297
gi|44662925|gb|AAS47543.1|       LFLGERLIASQWLAISVIILASAGTTLSIKSRSNIPELSAKD--- 297
gi|92112331|ref|YP_572259.1|     LFLSQLLISGLQWLAIGLIIVASIGTTLTVRRRDTFEIPAPD--- 305
gi|227325920|ref|ZP_03829944.1    LFLGEHLSFIQWMALIFIIIASIGATLTIKPAGGAKVTTQAE--- 295
```

FIG. 14S

```
gi|227115303|ref|ZP_03828959.1    LFLGEHLSFIQWMALIFIIIASIGATLTIKPAGGAKVTTQAE--- 295
gi|253687975|ref|YP_003017165.      LFLGEHLSFIQWMALIFIIIASIGATLTIKPAGAANVTTQTE--- 295
gi|50121689|ref|YP_050856.1|  LFLGEILAFIQWMALMFIIIASIGAFLTIKSAGAFKATTQAE--- 295
gi|255053893|ref|ZP_05306142.1      LFLGEHLSFIQWMALIFIFTASLGATLTIKSAEVRKEEK ----- 293
gi|251790084|ref|YP_003004805.      LFLNEIII.TI.IQWLALALAFIIMASLGTTLSVKKRAEPVVS------ 293
gi|258631979|ref|ZP_05724793.1      LFLNEIII.TLTQWLALALAFHEASLGTTLSVKTRSASATS------ 293
gi|242239813|ref|YP_002987994.      LFLNEHLSIAQWLALVFIII ASLGTTLSAKKSAA---------- 288
gi|123443052|ref|YP_001007026.      IFLNEIILTLIQWLALASIISASIGATLIIK-SKPQIDEIA----- 295
gi|238788823|ref|ZP_04632614.1      VFLNEIILTLIQWLALASIISASIGAILTIK-PKPHIDQVA----- 273
gi|170023846|ref|YP_001720351.      VFLNEHLTLWQWLALASHSASVGASETIK-PKPQLDQVT------ 295
gi|22125575|ref|NP_668998.1|  VFLNEHLTLVQWLALASIISASWGASLTIK-PKPQLDQVT ----- 295
gi|162418170|ref|YP_001606257.      VFLNEHLTLVQWLALASIISASVGASLTIK-PKPQLDQVT------ 293
gi|238793410|ref|ZP_04637035.1      VFLNEIILTWVQWLALASIISASKIAILTIK-PKPQLDQVA----- 295
gi|238761036|ref|ZP_04622013.1      VFLNEIILTFIQWLALASIISASKIATLTIK-PKPQIDKIA----- 276
gi|238798464|ref|ZP_04641944.1      VFLNEHLTLIQWLALASIICASIGATLTIR-PKPXLDNIN----- 283
gi|238784064|ref|ZP_04628079.1      VFLNEIILTLIQWMALASIICASIGATLTIR-PKPQLDNIT----- 278
gi|238749896|ref|ZP_04611400.1      IFLNEIILTLIQWLALVSIIIASIGATLTIR-PKPKIDTVA----- 276
gi|238757260|ref|ZP_04618447.1      VFLNEIILTMVQWLALASHCASIGATLTIK-PKPQLEQIT----- 283
gi|238753380|ref|ZP_04614743.1      IFLNEIILSLVQWLALASHCASIGATLTIRE-AKSKIEELA----- 273
gi|157369726|ref|YP_001477715.      LFLNEIILTIVQWLALAAHAASMGATLTIK-PKPQIEKLS------ 295
gi|258636050|ref|ZP_05728810.1      LFLGELLTLKQWVALLAHIASAGSTLTMKPKSPQINEVDLNPPQ 302
gi|188534331|ref|YP_001908128.      LFLGEVLTLVQWLALLAIIMASAGSTLTMRPAKAKITPIKEFNE- 298
gi|152969399|ref|YP_001334508.      IFLGETLKLSQTLALGAIIIASMGATLTMQ-RQSKVEQVDIN--- 295
gi|206577533|ref|YP_002239546       VFLGETLKLSQTLALGAIIIASMGATLTMP-RQSKIEQVDIN--- 295
gi|195940762|ref|ZP 03086144.1      IFLGETLTLVQILALCSHAASMGSTLTMII-PEPKVEKIDLN--- 295
gi|209907267|ref|ZP_03281751.1      IFLGETLIFTQTLALCSHAASMGSTLTMR-REPKVEKLDIN--- 284
gi|146310958|ref|YP_001176032.      VFLGESLTLVQVLALCSHAASMGSTLTMR-SEPKIKEIDIII--- 295
gi|227332340|ref|ZP_03835996.1      IFLGETLTLIQTLALGAIIAASMGSTLTIR-KEPQIKQVDVN--- 295
gi|237730791|ref|ZP_04561272.1      IFLGETLTLIQTLALGAIIAASMGSTLTIR-KEPQIKQVDVN--- 295
gi|16764194|ref|NP_459809.1   IFLGEILTGIQILALCAHAASMGSTLTIR-REPQIKQVDVK--- 295
gi|198245156|ref|YP_002214796.      ---GETLTGIQILALCAILAASMGSTLTIR-REPOIKQVDVK--- 286
```

FIG. 14T

```
gi|200389664|ref|ZP_03216275.1      IFLGETLTGIQILALCAILAASMGSTLTIR-REPQIKQVDVK--- 295
gi|62179398|ref|YP_215815.1|        IFLGETLTGIQILALCAILAASMGSTLTIR-REPQIKQVDVK--- 295
gi|6759750|ref|NP_455367.1|         IFLGETLIGIQILALCAIIAASMGSTLTIR-REPQIKQVDVK--- 295
gi|213022641|ref|ZP_03337088.1      IFLGETLTGIQILALCAIIAASMGSTLTIR-REPQIKQVDVK--- 279
gi|213163835|ref|ZP_03349545.1      IFLGETLTGIQILALCAIIAASMGSTLTIR-REPQIKQVDVK--- 295
gi|161504001|ref|YP_001571113.      IFLGETLTGVQIMALCAIIAASMGSTLTIR-REPQIKQVDVK--- 295
gi|157146546|ref|YP_001453865.      IFLGETLTLVQLLALAAHAASMGSTLTIR-RETKIQQVDIN--- 294
gi|440181|gb|AAA21854.1      IFLGETLTPIQLLRLGAILAASMGSTLTVR-KESKIKELDIN--- 295
gi|30062297|ref|NP_836468.1|        IFLGETLTPIQLLALGAILAASMGSTLTVR-KESKIKELDIN--- 295
gi|218704191|ref|YP_002411710.      IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 295
gi|26246789|ref|NP_752829.1|        IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 299
gi|170684270|ref|YP_001742917.      IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 295
gi|15800565|ref|NP_286577.1|        IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 295
gi|170020831|ref|YP_001725785.      IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 295
gi|215485900|rel|YP_002328331.      IFLGETLTPIQLLALGAIIAASMGSTLTVR-KERKIKELDIN--- 295
gi|227884223|ref|ZP_04002028.1      IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 288
gi|157158672|ref|YP_001462008.      IFLGETLTPIQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 295
gi|170769078|ref|ZP_02903531.1      IFLGETLAPVQLLALGAIIAASMGSTLTVR-KESKIKELDIN--- 295
gi|218548334|ref|YP_002382125.      IFLGETLTLVQLLALGAIIAASMGSTLTMR-QASKIKELDVN--- 295
gi|156934692|ref|YP_001438608.      IFLGETLTLVQWLALLSIIIASVGSTLTLR-RETQIKSLDID--- 295
gi|253990387|ref|YP_003041743.      VFLNEIILTIAQWLALFYTIFASK;STSTIK-GKPKIVEVDK---- 297
gi|37525482|ref|NP_928826.1|        VFLNEIILITTQWLALFCTIFASIGSTSTIK-RKPKITEVD------ 297
gi|183598233|ref|ZP_02959726.1      VFLQEIILTVTQWLALLCTVCASIGSTSTAR-PKTKIEEVS------ 295
gi|223990759|ref|ZP_03639931.1      VFLNEIILTMTQWLALLCTVCASIGSTSTAV-PKSKIEKVS------295
gi|212712149|ref|ZP_03320227.1      VFLQEIILTMTQWLALLCTVCASIGSTSTSV-PKSKIEKVS------ 295
gi|212708023|ref|ZP_03316151.1      VFLQEIILTMTQWLALLCTVCASIGSTSTAV-PKSKIKEVS------ 295
gi|197284529|ref|YP_002150401.      IFLIIEIILTLIQWVALAFIVLASIGSTATMK-RKTKIEKVE------ 296
gi|237807801|ref|YP_002892241.      IILGERLTLLEWGALGAVVVASAGATLTIK-TNK------------- 287
gi|238898963|ref|YP_002924545.      VFLNEYLTGVQWFGLAAIICASIGATLSIK-PKVELQELNS----- 307
gi|146307537|ref|YP_001188002.      LFLSEKLSWNQWLAIGAIILASAGAAATIR-PKS------------- 283
gi|108759302|ref|YP_628438.1|       LLLDERLSLVQWAAIGCIILASVGSSATSRKPVEAVAAS-------- 295
```

FIG. 14U

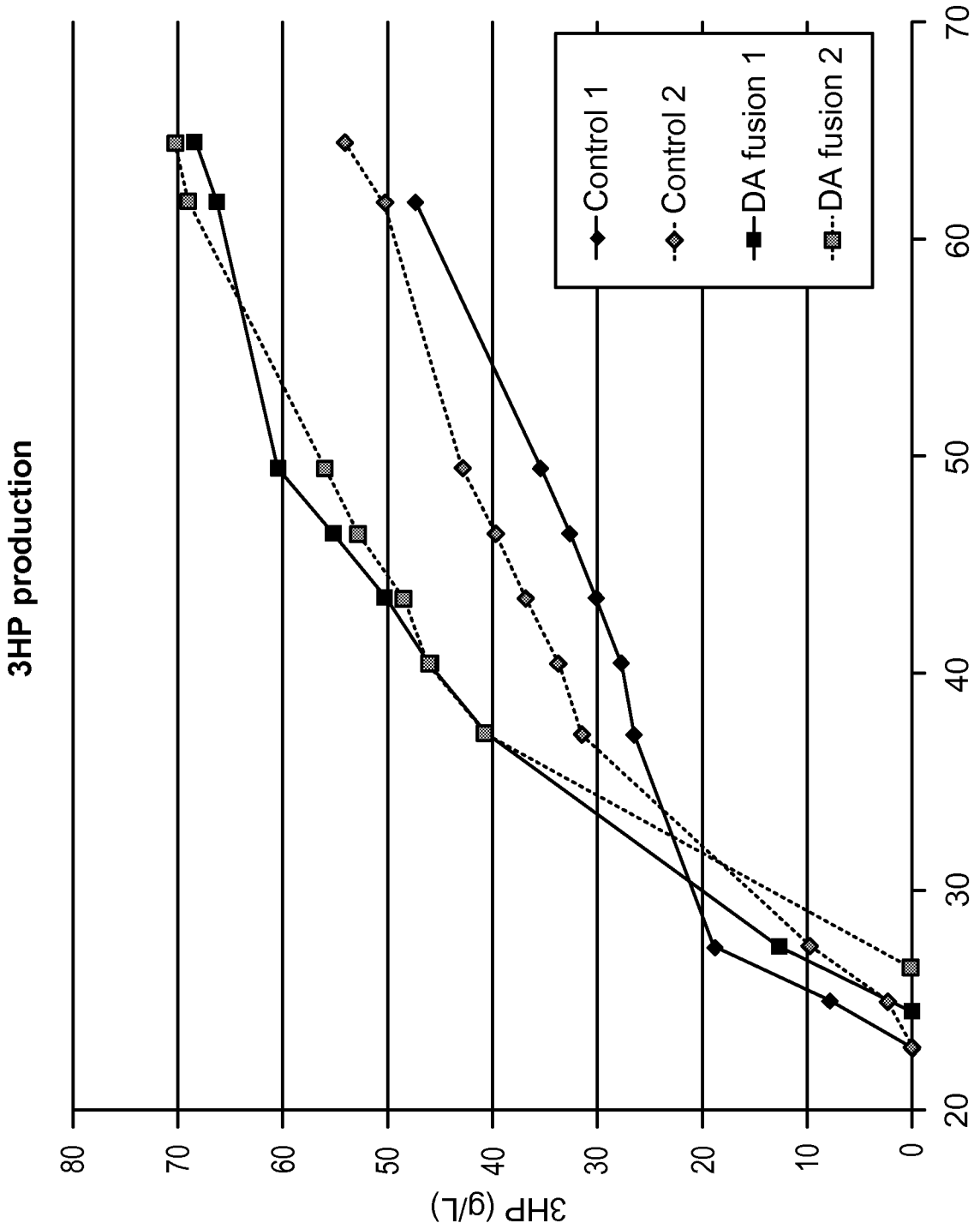

3HP production
3HP (g/L)
0
10
20
30
40
50
60
70
80
20
30
40
50
60
70
Timepoint
Control 1
Control 2
DA fusion 1
DA fusion 2

FIG. 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/030923

A. CLASSIFICATION OF SUBJECT MATTER

C12N 1/21(2006.01)i, C12N 15/52(2006.01)i, C12P 7/52(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 1/21; C12P 19/44; C12P 1/00; C12N 9/02; C12P 7/00; C12P 7/02; C12N 15/52; C12P 7/52

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: genetically modified organism, monofunctional malonyl-CoA, malonate semialdehyde, 3-hydroxypropionic acid, ydfG, mmsB, NDSD, rutE, nemA, not NADPH-dependent, NADH-dependent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012-129450 A1 (OPX BIOTECHNOLOGIES, INC.) 27 September 2012 See abstract; claims 1-45; and paragraphs [00263]-[00274], [00292]-[00325], [00533], [00837]-[00840].	1-3,9,11-13
A	KIM, K. S. et al. `The Rut pathway for pyrimidine degradation: novel chemistry and toxicity problems`, Journal of Bacteriology, 2010, Vol. 192, No. 16, pp. 4089-4102 See abstract; pages 4099-4100; and Table 1.	1-3,9,11-13
A	KR 10-2012-0108538 A (SAMSUNG ELECTRONICS CO., LTD.) 05 October 2012 See abstract; claims 1-20; and Figures 1-2, 3a-3b.	1-3,9,11-13
A	KR 10-2012-0136349 A (THE REGENTS OF THE UNIVERSITY OF COLORADO A BODY CORPORATE et al.) 18 December 2012 See abstract and claims 1-32.	1-3,9,11-13
A	US 8377666 B2 (HASELBECK, ROBERT et al.) 19 February 2013 See claims 1-11; and columns 91-92.	1-3,9,11-13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2014 (25.08.2014)	**26 August 2014 (26.08.2014)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701,
Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
HEO, Joo Hyung
Telephone No. +82-42-481-8150

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/030923

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-8,10,14-28
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/030923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012-129450 A1	27/09/2012	EP 2689020 A1	29/01/2014
KR 10-2012-0108538 A	05/10/2012	EP 2505656 A1	03/10/2012
		US 2012-0244588 A1	27/09/2012
KR 10-2012-0136349 A	18/12/2012	CA 2775390 A1	31/03/2011
		EP 2480673 A1	01/08/2012
		EP 2529023 A1	05/12/2012
		JP 2013-128494 A	04/07/2013
		JP 2013-505727 A	21/02/2013
		KR 10-2014-0015136 A	06/02/2014
		US 2013-0071893 A1	21/03/2013
		WO 2011-038364 A1	31/03/2011
		WO 2011-094457 A1	04/08/2011
US 8377666 B2	19/02/2013	AU 2010-306785 A1	26/04/2012
		EP 2488652 A1	22/08/2012
		EP 2488652 A4	09/04/2014
		JP 2013-507145 A	04/03/2013
		KR 10-2012-0083908 A	26/07/2012
		US 2011-0129899 A1	02/06/2011
		US 2011-229946 A1	22/09/2011
		US 2013-029381 A1	31/01/2013
		US 2013-189751 A1	25/07/2013
		US 8377667 B2	19/02/2013
		WO 2011-047101 A1	21/04/2011