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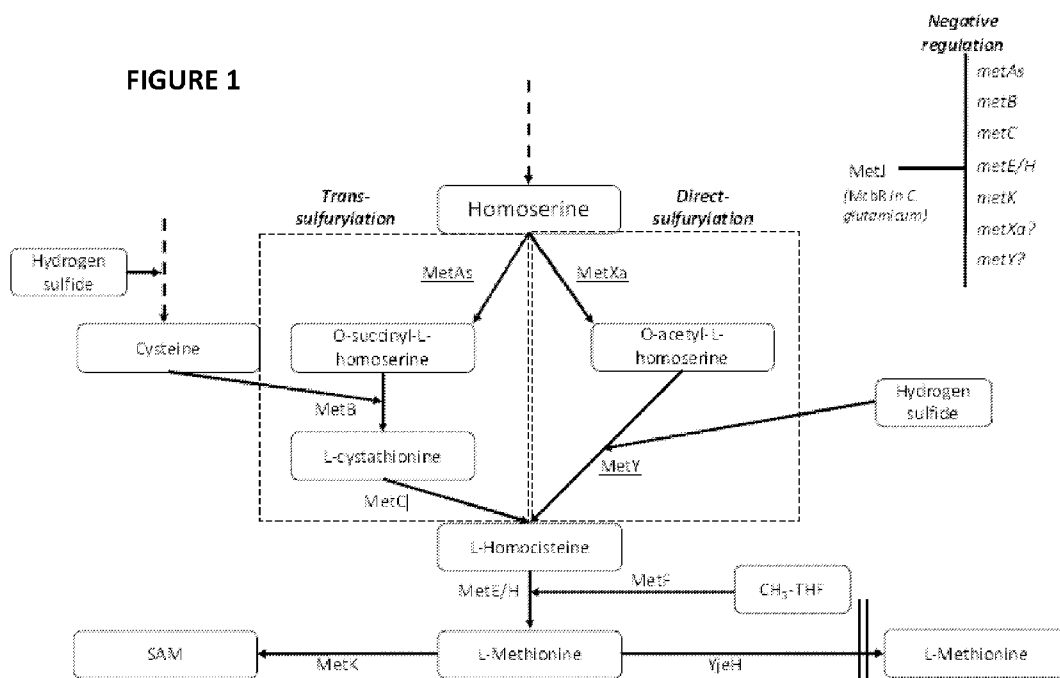
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(54) Title: BACTERIA AND A METHOD OF USING SAME FOR AMINO ACIDS BIOSYNTHESIS

FIGURE 1



(57) Abstract: The present invention provides is directed to a method of producing an amino acid, including: (a) providing a bacterial cell including an exogenous nucleic acid molecule or a plurality thereof encoding metX and metY; and culturing the bacterial cell from step (a) such that metX and metY encoded by an exogenous nucleic acid molecule are expressed, bacterial cell including: (i) an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (metX) and O-acetylhomoserine sulphydrylase (metY). Further provided are a composition comprising the bacterial cell, and a method for producing amino acids.

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BACTERIA AND A METHOD OF USING SAME FOR AMINO ACIDS
BIOSYNTHESIS

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[001] The contents of the electronic sequence listing (MIG-RMT-P-026-PCT ST26.xml; size: 79,562 bytes; and date of creation: November 23, 2023) is herein incorporated by reference in its entirety.

CROSS-REFERENCE TO RELATED-APPLICATIONS

[002] This application claims the benefit of priority of U.S. Provisional Patent Application No. 63/434,913, titled "BACTERIA AND A METHOD OF USING SAME FOR AMINO ACIDS BIOSYNTHESIS", filed on 22 December 2022, the contents of which are incorporated herein by reference in their entirety.

FIELD OF INVENTION

[003] The present invention is in the field of biosynthesis of amino acids in microorganisms.

BACKGROUND OF THE INVENTION

[004] Methionine is a sulfur-containing amino acid synthesized by plants, fungi and bacteria but not by vertebrates and thus considered an essential amino acid. Methionine plays a major role in mRNA translation initiation and is the precursor for the central metabolite S-adenosyl-methionine (SAM) that is part of the methionine biosynthesis regulation. Although methionine is a relatively nonabundant amino acid in proteins, it is a hydrophobic building block that stabilizes the overall structure of proteins. Besides being the first amino acid in protein synthesis, it is a key factor for the synthesis of a wide range of metabolites. Thus, L-Methionine is an important molecule for food and feed purposes as well as for the pharmaceutical industry. Despite the growing need for L-methionine, the main approach to obtain methionine is by chemical synthesis, resulting in a racemic mixture of the D, L forms of methionine.

[005] The metabolic cost of methionine biosynthesis in microorganisms that utilizes the trans-sulfurylation pathway is high and its synthesis and consumption are under strict regulation.

Therefore, production of L-methionine beyond physiological levels is challenging. There is an unmet need for developing an efficient bio-fermentation process for L-methionine synthesis.

SUMMARY OF THE INVENTION

[006] According to one aspect, there is provided a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (metX) and O-acetylhomoserine sulfhydrylase (metY), and any one of: (ii) inactive homoserine O-succinyltransferase (metA) and cystathionine gamma-synthase (metB) genes; (iii) an inactive metJ methionine repressor gene, or both (ii) and (iii).

[007] According to another aspect, there is provided a composition comprising the bacterial cell disclosed herein, and a biologically accepted carrier.

[008] According to another aspect, there is provided a method of producing an amino acid, the method comprising: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding metX and metY, and (ii) inactive metA and metB genes; and, (b) culturing the bacterial cell from step (a) such that metX and metY encoded by the exogenous nucleic acid molecule are expressed, thereby, producing the amino acid.

[009] In some embodiments, any one of the inactive genes is knocked out, knocked down, mutated, or chemically inhibited.

[010] In some embodiments, the bacterial cell further comprising one or more inhibitory nucleic acid sequences being complementary to any one of: metA, metB, and metJ genes.

[011] In some embodiments, the inactive gene being mutated comprises a nucleic acid sequence comprising a premature stop codon compared to a wildtype form of the gene.

[012] In some embodiments, the encoded metX comprises the amino acid sequence set forth in any one of SEQ ID Nos: 1-6, and 50.

[013] In some embodiments, the encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 7-12.

[014] In some embodiments, the exogenous nucleic acid molecule or a plurality thereof, comprises a nucleic acid sequence being codon optimized for expression in the bacterial cell.

[015] In some embodiments, the bacterial cell comprises an Escherichia coli cell.

[016] In some embodiments, the exogenous nucleic acid molecule encoding metX comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 13-18.

[017] In some embodiments, the exogenous nucleic acid molecule encoding metY comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 19-24.

[018] In some embodiments, the encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 7-12.

[019] In some embodiments, the encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 9 and 11.

[020] In some embodiments, the exogenous nucleic acid molecule or plurality thereof encoding metX and metY are operably linked.

[021] In some embodiments, the exogenous nucleic acid molecule or plurality thereof, is: (i) integrated into the genome of the bacterial cell; or is comprised within a plasmid or an expression vector.

[022] In some embodiments, the exogenous nucleic acid molecule or a plurality thereof comprises the nucleic acid sequence set forth in any one of SEQ ID Nos: 25-30.

[023] In some embodiments, the encoded metX, comprises the amino acid sequence set forth in any one of SEQ ID Nos: 3 and 5, and the encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 9 and 11.

[024] In some embodiments, the exogenous nucleic acid molecule encoding the metX comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 15 and 16, and the exogenous nucleic acid molecule encoding the metY comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 21 and 22.

[025] In some embodiments, the bacterial cell further comprises an inactive metJ.

[026] In some embodiments, the bacterial cell is further characterized by expression of the yjeH gene.

[027] In some embodiments, the bacterial cell comprises or further comprises an L-methionine and branched chain amino acid (BCAA) exporter protein.

[028] In some embodiments, the amino acid comprises an essential amino acid.

[029] In some embodiments, the essential amino acid is selected from the group consisting of: methionine, alanine, valine, leucine, threonine, isoleucine, glutamate, and any combination thereof.

[030] In some embodiments, culturing comprises supplementing the bacterial cell with a culture medium comprising an effective amount of a sulfur compound.

[031] In some embodiments, the produced levels of the L-methionine being produced by the bacterial cell are at least 5-fold greater than levels of L-methionine being produced by a control bacterial cell.

[032] In some embodiments, produced levels comprises intracellular levels, extracellular levels, or both.

[033] In some embodiments, the produced levels of the amino acid comprises intracellular levels, extracellular levels, or both, of the amino acid.

[034] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

[035] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[036] **Figure 1** includes a schematic illustration of direct- and trans-sulfurylation of methionine biosynthesis in bacteria. The first step involves the activation of homoserine through an acylation step. Two enzymes can process the activated homo-serine. The enzyme L-homoserine O-succinyl transferase (HST; EC 2.3.1.46) converts homoserine and succinyl-CoA to O-succinyl-L-homoserine (OSH). The enzyme L-homoserine O-acetyltransferases (HAT; EC 2.3.1.31) converts homoserine and acetyl-CoA to generate O-acetyl-L-homoserine (OAH). These enzymes are encoded by the MetA and MetX genes. In the trans-sulfurylation pathway the sulfur donor cysteine together with OSH/OAH are converted to cystathionine. This step is catalyzed by the cystathionine gamma synthase (CgS; EC 2.5.1.48) encoded by the MetB gene. Cystathionine is further converted to homocysteine by the enzyme cystathionine beta lyase (CbL; EC 4.4.1.13) encoded by MetC. In the direct sulfurylation path, OAH is converted to homocysteine within a single step. This reaction is catalyzed by the enzyme O-acetylhomoserine sulfhydrylase (OAHS; EC 2.5.1.49) encoded by MetY. Blue boxes - common metabolites; Green boxes - step in trans-sulfurylation pathway; Brown – Direct sulfurylation steps. MetJ and mcbR are master negative regulators in *E. coli* and *C. glutamicum*, respectively.

[037] **Figure 2** includes curve graphs demonstrating the growth rate of auxotroph *E. coli* Δ MetAB with variable methionine concentrations (indicated in the legend in μ M).

[038] **Figures 3A-3D** include images and graphs demonstrating the complementation of engineered *E. coli* with MetX/Y. **(3A)** Synthetic metYX operon on a low copy plasmid. A synthetic operon consisting of the metY and metX genes was constructed by adding a constitutive promoter, RBS and a synthetic terminator for each gene. Restriction sites were included to facilitate the rearrangement and analysis of mutant genes. **(3B)** Growth curves of the complemented Δ MetAB strains on methionine-depleted minimal media. **(3C)** Growth of the complemented Δ MetAB strains on a methionine-depleted minimal media agar plate. **(3D)** Intracellular (*left*) methionine accumulated by WT *E. coli*, Δ metAB-DG and Δ metAB-CM, reported as μ g/ml, and extracellular (*right*) methionine accumulated in the growth media by WT *E. coli*, Δ metAB-DG and Δ metAB-CM, reported as μ g/ml. The results are presented as means $\pm$ SD of three to four replicates for each sample. Significance between WT and the different bacterial strain was calculated according to the Student's t-test ($P < 0.05$) and is identified by an asterisk. The numbers on top of the bars indicate the fold increase relative to the WT in each panel.

[039] **Figures 4A-4C** include bar graphs showing production of methionine by Δ metABJ overexpressing YjeH and complemented by metY/X from CM or DG. Comparison of: **(4A)** intracellular; **(4B)** extracellular; and **(4C)** total methionine levels that were quantified by GC-MS. Peak areas were normalized to Norleucine internal control, and total methionine levels were calculated according to the standard calibration curves. The results are presented as means $\pm$ SD of three or four replicates for each sample. Significance between bacterial strains was calculated according to the Turkey-Kramer HSD test ($p < 0.05$) and is identified by different small letters. Significance between WT and the different bacterial strains was calculated according to the Student's t-test ($P < 0.05$) and is identified by an asterisk. The numbers on top of the bars in each panel indicate the fold-increase relative to the WT in each panel.

[040] **Figures 5A-5C** include a non-limiting scheme and curves showing growth of methionine-auxotroph *E. coli* in spent medium of each strain. **(5A)** Non-limiting experimental design used to evaluate methionine level in the medium following the growth of each strain. **(5B)** Growth curves in medium from DG strains. **(5C)** Growth curves in medium from CM strains. All curves show the growth of the auxotroph *E. coli* Δ metAB in fresh MOPS minimal medium supplemented with spent medium filtered following the growth of the indicated strains.

[041] **Figures 6A-6B** include growth curves of methionine-auxotroph *E. coli* in spent medium of modified strains. **(6A)** Growth curves of a methionine auxotroph *E. coli* strain (Δ metAB) supplemented with spent medium retrieved from cultivation of an *E. coli* strain deleted of metJ and

expressing metXmetY from a plasmid (metJ+DG), and of an *E. coli* strain deleted of metABJ and expressing metXmetY from a plasmid (metABJ+DG). **(6B)** Growth curves of a methionine auxotroph *E. coli* strain (DmetAB) supplemented with the spent medium following the cultivation of an *E. coli* strain deleted of metJ and expressing yjeH and metXmetY from a plasmid (J DG yjeH), of an *E. coli* strain deleted of metABJ and expressing yjeH and MetYX from a plasmid (ABJ DG yjeH), or supplemented with 0, 2, and 5 mg/ml free methionine (met0, met2 and met5, respectively). metJ – knockout of the metJ gene; metAB – knockout of metA and metB genes; metABJ - knockout of metA, metB and metJ genes; DG – metX and metY genes from *D. geothermalis* expressed from a plasmid; and yjeH – a gene encoding for methionine transporter expressed from a plasmid.

DETAILED DESCRIPTION OF THE INVENTION

[042] The present invention, in some embodiments, is partially based on the finding that inactivating metA and metB genes of *E. coli*, and replacing it with metX and metY genes, successfully restored L- methionine production ability, by converting its methionine biosynthesis pathway from trans- to direct-sulfurylation (**Figures 3B and 3C**). The invention is further based on the surprising finding, that insertion of metX and metY genes, from several bacteria strains, comprising *Cyclobacterium marinum* (CM) and *Deinococcus geothermalis* (DG), are more effective in converting *E. coli* to an effective L- methionine producer. In some embodiments it was discovered that additional inactivation of the metJ gene, overexpression of the yjeH gene encoding a L-methionine exporter, or both, further improved L-methionine production ability (**Figures 4 and 5**).

[043] According to a first aspect, there is provided a microorganism comprising an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (HAT) and O-acetylhomoserine sulphydrylase (OAHS). In some embodiments, the microorganism further comprises inactive genes encoding homoserine O-succinyltransferase (HST) and cystathionine gamma-synthase (CgS) genes. In some embodiments, HAT is encoded by the metX gene. In some embodiments, OAHS is encoded by the metY gene. In some embodiments, HST is encoded by the metA gene. In some embodiments, CgS is encoded by the metB gene. In some embodiments, the microorganism comprises a bacterial cell. In some embodiments, the bacterial cell is characterized by increased amino acid production, secretion, or both, compared to a wild type, genetic reference or background, control bacterial cell. In some embodiments, a wild type, genetic reference or background, control bacterial cell comprises: an active metA gene, an active metB gene, an active metJ gene, or any combination thereof. In some embodiments, a wild type, genetic reference or

background, control bacterial cell is devoid of metX gene, metY gene, or both. In some embodiments, a wild type, genetic reference or background, control bacterial cell comprises inactive metX gene, inactive metY gene, or both.

[044] According to another aspect, there is provided a bacterial cell comprising: an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (metX) and O-acetylhomoserine sulfhydrylase (metY).

[045] In some embodiments, the bacterial cell further comprises inactive homoserine O-succinyltransferase (metA) gene and/or inactive cystathionine gamma-synthase (metB) gene and/or inactive metJ methionine repressor gene. In some embodiments, the bacterial cell further comprises inactive homoserine O-succinyltransferase (metA) gene, inactive cystathionine gamma-synthase (metB) gene, and inactive metJ methionine repressor gene.

[046] For simplicity of the invention description, in several parts of the application, the terms: HAT and metX are interchangeably used. This also applies to OAHS and metY, HST and metA and to CgS and metB.

[047] In some embodiments, the bacterial cell disclosed herein comprises an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (HAT).

[048] Homoserine O-acetyltransferase (EC no. 2.3.1.31) is an enzyme that belongs to the family of transferases and catalyzes the chemical reaction $\text{acetyl-CoA} + \text{L-homoserine} \rightarrow \text{CoA} + \text{O-acetyl-L-homoserine}$. The systematic name of this enzyme class is acetyl-CoA:L-homoserine O-acetyltransferase. Other names include homoserine acetyltransferase, homoserine transacetylase, homoserine-O-transacetylase, and L-homoserine O-acetyltransferase. This enzyme participates in methionine metabolism and sulfur metabolism. In some embodiments, HAT enzyme participates in methionine metabolism and sulfur metabolism. In some embodiments, HAT enzyme is encoded by the metX gene.

[049] In some embodiments, the bacterial cell disclosed herein comprises an exogenous nucleic acid molecule or a plurality thereof, encoding O-acetylhomoserine sulfhydrylase (OAHS).

[050] O-acetylhomoserine sulfhydrylase (OAHS) or O-acetylhomoserine aminocarboxypropyltransferase (EC no. 2.5.1.49) is an enzyme that belongs to the family of transferases and catalyzes the chemical reaction $\text{O-acetyl-L-homoserine} + \text{methanethiol} \rightarrow \text{L-methionine} + \text{acetate}$. The systematic name of this enzyme class is O-acetyl-L-homoserine:methanethiol 3-amino-3-carboxypropyltransferase. Other names include O-acetyl-L-homoserine acetate-lyase (adding methanethiol), O-acetyl-L-homoserine sulfhydrylase, O-acetylhomoserine (thiol)-lyase, O-acetylhomoserine sulfhydrylase, and methionine synthase. In

some embodiments, OAHS enzyme participates in methionine metabolism and cysteine metabolism. In some embodiments, OAHS enzyme is encoded by the metY gene.

[051] In some embodiments, the bacterial cell disclosed herein comprises an inactive homoserine O-succinyltransferase (HST).

[052] Homoserine O-succinyltransferase (EC no. 2.3.1.46), is an enzyme that belongs to the family of transferases and catalyzes the chemical reaction $\text{succinyl-CoA} + \text{L-homoserine} \rightarrow \text{CoA} + \text{O-succinyl-L-homoserine}$. Other names include: succinyl-CoA:L-homoserine O-succinyltransferase, homoserine O-transsuccinylase, or homoserine succinyltransferase. In some embodiments, HST enzyme participates in methionine metabolism and sulfur metabolism. In some embodiments, HST is encoded by the metA gene. In some embodiments, HST catalyzes the activation of homoserine. In some embodiments, activation of homoserine by succinylation allows the trans-sulfurylation to occur. Several bacteria strains were reported to express HST, particularly proteobacteria. In some embodiments, the bacterial cell that expresses HST comprises at least one of: proteobacteria and actinobacteria. In some embodiments, the proteobacteria comprises gammaproteobacteria or zetaproteobacteria. In some embodiments, gammaproteobacteria comprises E.coli .

[053] In some embodiments, the bacterial cell disclosed herein comprises an inactive cystathionine gamma-synthase (CgS).

[054] Cystathionine gamma-synthase, (EC no. 2.5.1.48) is an enzyme that belongs to the family of transferases and catalyzes the formation of cystathionine from cysteine and an activated derivative of homoserine (e.g. $\text{O4-succinyl-L-homoserine} + \text{L-cysteine} \rightarrow \text{L-cystathionine} + \text{succinate}$).

[055] In some embodiments, the activated substrate of CgS is O4-succinyl-L-homoserine or O4-acetyl-L-homoserine. The systematic name of CgS class is O4-succinyl-L-homoserine:L-cysteine S-(3-amino-3-carboxypropyl)transferase. Other names include O-succinyl-L-homoserine succinate-lyase (adding cysteine), O-succinylhomoserine (thiol)-lyase, homoserine O-transsuccinylase, O-succinylhomoserine synthase, O-succinylhomoserine synthetase, cystathionine synthase, cystathionine synthetase, homoserine transsuccinylase, 4-O-succinyl-L-homoserine:L-cysteine, and S-(3-amino-3-carboxypropyl)transferase. In some embodiments, CgS enzyme participates in metabolic pathways such as: methionine metabolism, cysteine metabolism, selenoamino acid metabolism, and sulfur metabolism. In some embodiments, CgS enzyme is encoded by metB gene. In some embodiments, CgS is encoded by metB in E. coli. In some embodiments, CgS is encoded by metI in B. subtilis. In some embodiments, metB is naturally present in actinobacteria and/or proteobacteria, comprising: alphaproteobacteria, and gammaproteobacteria. In some embodiments, gammaproteobacteria comprises E. coli.

[056] In some embodiments, there is provided a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (metX) and O-acetylhomoserine sulfhydrylase (metY), and wherein the cell is devoid of homoserine O-succinyltransferase (metA) and/or cystathionine gamma-synthase (metB) genes.

[057] In some embodiments, there is provided a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (metX) and O-acetylhomoserine sulfhydrylase (metY), and wherein the cell comprises non-functional homoserine O-succinyltransferase (metA) and/or cystathionine gamma-synthase (metB) genes.

[058] In some embodiments, non-functional genes, e.g., metA and/or metB, as disclosed herein, comprise a premature stop codon ('nonsense mutation'), a frame shift, a missense mutation, or any combination thereof, rendering a protein product encoded from a gene non-functional. In some embodiments, non-functional comprises at least partially non-functional or completely non-functional.

[059] In some embodiments, the microorganism disclosed herein is a cell that naturally (i.e. its counterpart wild-type) utilizes the trans-sulfurylation pathway to produce L-methionine.

[060] As used herein, the term "trans-sulfurylation pathway" refers to a metabolic pathway involving the interconversion of cysteine and homocysteine through the intermediate cystathionine. In some embodiments, the trans-sulfurylation pathway is the forward trans-sulfurylation pathway. In some embodiments, the product of the forward trans-sulfurylation pathway comprises L-homocysteine.

[061] In some embodiments, the forward pathway is present in several bacteria, comprising *escherichia coli* (E. coli) and *bacillus subtilis* (B. subtilis).

[062] As used herein, the forward trans-sulfurylation pathway refers to the transfer of a thiol group from cysteine to homocysteine, by the γ -replacement of the acetyl or succinyl group of a homoserine with cysteine to form cystathionine. Cystathionine is cleaved by β -elimination of its homocysteine portion. In some embodiments, the transfer from cystathionine to homocysteine is catalyzed by cystathionine β -lyase (CbL). In some embodiments, the CbL is encoded by metC gene. In some embodiments, the trans-sulfurylation pathway comprises two steps: (i) production of cystathionine and succinate from O-succinylhomoserine and cysteine; and, (ii) cleavage of cystathionine to form homocysteine, pyruvate and ammonia. In some embodiments, step (i) is catalyzed by cystathionine γ -synthase (CgS). In some embodiments, CgS is encoded by metB. In some embodiments, step (ii) is catalyzed by cystathionine β -lyase (CbL). In some embodiments, CbL is encoded by metC. In some embodiments, the production of homocysteine through trans-sulfuration allows the conversion of this intermediate to methionine, through a methylation reaction

carried out by methionine synthase. In some embodiments, the trans-sulfurylation pathway comprises the catalytic activity of CgS and/or CbL. In some embodiments, the trans-sulfurylation pathway comprises the catalytic activity of the enzymes encoded by metB and/or metC.

[063] In some embodiments, the microorganism disclosed herein is a cell that naturally (i.e., its wild-type counterpart) comprises at least one of: HST (metA) and CgS (metB). In some embodiments, the microorganism disclosed herein is a cell that its wild-type counterpart comprises HST (metA) and CgS (metB). In some embodiments, the microorganism comprises a bacterial cell. In some embodiments, the bacterial cell disclosed herein is a bacterial cell that its counterpart wild-type comprises HST (metA) and CgS (metB). In some embodiments, the bacterial cell is selected from: actinobacteria and proteobacteria. In some embodiments, the proteobacteria is selected from: gammaproteobacterial, alphaproteobacterial and zetaproteobacteria. In some embodiments, gammaproteobacterial cell comprises *E. coli*. In some embodiments, the bacterial cell disclosed herein is selected from *E. coli* and *B. subtilis*.

[064] In some embodiments, there is provided a bacterial cell comprising an exogenous nucleic acid molecule or a plurality thereof comprising metX and metY genes. In some embodiments, the exogenous nucleic acid molecule or a plurality thereof comprises metX. In some embodiments, the exogenous nucleic acid molecule or a plurality thereof comprises metY. In some embodiments at least one of the genes: metA and metB, is inactivated. In some embodiments, metA and metB are inactivated.

[065] In some embodiments, the bacteria disclosed herein comprises an exogenous nucleic acid molecule or a plurality thereof, comprising metX, and at least one inactivated gene selected from: metA and metB. In some embodiments, the exogenous nucleic acid molecule or a plurality thereof, comprises metY, and at least one of the genes selected from: metA and metB, is inactivated. In some embodiments, the exogenous nucleic acid molecule or a plurality thereof comprises metX and metY, and at least one of the genes selected from: metA and metB is inactivated. In some embodiments, the exogenous nucleic acid molecule or a plurality thereof comprises metX and metY, and inactivated metA and metB genes.

[066] In some embodiments, the bacterial cell further comprises an inactive MetJ gene and/or a protein product thereof. In some embodiments, MetJ is a protein that functions as a repressor for methionine synthesis. In some embodiments, MetJ is a homodimer that interacts with DNA bases via a ribbon-helix-helix (RHH) motif. In some embodiments, MetJ regulates the methionine synthesis by binding to the DNA operator site of a “Met box”, halting transcription of genes involved in methionine synthesis. In some embodiments, MetJ is transcribed by metJ gene. In some embodiments, the bacterial cell disclosed herein further comprises an inactive metJ gene.

[067] In some embodiments, any one of the inactive genes is knocked out, knocked down, mutated, or chemically inhibited. As used herein, the terms “knocked out” and “knockout” are used interchangeably, and refer to a gene that is inactive. In some embodiments, the inactivation is by a genetic technique. In some embodiments, the genetic technique comprises a method in which the gene, or at least part of it (e.g. the operon or the protein coding sequence), is taken out from the organism’s genome.

[068] Methods for generation of knocked out genes are well known in the art. Non-limiting examples comprise: homologous recombination, site-specific nucleases, zinc finger nuclease, transcription activator-like effector nuclease (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9.

[069] In some embodiments, any one of the inactive genes is knocked down. As used herein, the term “knocked down”, or “knockdown”, refers to a condition in which the expression of the gene is reduced. In some embodiments, the transcription of the gene is reduced. In some embodiments, the translation of the gene is reduced. In some embodiments, the expression of the gene is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or by at least 99%. Each embodiment refers to a separate embodiment of the invention. In some embodiments, the reduction can occur either through a genetic modification or by treatment with a reagent such as a short DNA or RNA oligonucleotide that has a nucleic acid sequence complementary to either gene or a mRNA transcript.

[070] In some embodiments, the knocked down gene is achieved by a genetic modification or mutation, leading to gene silencing. In some embodiments, the knocked down gene is by a stable knockdown, referring to constituent reduced gene expression. In some embodiments, the gene reduced expression is caused by an oligonucleotide binding to mRNA or temporarily binding to a gene. In some embodiments, the knocked down gene is a transient knockdown referring to a temporary change in gene expression that does not modify the chromosomal DNA. In some embodiments, the transient knockdown, is achieved by at least one of: blocking or reducing transcription, degradation of the mRNA transcript, blocking or reducing mRNA translation, blocking or reducing the binding of pre-mRNA splicing sites, or nuclease cleavage sites used for maturation of other functional RNAs, including miRNA. In some embodiments, blocking or reducing transcription can be achieved by small interfering RNA (siRNA) or RNase-H dependent antisense. Methods for generation of knockdown genes are known in the art, comprising RNA interference, CRISPRs, and TALENs.

[071] In some embodiments, the bacterial cell comprises one or more inhibitory nucleic acid sequences being complementary to any one of: metA, metB, and metJ genes. In some embodiments,

the inhibitory nucleic acid molecule comprises at least one of: small interfering RNA (siRNA), microRNA (miRNA), or piwi-associated RNA (piRNA). In some embodiment the bacterial cell comprises a ribozyme (i.e., a catalytic RNA molecule that cleaves mRNA molecule).

[072] In some embodiments inactivation of a gene is by a mutation. In some embodiments the mutation comprises a nucleic acid sequence comprising a premature stop codon compared to a wildtype form of the gene. As used herein, the terms “premature stop codon” and “premature termination codon” (PTC) are used interchangeably. In some embodiments, PTC is derived from a nucleotide mutation that converts a canonical triplet nucleotide codon into one of three stop codons, (e.g., TAG, TGA, or TAA). In some embodiments, PTC results in the loss of protein expression.

[073] In some embodiments, the gene is inactivated by a chemical inhibition. In some embodiments, the chemical inhibition is by a small molecule (e.g., by a small organic molecule). In some embodiments, the small molecule inhibits gene transcription. In some embodiments, the small molecule inhibits mRNA translation. The term “small molecule” or micromolecule refers to a low molecular weight (≤ 1000 daltons) organic compound that can regulate a biological process.

[074] In some embodiments, inactivation of a gene, or reduced activation of a gene, is induced, and dependent upon addition of an inducer supplementary. In other embodiments, inactivation of a gene, or reduced activation of a gene is constituent.

[075] In some embodiments, the amino acid sequence of at least one homoserine O-acetyltransferase (HAT), encoded by metX, and O-acetylhomoserine sulfhydrylase (OAHS), encoded by metY, is selected from: leptospira interrogans (LI), corynebacterium glutamicum (CG), deinococcus geothermalis (DG), sulfurimonas autotrophica (SA), cyclobacterium marinum (CM) and thermus thermophilus (TT).

[076] In some embodiments, at least one of: HAT and OAHS, is from the phylum spirochaetota. In some embodiments, at least one of: HAT and OAHS, is from the class spirochaetia. In some embodiments, at least one of: HAT and OAHS, is from the order leptospirales. In some embodiments, at least one of: HAT and OAHS, is from the family leptospiraceae. In some embodiments, at least one of: HAT and OAHS, is from the genus leptospira. In some embodiments, at least one of: HAT and OAHS, is from the species leptospira interrogans (LI).

[077] In some embodiments, at least one of: HAT and OAHS, is from the phylum actinomycetota. In some embodiments, at least one of: HAT and OAHS, is from the class actinomycetia. In some embodiments, at least one of: HAT and OAHS, is from the order mycobacteriales. In some embodiments, at least one of: HAT and OAHS, is from the family corynebacteriaceae. In some embodiments, at least one of: HAT and OAHS, is from the genus Corynebacterium. In some

embodiments, at least one of: HAT and OAHS, is from the species *corynebacterium glutamicum* (CG).

[078] In some embodiments, at least one of: HAT and OAHS, is from the phylum *deinococcota*.

In some embodiments, at least one of: HAT and OAHS, is from the class *deinococci*. In some embodiments, at least one of: HAT and OAHS, is from the order *deinococcales*. In some embodiments, at least one of: HAT and OAHS, is from the family *deinococcaceae*. In some embodiments, at least one of: HAT and OAHS, is from the genus *deinococcus*. In some embodiments, at least one of: HAT and OAHS, is from the species *deinococcus geothermalis* (DG).

[079] In some embodiments, at least one of: HAT and OAHS, is from the phylum *campylobacterota*. In some embodiments, at least one of: HAT and OAHS, is from the class *campylobacteria*. In some embodiments, at least one of: HAT and OAHS, is from the order *campylobacterales*. In some embodiments, at least one of: HAT and OAHS, is from the family *helicobacteraceae*. In some embodiments, at least one of: HAT and OAHS, is from the genus *sulfurimonas*. In some embodiments, at least one of: HAT and OAHS, is from the species *sulfurimonas autotrophica* (SA).

[080] In some embodiments, at least one of: HAT and OAHS, is from the phylum *bacteroidota*. In some embodiments, at least one of: HAT and OAHS, is from the class *cytophagia*. In some embodiments, at least one of: HAT and OAHS, is from the order *cytophagales*. In some embodiments, at least one of: HAT and OAHS, is from the family *cyclobacteriaceae*. In some embodiments, at least one of: HAT and OAHS, is from the genus *cyclobacterium*. In some embodiments, at least one of: HAT and OAHS, is from the species *cyclobacterium marinum* (CM).

[081] In some embodiments, at least one of: HAT and OAHS, is from the phylum *deinococcota*. In some embodiments, at least one of: HAT and OAHS, is from the class *deinococci*. In some embodiments, at least one of: HAT and OAHS, is from the order *thermals*. In some embodiments, at least one of: HAT and OAHS, is from the family *thermaceae*. In some embodiments, at least one of: HAT and OAHS, is from the genus *thermus*. In some embodiments, at least one of: HAT and OAHS, is from the *thermus thermophilus* (TT).

[082] In some embodiments, the bacterial cell comprising an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (HAT), encoded by *metX*, comprising the amino acid sequence:
MNETGSIGIIETKYAEFKELILNNGSVLSPVVIAYETYGTLSSSKNNAILICHALSGDAHAA
GYHSGSDKKPGWWDDYIGPGKSFDTNQYFIICSNVIGGCKGSSGPLSIHPETSTPYGSRFP

FVSIQDMVKAQKLLVESLGIEKLFCVAGGSMGGMQALEWSIAYPNSLSNCIVMASTAEH
SAMQIAFNEVGRQAILSDPNWKNGLYDENS PRKGLALARMVGHITYLSDDKMREKFGR
NPPRGNILSTDFAVGSYLIYQGSEFVDRFDANSYIYVTKALDHYS LGKGKELTAALS NAT
CRFLVVSYS SDWLYPPAQSREIVKSLEAADKRVFYVELQSGEGHDSFLLKNPKQIEILKGF
LENPN (SEQ ID NO: 1).

[083] In some embodiments, homoserine O-acetyltransferase (HAT encoded by metX), comprises the amino acid sequence:

MPTLAPSGQLEIQAIGDVSTEAGAIKNAEIA YHRWGEYRVDKEGRSNVVLIEHALTGDS
NAADWWADLLGPGKAINTDIYCVICTNVIGGCNGSTGPGSMHPDGNFWGNRFPATSIRD
QVNAEKQFLDALGITTVA AVLGGSMGGARTLEWAAMYPEIVGAAAVLAVSARASAWQI
GIQSAQIKAIENDHHWHEGNYYESGCNPATGLGAARRIAHLTYRGELEIDERFGTKAQKN
ENPLGPYRKPDQRF AVESYLDYQADKLVRFDAGSYVLLTDALNRHDIGRDRGGLNKA
LESIKVPVLVAGVDTDILYLHQEHLSRNLGNLLAMAKIVSPVGHDAFLTESRQMDRIV
RNFFSLISPDENNPSTYIEFYI (SEQ ID NO: 2).

[084] In some embodiments, homoserine O-acetyltransferase (HAT), encoded by metX, comprises the amino acid sequence:

MTALISQPDLLPPPAPERCPPQQTARLFRETPLLLDCGQVVQDVRVAYHTYGTSPDHAIL
VLHALTGTS AVHEWWPDLFGEGKPLDPTRDYIVCANVLGGCAGSTGPAELPRVNGEDPP
LTLRDMARVGRALLEELGVRRVSVIGASMGMLAYAWLLECPDLVDRAVIIGAPARHSP
WAIGLNTAARNAIRAAPGGEGLKVARQIAMLSYRSPESFALTQSGWGTRRPGTPDITTYL
EHQGEKLSTRFCERSYLALTGAMDRFQPTDAELRSIRVPVLVVGISSDVLYPPAEVRTYA
GLLPRGQYLELQSPHGHDAFLIDPQGLPEAAAFLHGA (SEQ ID NO: 3).

[085] In some embodiments, homoserine O-acetyltransferase (HAT), encoded by metX, comprises the amino acid sequence:

MSLNLQTYTEHFTNPLYLESGRILEPYDITYETYGT MNEDKSNV VVVCHALTGSHHAAG
LYEDET KPGWWDGFIGSGKAIDTDKYFVICS NVIGSCFGSTGPM SLQHPYQEPYRYKFPV
VSIKDMVKAQRILFDRLDIHRVHAIVGGSMGGMQALQFAIHYPNFANKIIALATTHATQP
WAIAFNKVAQESILNDPDFKQGYYPDLLKEQGLSGMAVGRMAGHISFLSHESMREKFG
RDYKLT DGLYELFGKFQVESYLEYNGYNFTKWFDPLAYLYITKAINIYDL SRGFDSLAEA
LKRVTSALYLV SFKNDLLFKNFEMKEIADEL DKIGNKNHSYIDVKSDYGHDAFLVELNKF
ENHVKDALNG (SEQ ID NO: 4).

[086] In some embodiments, homoserine O-acetyltransferase (HAT), encoded by metX, comprises the amino acid sequence:

MNLQSPHLTIEMTQEIFYCQEALSLESGESFPEFQLSFTTQGQLNANKDNVIWVLHALTG

DANPHEWWWSGLIGEDKFFDPSKYFIVCANFLGSCYGSTQPLSNNPNNGKPYYYDFPNITT
 RDIASALDKLRIHLGLEKINTVIGGSLGGQVGLWAVSLGEKLENAIIVASNAKASPWIIG
 FNETQRMALIESDSTWGKTQPEAGKKGLETARAIGMLSYRHPMTFLQNQSETEEKRDDFK
 ISSYLRQGLKLANRFNAMS YWILSKAMDSDHIGRGRGGTPVALSNIKCKVLSIGVDTDI
 LFTSEESRYISKHVPKGTREISSIYGHDAFLIEYEQLQYILKSFYLENNG (SEQ ID NO: 5).

[087] In some embodiments, homoserine O-acetyltransferase (HAT), encoded by metX, comprises the amino acid sequence:

MSEIALEAWGEHEALLLKPPRSPLSIPPPKPRTA VLFPRREGFYTELGGYLPEVRLRFETYG
 TLSRRRDNAVLVFHALTGSAHLAGTYDEETFRSLSPLEQAFGREGWWDSL VGPGRILDP
 ALYYVVSANHLGSCYGSTGPLSLDPHTGRPYGRDFPPLTIRDLARAQARLLDHLGVEKAI
 VIGGSLGGMVALEFALMYPERVKKL VVLAAPARHGPWARAFNHLSRQAILQDPEYQKG
 NPAPKGMALARGIAMMSYRAPEGFEARWGAEPDELGETYLDYQGEKFLRRFHAESYLV
 SRAMDTHDVGRGRGGVEEALKRLRAIPSLFVGIDTDLLYPaweVRQA AKAAGARYREIK
 SPHGHDAFLIETDQVEEILDAFLP (SEQ ID NO: 6).

[088] In some embodiments, homoserine O-acetyltransferase (HAT), encoded by metX, comprises the amino acid sequence:

PTLAPSGQLEIQAIGDVSTEAGAIKNAEIA YHRWGEYRVDKEGRSNVVLIEHALTGDSNA
 ADWWADLLGPGKAINTDIYCVICTNVIGGCNGSTGPGSMHPDGNFWGNRFPATSIRDQV
 NAEKQFLDALGITTVA AVLGGSMGGARTLEWAAMYPEIVGAAAVLAVSARASAWQIGI
 QSAQIKAIENDHHWHEGNYYESGCNPATGLGAARRIAHLTYRGELEIDERFGTKAQKNE
 NPLGPYRKPDQRF AVESYLDYQADKL VQRFDAGSYVLLTDALNRHDIGRDRGGLNKAL
 ESIKVPVLVAGVDTDILYPYHQEHL SRNLGNLLAMAKIVSPVGHDAFLTESRQMDRIVR
 NFFSLSPDENNPSTYIEFYI (SEQ ID NO: 50).

[089] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof encoding an amino acid sequence selected from SEQ ID Nos: 1-6, and 50.

[090] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof encoding the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

[091] In some embodiments, HAT sequence from LI comprises SEQ ID NO: 1. In some embodiments, HAT sequence from CG comprises SEQ ID NO: 2. In some embodiments, HAT sequence from DG comprises SEQ ID NO: 3. In some embodiments, HAT sequence from SA comprises SEQ ID NO: 4. In some embodiments, HAT sequence from CM comprises SEQ ID NO: 5. In some embodiments, HAT sequence from TT comprises SEQ ID NO: 6.

[092] In some embodiments, the bacterial cell comprises a nucleic acid or a plurality thereof, encoding a functional analog of any one of SEQ IN NOs: 1-6, and 50. In some embodiments, the

bacterial cell comprises a nucleic acid molecule or a plurality thereof, encoding a functional analog of SEQ IN Nos: 3 or 5. The term “analog” as used herein, refers to a polypeptide that is similar, but not identical, to the polypeptide of HAT, and that is still capable of catalyzing the reaction of homoserine O-acetyltransferase. An analog may have deletions or mutations that result in an amino acids sequence that is different than the amino acid sequence of the polypeptide of the invention. It should be understood that all analogs of the polypeptide of the invention would still be capable of catalyzing the formation of acetyl-L-homoserine from L-homoserine. Further, an analog may be analogous to a fragment of the polypeptide of the invention, however, in such a case the fragment must comprise at least 100 consecutive amino acids of the polypeptide of the invention.

[093] In some embodiments, an analog to the polypeptide disclosed herein comprises an amino acid sequence with at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% homology to the amino acid sequence presented in SEQ ID NOs: 1-6, and 50. Each embodiment refers to a separate embodiment of the invention.

[094] In some embodiments, the bacterial cell comprising an exogenous nucleic acid molecule or a plurality thereof, encoding O-acetylhomoserine sulfhydrylase (OAHS encoded by metY), comprises the amino acid sequence:

MPRNYKPETIALHGGQSPDPSTLSRAVPIYQTTSYVFKNTEHAAKLFGLQEFNGIYTRIMN
PTTDVLEQRIAALEGGVAALATASGQAAETLALLNIVEAGQEIVASSSLYGGTYNLLHYT
FPKGILKVHFVDPSPDENFRKAVNDKTRAFYAETLGNPKLDTLNLEAIAKVAHDSEVPLII
DNTLPSPYLVNPIEHGADIVVHSLTKFLGGHGTSSIGGIIVDSGKFNWGNGKFKNFTEPDPS
YHGLKFWFVFGKFEPFGGVNIAIYIIKAKVQGLRDMGASISPFNAWQILQGVETLPLRMRK
HSENALAVAEYLTKHTKVSWNYPGLKMDKNYSKAKYHKKDLYGAILGFGIKGGAV
EAKKFIDGLELFSLLANVGDAKSLVIHPASTTHQQLTPEEQLSAGVTPDFVRLSVGLENIE
DILFDLEEALKKV (SEQ ID NO: 7).

[095] In some embodiments, O-acetylhomoserine sulfhydrylase (OAHS) encoded by metY, comprises the amino acid sequence:

MPKYDNSNADQWGFETRSHAGQSVDAQTSARNLPYQSTAFVFDSAETHAKQRFALEDL
GPVYSRLTNPTVEALENRIASLEGGVHAVAFFSSGQAATTNAILNLGAGDHIIVTSPRLYG
GTETLFLITLNLRLGIDVSFVENPDDPESWQAAVQPNTKAFFGETFANPQADVLDIPAVAE
VAHRNSVPLIIDNTIATAALVRPLELGADVVASLTKFYTGNGSGLGGVLIDGGKFDWTV
EKDGKSVFPYFVTPDAAYHGLKYADLGAPAFGLKVRVGLLRDTGSTLSAFNAWAAVQGG
IDTLRLRLERHNENAIKVAEFLNNHEKVEKVNFAGLKDSPWYATKEKLGLKYTGSVLTF
EIKGGKDEAWAFIDALKLHSNLANIGDVRSLVVHPATTTHSQSDEAGLARAGVTQSTVR
LSVGIIETIDDIADLEGGFAAI (SEQ ID NO: 8).

[096] In some embodiments, O-acetylhomoserine sulphydrylase (OAHS) encoded by metY, comprises the amino acid sequence:

MASNTLHFETLQVHAGQHDPATGAQAVPIYATNAYVFESPEHAADLFGRLAFGNIYSRI
MNPTNAVLEERIAALEGGVGALAVASGHAAQFLAITTVAQAGDNIVSTPNLYGGTVNQF
RVTLRLRGIEVRFTSKDERPEEFAALIDDRTRAVYLETLGNPALNVPDFEGIAEVAHARG
VAVFVDNTFGAGGYCQPLRHGADVVLHSASKWIGGHGNGIGGLLVDGGTFDWGNR
YPLLTEPSPSYHGLSFWEAFGEGNALGLPNIAFITRARTEGRLDLGPTLAPQQAWQFLQG
VETLSLRAERHAQNALALASWLSGHPDVSRTYPGLSNHPHYDRAQTYLPRGAGAVLTF
ELRGGRAAGEAFIGAVRLAQHVANVGDRTRLVIHPASTTHSQLDEAAQAAAGVTPGLVR
VSVGIEHIDDIREDFAQALATALVDAEGA (SEQ ID NO: 9).

[097] In some embodiments, O-acetylhomoserine sulphydrylase (OAHS) encoded by metY, comprises the amino acid sequence:

MDLQTLALHAGYEKDSQRTMAVPIYQTTAYEFRDVEHAANLFALKELGNIYTRLNNPTT
DVFEKRFTALEGGEEAIIATASGMSAIIFFALANAAQAGDNIICANQLYGGSLTLSTHTLKR
FGIEARFFDVHKPQQIEVLIDEKTKVIFFESLTNPSIDVADIEALTAIANKHNILTIVDNTVA
TPVLCRPFEGADITVHSASKYTTGQGLAIGGILVERKGLVDKLNPNRYSHFNEPDASY
HGLVYTDGTGLPPYTLRARLSLLRDLGAVVSPFNSWLFIQGIETLSLRMKEHKSNAFMLAE
FLESHKKVKKVNYPGLKSNANYKNAQKYFQDGLCSGLLSFEVEDFDEATKIVDATKLYS
LVVNIGDSKSIITHPASTTHQQLSHEELIACGVPEGLIRISCGLESVKDLIEDLKQALEA
(SEQ ID NO: 10).

[098] In some embodiments, O-acetylhomoserine sulphydrylase (OAHS) encoded by metY, comprises the amino acid sequence:

MSKNYRFETLQVHGGQEVDPPTNSRAVPIYQTSSYVFNSAEHGANLFALKEFGNIYTRIM
NPTSDVFEKRMAALEGGVAAVATASGQAAQFLALNNFLSVGDNFVTSPFLYGGSYNQF
KVSFKRIGIEARFAKSDKVDDLAAEINDKTKAIYVETIGNPEFNVPDFEAI AALAKKHDIPL
VVDNTFGAGGYLCQPIKHGANIVTSSATKWIGGHGTSIGGIIVDGGNYNWGNGKFPQFSE
PSEGYHGLNFWETFGDNNPLGLPNIAFAIRARVEGLRDFGPAISPFNSFLLLQGLETLRLV
QRTVDNALELAKWLEAHPKVKS VNYPGLTNSPYHATAKKYLTHGFGGVLSFEIEGDKET
ASNFINNLELISHLANVGDAKTIIQPSATTHQQLSDEAQIAAGVTPSLLRISSGIEHIEDLK
ADLTAAFDKI (SEQ ID NO: 11).

[099] In some embodiments, O-acetylhomoserine sulphydrylase (OAHS) encoded by metY, comprises the amino acid sequence:

MRFETLQLHAGYEPEPTTLRQVPIYPTTSYVFKSPEHAANLFALKEFGNIYSRIMNPTVD
VLEKRLAALEGGKAALATASGHAAQFLALTTLAQAGDNIVSTPNLYGGTFNQFKVTLKR

LGIEVRFTSREERPEEFLALTDERTRAWWVESIGNPALNIPDLEALAQAAREKGVALIVDN
TFGMGGYLLRPLAWGAALVTHSLTKWVGGHGAVIAGAIVDGGSPWEGGRYPLLTEPQ
PGYHGLRLTEAFGELAFIVKARVDGLRDQGGQALGPFEAWVVLLGMETLSLRAERHVENT
LHLAHWLLEQPQVAWVNYPGLPHHPHHDRAQKYFKGKPGAVLTFGLKGGYEAAKRFIS
RLKLISHLANVGDTRTLAIHPASTTHSQLSPEEQAQAGVSPPEMVRLSVGLEHVEDLKAEL
KEALA (SEQ ID NO: 12).

[0100] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding O-acetylhomoserine sulfhydrylase (OAHS) encoded by metY, comprising the amino acid sequence selected from SEQ ID NOs: 7-12.

[0101] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof encoding O-acetylhomoserine sulfhydrylase (OAHS) encoded by metY, comprising the amino acid sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 11.

[0102] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 1, and SEQ ID NO: 7. In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 2, and SEQ ID NO: 8. In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 3, and SEQ ID NO: 9. In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 4, and SEQ ID NO: 10. In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 5, and SEQ ID NO: 11. In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 6, and SEQ ID NO: 12.

[0103] In some embodiments, OAHS sequence from LI comprises SEQ ID NO: 7. In some embodiments, OAHS sequence from CG comprises SEQ ID NO: 8. In some embodiments, OAHS sequence from DG comprises SEQ ID NO: 9. In some embodiments, OAHS sequence from SA comprises SEQ ID NO: 10. In some embodiments, OAHS sequence from CM comprises SEQ ID NO: 11. In some embodiments, OAHS sequence from TT comprises SEQ ID NO: 12.

[0104] In some embodiments, the bacterial cell comprises a nucleic acid or a plurality thereof, encoding a functional analog of any one of SEQ IN NOs: 7-12. In some embodiments, the bacterial cell comprises a nucleic acid molecule or a plurality thereof, encoding a functional analog set forth in SEQ IN Nos: 9 or 11. The term “analog” as used herein, refers to a polypeptide that is similar, but not identical, to the polypeptide of OAHS, and that still is capable of catalyzing the reaction of O-acetylhomoserine sulfhydrylase. An analog may have deletions or mutations that result in an amino acids sequence that is different than the amino acid sequence of the polypeptide of the

invention. It should be understood that all analogs of the polypeptide of the invention would still be capable of catalyzing the formation of L-homocysteine. Further, an analog may be analogous to a fragment of the polypeptide of the invention, however, in such a case the fragment must comprise at least 100 consecutive amino acids of the polypeptide of the invention.

[0105] In some embodiments, an analog to the polypeptide sequence disclosed herein comprises an amino acid sequence with at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% homology to the amino acid sequence presented in SEQ ID NOs: 7-12. Each embodiment refers to a separate embodiment of the invention.

[0106] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, comprising a nucleic acid sequence being codon optimized for expression in the bacterial cell. As used herein, the term “codon optimized” describes a sequence that encodes identical amino acids to those encoded by a non-optimized codon sequence (synonymous codon), however, at least one of: translation rate of the codon optimized sequence, protein product amount, duration of protein structure stability, or any combination thereof, is increased, compared to the non-optimized codon. An ordinary skill in the art will know how to optimize a codon sequence for its expression in the desired cell, using a codon optimization gene engineering tool, comprising, but not limited to, algorithms that analyze codon optimization based on the codon frequencies in the desired cell/species. In some embodiments, increased one of: translation rate, protein product amount, and duration of structure stability, is by at least by 30%.

[0107] In some embodiments, the bacterial cell comprises a nucleic acid sequence being codon optimized for expression in a bacterial cell that naturally (or its wildtype counterpart) uses the forward trans- sulfurylation pathway for production of L-methionine. In some embodiments, the bacterial cell comprises a nucleic acid sequence being codon optimized for expression in *E. coli*.

[0108] In some embodiments, the bacterial comprises an exogenous nucleic acid molecule, of metX or metX optimized codon.

[0109] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGAACGAGACCGGTAGCATCGGCATCATTGAAACCAAGTACGCGGAGTTCAAAGA
 ACTGATCCTGAACAACGGTAGCGTGCTGAGCCCGGTGGTTATTGCGTACGAGACCTA
 TGGCACCCTGAGCAGCAGCAAGAACAACGCGATCCTGATTTGCCATGCGCTGAGCGG
 TGATGCGCATGCGGCGGGTTATCACAGCGGCAGCGATAAGAAACCGGGTTGGTGGG
 ACGATTACATTGGTCCGGGCAAGAGCTTCGACACCAACCAGTATTTTATCATTTGCAG
 CAACGTTATCGGTGGCTGCAAAGGTAGCAGCGGCCCGCTGAGCATTCATCCGGAGAC
 CAGCACCCCGTATGGTAGCCGTTTCCCGTTTGTGAGCATCCAGGATATGGTTAAGGC

GCAAAAGCTGCTGGTTGAGAGCCTGGGTATTGAAAACTGTTCTGCGTTGCGGGTGG
CAGCATGGGTGGCATGCAAGCGCTGGAATGGAGCATCGCGTATCCGAACAGCCTGA
GCAACTGCATTGTTATGGCGAGCACCGCGGAGCACAGCGCGATGCAGATCGCGTTTA
ACGAAGTTGGTCGTCAAGCGATTCTGAGCGACCCGAACTGGAAGAACGGCCTGTACG
ATGAGAACAGCCCGCGTAAAGGTCTGGCGCTGGCGCGTATGGTGGGTACATCACCT
ATCTGAGCGACGATAAGATGCGTGAAAAATTCGGTCGTAACCCGCCGCGTGGCAACA
TCCTGAGCACCGACTTTGCGGTTGGTAGCTACCTGATTTATCAGGGCGAGAGCTTCGT
GGACCGTTTTGATGCGAACAGCTACATCTATGTTACCAAGGCGCTGGACCACTACAG
CCTGGGCAAGGGCAAAGAACTGACCGCGGCGCTGAGCAACGCGACCTGCCGTTTCCT
GGTGGTTAGCTACAGCAGCGATTGGCTGTATCCGCCGGCGCAAAGCCGTGAGATTGT
GAAGAGCCTGGAAGCGGGCGACAAACGTGTGTTCTACGTTGAGCTGCAAAGCGGTG
AAGGCCACGATAGCTTTCTGCTGAAGAACCCGAAACAAATCGAGATTCTGAAAGGTT
TTCTGGAAAACCCGAACTAA (SEC ID NO: 13).

[0110] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGAGCCTGAACCTGCAAACCTATACCGAGCACTTCACCAACCCGCTGTACCTGGAG
AGCGGTCGTATCCTGGAACCGTACGATATTACCTACGAGACCTATGGCACCATGAAC
GAAGACAAGAGCAACGTGGTTGTGGTTTGCCATGCGCTGACCGGTAGCCACCATGCG
GCGGGTCTGTATGAGGATGAAACCAAAACCGGGTTGGTGGGACGGCTTCATCGGTAGC
GGCAAGGCGATTGACACCGATAAATACTTCGTGATTTGCAGCAACGTTATTGGTAGC
TGCTTTGGTAGCACCGGCCCGATGAGCCTGCAACACCCGTATCAAGAACCGTACCGT
TATAAGTTCCCGGTGGTTAGCATCAAGGATATGGTGAAAGCGCAACGTATTCTGTTT
GACCGTCTGGATATCCACCGTGTGCACGCGATTGTTGGTGGCAGCATGGGTGGCATG
CAGGCGCTGCAATTCGCGATCCACTACCCGAACTTTGCGAACAAGATCATTGCGCTG
GCGACCACCCATGCGACCCAGCCGTGGGCGATCGCGTTCAACAAAGTGGCGCAAGA
GAGCATTCTGAACGACCCGGATTTTAAGCAGGGTTACTATGACCCGGATCTGCTGAA
AGAACAAGGTCTGAGCGGTATGGCGGTTGGTCGTATGGCGGGTCACATCAGCTTCCT
GAGCCACGAGAGCATGCGTGAAAAGTTTGGTCGTGATTATAAACTGACCGACGGTCT
GTACGAGCTGTTTCGGCAAGTTTCAGGTGGAGAGCTACCTGGAATATAACGGCTACAA
CTTCACCAAGTGGTTTGATCCGCTGGCGTACCTGTACATACCAAGGCGATCAACATC
TACGATCTGAGCCGTGGTTTTCGACAGCCTGGCGGAGGCGCTGAAGCGTGTGACCAGC
GCGCTGTACCTGGTTAGCTTTAAAAACGACCTGCTGTTCAAGAACTTTGAGATGAAA
GAAATCGCGGACGAACTGGATAAGATTGGTAACAAAAACCACAGCTATATCGACGTT

AAAAGCGATTACGGCCACGACGCGTTCCTGGTGGAGCTGAACAAGTTTGAAAACCAC
GTTAAAGACGCGCTGAACGGCTAA (SEQ ID NO: 14).

[0111] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGCGTATCCAGCGTTACATTCTGATGACCGCGCTGATCAGCCAACCGGACCTGCTG
CCGCCGCCGGCGCCGGAGCGTTGCCCCGCCGAGCAAACCGCGCGTCTGTTCCGTGAA
ACCCCGCTGCTGCTGGACTGCGGTCAGGTGGTTCAAGATGTGCGTGTTGCGTACCAC
ACCTATGGCACCCCGAGCGACCATGCGATCCTGGTGCTGCATGCGCTGACCGGCACC
AGCGCGGTTTCATGAGTGGTGGCCGATTTTCTGGGTGAAGGCAAGCCGCTGGACCCG
ACCCGTGATTATATTGTTTGCGCGAACGTTCTGGGTGGCTGCGCGGGTAGCACCGGTC
CGGCGGAGCTGCCGCGTGTGAACGGTGAAGACCCGCCGCTGACCCTGCGTGATATGG
CGCGTGTGGGTTCGTGCGCTGCTGGAGGAACTGGGCGTTCGTCGTGTGAGCGTTATTG
GTGCGAGCATGGGTGGCATGCTGGCGTATGCGTGGCTGCTGGAGTGCCCGGACCTGG
TGGATCGTGCGGTTATCATTGGTGCGCCGGCGCGTACAGCCCGTGGGCGATTGGTC
TGAACACCGCGGCGCGTAACGCGATTCGTGCGGCGCCGGGTGGCGAGGGTCTGAAG
GTTGCGCGTCAGATCGCGATGCTGAGCTATCGTAGCCCGGAGAGCTTCGCGCTGACC
CAGAGCGGTTGGGGCACCCGTCGTCCGGGCACCCCGGACATTACCACCTACCTGGAG
CACCAGGGTGAAAACTGAGCACCCGTTTCTGCGAGCGTAGCTATCTGGCGCTGACC
GGCGCGATGGACCGTTTTCAACCGACCGATGCGGAACTGCGTAGCATCCGTGTGCCG
GTTCTGGTGGTTGGTATTAGCAGCGATGTGCTGTACCCGCCGGCGGAAGTGCGTACC
TATGCGGGTCTGCTGCCGCGTGGCCAGTACCTGGAAGTCAAAGCCCGCACGGCCAT
GATGCGTTCCTGATCGATCCGCAGGGTCTGCCGGAAGCGGCGGCGGCGTTCTGCAC
GGTGCGTAA (SEQ ID NO: 15).

[0112] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGAACCTGCAAAGCCCGCACCTGACCATCGAGATGACCCAGGAAATTTCTACTGC
CAAGAGGCGCTGAGCCTGGAGAGCGGCGAGAGCTTCCCGGAATTTCAACTGAGCTTT
ACCACCCAGGGCCAACTGAACGCGAACAAGGACAACGTGATCTGGGTTCTGCACGC
GCTGACCGGTGATGCGAACCCGCACGAGTGGTGGAGCGGTCTGATCGGCGAAGACA
AGTTCTTTGATCCGAGCAAATATTTTCATTGTGTGCGCGAACTTTCTGGGTAGCTGCTA
CGGCAGCACCCAGCCGCTGAGCAACAACCCGAACAACGGTAAACCGTACTATTACG
ACTTCCCGAACATCACCACCCGTGACATTGCGAGCGCGCTGGATAAGCTGCGTATCC
ACCTGGGCCTGGAGAAAATCAACACCGTGATTGGTGGCAGCCTGGGTGGCCAAGTGG
GTCTGGAATGGGCGGTTAGCCTGGGCGAGAAGCTGGAAAACGCGATCATTGTTGCGA

GCAACGCGAAAGCGAGCCCGTGGATCATTGGTTTTAACGAGACCCAGCGTATGGCGA
TCGAAAGCGATAGCACCTGGGGCAAGACCCAACCGGAGGCGGGTAAGAAAGGCCTG
GAAACCGCGCGTGCGATTGGTATGCTGAGCTATCGTCACCCGATGACCTTCCTGCAA
AACCAAAGCGAGACCGAGGAAAAGCGTGACGATTTTAAAATCAGCAGCTATCTGCG
TTACCAGGGCCTGAAGCTGGCGAACCGTTTCAACGCGATGAGCTACTGGATTCTGAG
CAAAGCGATGGACAGCCACGATATTGGTCGTGGTCGTGGTGGCACCCCGGTGGCGCT
GAGCAACATCAAGTGCAAAGTGCTGAGCATCGGTGTTGACACCGATATTCTGTTTAC
CAGCGAGGAAAGCCGTTATATTAGCAAGCACGTTCCGAAAGGCACCTATCGTGAGAT
CAGCAGCATTTACGGCCACGACGCGTTCCTGATCGAGTATGAACAGCTGCAATACAT
TCTGAAGAGCTTCTACCTGGAAAACAACGGCTAA (SEQ ID NO: 16).

[0113] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGCCGACCCTGGCGCCGAGCGGTCAGCTGGAGATCCAAGCGATTGGTGACGTTAGC
ACCGAGGCGGGCGCGATCATTAAAGAACGCGGAAATTGCGTACCACCGTTGGGGTGA
GTATCGTGTGGACAAAGAAGGCCGTAGCAACGTGGTTCTGATCGAACACGCGCTGAC
CGGTGATAGCAACGCGGCGGACTGGTGGGCGGATCTGCTGGGTCCGGGCAAGGCGA
TCAACACCGACATTTACTGCGTTATCTGCACCAACGTGATCGGTGGCTGCAACGGCA
GCACCGGTCCGGGCAGCATGCACCCGGATGGTAACTTCTGGGGCAACCGTTTTCCGG
CGACCAGCATTCGTGACCAGGTAAACGCGGAGAAACAATTCCTGGATGCGCTGGGT
TTACCACCGTTGCGGCGGTGCTGGGTGGCAGCATGGGTGGCGCGCGTACCCTGGAGT
GGGCGGCGATGTATCCGGAATCGTTGGTGCGGCGGCGGTGCTGGCGGTTAGCGCGC
GTGCGAGCGCGTGGCAGATCGGCATTCAGAGCGCGCAAATCAAGGCGATTGAAAAC
GATCACCCTGGCACGAGGGTAACTACTATGAAAGCGGCTGCAACCCGGCGACCGGT
CTGGGTGCGGCGCGTTCGTATTGCGCACCTGACCTACCGTGGTGAGCTGGAAATCGAC
GAGCGTTTTTGGCACCAAGGCGCAGAAAAACGAAAACCCGCTGGGTCCGTATCGTAA
GCCGGATCAACGTTTCGCGGTTGAGAGCTACCTGGACTATCAGGCGGATAAACTGGT
TCAACGTTTTTGACGCGGGTAGCTACGTGCTGCTGACCGATGCGCTGAACCGTCACGA
CATTGGCCGTGATCGTGGTGGCCTGAACAAGGCGCTGGAGAGCATTAAAGTGCCGGT
TCTGGTGGCGGGCGTTGACACCGATATCCTGTACCTGTATCACCAGCAAGAACACCT
GAGCCGTAACCTGGGTAACCTGCTGGCGATGGCGAAAATCGTTAGCCCGGTGGGTCA
TGATGCGTTCCTGACCGAAAGCCGTCAAATGGATCGTATTGTGCGTAACTTCTTTAGC
CTGATCAGCCCGGACGAGAACAAACCCGAGCACCTACATTGAATTTTATATCTAA (SEQ
ID NO: 17).

[0114] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGCGTATCCAGCGTTACATTCTGATGAGCGAGATCGCGCTGGAAGCGTGGGGTGAG
CATGAAGCGCTGCTGCTGAAGCCGCCGCGTAGCCCGCTGAGCATCCCGCCGCCGAAG
CCGCGTACCGCGGTGCTGTTCCCGCGTCGTGAGGGTTTTTACACCGAGCTGGGTGGTT
ACCTGCCGGAAGTGCGTCTGCGTTTTCGAAACCTACGGCACCCCTGAGCCGTCGTCTGTG
ATAACGCGGTGCTGGTTTTTTCATGCGCTGACCGGTAGCGCGCACCTGGCGGGCACCT
ATGATGAGGAAACCTTCCGTAGCCTGAGCCCGCTGGAGCAGGCGTTTGGTCGTGAAG
GTTGGTGGGATAGCCTGGTGGGTCCGGGTCGTATCCTGGACCCGGCGCTGTACTATG
TGGTTAGCGCGAACCACCTGGGTAGCTGCTATGGTAGCACCGGTCCGCTGAGCCTGG
ACCCGCACACCGGTCGTCCGTATGGTCGTGATTTCCCGCCGCTGACCATTCTGTGATCT
GGCGCGTGCGCAAGCGCGTCTGCTGGACCACCTGGGTGTGGAGAAGGCGATCGTTAT
TGGTGGCAGCCTGGGTGGCATGGTGGCGCTGGAGTTCGCGCTGATGTACCCGGAACG
TGTTAAGAAACTGGTGGTTCTGGCGGCGCCGGCGCGTCACGGCCCGTGGGCGCGTGC
GTTTAACCACCTGAGCCGTCAGGCGATCCTGCAAGATCCGGAATACCAGAAGGGTAA
CCCGGCGCCGAAGGGTATGGCGCTGGCGCGTGGTATTGCGATGATGAGCTATCGTGC
GCCGGAAGGTTTTGAAGCGCGTTGGGGTGCGGAGCCGGAAGTGGGCGAGACCTACCT
GGACTATCAAGGTGAAAAGTTCCTGCGTCGTTTTACGCGGAGAGCTATCTGGTGCT
GAGCCGTGCGATGGACACCCATGATGTGGGTCTGTTGGTCTGGTGGCGTTGAGGAAGC
GCTGAAACGTCTGCGTGCGATCCCGAGCCTGTTTGTGGGCATTGACACCGATCTGCTG
TACCCGGCGTGGGAAGTTCGTCAGGCGGCGAAGGCGGCGGGTGCGCGTTATCGTGAG
ATCAAAAGCCCGCACGGCCATGATGCGTTCCTGATCGAAACCGATCAAGTTGAGGAA
ATTCTGGACGCGTTTCTGCCGTAA (SEQ ID NO: 18).

[0115] In some embodiments, metX comprises metX of LI, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 13.

[0116] In some embodiments, metX comprises metX of SA, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 14.

[0117] In some embodiments, metX comprises metX of DG, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 15.

[0118] In some embodiments, metX comprises metX of CM, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 16.

[0119] In some embodiments, metX comprises metX of GC, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 17.

[0120] In some embodiments, metX comprises metX of TT, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 18.

[0121] In some embodiments, a nucleic acid sequence as set forth in SEQ ID Nos: 13-18 comprises a codon optimized nucleic acid sequence, such as, but not limited to expression of metX as disclosed herein.

[0122] In some embodiments, codon optimized is for optimized expression, e.g., transcription, translation, or both, of a protein product of metX, in a cell.

[0123] In some embodiments, a bacterial cell comprises an exogenous nucleic acid molecule encoding metX. In some embodiments, a bacterial cell comprises an exogenous nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID Nos: 15 or 16.

[0124] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, of metY or metY optimized codon.

[0125] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGGGTCCGCGTAACTATAAACCGGAGACCATTGCGCTGCACGGTGGCCAGAGCCCG
GACCCGAGCACCCCTGAGCCGTGCGGTGCCGATTTACCAAACCACCAGCTATGTTTTTC
AAGAACACCGAGCACGCGGCGAAACTGTTTGGTCTGCAAGAGTTCGGCAACATCTAC
ACCCGTATTATGAACCCGACCACCGATGTTCTGGAGCAACGTATCGCGGCGCTGGAA
GGTGGCGTGGCGGCGCTGGCGACCGCGAGCGGTCAGGCGGCGGAAACCCTGGCGCT
GCTGAACATCGTGGAGGCGGGCCAAGAAATTGTTGCGAGCAGCAGCCTGTACGGTG
GCACCTATAACCTGCTGCACTATACCTTTCCGAAGCTGGGTATCAAAGTGCACTTCGT
TGACCCGAGCGATCCGGAGAACTTTCGTAAGGCGGTTAACGACAAAACCCGTGCGTT
TTACGCGGAAACCCTGGGCAACCCGAAGCTGGATACCCTGAACCTGGAGGCGATTGC
GAAAGTGGCGCACGACAGCGAAGTTCCGCTGATCATTGATAACACCCTGCCGAGCCC
GTACCTGGTTAACCCGATCGAGCACGGTGCGGACATTGTGGTTCACAGCCTGACCAA
GTTCTGGGTGGTCACGGCACCAGCATCGGTGGCATCATTGTGGACAGCGGCAAATT
TAACTGGGGTAACGGCAAGTTCAAAAACCTTTACCGAACCGGACCCGAGCTATCACGG
TCTGAAGTTCTGGGAAGTGTTTCGGCAAATTTGAACCGTTCGGTGGCGTTAACATCGC
GTACATCATTAAAGGCGAAAGTGCAGGGTCTGCGTGATATGGGCGCGAGCATCAGCCC
GTTTAACGCGTGGCAGATTCTGCAAGGTGTTGAGACCCTGCCGCTGCGTATGCGTAA
ACACAGCGAGAACGCGCTGGCGGTGGCGGAATATCTGACCAAGCACACCAAAGTGA
GCTGGGTAACTACCCGGGTCTGAAGATGGACAAAACTACAGCCTGGCGAAGAAA
TATCACAAGAAAGATCTGTACGGCGCGATCCTGGGTTTCGGCATTAAGGGTGGCGCG
GTGGAGGCGAAGAAATTTATCGACGGTCTGGAAGTTCAGCCTGCTGGCGAACGTG

GGCGATGCGAAAAGCCTGGTTATTCACCCGGCGAGCACCACCCACCAGCAACTGACC
CCGGAGGAACAACCTGAGCGCGGGTGTACCCCGGACTTCGTGCGTCTGAGCGTTGGC
CTGGAGAACATCGAAGACATTCTGTTTGATCTGGAGGAAGCGCTGAAGAAAGTGTA
(SEQ ID NO: 19).

[0126] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGGACCTGCAAACCCTGGCGCTGCATGCGGGTTACGAAAAGGATAGCCAGCGTACG
ATGGCGGTGCCGATCTACCAAACCACCGCGTATGAGTTCCGTGACGTTGAACACGCG
GCGAACCTGTTTGCGCTGAAGGAACTGGGCAACATTTATACCCGTCTGAACAACCCG
ACCACCGATGTGTTTCGAGAAACGTTTTACCGCGCTGGAGGGTGGCGAAGCGGCGATT
GCGACCGCGAGCGGTATGAGCGCGATTTTCTTTGCGCTGGCGAACGCGGCGCAGGCG
GGTGACAACATCATTTGCGCGAACCAACTGTACGGTGGCAGCCTGACCCTGAGCACC
CACACCCTGAAGCGTTTCGGTATCGAAGCGCGTTTCTTTGACGTTACAAACCGCAGC
AAATCGAGGTGCTGATCGATGAAAAGACCAAGGTTATCTTCTTTGAAAGCCTGACCA
ACCCGAGCATCGACGTGGCGGATATTGAGGCGCTGACCGCGATTGCGAACAACAC
AACATCCTGACCATTGTGGACAACACCGTTGCGACCCCGGTGCTGTGCCGTCCGTTTG
AGTTTGGTGCGGATATCACCGTTCACAGCGCGAGCAAGTATACCACCGGTCAGGGCC
TGGCGATCGGTGGCATTCTGGTTGAACGTAAAGGCCTGGTGGACAAGCTGAAAAACA
ACCCGCGTTACAGCCACTTCAACGAGCCGGACGCGAGCTACCACGGTCTGGTTTATA
CCGATACCGGTCTGCCGCCGTATACCCTGCGTGCGCGTCTGAGCCTGCTGCGTGATCT
GGGTGCGGTGGTTAGCCCGTTCAACAGCTGGCTGTTTATCCAAGGCATTGAAACCCT
GAGCCTGCGTATGAAGGAGCACAGCAAAAACGCGCTGATGCTGGCGGAGTTCCTGG
AAAGCCACAAGAAAGTGAAGAAAGTTAACTACCCGGGTCTGAAGAGCAACGCGAAC
TACAAGAACGCGCAGAAATATTTTCAAGATGGTCTGTGCAGCGGCCTGCTGAGCTTC
GAGGTGGAAGACTTTGATGAAGCGACCAAGATCGTTGACGCGACCAAACTGTATAGC
CTGGTGGTTAACATTGGTGATAGCAAAAGCATCATTACCCATCCGGCGAGCACCACC
CACCAGCAACTGAGCCACGAGGAACTGATTGCGTGCGGTGTGCCGGAGGGTCTGATC
CGTATTAGCTGCGGCCTGGAGAGCGTTAAGGACCTGATTGAAGATCTGAAACAAGCG
CTGGAGGCGTAA (SEQ ID NO: 20).

[0127] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGGCGAGCAACACCCTGCACTTCGAGACCCTGCAAGTGACGCGGGTCAACATCCG
GACCCGGCGACCGGCGCGCAAGCGGTGCCGATTACGCGACCAACGCGTATGTTTTT
GAAAGCCCGGAACATGCGGCGGACCTGTTTCGGTCTGCGTGCGTTTGGCAACATTAC

AGCCGTATCATGAACCCGACCAACGCGGTTCTGGAGGAACGTATTGCGGCGCTGGAA
GGTGGCGTGGGTGCGCTGGCGGTTGCGAGCGGTCATGCGGCGCAGTTCCTGGCGATT
ACCACCGTGGCGCAAGCGGGTGATAACATCGTTAGCACCCCGAACCTGTACGGTGGC
ACCGTGAACCAATTTCTGTGTACCCTGCGTCGTCTGGGCATTGAAGTGCGTTTCACCA
GCAAGGACGAACGTCCGGAGGAATTTGCGGCGCTGATCGACGATCGTACCCGTGCGG
TTTATCTGGAGACCCTGGGTAAACCCGGCGCTGAACGTGCCGGACTTTGAGGGTATTG
CGGAAGTTGCGCATGCGCGTGGCGTGGCGGTTTTTGTGGATAACACCTTTGGTGCGG
GTGGCTACTATTGCCAACCGCTGCGTCACGGCGCGGATGTGGTTCTGCACAGCGCGA
GCAAATGGATTGGTGGCCACGGTAACGGCATCGGTGGTCTGCTGGTTGACGGTGGCA
CCTTTGATTGGGGTAACGGCCGTTACCCGCTGCTGACCGAGCCGAGCCCGAGCTATC
ACGGTCTGAGCTTCTGGGAGGCGTTTGGTGAGGGTAACGCGCTGGGTCTGCCGAACA
TTGCGTTCATTACCCGTGCGCGTACCGAAGGTCTGCGTGATCTGGGTCCGACCCTGGC
GCCGCAGCAAGCGTGGCAGTTTCTGCAAGGTGTGGAGACCCTGAGCCTGCGTGCGGA
ACGTCATGCGCAGAACGCGCTGGCGCTGGCGAGCTGGCTGAGCGGTCACCCGGATGT
GAGCCGTGTTACCTATCCGGGCCTGAGCAACCACCCGCACTACGATCGTGCGCAAAC
CTATCTGCCGCGTGGTGCGGGTGCGGTTCTGACCTTTGAGCTGCGTGGTGGCCGTGCG
GCGGGTGAAGCGTTTATTGGTGCGGTGCGTCTGGCGCAGCATGTGGCGAACGTTGGT
GACACCCGTACCCTGGTTATTCATCCGGCGAGCACCAACCCACAGCCAGCTGGATGAA
GCGGCGCAAGCGGCGGCGGGTGTGACCCCGGGCCTGGTTCGTGTGAGCGTTGGTATC
GAGCACATTGACGATATCCGTGAAGATTTTGGCGAGGCGCTGGCGACCGCGCTGGTT
GATGCGGAGGGTGCGTAA (SEQ ID NO: 21).

[0128] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGGGTAGCAAGAACTACCGTTTCGAGACCCTGCAAGTGACGGTGGCCAAGAAGTT
GACCCGACCACCAACAGCCGTGCGGTGCCGATCTACCAGACCAGCAGCTATGTTTTT
AACAGCGCGGAGCATGGTGCGAACCTGTTTCGCGCTGAAGGAATTTGGCAACATCTAT
ACCCGTATTATGAACCCGACCAGCGACGTTTTTCGAGAAACGTATGGCGGCGCTGGAA
GGTGGCGTGGCGGCGGTTGCGACCGCGAGCGGTCAGGCGGCGCAATTCCTGGCGCTG
AACAACTTTCTGAGCGTGGGCGATAACTTCGTTACCAGCCCGTTTCTGTACGGTGGCA
GCTATAACCAATTCAAAGTGAGCTTTAAACGTATCGGTATTGAGGCGCGTTTTTGCGA
AGAGCGACAAAGTTGACGATCTGGCGGCGGAAATCAACGATAAGACCAAAGCGATC
TACGTGGAGACCATTGGCAACCCGGAGTTCAACGTTCCGGACTTTGAAGCGATCGCG
GCGCTGGCGAAGAAACACGACATTCGCTGGTTGTGGATAACACCTTCGGTGCGGGT
GGCTATCTGTGCCAGCCGATCAAGCACGGCGCGAACATTGTGACCAGCAGCGCGACC

AAATGGATCGGTGGTCACGGCACCAGCATTGGTGGCATCATTGTTGATGGTGGCAAC
TACAACTGGGGTAACGGCAAGTTCCCGCAATTTAGCGAGCCGAGCGAAGGTTATCAC
GGCCTGAACTTCTGGGAGACCTTTGGTGACAACAACCCGCTGGGTCTGCCGAACATT
GCGTTCGCGATTCTGTGCGCGTGTGGAAGGTCTGCGTGATTTTGGCCCGGCGATCAGC
CCGTTCAACAGCTTTCTGCTGCTGCAAGGTCTGGAGACCCTGAGCCTGCGTGTGCAAC
GTACCGTTGACAACGCGCTGGAGCTGGCGAAATGGCTGGAAGCGCACCCGAAGGTG
AAAAGCGTTAACTATCCGGGTCTGACCAACAGCCCGTACCACGCGACCGCGAAGAA
ATATCTGACCCACGGTTTCGGTGGCGTGCTGAGCTTTGAGATTGAAGGCGATAAGGA
GACCGCGAGCAACTTTATCAACAACCTGGAAGTATTAGCCACCTGGCGAACGTTGG
TGACGCGAAAACCCTGATCATTAGCCGAGCGCGACCAACCCACCAGCAACTGAGCGA
TGAAGCGCAAATTGCGGCGGGTGTGACCCCGAGCCTGCTGCGTATTAGCAGCGGCAT
CGAGCACATTGAAGACCTGAAGGCGGATCTGACCGCGGCGTTTCGATAAAATCTAA
(SEQ ID NO: 22).

[0129] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGGGTCCGAAGTACGACAACAGCAACGCGGATCAGTGGGGCTTCGAGACCCGTAG
CATCCACGCGGGTCAGAGCGTTGACGCGCAAACCAGCGCGCGTAACCTGCCGATTTA
CCAGAGCACCGCGTTTCGTGTTTGACAGCGCGGAGCACGCGAAACAACGTTTCGCGCT
GGAAGATCTGGGCCCCGGTTTATAGCCGTCTGACCAACCCGACCGTGGAGGCGCTGGA
AAACCGTATTGCGAGCCTGGAGGGTGGCGTTCATGCGGTGGCGTTTAGCAGCGGTCA
GGCGGCGACCAACGCGATCCTGAACCTGGCGGGTGCGGGTGACCACATTGTTAC
CAGCCCGCGTCTGTATGGTGGCACCGAAACCCTGTTCTGATCACCTGAACCGTCTG
GGCATTGATGTTAGCTTTGTGGAGAACCCGGATGATCCGGAAGCTGGCAGGCGGCG
GTTCAACCGAACACCAAGGCGTTCTTTGGCGAGACCTTTGCGAACCCGCAAGCGGAC
GTGCTGGATATCCCGGCGGTTGCGGAAGTGGCGCACCGTAACAGCGTTCCGCTGATC
ATTGACAACACCATTTGCGACCGCGGCGCTGGTTCGTCCGCTGGAGCTGGGTGCGGAT
GTGGTTGTGGCGAGCCTGACCAAGTTCTACACCGGTAACGGCAGCGGTCTGGGTGGC
GTTCTGATCGACGGTGGCAAATTTGATTGGACCGTGGAAAAGGACGGCAAAAGCGTT
TTCCCGTATTTTGTTACCCCGGATGCGGCGTACCACGGTCTGAAGTATGCGGATCTGG
GTGCGCCGGCGTTTGGTCTGAAAGTTCGTGTGGGCCTGCTGCGTGACACCGGTAGCA
CCCTGAGCGCGTTTAAACGCGTGGGCGGCGGTTCAAGGCATCGATACCCTGAGCCTGC
GTCTGGAGCGTCACAACGAAAACGCGATTAAGGTGGCGGAGTTCCTGAACAACCAC
GAGAAGGTTGAAAAAGTGAACCTTTGCGGGTCTGAAGGATAGCCCGTGGTACGCGAC
CAAGGAAAAACTGGGCCTGAAATATACCGGTAGCGTGCTGACCTTCGAGATCAAGG

GTGGCAAAGACGAAGCGTGGGCGTTTATTGATGCGCTGAAACTGCACAGCAACCTGG
CGAACATCGGCGACGTTTCGTAGCCTGGTTGTGCATCCGGCGACCACCACCCATAGCC
AAAGCGATGAGGCGGGCCTGGCGCGTGCGGGTGTGACCCAAAGCACCGTTCGTCTGA
GCGTGGGTATCGAGACCATTGACGATATCATTGCGGACCTGGAAGGTGGCTTCGCGG
CGATTTAA (SEQ ID NO: 23).

[0130] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule, comprising the nucleotide sequence:

ATGGGTCGTTTCGAGACCCTGCAACTGCATGCGGGTATGAGCCGGAACCGACCACC
CTGAGCCGTCAAGTTCCGATCTACCCGACCACCAGCTATGTGTTTAAAAGCCCGGAG
CATGCGGCGAACCTGTTTCGCGCTGAAGGAATTTGGTAACATCTATAGCCGTATTATG
AACCCGACCGTGGACGTTCTGGAGAAACGTCTGGCGGCGCTGGAAGGTGGCAAAGC
GGCGCTGGCGACCGCGAGCGGTCATGCGGCGCAGTTTCTGGCGCTGACCACCCTGGC
GCAAGCGGGCGATAACATCGTTAGCACCCCGAACCTGTACGGTGGCACCTTCAACCA
GTTTAAGGTTACCCTGAAACGTCTGGGCATTGAAGTGCGTTTCACCAGCCGTGAGGA
ACGTCCGGAGGAATTTCTGGCGCTGACCGATGAGCGTACCCGTGCGTGGTGGGTGGA
AAGCATCGGTAACCCGGCGCTGAACATTCCGGACCTGGAGGGCGCTGGCGCAAGCGG
CGCGTGAAAAAGGTGTGGCGCTGATCGTTGATAACACCTTCGGCATGGGTGGCTATC
TGCTGCGTCCGCTGGCGTGGGGCGCGGCGCTGGTTACCCACAGCCTGACCAAGTGGG
TGGGTGGCCACGGTGCGGTTATTGCGGGTGCGATTGTGGATGGTGGCAGCTTTCGT
GGGAGGGTGGCCGTTACCCGCTGCTGACCGAACCGCAGCCGGGTTATCACGGCCTGC
GTCTGACCGAGGCGTTCGGTGAACTGGCGTTTATTGTGAAAGCGCGTGTTGACGGCC
TGCGTGATCAAGGTCAAGCGCTGGGTCCGTTTGAGGCGTGGGTGGTTCTGCTGGGTA
TGGAACCCCTGAGCCTGCGTGCGGAGCGTCATGTGGAAAACACCTGCATCTGGCGC
ACTGGCTGCTGGAGCAGCCGCAAGTGGCGTGGGTAACTATCCGGGTCTGCCGCACC
ACCCGCACCACGACCGTGCGCAGAAGTATTTCAAGGGTAAACCGGGCGCGGTTCTGA
CCTTTGGCCTGAAGGGTGGCTACGAAGCGGCGAAACGTTTTATCAGCCGTCTGAAGC
TGATTAGCCACCTGGCGAACGTGGGTGACACCCGTACCCTGGCGATTACCCGGCGA
GCACCACCCACAGCCAACTGAGCCCGGAGGAACAGGCGCAAGCGGGTGTTAGCCCG
GAGATGGTGCGTCTGAGCGTTGGCCTGGAGCACGTGGAAGATCTGAAGGCGGAGCT
GAAAGAAGCGCTGGCGTAA (SEQ ID NO: 24).

[0131] In some embodiments, metY comprises metY of LI, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 19.

[0132] In some embodiments, metY comprises metY of SA, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 20.

[0133] In some embodiments, metY comprises metY of DG, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 21.

[0134] In some embodiments, metY comprises metY of CM, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 22.

[0135] In some embodiments, metY comprises metY of GC, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 23.

[0136] In some embodiments, metY comprises metY of TT, as disclosed herein, and comprises a nucleic acid sequence as set forth in SEQ ID NO: 24.

[0137] In some embodiments, a nucleic acid sequence as set forth in SEQ ID Nos: 19-24 comprises a codon optimized nucleic acid sequence, such as, but not limited to expression of metY as disclosed herein.

[0138] In some embodiments, a bacterial cell comprises an exogenous nucleic acid molecule encoding metY. In some embodiments, a bacterial cell comprises an exogenous nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID Nos: 21 or 22.

[0139] In some embodiments, the nucleic acid or plurality thereof disclosed herein comprises a synonymous mutation (e.g., not affecting the polypeptide sequence) for any one of SEQ ID NOs: 13-24. In some embodiments, the nucleic acid or plurality thereof comprises a nonsynonymous mutation for any one of SEQ ID NOs: 13-24, however in this case, the nucleic acid comprising the nonsynonymous mutation would still be translated to a functional analog, as disclosed herein.

[0140] In some embodiments, the nucleic acid molecule encoding HAT (metX) and the nucleic acid molecule encoding OHS (metY) are operably linked. The term “operably linked” is intended to mean that the nucleotide sequence encoding HAT (metX) and the nucleotide sequence encoding OHS (metY) are present in the same nucleotide molecule and are both linked to a regulatory element or elements in a manner that allows for expression of their nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell).

[0141] In some embodiments, metX encoding or encoded sequence or metY encoding or encoded sequence being derived from the same microorganism. In some embodiments, the operably linked nucleic acid molecule disclosed herein comprises at least one of: (a) SEQ ID NO: 19 and SEQ ID NO: 13, (b) SEQ ID NO: 20 and SEQ ID NO: 14, (c) SEQ ID NO: 21 and SEQ ID NO: 15, (d) SEQ ID NO: 22 and SEQ ID NO: 16, (e) SEQ ID NO: 23 and SEQ ID NO: 17; and (f) SEQ ID NO: 24, and SEQ ID NO: 18.

[0142] In some embodiments, a bacterial cell comprises a nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 19, SEQ ID NO: 13, or both. In some embodiments, the bacterial cell comprises a nucleic acid molecule or a plurality thereof, encoding SEQ ID NO: 20 and SEQ

ID NO: 14. In some embodiments, the bacterial cell comprises a nucleic acid or plurality thereof, encoding SEQ ID NO: 21 and SEQ ID NO: 15. In some embodiments, the bacterial cell comprises a nucleic acid or plurality thereof, encoding SEQ ID NO: 22 and SEQ ID NO: 16. In some embodiments, the bacterial cell comprises a nucleic acid or plurality thereof, encoding SEQ ID NO: 23 and SEQ ID NO: 17. In some embodiments, the bacterial cell comprises a nucleic acid or plurality thereof, encoding SEQ ID NO: 24 and SEQ ID NO: 18.

[0143] In some embodiments, the nucleic acid molecule comprises or is operably linked to a promoter. In some embodiments, the promoter comprises a single promoter. In some embodiments, the promoter comprises or consists of 35-50 nucleotides. In some embodiments, the promoter comprises the nucleotide sequence: TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGA (SEQ ID NO: 25).

[0144] In some embodiments, the nucleotide sequence of the promoter is positioned or located upstream to the nucleotide sequence of metY. In some embodiments, the nucleotide sequence of metY is positioned or located between the nucleotide sequence of the promoter and the nucleotide sequence of metX. In some embodiments, any one of SEQ ID NOs: 19-24, is positioned or located between SEQ ID NO: 25, and any one of SEQ ID NOs: 13-18.

[0145] In some embodiments, nucleic acid molecule further comprises a terminator sequence.

[0146] As used herein, the term "terminator sequence" refers to a sequence in that signals, dictates, promotes, induces, required, or any combination thereof, for termination of transcription. In some embodiments, the terminator sequence comprises 25-35 nucleotides. In some embodiments, the terminator sequence comprises the nucleotide sequence: CAACCTGGAGGCGGGCGCAGGCCCGCCTTTT (SEQ ID NO: 26) or a functional analog thereof having at 80-100% sequence identity or homology thereto.

[0147] In some embodiments, the terminator sequence is located or position downstream to the nucleotide sequence of metX. In some embodiments, the nucleotide sequence of metX is positioned or located between the nucleotide sequence of metY and the nucleotide sequence of the terminator. In some embodiments, any one of SEQ ID NOs: 13-18, is positioned or located between any one of SEQ ID NOs: 19-24, and SEQ ID NO: 26.

[0148] In some embodiments, the nucleic acid molecule further comprises a ribosome binding site (RBS).

[0149] As used herein, the term "ribosome binding site" or "RBS" refers to a nucleotide sequence located or positioned upstream of a start codon of or in a messenger RNA (mRNA) transcript that is responsible for the recruitment of a ribosome during the initiation of translation. In some embodiments, the nucleic acid molecule further comprises a RBS sequence located or positioned

upstream to at least one of the sequences comprising metX and metY. In some embodiments, the nucleic acid molecule comprises RBS sequence located or positioned between the promotor and sequence comprising metY. In some embodiments, the nucleic acid molecule comprises RBS sequence located or positioned between the sequence comprising metY and the sequence comprising metX. In some embodiments, the nucleic acid molecule comprises RBS sequence located or positioned between the promotor and sequence comprising metY and RBS sequence between the sequence comprising metY and the sequence comprising metX. In some embodiments the RBS sequence consists of or comprises 10-15 nucleotides. In some embodiments the RBS sequence comprises the sequence: AGAGGTATATATTA (SEQ ID NO: 27).

[0150] In some embodiments, the nucleic acid molecule comprises SEQ ID NO: 27, located or positioned between SEQ ID NO: 25 and any one of SEQ ID NOs: 19-24. In some embodiments, the nucleic acid molecule comprises SEQ ID NO: 27, located or positioned between any one of SEQ ID NOs: 19-24, and any one of SEQ ID NOs: 13-18. In some embodiments, the nucleic acid molecule comprises SEQ ID NO: 27, located or positioned between SEQ ID NO: 25 and any one of SEQ ID NOs: 19-24, and further comprises SEQ ID NO: 27, located or positioned between any one of SEQ ID NOs: 19-24, and any one of SEQ ID NOs: 13-18.

[0151] In some embodiments, the nucleic acid comprises 25-35 nucleotides between the promotor sequence and the sequence encoding metY. In some embodiments, the nucleic acid comprises 15-40 nucleotides between the promotor sequence and the sequence encoding metY. In some embodiments, the sequence located or positioned between SEQ ID NO: 25, and any one of SEQ ID NOs: 19-24, comprises or consists of the sequence: GTTAACTTTAAGAGAGGTATATATTACC (SEQ ID NO: 34).

[0152] In some embodiments, the nucleic acid molecule comprises 15-25 nucleotides located or positioned between the sequence encoding metY and the sequence encoding metX. In some embodiments, the nucleic acid comprises 15-35 nucleotides located or positioned between the sequence encoding metY and the sequence encoding metX. In some embodiments, the sequence located or positioned between any one of SEQ ID NOs: 19-24, and any one of SEQ ID NOs: 13-18, comprises or consists of the sequence: GGATCCAGAGGTATATATTA (SEQ ID NO: 35).

[0153] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, comprising the sequence:
 TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTTAACTTTAAGAGA
 GGTATATATTACCATGGGTCCGCGTAACTATAAACCGGAGACCATTGCGCTGCACGG
 TGGCCAGAGCCCGGACCCGAGCACCCCTGAGCCGTGCGGTGCCGATTTACCAAACCAC
 CAGCTATGTTTTCAAGAACACCGAGCACGCGGCGAACTGTTTGGTCTGCAAGAGTT

CGGCAACATCTACACCCGTATTATGAACCCGACCACCGATGTTCTGGAGCAACGTAT
CGCGGCGCTGGAAGGTGGCGTGGCGGCGCTGGCGACCGCGAGCGGTCAGGCGGCGG
AAACCCTGGCGCTGCTGAACATCGTGGAGGCGGGCCAAGAAATTGTTGCGAGCAGC
AGCCTGTACGGTGGCACCTATAACCTGCTGCACTATACCTTTCCGAAGCTGGGTATCA
AAGTGCACCTTCGTTGACCCGAGCGATCCGGAGAACTTTTCGTAAGGCGGTTAACGACA
AAACCCGTGCGTTTTTACGCGGAAACCCTGGGCAACCCGAAGCTGGATACCCTGAACC
TGGAGGCGATTGCGAAAGTGGCGCACGACAGCGAAGTTCCGCTGATCATTGATAACA
CCCTGCCGAGCCCGTACCTGGTTAACCCGATCGAGCACGGTGC GGACATTGTGGTTC
ACAGCCTGACCAAGTTCCTGGGTGGTCACGGCACCAGCATCGGTGGCATCATTGTGG
ACAGCGGCAAATTTAACTGGGGTAACGGCAAGTTCAAAAACCTTTACCGAACCGGACC
CGAGCTATCACGGTCTGAAGTTCTGGGAAGTGTTTCGGCAAATTTGAACCGTTCGGTG
GCGTTAACATCGCGTACATCATTAAGGCGAAAGTGCAGGGTCTGCGTGATATGGGCG
CGAGCATCAGCCCGTTTAAACGCGTGGCAGATTCTGCAAGGTGTTGAGACCCTGCCGC
TGCGTATGCGTAAACACAGCGAGAACGCGCTGGCGGTGGCGGAATATCTGACCAAG
CACACCAAAGTGAGCTGGGTAACTACCCGGGTCTGAAGATGGACAAAAACTACAG
CCTGGCGAAGAAATATCACAAGAAAGATCTGTACGGCGCGATCCTGGGTTTTCGGCAT
TAAGGGTGGCGCGGTGGAGGCGAAGAAATTTATCGACGGTCTGGAACGTGTTACGCCT
GCTGGCGAACGTGGGCGATGCGAAAAGCCTGGTTATTCACCCGGCGAGCACCAACCA
CCAGCAACTGACCCCGGAGGAACAACCTGAGCGCGGGTGTACCCCGGACTTCGTGCG
TCTGAGCGTTGGCCTGGAGAACATCGAAGACATTCTGTTTGATCTGGAGGAAGCGCT
GAAGAAAGTGTAAGGATCCAGAGGTATATATTAATGAACGAGACCGGTAGCATCGG
CATCATTGAAACCAAGTACGCGGAGTTCAAAGAACTGATCCTGAACAACGGTAGCGT
GCTGAGCCCGGTGGTTATTGCGTACGAGACCTATGGCACCCCTGAGCAGCAGCAAGAA
CAACGCGATCCTGATTTGCCATGCGCTGAGCGGTGATGCGCATGCGGCGGGTTATCA
CAGCGGCAGCGATAAGAAACCGGGTGGTGGGACGATTACATTGGTCCGGGCAAGA
GCTTCGACACCAACCAGTATTTTATCATTTGCAGCAACGTTATCGGTGGCTGCAAAGG
TAGCAGCGGCCCGCTGAGCATTCATCCGGAGACCAGCACCCCGTATGGTAGCCGTTT
CCCGTTTGTGAGCATCCAGGATATGGTTAAGGCGCAAAAGCTGCTGGTTGAGAGCCT
GGGTATTGAAAAACTGTTCTGCGTTGCGGGTGGCAGCATGGGTGGCATGCAAGCGCT
GGAATGGAGCATCGCGTATCCGAACAGCCTGAGCAACTGCATTGTTATGGCGAGCAC
CGCGGAGCACAGCGCGATGCAGATCGCGTTTAAACGAAGTTGGTCGTCAAGCGATTCT
GAGCGACCCGAACCTGGAAGAACGGCCTGTACGATGAGAACAGCCCGCGTAAAGGTC
TGGCGCTGGCGCGTATGGTGGGTACATCACCTATCTGAGCGACGATAAGATGCGTG
AAAAATTCGGTCGTAACCCGCCGCGTGGCAACATCCTGAGCACCGACTTTGCGGTTG

GTAGCTACCTGATTTATCAGGGCGAGAGCTTCGTGGACCGTTTTGATGCGAACAGCT
ACATCTATGTTACCAAGGCGCTGGACCACTACAGCCTGGGCAAGGGCAAAGAACTGA
CCGCGGCGCTGAGCAACGCGACCTGCCGTTTCCTGGTGGTTAGCTACAGCAGCGATT
GGCTGTATCCGCCGGCGCAAAGCCGTGAGATTGTGAAGAGCCTGGAAGCGGCGGAC
AAACGTGTGTTCTACGTTGAGCTGCAAAGCGGTGAAGGCCACGATAGCTTTCTGCTG
AAGAACCCGAAACAAATCGAGATTCTGAAAGGTTTTCTGGAAAACCCGAACTAACTC
GAGCAACCTGGAGGCGGGCGCAGGCCCGCCTTTT (SEQ ID NO: 28).

[0154] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or
a plurality thereof, comprising the sequence:

TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTTAACTTTAAGAGA
GGTATATATTACCATGGACCTGCAAACCCTGGCGCTGCATGCGGGTTACGAAAAGGA
TAGCCAGCGTACGATGGCGGTGCCGATCTACCAAACCACCGCGTATGAGTTCCGTGA
CGTTGAACACGCGGCGAACCTGTTTGCGCTGAAGGAACTGGGCAACATTTATACCCG
TCTGAACAACCCGACCACCGATGTGTTTCGAGAAACGTTTTACCGCGCTGGAGGGTGG
CGAAGCGGCGATTGCGACCGCGAGCGGTATGAGCGCGATTTTCTTTGCGCTGGCGAA
CGCGGCGCAGGCGGGTGACAACATCATTTGCGCGAACCAACTGTACGGTGGCAGCCT
GACCCTGAGCACCCACACCCTGAAGCGTTTCGGTATCGAAGCGCGTTTCTTTGACGTT
CACAAACCGCAGCAAATCGAGGTGCTGATCGATGAAAAGACCAAGGTTATCTTCTTT
GAAAGCCTGACCAACCCGAGCATCGACGTGGCGGATATTGAGGCGCTGACCGCGATT
GCGAACAACACAACATCCTGACCATTGTGGACAACACCGTTGCGACCCCGGTGCTG
TGCCGTCCGTTTGAGTTTGGTGCGGATATCACCGTTCACAGCGCGAGCAAGTATACC
ACCGGTCAGGGCCTGGCGATCGGTGGCATTCTGGTTGAACGTAAAGGCCTGGTGGAC
AAGCTGAAAAACAACCCGCGTTACAGCCACTTCAACGAGCCGGACGCGAGCTACCA
CGGTCTGGTTTATACCGATACCGGTCTGCCGCCGTATACCCTGCGTGCGCGTCTGAGC
CTGCTGCGTGATCTGGGTGCGGTGGTTAGCCCGTTCAACAGCTGGCTGTTTATCCAAG
GCATTGAAACCTGAGCCTGCGTATGAAGGAGCACAGCAAAAACGCGCTGATGCTG
GCGGAGTTCCTGGAAAGCCACAAGAAAGTGAAGAAAGTTAACTACCCGGGTCTGAA
GAGCAACGCGAACTACAAGAACGCGCAGAAATATTTTCAAGATGGTCTGTGCAGCG
GCCTGCTGAGCTTCGAGGTGGAAGACTTTGATGAAGCGACCAAGATCGTTGACGCGA
CCAAACTGTATAGCCTGGTGGTTAACATTGGTGATAGCAAAAGCATCATTACCCATC
CGGCGAGCACCCACCAGCAACTGAGCCACGAGGAACTGATTGCGTGCGGTGTGC
CGGAGGGTCTGATCCGTATTAGCTGCGGCCTGGAGAGCGTTAAGGACCTGATTGAAG
ATCTGAAACAAGCGCTGGAGGCGTAAGGATCCAGAGGTATATATTAATGAGCCTGAA
CCTGCAAACCTATACCGAGCACTTCACCAACCCGCTGTACCTGGAGAGCGGTCTGTAT

CCTGGAACCGTACGATATTACCTACGAGACCTATGGCACCATGAACGAAGACAAGAG
CAACGTGGTTGTGGTTTGGCCATGCGCTGACCGGTAGCCACCATGCGGCGGGTCTGTA
TGAGGATGAAACCAAACCGGGTTGGTGGGACGGCTTCATCGGTAGCGGCAAGGCGA
TTGACACCGATAAATACTTCGTGATTTGCAGCAACGTTATTGGTAGCTGCTTTGGTAG
CACCGGCCCGATGAGCCTGCAACACCCGTATCAAGAACCGTACCGTTATAAGTTCCC
GGTGGTTAGCATCAAGGATATGGTGAAAGCGCAACGTATTCTGTTTGACCGTCTGGA
TATCCACCGTGTGCACGCGATTGTTGGTGGCAGCATGGGTGGCATGCAGGCGCTGCA
ATTCGCGATCCACTACCCGAACCTTTCGGAACAAGATCATTGCGCTGGCGACCACCCA
TGCGACCCAGCCGTGGGCGATCGCGTTCAACAAAGTGCGCAAGAGAGCATTCTGAA
CGACCCGGATTTTAAGCAGGGTTACTATGACCCGGATCTGCTGAAAGAACAAGGTCT
GAGCGGTATGGCGGTTGGTCGTATGGCGGGTCACATCAGCTTCCTGAGCCACGAGAG
CATGCGTGAAAAGTTTGGTCGTGATTATAAACTGACCGACGGTCTGTACGAGCTGTT
CGGCAAGTTTCAGGTGGAGAGCTACCTGGAATATAACGGCTACAACCTCACCAAGTG
GTTTGATCCGCTGGCGTACCTGTACATCACCAAGGCGATCAACATCTACGATCTGAG
CCGTGGTTTCGACAGCCTGGCGGAGGCGCTGAAGCGTGTGACCAGCGCGCTGTACCT
GGTTAGCTTTAAAAACGACCTGCTGTTCAAGAACTTTGAGATGAAAGAAATCGCGGA
CGAACTGGATAAGATTGGTAACAAAAACCACAGCTATATCGACGTTAAAAGCGATTA
CGGCCACGACGCGTTCCTGGTGGAGCTGAACAAGTTTGAAAACCACGTTAAAGACGC
GCTGAACGGCTAACTCGAGCAACCTGGAGGCGGGCGCAGGCCCGCCTTTT (SEQ ID
NO: 29).

[0155] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, comprising the sequence:

TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTTAACTTTAAGAGA
GGTATATATTACCATGGCGAGCAACACCCTGCACTTCGAGACCCTGCAAGTGACGCG
GGGTCAACATCCGGACCCGGCGACCGGCGCGCAAGCGGTGCCGATTTACGCGACCA
ACGCGTATGTTTTTGAAAGCCCGGAACATGCGGCGGACCTGTTTCGGTCTGCGTGCGTT
TGGCAACATTTACAGCCGTATCATGAACCCGACCAACGCGGTTCTGGAGGAACGTAT
TGCGGCGCTGGAAGGTGGCGTGGGTGCGCTGGCGGTTGCGAGCGGTCATGCGGCGCA
GTTCTTGGCGATTACCACCGTGGCGCAAGCGGGTGATAACATCGTTAGCACCCCGAA
CCTGTACGGTGGCACCGTGAACCAATTTTCGTGTTACCCTGCGTCGTCTGGGCATTGAA
GTGCGTTTCACCAGCAAGGACGAACGTCCGGAGGAATTTGCGGCGCTGATCGACGAT
CGTACCCGTGCGGTTTATCTGGAGACCCTGGGTAAACCCGGCGCTGAACGTGCCGGAC
TTTGAGGGTATTGCGGAAGTTGCGCATGCGCGTGGCGTGGCGGTTTTTGTGGATAAC
ACCTTTGGTGCGGGTGGCTACTATTGCCAACCGCTGCGTCACGGCGCGGATGTGGTTTC

TGCACAGCGCGAGCAAATGGATTGGTGGCCACGGTAACGGCATCGGTGGTCTGCTGG
TTGACGGTGGCACCTTTGATTGGGGTAACGGCCGTTACCCGCTGCTGACCGAGCCGA
GCCCCGAGCTATCACGGTCTGAGCTTCTGGGAGGCGTTTGGTGAGGGTAACGCGCTGG
GTCTGCCGAACATTGCGTTCATTACCCGTGCGCGTACCGAAGGTCTGCGTGATCTGGG
TCCGACCCTGGCGCCGCAGCAAGCGTGGCAGTTTCTGCAAGGTGTGGAGACCCTGAG
CCTGCGTGCGGAACGTCATGCGCAGAACGCGCTGGCGCTGGCGAGCTGGCTGAGCGG
TCACCCGGATGTGAGCCGTGTTACCTATCCGGGCCTGAGCAACCACCCGCACTACGA
TCGTGCGCAAACCTATCTGCCGCGTGGTGCGGGTGCGGTTCTGACCTTTGAGCTGCGT
GGTGGCCGTGCGGCGGGTGAAGCGTTTATTGGTGCGGTGCCTGCTGGCGCAGCATGTG
GCGAACGTTGGTGACACCCGTACCCTGGTTATTCATCCGGCGAGCACCAACCCACAGC
CAGCTGGATGAAGCGGCGCAAGCGGCGGCGGGTGTGACCCCGGGCCTGGTTCGTGTG
AGCGTTGGTATCGAGCACATTGACGATATCCGTGAAGATTTTGCGCAGGCGCTGGCG
ACCGCGCTGGTTGATGCGGAGGGTGCCTAAGGATCCAGAGGTATATATTAATGCGTA
TCCAGCGTTACATTCTGATGACCGCGCTGATCAGCCAACCGGACCTGCTGCCGCCGC
CGGCGCCGGAGCGTTGCCCGCCGCAGCAAACCGCGCGTCTGTTCCGTGAAACCCCGC
TGCTGCTGGACTGCGGTCAGGTGGTTCAAGATGTGCGTGTTGCGTACCACACCTATG
GCACCCCGAGCGACCATGCGATCCTGGTGTGCTGCATGCGCTGACCGGCACCAGCGCGG
TTCATGAGTGGTGGCCGGATTTTCTGGGTGAAGGCAAGCCGCTGGACCCGACCCGTG
ATTATATTGTTTGCGCGAACGTTCTGGGTGGCTGCGCGGGTAGCACCGGTCCGGCGG
AGCTGCCGCGTGTGAACGGTGAAGACCCGCCGCTGACCCTGCGTGATATGGCGCGTG
TGGGTCGTGCGCTGCTGGAGGAACTGGGCGTTCGTCTGTGAGCGTTATTGGTGCGA
GCATGGGTGGCATGCTGGCGTATGCGTGGCTGCTGGAGTGCCCGGACCTGGTGGATC
GTGCGGTTATCATTGGTGCGCCGGCGCGTCACAGCCCGTGGGCGATTGGTCTGAACA
CCGCGGCGCGTAACGCGATTCTGTGCGGCGCCGGGTGGCGAGGGTCTGAAGGTTGCGC
GTCAGATCGCGATGCTGAGCTATCGTAGCCCGGAGAGCTTCGCGCTGACCCAGAGCG
GTTGGGGCACCCGTCGTCCGGGCACCCCGGACATTACCACCTACCTGGAGCACCAGG
GTGAAAACTGAGCACCCGTTTCTGCGAGCGTAGCTATCTGGCGCTGACCGGCGCGA
TGGACCGTTTTCAACCGACCGATGCGGAACTGCGTAGCATCCGTGTGCCGGTTCTGGT
GGTTGGTATTAGCAGCGATGTGCTGTACCCGCCGGCGGAAGTGCGTACCTATGCGGG
TCTGCTGCCGCGTGGCCAGTACCTGGAAGTGCAGCCCGCACGGCCATGATGCGTT
CCTGATCGATCCGCAGGGTCTGCCGGAAGCGGCGGCGGCGTTCCTGCACGGTGCCTA
ACTCGAGCAACCTGGAGGCGGGCGCAGGCCCGCCTTTT (SEQ ID NO: 30).

[0156] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, comprising the sequence:

TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTTAACTTTAAGAGA
GGTATATATTACCATGGGTAGCAAGAACTACCGTTTCGAGACCCTGCAAGTGCACGG
TGGCCAAGAAGTTGACCCGACCACCAACAGCCGTGCGGTGCCGATCTACCAGACCAG
CAGCTATGTTTTTAAACAGCGCGGAGCATGGTGCGAACCTGTTTCGCGCTGAAGGAATT
TGGCAACATCTATACCCGTATTATGAACCCGACCAGCGACGTTTTTCGAGAAACGTAT
GGCGGCGCTGGAAGGTGGCGTGGCGGCGGTTGCGACCGCGAGCGGTCAGGCGGCGC
AATTCCTGGCGCTGAACAACTTTCTGAGCGTGGGCGATAACTTCGTTACCAGCCCGTT
TCTGTACGGTGGCAGCTATAACCAATTCAAAGTGAGCTTTAAACGTATCGGTATTGA
GGCGCGTTTTTGCGAAGAGCGACAAAGTTGACGATCTGGCGGCGGAAATCAACGATA
AGACCAAAGCGATCTACGTGGAGACCATTGGCAACCCGGAGTTCAACGTTCCGGACT
TTGAAGCGATCGCGGCGCTGGCGAAGAAACACGACATTCCGCTGGTTGTGGATAACA
CCTTCGGTGC GGGTGGCTATCTGTGCCAGCCGATCAAGCACGGCGCGAACATTGTGA
CCAGCAGCGCGACCAAATGGATCGGTGGTCACGGCACCAGCATTGGTGGCATCATTG
TTGATGGTGGCAACTACAACCTGGGGTAACGGCAAGTTCCCGCAATTTAGCGAGCCGA
GCGAAGGTTATCACGGCCTGAACTTCTGGGAGACCTTTGGTGACAACAACCCGCTGG
GTCTGCCGAACATTGCGTTCGCGATTTCGTGCGCGTGTGGAAGGTCTGCGTGATTTTGG
CCCGGCGATCAGCCCGTTCAACAGCTTTCTGCTGCTGCAAGGTCTGGAGACCCTGAG
CCTGCGTGTGCAACGTACCGTTGACAACGCGCTGGAGCTGGCGAAATGGCTGGAAGC
GCACCCGAAGGTGAAAAGCGTTAACTATCCGGGTCTGACCAACAGCCCGTACCACGC
GACCGCGAAGAAATATCTGACCCACGGTTTCGGTGGCGTGCTGAGCTTTGAGATTGA
AGGCGATAAGGAGACCGCGAGCAACTTTATCAACAACCTGGAACCTGATTAGCCACCT
GGCGAACGTTGGTGACGCGAAAACCTGATCATTACGCCGAGCGCGACCACCCACCA
GCAACTGAGCGATGAAGCGCAAATTGCGGCGGGTGTGACCCCGAGCCTGCTGCGTAT
TAGCAGCGGCATCGAGCACATTGAAGACCTGAAGGCGGATCTGACCGCGGCGTTTGA
TAAAATCTAAGGATCCAGAGGTATATATTAATGAACCTGCAAAGCCCGCACCTGACC
ATCGAGATGACCCAGGAAATTTTCTACTGCCAAGAGGCGCTGAGCCTGGAGAGCGGC
GAGAGCTTCCCGGAATTTCAACTGAGCTTTACCACCCAGGGCCAACCTGAACGCGAAC
AAGGACAACGTGATCTGGGTTCTGCACGCGCTGACCGGTGATGCGAACCCGCACGAG
TGGTGGAGCGGTCTGATCGGCGAAGACAAGTTCTTTGATCCGAGCAAATATTTTATT
GTGTGCGCGAACTTTCTGGGTAGCTGCTACGGCAGCACCCAGCCGCTGAGCAACAAC
CCGAACAACGGTAAACCGTACTATTACGACTTCCCGAACATCACCACCCGTGACATT
GCGAGCGCGCTGGATAAGCTGCGTATCCACCTGGGCCTGGAGAAAATCAACACCGTG
ATTGGTGGCAGCCTGGGTGGCCAAGTGGGTCTGGAATGGGCGGTTAGCCTGGGCGAG
AAGCTGGAAAACGCGATCATTGTTGCGAGCAACGCGAAAGCGAGCCCGTGGATCATT

GGTTTTAACGAGACCCAGCGTATGGCGATCGAAAGCGATAGCACCTGGGGCAAGAC
CCAACCGGAGGCGGGTAAGAAAGGCCTGGAAACCGCGCGTGCGATTGGTATGCTGA
GCTATCGTCACCCGATGACCTTCCTGCAAAACCAAAGCGAGACCGAGGAAAAGCGTG
ACGATTTTAAAATCAGCAGCTATCTGCGTTACCAGGGCCTGAAGCTGGCGAACCGTT
TCAACGCGATGAGCTACTGGATTCTGAGCAAAGCGATGGACAGCCACGATATTGGTC
GTGGTCGTGGTGGCACCCCGGTGGCGCTGAGCAACATCAAGTGCAAAGTGCTGAGCA
TCGGTGTTGACACCGATATTCTGTTTACCAGCGAGGAAAGCCGTTATATTAGCAAGC
ACGTTCCGAAAGGCACCTATCGTGAGATCAGCAGCATTTACGGCCACGACGCGTTCC
TGATCGAGTATGAACAGCTGCAATACATTCTGAAGAGCTTCTACCTGGAAAACAACG
GCTAACTCGAGCAACCTGGAGGCGGGCGCAGGCCCGCCTTTT (SEQ ID NO: 31).

[0157] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, comprising the sequence:

TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTTAACTTTAAGAGA
GGTATATATTACCATGGGTCCGAAGTACGACAACAGCAACGCGGATCAGTGGGGCTT
CGAGACCCGTAGCATCCACGCGGGTCAGAGCGTTGACGCGCAAACCAGCGCGCGTA
ACCTGCCGATTTACCAGAGCACCGCGTTCTGTGTTTGACAGCGCGGAGCACGCGAAAC
AACGTTTCGCGCTGGAAGATCTGGGCCCCGGTTTATAGCCGTCTGACCAACCCGACCG
TGGAGGCGCTGGAAAACCGTATTGCGAGCCTGGAGGGTGGCGTTCATGCGGTGGCGT
TTAGCAGCGGTCAGGCGGCGACCAACGCGATCCTGAACCTGGCGGGTGCGGGTG
ACCACATTGTTACCAGCCCCGCGTCTGTATGGTGGCACCGAAACCCTGTTCCCTGATCAC
CCTGAACCGTCTGGGCATTGATGTTAGCTTTGTGGAGAACCCGGATGATCCGGAAAG
CTGGCAGGCGGCGGTTCAACCGAACACCAAGGCGTTCTTTGGCGAGACCTTTGCGAA
CCCGCAAGCGGACGTGCTGGATATCCCGGCGGTTGCGGAAGTGGCGCACCGTAACAG
CGTTCCGCTGATCATTGACAACACCATTGCGACCGCGGCGCTGGTGCCTCCGCTGGA
GCTGGGTGCGGATGTGGTTGTGGCGAGCCTGACCAAGTTCTACACCGGTAACGGCAG
CGGTCTGGGTGGCGTTCTGATCGACGGTGGCAAATTTGATTGGACCGTGGAAGGA
CGGCAAAAGCGTTTTCCCGTATTTTGTACCCCGGATGCGGCGTACCACGGTCTGAAG
TATGCGGATCTGGGTGCGCCGGCGTTTGGTCTGAAAGTTCGTGTGGGCCTGCTGCGTG
ACACCGGTAGCACCTGAGCGCGTTTAACGCGTGGGCGGCGGTTCAAGGCATCGATA
CCCTGAGCCTGCGTCTGGAGCGTCACAACGAAAACGCGATTAAGGTGGCGGAGTTCC
TGAACAACCACGAGAAGGTTGAAAAAGTGAACCTTTGCGGGTCTGAAGGATAGCCCG
TGGTACGCGACCAAGGAAAAACTGGGCCTGAAATATACCGGTAGCGTGCTGACCTTC
GAGATCAAGGGTGGCAAAGACGAAGCGTGGGCGTTTATTGATGCGCTGAAACTGCA
CAGCAACCTGGCGAACATCGGCGACGTTCTGTAGCCTGGTTGTGCATCCGGCGACCAC

CACCCATAGCCAAAGCGATGAGGCGGGCCTGGCGCGTGCGGGTGTGACCCAAAGCA
CCGTTTCGTCTGAGCGTGGGTATCGAGACCATTGACGATATCATTGCGGACCTGGAAG
GTGGCTTCGCGGCGATTTAAGGATCCAGAGGTATATATTAATGCCGACCCTGGCGCC
GAGCGGTCAGCTGGAGATCCAAGCGATTGGTGACGTTAGCACCGAGGCGGGCGCGA
TCATTAAGAACGCGGAAATTGCGTACCACCGTTGGGGTGAGTATCGTGTGGACAAAG
AAGGCCGTAGCAACGTGGTTCTGATCGAACACGCGCTGACCGGTGATAGCAACGCGG
CGGACTGGTGGGCGGATCTGCTGGGTCCGGGCAAGGCGATCAACACCGACATTTACT
GCGTTATCTGCACCAACGTGATCGGTGGCTGCAACGGCAGCACCGGTCCGGGCAGCA
TGCACCCGGATGGTAACTTCTGGGGCAACCGTTTTCCGGCGACCAGCATTCGTGACC
AGGTAAACGCGGAGAAACAATTCCTGGATGCGCTGGGTATTACCACCGTTGCGGCGG
TGCTGGGTGGCAGCATGGGTGGCGCGCGTACCCTGGAGTGGGCGGCGATGTATCCGG
AAATCGTTGGTGCGGCGGCGGTGCTGGCGGTTAGCGCGCGTGCGAGCGCGTGCGCAGA
TCGGCATTTCAGAGCGCGCAAATCAAGGCGATTGAAAACGATCACCCTGGCACGAG
GGTAACTACTATGAAAGCGGCTGCAACCCGGCGACCGGTCTGGGTGCGGCGCGTCGT
ATTGCGCACCTGACCTACCGTGGTGAGCTGGAAATCGACGAGCGTTTTGGCACCAAG
GCGCAGAAAAACGAAAACCCGCTGGGTCCGTATCGTAAGCCGGATCAACGTTTCGCG
GTTGAGAGCTACCTGGACTATCAGGCGGATAAACTGGTTCAACGTTTTGACGCGGGT
AGCTACGTGCTGCTGACCGATGCGCTGAACCGTCACGACATTGGCCGTGATCGTGGT
GGCCTGAACAAGGCGCTGGAGAGCATTAAGTGCCGGTTCTGGTGGCGGGCGTTGAC
ACCGATATCCTGTACCTGTATCACCAGCAAGAACACCTGAGCCGTAACCTGGGTAAC
CTGCTGGCGATGGCGAAAATCGTTAGCCCGGTGGGTCATGATGCGTTCCTGACCGAA
AGCCGTCAAATGGATCGTATTGTGCGTAACTTCTTTAGCCTGATCAGCCCGGACGAG
AACAAACCCGAGCACCTACATTGAATTTTATATCTAACTCGAGCAACCTGGAGGCGGG
CGCAGGCCCGCCTTTT (SEQ ID NO: 32).

[0158] In some embodiments, the bacterial cell comprises an exogenous nucleic acid molecule or a plurality thereof, comprising the sequence:

TTTATTCTTGACACTAGTCGGCCAAAATGATATAATACCTGAGTTTAACTTTAAGAGA
GGTATATATTACCATGGGTCTGTTTCGAGACCCTGCAACTGCATGCGGGTTATGAGCC
GGAACCGACCACCCTGAGCCGTCAAGTTCGATCTACCCGACCACCAGCTATGTGTT
TAAAAGCCCGGAGCATGCGGCGAACCTGTTCGCGCTGAAGGAATTTGGTAACATCTA
TAGCCGTATTATGAACCCGACCGTGGACGTTCTGGAGAAACGTCTGGCGGGCGCTGGA
AGGTGGCAAAGCGGCGCTGGCGACCGCGAGCGGTCATGCGGCGCAGTTTCTGGCGCT
GACCACCCTGGCGCAAGCGGGCGATAACATCGTTAGCACCCCGAACCTGTACGGTGG
CACCTTCAACCAGTTTAAGGTTACCCTGAAACGTCTGGGCATTGAAGTGCGTTTCACC

AGCCGTGAGGAACGTCCGGAGGAATTTCTGGCGCTGACCGATGAGCGTACCCGTGCG
TGGTGGGTGGAAAGCATCGGTAACCCGGCGCTGAACATTCCGGACCTGGAGGCGCTG
GCGCAAGCGGCGCGTGAAAAAGGTGTGGCGCTGATCGTTGATAACACCTTCGGCATG
GGTGGCTATCTGCTGCGTCCGCTGGCGTGGGGCGCGGCGCTGGTTACCCACAGCCTG
ACCAAGTGGGTGGGTGGCCACGGTGCGGTTATTGCGGGTGCGATTGTGGATGGTGGC
AGCTTTCCGTGGGAGGGTGGCCGTTACCCGCTGCTGACCGAACCGCAGCCGGGTTAT
CACGGCCTGCGTCTGACCGAGGCGTTCGGTGAACCTGGCGTTTATTGTGAAAGCGCGT
GTTGACGGCCTGCGTGATCAAGGTCAAGCGCTGGGTCCGTTTGAGGCGTGGGTGGTT
CTGCTGGGTATGGAAACCCTGAGCCTGCGTGCGGAGCGTCATGTGGAAAACACCCTG
CATCTGGCGCACTGGCTGCTGGAGCAGCCGCAAGTGGCGTGGGTAACTATCCGGGT
CTGCCGCACCACCCGCACCACGACCGTGCGCAGAAGTATTTCAAGGGTAAACCGGGC
GCGGTTCTGACCTTTGGCCTGAAGGGTGGCTACGAAGCGGCGAAACGTTTTATCAGC
CGTCTGAAGCTGATTAGCCACCTGGCGAACGTGGGTGACACCCGTACCCTGGCGATT
CACCCGGCGAGCACCACCCACAGCCAACTGAGCCCGGAGGAACAGGCGCAAGCGGG
TGTTAGCCCGGAGATGGTGCGTCTGAGCGTTGGCCTGGAGCACGTGGAAGATCTGAA
GGCGGAGCTGAAAGAAGCGCTGGCGTAAGGATCCAGAGGTATATATTAATGCGTATC
CAGCGTTACATTCTGATGAGCGAGATCGCGCTGGAAGCGTGGGGTGAGCATGAAGCG
CTGCTGCTGAAGCCGCCGCGTAGCCCGCTGAGCATCCCGCCGCCGAAGCCGCGTACC
GCGGTGCTGTTCCCGCGTCGTGAGGGTTTTTACACCGAGCTGGGTGGTTACCTGCCGG
AAGTGCGTCTGCGTTTCGAAACCTACGGCACCTGAGCCGTCGTCGTGATAACGCGG
TGCTGGTTTTTCATGCGCTGACCGGTAGCGCGCACCTGGCGGGCACCTATGATGAGG
AAACCTTCCGTAGCCTGAGCCCGCTGGAGCAGGCGTTTGGTCGTGAAGGTTGGTGGG
ATAGCCTGGTGGGTCCGGGTCGTATCCTGGACCCGGCGCTGTACTATGTGGTTAGCG
CGAACCACCTGGGTAGCTGCTATGGTAGCACCGGTCCGCTGAGCCTGGACCCGCACA
CCGGTCGTCCGTATGGTCGTGATTTCCCGCCGCTGACCATTCTGTGATCTGGCGCGTGC
GCAAGCGCGTCTGCTGGACCACCTGGGTGTGGAGAAGGCGATCGTTATTGGTGGCAG
CCTGGGTGGCATGGTGGCGCTGGAGTTCGCGCTGATGTACCCGGAACGTGTTAAGAA
ACTGGTGGTTCTGGCGGCGCCGGCGCGTCACGGCCCGTGGGCGCGTGCGTTTAACCA
CCTGAGCCGTCAGGCGATCCTGCAAGATCCGGAATACCAGAAGGGTAACCCGGCGCC
GAAGGGTATGGCGCTGGCGCGTGGTATTGCGATGATGAGCTATCGTGCGCCGGAAGG
TTTTGAAGCGCGTTGGGGTGCGGAGCCGGAACCTGGGCGAGACCTACCTGGACTATCA
AGGTGAAAAGTTCCTGCGTCGTTTTACGCGGAGAGCTATCTGGTGCTGAGCCGTGC
GATGGACACCCATGATGTGGGTCTGGTCTGGTGGCGTTGAGGAAGCGCTGAAACG
TCTGCGTGCGATCCCGAGCCTGTTTGTGGGCATTGACACCGATCTGCTGTACCCGGCG

TGGGAAGTTCGTCAGGCGGCGAAGGCGGCGGGTGCGCGTTATCGTGAGATCAAAAG
 CCCGCACGGCCATGATGCGTTCCTGATCGAAACCGATCAAGTTGAGGAAATTCTGGA
 CGCGTTTCTGCCGTAACCTCGAGCAACCTGGAGGCGGGCGCAGGCCCGCCTTTT (SEQ
 ID NO: 33).

[0159] In some embodiments, the exogenous nucleic acid molecule or a plurality thereof is integrated into the genome of the bacterial cell. In other embodiments, the exogenous nucleic acid molecule or a plurality thereof is comprised within a plasmid or other expression vector. In some embodiments, an expression vector comprises a viral expression vector. In some embodiments, the plasmid or the expression vector further comprises one or more nucleic acid sequences encoding: a tag, a linker, or a combination thereof. In some embodiments, the tag may be suitable for protein isolation and/or purification. In some embodiments, the linker is a flexible linker.

[0160] A vector nucleic acid sequence generally contains at least an origin of replication for propagation in a cell and optionally additional elements, such as a heterologous polynucleotide sequence, expression control element (e.g., a promoter, enhancer), selectable marker (e.g., antibiotic resistance), or poly- Adenine sequence. The vector may be a DNA plasmid delivered via non-viral methods or via viral methods. The viral vector may be a retroviral vector, a herpesviral vector, an adenoviral vector, an adeno- associated viral vector, a virgaviridae viral vector, or a poxviral vector. The barley stripe mosaic virus (BSMV), the tobacco rattle virus and the cabbage leaf curl geminivirus (CbLCV) may also be used. The promoters may be a viral promoter.

[0161] Methods for gene expression in bacteria are well known in the art. An ordinary skilled in the art would know how to express the polypeptide of the invention, A large number of E. coli expression plasmids, or B. subtilis plasmids, are available for a wide variety of needs. A heterologous polypeptide can be expressed in the cytoplasm of the bacterial cell. Non- limiting examples for E. coli expression vectors are the pCCL, pGEX and the pET series of vectors. In some embodiments, the expression vector, or plurality thereof, comprises a sequence encoding an antibiotic for positive selection of the bacterial cell expressing it.

[0162] In some embodiments, the bacterial cell further comprises L-methionine exporter. In some embodiments, the L-methionine exporter is encoded by the yjeH gene. YjeH is a member of the amino acid efflux (AAE) family within the amino acid-polyamine-organocation (APC) superfamily of transporters. In some embodiments, yjeH encodes a L-methionine and branched chain amino acid (BCAA) efflux transporter in E. coli. In some embodiments, yjeH is overexpressed in the bacterial cell disclosed herein. In some embodiments, a cell as disclosed herein, characterized by overexpression of yjeH comprises one or more copies of the yjeH gene. In some embodiments, one or more copies of the yjeH gene are located within the genome of a cell as disclosed herein, extra-

genomically, e.g., plasmid, etc., or both. In some embodiments, overexpression of yjeH results in increased tolerance to L-methionine. In some embodiments, overexpression of yjeH is associated with increased export of methionine and the BCAAs when the cell is grown in the presence of at least one of: Met-Met, Ile-Ile, Leu-Leu or Val-Val dipeptides. In some embodiments, absence or reduced expression of yjeH results in increased levels of intracellular methionine. In some embodiments, expression of yjeH is not regulated by the transcriptional repressor, metJ.

[0163] In some embodiments, there is provided a composition comprising the bacterial cell disclosed herein and a biologically accepted carrier.

[0164] As used herein, the term “carrier,” refers to any component of a composition that is not the active agent. In some embodiments, the carrier comprises a culture liquid medium. In some embodiments, the carrier comprises a solid medium (e.g., agar).

[0165] In some embodiments, there is provided a method for producing an amino acid.

[0166] In some embodiments, the method comprises: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding metX and metY, and (ii) inactive metA and metB genes; and, (b) culturing the bacterial cell from step (a) such that the metX and metY encoded by the exogenous nucleic acid molecule are expressed, thereby, producing the amino acid.

[0167] In some embodiments, culturing is in a culture or a growth medium. Types of culture media are common and would be apparent to one of ordinary skill in the art of microbiology.

[0168] In some embodiments, the amino acid comprises an essential amino acid.

[0169] In some embodiments, the essential amino acid is selected from the group consisting of: alanine, valine, leucine, threonine, isoleucine, homoserine, methionine, aspartate, phenylalanine, glutamate, and any combination thereof.

[0170] In some embodiments, the amino acid comprises methionine. In some embodiments, the amino acid comprises or consists of L-methionine.

[0171] In some embodiments, there is provided a method for producing L-methionine.

[0172] In some embodiments, the method is for increasing production of an amino acid. In some embodiments, increasing it compared to a control cell. In some embodiments, a control cell comprises active metA, active metB, or both. In some embodiments, a control cell is devoid of metX, met Y, or both. In some embodiments, a control cell comprises an active metJ. In some embodiments, a control cell comprises active metA, active metB, active metJ and is devoid of metX and metY. In some embodiments, a control cell comprises a bacterial cell. In some embodiments, a control cell comprises an E. coli cell. In some embodiments, a control cell comprises an E. coli cell comprising active metA, active metB, active metJ and being devoid of metX and metY.

[0173] In some embodiments, the produced levels of an amino acid being produced by a bacterial cell as disclosed herein are at least 1.5-fold, at least 2-fold, at least 5-fold, at least 10-fold, or at least 20-fold greater than levels of amino acid being produced by a control bacterial cell, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[0174] In some embodiments, the produced levels of an amino acid being produced by a bacterial cell as disclosed herein are 1.5-fold to 20-fold, 5-fold to 50-fold, 1.5-fold to 100-fold, 2-fold to 80-fold, 4-fold to 30-fold, 10-fold to 100-fold, 5-fold to 200-fold greater than levels of amino acid being produced by a control bacterial cell. Each possibility represents a separate embodiment of the invention.

[0175] In some embodiments, the produced levels comprises intracellular levels, extracellular levels, or both. In some embodiments, the produced levels are determined intracellularly, e.g., from an intracellular fraction of the bacterial cell, extracellularly, e.g., from an extracellular fraction wherein the bacterial cell is cultured, or both.

[0176] In some embodiments, the method further comprises a determining step, comprising determining the intracellular production level of the amino acid, the extracellular production level of the amino acid, or both.

[0177] In some embodiments, the method further comprises extracting the intracellular content of the bacterial cell. Methods and means for extracting bacterial cells are common and would be apparent to one of ordinary skill in the art.

[0178] In some embodiments, the method further comprises collecting or obtaining the cultured medium wherein the bacterial cell disclosed herein is cultured. In some embodiments, the collected culture medium comprises an extracellular fraction produced and/or secreted from the bacterial cell disclosed herein. In some embodiments, the method further comprises extracting the collected culture medium.

[0179] In some embodiments, there is provided an extract of the bacterial cell disclosed herein. In some embodiments, the extract comprises an amino acid. In some embodiments, the extract comprises L-methionine.

[0180] In some embodiments, the extract comprises intracellular produce, extracellular produce, or both, of the bacterial cell disclosed herein

[0181] In some embodiments, there is provided a method for producing L-methionine, the method comprising: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding metX and metY, and (ii) inactive metA and metB genes; and (b) culturing the bacterial cell from step (a) such that metX and metY encoded by the exogenous nucleic acid molecule are expressed, thereby, producing L- methionine.

[0182] In some embodiments, the method comprises: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding at least one of: SEQ ID NOs: 1-6, and 50, and SEQ ID NOs: 7-12, and (ii) inactive metA and metB genes; and, (b) culturing the bacterial cell from step (a) such that at least one of: SEQ ID NOs: 1-6, and 50, and at least one of: SEQ ID NOs: 7-12, are expressed and/or translated.

[0183] In some embodiments, the method comprises: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding at least one of: SEQ ID NOs: 1-6, and 50, and SEQ ID NOs: 7-12, and (ii) inactive metA, metB, and metJ genes; and, (b) culturing the bacterial cell from step (a) such that at least one of: SEQ ID NOs: 1-6, and 50, and at least one of: SEQ ID NOs: 7-12, is expressed, e.g., transcribed and/or translated.

[0184] In some embodiments, the method comprises: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding at least one of: SEQ ID NOs: 1-6, and 50, and SEQ ID NOs: 7-12, (ii) inactive metA, metB, and metJ genes, and (iii) one or more copies of yjeH gene; and, (b) culturing the bacterial cell from step (a) such that at least one of: SEQ ID NOs: 1-6, and 50, and at least one of: SEQ ID NOs: 7-12, is expressed, e.g., transcribed and/or translated.

[0185] In some embodiments, the method comprises: (a) providing a bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof encoding at least one of: SEQ ID NO: 3 or 5, and SEQ ID NOs: 9 or 11, (ii) inactive metA, metB, and metJ genes, and (iii) one or more copies of the yjeH gene; and, (b) culturing the bacterial cell from step (a) such that at least one of: SEQ ID NOs: 3 or 5, and at least one of: SEQ ID NOs: 9 or 11, is expressed, e.g., transcribed and/or translated.

[0186] In some embodiments, the bacterial cell is cultured in media comprising MOPS (3-(N-morpholino) propanesulfonic acid). In some embodiments, the media comprises an effective amount of a sulfur compound. Examples for sulfur compounds that can be provided in the media are K_2SO_4 and $FeSO_4$.

[0187] In some embodiments, transformed cells are cultured under effective conditions, which allow for the expression of high amounts of recombinant polypeptide. In some embodiments, effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. In one embodiment, an effective medium refers to any medium in which a cell is cultured to produce the recombinant polypeptide of the present invention. In some embodiments, a medium typically includes an aqueous solution having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals, and other nutrients, such as vitamins. In some embodiments, cells of the present invention can be

cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes and petri plates. In some embodiments, culturing is carried out at a temperature, pH, and oxygen content appropriate for a recombinant cell. In some embodiments, culturing conditions are within the expertise of one of ordinary skill in the art.

[0188] A "nucleic acid" as used herein will generally refer to a molecule (i.e., a strand) of DNA, RNA or a derivative or analog thereof, comprising a nucleobase. A nucleobase includes, for example, a naturally occurring purine or pyrimidine base found in DNA (e.g., an adenine "A," a guanine "G," a thymine "T" or a cytosine "C") or RNA (e.g., an A, a G, an uracil "U" or a C).

[0189] As used herein, the term "encoding" refers to molecule comprising a DNA sequence which can be transcribed into an RNA sequence which can be translated into the encoded protein or a molecule comprising the RNA sequence which can be translated into the encoded protein. In some embodiments, the molecule is a DNA molecule. In some embodiments, the molecule is an RNA molecule. In some embodiments, the DNA is cDNA. In some embodiments, the molecule is a DNA/RNA hybrid. In some embodiments, the molecule comprises non-naturally occurring nucleotides.

[0190] Expressing of a polynucleotide within a cell is well known to one skilled in the art. It can be carried out by, among many methods, transfection, viral infection, or direct alteration of the cell's genome.

[0191] In some embodiments, the vector is introduced into the cell by standard methods including electroporation (e.g., as described in From et al., Proc. Natl. Acad. Sci. USA 82, 5824 (1985)), heat shock, infection by viral vectors, high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., Nature 327. 70-73 (1987)), such as biolistic use of coated particles, and needle-like particles, Agrobacterium Ti plasmids and/or the like. [096] The term "promoter" as used herein refers to a group of transcriptional control modules that are clustered around the initiation site for an RNA polymerase i.e., RNA polymerase II. Promoters are composed of discrete functional modules, each consisting of approximately 7-20 bp of DNA, and containing one or more recognition sites for transcriptional activator or repressor proteins. The promoter may extend upstream or downstream of the transcriptional start site and may be any size ranging from a few base pairs to several kilobases.

[0192] In some embodiments, the polynucleotide is transcribed by RNA polymerase II (RNAP II and Pol II). RNAP II is an enzyme found in eukaryotic cells, known to catalyze the transcription of DNA to synthesize precursors of mRNA and most snRNA and microRNA.

[0193] In some embodiments, a plant expression vector is used. In one embodiment, the expression of a polypeptide coding sequence is driven by a number of promoters. In some embodiments, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al., *Nature* 310:511-514 (1984)], or the coat protein promoter to TMV [Takamatsu et al., *EMBO J.* 6:307-311 (1987)] are used. In another embodiment, plant promoters are used such as, for example, the small subunit of RUBISCO [Coruzzi et al., *EMBO J.* 3: 1671-1680 (1984); and Brogli et al., *Science* 224:838-843 (1984)] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al., *Mol. Cell. Biol.* 6:559-565 (1986)]. In one embodiment, constructs are introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for example, Weissbach & Weissbach [Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463 (1988)]. Other expression systems such as insects and mammalian host cell systems, which are well known in the art, can also be used by the present invention.

[0194] Various methods can be used to introduce the expression vector of the present invention into cells. Such methods are generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, Mich. (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor Mich. (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston Mass. (1988) and Gilboa et al. [*Biotechniques* 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation, agrobacterium Ti plasmids and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

[0195] It will be appreciated that other than containing the necessary elements for the transcription and translation of the inserted coding sequence (encoding the polypeptide), the expression construct of the present invention can also include sequences engineered to optimize stability, production, purification, yield, or activity of the expressed polypeptide.

General

[0196] Any concentration ranges, percentage range, or ratio range recited herein are to be understood to include concentrations, percentages, or ratios of any integer within that range and fractions thereof, such as one tenth and one hundredth of an integer, unless otherwise indicated.

[0197] Any number range recited herein relating to any physical feature, such as polynucleotides and polypeptides, size, weight, or length, are to be understood to include any integer within the recited range, unless otherwise indicated.

[0198] In the discussion unless otherwise stated, adjectives such as “substantially” and “about” modifying a condition or relationship characteristic of a feature or features of an embodiment of the invention, are understood to mean that the condition or characteristic is defined to within tolerances that are acceptable for operation of the embodiment for an application for which it is intended. Unless otherwise indicated, the word “or” in the specification and claims is considered to be the inclusive “or” rather than the exclusive or, and indicates at least one of, or any combination of items it conjoins.

[0199] It should be understood that the terms “a” and “an” as used above and elsewhere herein refer to “one or more” of the enumerated components. It will be clear to one of ordinary skill in the art that the use of the singular includes the plural unless specifically stated otherwise. Therefore, the terms “a”, “an”, and “at least one”, are used interchangeably in this application.

[0200] The descriptions of the various embodiments of the present invention have been presented for purposes of illustration but are not intended to be exhaustive or limited to the embodiments disclosed. Many modifications and variations will be apparent to those of ordinary skill in the art without departing from the scope and spirit of the described embodiments. The terminology used herein was chosen to best explain the principles of the embodiments, the practical application or technical improvement over technologies found in the marketplace, or to enable others of ordinary skill in the art to understand the embodiments disclosed herein.

[0201] For purposes of better understanding the present teachings and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0202] In the description and claims of the present application, each of the verbs, “comprise”, “include” and “have” and conjugates thereof, are used to indicate that the object or objects of the verb are not necessarily a complete listing of components, elements or parts of the subject or subjects of the verb. Other terms as used herein are meant to be defined by their well-known meanings in the art.

[0203] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[0204] All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by references into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

EXAMPLES

[0205] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include immunological, chemical, molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current

Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document.

Material and Methods

Bacterial strains and growth conditions

[0206] *E. coli* MG1655 strain was used in this study. Bacteria were routinely grown in Luria-Bertani (LB) media at 37°C. For screening of the enzymatic variants bacteria were grown in liquid or solid (supplemented with 1.5 % agar) MOPS media (8.73 g/L MOPS, 0.71 g/L tricine, 0.51 g/L NH₄Cl, 0.05 g/L K₂SO₄, 2.92 g/L NaCl, 2.8 mg/L FeSO₄, 0.074 mg/L, 0.1 g/L MgCl₂ 1 ml/L trace elements, K₂HPO₄ 30 mg/L, Glucose 2g/L, pH 7.3).

Generation of methionine auxotrophic mutants

[0207] Target deletion mutations were generated in MG1655 by the lambda red recombinase procedure (Datsenko and Wanner, 2000) using primers listed in **Table 1**, with pkD4 plasmid carrying Kn^R cassette serving as a template for PCR reactions. Mutations were verified using nearby locus-specific primers (**Table 1**) with the respective primers k2 or kt (Datsenko and Wanner, 2000). Afterwards the cassette was removed, and double/ triple mutations were generated in single/double mutant strains as described by Datsenko and Wanner (2000). The mutations were generated by following scheme $\Delta metA \rightarrow \Delta metAB \rightarrow \Delta metABJ$.

Table 1. Primers used in this study

Primer name	Primer sequence	Description
MetA-D-For	CGAGCTACCCGCCGTCAATTTCTT GCGTGAAGAAAACGTCTTTGTGAT GACATATGAATATCCTCCTTAG (SEQ ID NO: 36)	Generation of $\Delta metA$ mutation
MetA-D-Rev	TGTGCCGTAGATCGTATGGCGTG ATCTGGTAGACGTAATAGTTGAGC	Generation of $\Delta metA$ mutation

	<u>CAGTGTAGGCTGGAGCTGCTTCG</u> (SEQ ID NO: 37)	
MetB-D-For	ACAGGCCACCATCGCAGTGCCTAGC GGGTAAATGACGACGAACAGTATG <u>CATATGAATATCCTCCTTAG</u> (SEQ ID NO: 38)	Generation of $\Delta metB$ mutation
MetB-D-Rev	CGGAAGCCATTTTCCAGGTCGGCAA TTAAATCTTCGCCATCTTCAATACCT <u>GTAGGCTGGAGCTGCTTCG</u> (SEQ ID NO: 39)	Generation of $\Delta metB$ mutation
MetJ-D-For	GGAGCGGCGAATATATCAGCCCA TACGCTGAGCACGGCAAGAAGAG TGAATGTAGGCTGGAGCTGCTTCG (SEQ ID NO: 40)	Generation of $\Delta metJ$ mutation
MetJ-D-Rev	CACGTCTCCGGGTAAATCCCCATC TCACGCATGATCTCTTTTGCCGCT TCCATATGAATATCCTCCTTAG (SEQ ID NO: 41)	Generation of $\Delta metJ$ mutation
MetA-For	GTTATGCCGATTCGTGTGCC (SEQ ID NO: 42)	Verification of $\Delta metA$ mutation
MetA-Rev	AGGTAAGGTGCTGAATCGCT (SEQ ID NO: 43)	Verification of $\Delta metA$ mutation
MetB-For	ACATTTACCGACAAAGCCC (SEQ ID NO: 44)	Verification of $\Delta metB$ mutation
MetB-Rev	TTTACCCCTTGTTTGCAGCC (SEQ ID NO: 45)	Verification of $\Delta metB$ mutation
MetJ-For	ACTGTGTGGTCTGGTCTCAA (SEQ ID NO: 46)	Verification of $\Delta metJ$ mutation

MetJ-Rev	CTGATAAGCGTAGCGCATCA (SEQ ID NO: 47)	Verification of $\Delta metJ$ mutation
yjeH-For	GCGCCATGGATGAGTGGACTCAAAC AAGAAC (SEQ ID NO: 48)	Amplification of genomic copy of yjeH and addition of a NcoI site for cloning (underlined)
yjeH-Rev	GCGCTCGAGTTATGTGGTTATGCCA TTTTCC (SEQ ID NO: 49)	Amplification of genomic copy of yjeH and addition of a XhoI site for cloning (underlined)

Underlined sequences refer to sequences derived from pKD4 plasmid (Datsenko and Wanner, 2000)

Complementation with MetXY constructs and activity validation

[0208] pCCI plasmid carrying metXY synthetic operons were transformed to electrocompetent $\Delta metAB/\Delta metABJ$ mutants. Following transformation, several colonies growing on LB agar plates supplemented with 30 μ g/ml chloramphenicol, were tested for the presence of the correct plasmid by colony PCR with M13F and M13R primers. Positive clones were further screened for the growth pattern in methionine-depleted minimal media (MOPS). Briefly, Cultures were prepared by inoculation of a single colony grown on LB into 5 ml MOPS media and incubated overnight at 37 °C, 200 rpm. The culture was diluted 1000x fold in fresh MOPS media and 200 μ l were placed in each well of 96-well plate (Costar). Bacteria were grown for 20 h at 37 °C (constant orbital shaking 280 rpm) through interval measuring of OD(600nm) every 16 minutes using Infinite M200 Plate Reader (Tecan). Each sample was tested in triplicates. For control, MOPS media was supplemented with 5-50 μ g/ml of methionine (Merck) or 0.1-0.5mg/ml of norleucine (Merck). Alternatively, a single colony grown on Luria-Bertani (LB) media was spread on MOPS solid media and visual growth following 24-72h at 37 °C was detected.

Construction of a yjeH overexpression plasmid

[0209] The yjeH gene was amplified from genomic DNA extracted from E. coli MG1655 using a forward primer that adds an NcoI restriction site

(GCGCCATGGATGAGTGGACTCAAACAAGAAC; SEQ ID NO: 48), and a reverse primer adding an XhoI restriction site GCGCTCGAGTTATGTGGTTATGCCATTTTCC; SEQ ID NO: 49). The purified PCR product was digested with NcoI and XhoI and inserted into pTrcHis-a digested with the same enzymes. The correct construct was validated by sequencing.

[0210] **Table 2** summarizes the terminology and the strains that were used in the study.

Table 2. Summary of terminology and strains

Name	Description
Wildtype, WT	<i>E. coli</i> MG1655
Δ metAB	<i>E. coli</i> MG1655 with a deletion of the metA and metB genes
Δ metABJ	<i>E. coli</i> MG1655 with a deletion of the metA, metB and metJ genes
Δ metABJ-Y	<i>E. coli</i> MG1655 with a deletion of the metA, metB and metJ genes overexpressing the methionine exporter YjeH
DG/CM/LI/CG	Complementation with a pCCI plasmid expressing metA and metB of the noted bacterial strain

Qualitative evaluation of extracellular methionine levels

[0211] For qualitative analysis of methionine concentration in the media, bacteria were grown over-night at 37 °C in 5 ml of MOPS media and filtered through a 0.22 membrane to remove bacterial cells. The filtered media was diluted two-fold in fresh MOPS media and the growth of Δ metAB mutant in pre-cultured media was determined by OD_(600nm) measurement as described.

Methionine extraction from lysate and medium to evaluate intra- and extra-cellular methionine levels

[0212] To evaluate the intra-cellular level of methionine, amino acids were extracted from 1 ml bacteria pellet using methanol/water/chloroform at ratio of 1:1:2.5 respectively. After centrifugation, the crude extract was separated to polar and non-polar phase with the addition of 300 μ l water and 300 μ l chloroform and centrifuge for 10 min. Four hundred (400) μ l from the top polar phase were vacuum dried. In addition, to evaluate extra-cellular methionine level, amino acids were extracted from 500 μ l of the growth medium by adding 500 μ l of chloroform and centrifuge for 10 min. Four hundred (400) μ l from the upper polar phase was vacuum dried. The vacuum-dried fraction was dissolved in 40 μ l of 20 mg of methoxyamine hydrochloride in 1 ml pyridine and incubated at 37 °C for 2 h with vigorous shaking, followed by derivatization for 30 min in N-methyl-N(trimethylsilyl)-trifluoroacetamide at 37 °C. One μ l of

the sample was injected into a BP5MS capillary column (SEG; 30 m, 0.25-mm i.d., and 0.25-mm thickness).

Evaluation of methionine levels by GC-MS

[0213] All analyses were carried out on a GC-MS system (Agilent 7890A) coupled with a mass selective detector (Agilent 5975c) and a Gerstel multipurpose sampler (MPS2). For free amino acid detection, the single-ion mass method was used. Amino acid standards of 5, 10, 25, 50, 100, and 200 μM were used to generate standard calibration curves, and ribitol (2 mg in 1 ml HPLC grade water) was used as internal standards. The peak areas were calculated from the standard calibration curves and normalized to the ribitol signal.

EXAMPLE 1

Engineering of methionine auxotroph *E. coli*

[0214] Whereas most microorganisms rely on sulfur assimilation via the direct-sulfurylation methionine biosynthesis pathway, several bacteria, such as *Escherichia coli* (*E. coli*), utilizes the less efficient and highly regulated trans-sulfurylation pathway. In the trans-sulfurylation pathway in *E. coli*, homoserine is converted to L-homocysteine in three steps catalyzed by enzymes encoded by *metA*, *metB* and *metC* genes. Sulfur is assimilated into the intermediate O-succinyl or O-acetyl L-homoserine from cysteine by an enzyme encoded by *metB* to form cystathionine. The enzyme encoded by *metC* is a lyase converting cystathionine to L-homocysteine. In the direct sulfurylation pathway, L-homoserine is converted to L-homocysteine in two steps catalyzed by the enzymes encoded by *metX* and *metY*. Sulfur in the form of hydrogen sulfide is assimilated into O-acetyl L-homoserine by the enzyme encoded by *metY* to directly form L-homocysteine. **Figure 1** illustrates the two orthogonal pathways.

[0215] The trans-sulfurylation pathway is less parsimonious in terms of step numbers and is dependent on the type of the sulfur donor molecule. While *E. coli* is a valuable work-horse in synthetic biology, the trans-sulfurylation pathway is a limiting step in the bioproduction of methionine.

[0216] To exploit the option of converting the methionine biosynthesis pathway from trans- to direct- sulfurylation, the inventors deleted the two essential genes in the methionine pathway of *E. coli*, *metA* and *metB*, encoding the enzymes HST and CgS, respectively. The deletion generated a methionine auxotroph *E. coli* strain (ΔmetAB). **Figure 2** shows growth curves of the ΔmetAB in MOPS minimal media containing glucose and ammonium chloride as the sole carbon and nitrogen source, respectively, and K_2SO_4 as the sulfur source. To test the effect of

methionine on the growth rate, variable concentrations of external methionine were supplemented as indicated (**Figure 2**, legend in μM). While a normal growth rate is observed for the wild-type bacteria, the ΔmetAB auxotroph is incapable of growing without supplementation of methionine. However, the addition of methionine rescues bacterial growth, reaching maximal growth and cell density resembling the WT bacteria at a concentration of 25 μM and showing that indeed methionine is the limiting growth factor.

EXAMPLE 2

Complementation of *E. coli* ΔMetAB by MetX and MetY genes of various bacterial genomes

[0217] Aiming to explore the ability of *E. coli* to synthesize methionine via the direct-sulfurylation enzymes metX and metY, the inventors cloned four metY/X pairs of genes to complement the ΔMetAB auxotroph bacteria. It was expected that such complementation would enable *E. coli* to grow without supplementation of external methionine. The inventors applied two complemented selection criteria. The first was metX enzymes showing a range of catalytic activity of 10^3 - 10^4 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$. The second criteria was that the relevant bacterial genome for the sequence of the counterpart metY gene is known. Based on this rationale, the inventors selected the metY/X pairs from the following four strains: i) *Corynebacterium glutamicum* (CG), ii) *Leptospira interrogans* (LI), iii) *Cyclobacterium marinum* (CM) and iv) *Deinococcus geothermalis* (DG). Interestingly, the sequence identity between the 4 selected metX genes ranged from 33.5% to 37% and between the 4 selected metY genes from 43.9% to 56.7%. Since the arrangement of the genes in each of the selected bacteria is different and in order to design a uniform construct, the metY/X genes were cloned into a pCC1 plasmid. The latter enabled to maintain the inserted genes at low to a single copy, similar to genomic sequences of the corresponding genes. The genes were codon-optimized for *E. coli* expression and synthesized and cloned with a preceding ribosomal binding site (RBS) to the plasmid. **Figure 3A** shows a schematic illustration of the operon that was constructed. The ability of the complemented ΔmetAB to grow on liquid minimal media was then tested. The ΔMetAB bacteria cloned with the metY/X of DG and CM (ΔMetAB -DG and ΔMetAB -CM in **Figure 3B**), successfully complemented the auxotroph bacteria and after about 800 min reached the same growth rate and similar order of cell density as the WT. The complemented bacteria having metY/X of LI and CG (ΔMetAB -LI and ΔMetAB -CG in **Figure 3B**) did not grow under these conditions. The ability of these complemented bacteria to grow on minimal media agar

plates was also tested (**Figure 3C**) and a similar pattern was observed in both assays. The ability of the CM and DG strains to grow on a minimal medium indicates that they can produce methionine at a level sufficient to maintain their growth. To quantify intracellular and extracellular methionine levels in the complemented bacteria, the methionine levels were evaluated using GC-MS and compared to those of the WT bacteria. The Δ metAB-DG and Δ metAB-CM strains exhibited a five-fold enhancement of intracellular methionine levels compared to WT (**Figure 3D, left**). Analysis of the extracellular methionine in the growth medium indicated that Δ metAB-DG exhibited significantly enhanced accumulation of extracellular methionine as compared to WT (18-fold). Although the difference in methionine accumulation was not significant, the Δ metAB-CM also showed a five-fold increase compared to the WT (**Figure 3D, right**).

EXAMPLE 3

Evaluation of intra- and extra-cellular methionine of engineered *E.coli* with deletion of metJ and over-expression of the transporter YjeH

[0218] Having the transgenic *E. coli* strains that expressed the metY/X of DG and CM in Δ metAB, the inventors further explored the effect of additional genetic variations related to the methionine biosynthetic pathway. To this end, the metJ repressor that is known to strictly regulate the transcription of multiple genes in the pathway (**Figure 1**) was deleted. The *E. coli* methionine exporter protein YjeH that was also shown to have a strong positive effect on extracellular methionine accumulation was cloned to facilitate overexpression and enable methionine export. To evaluate the ability of the engineered strains to produce and export methionine, the bacteria were cultured in minimal media until reaching $OD_{(600nm)} = 2.5$. The intracellular and extracellular levels of methionine were evaluated using GC-MS (**Figure 4**). By deleting MetJ at WT background, the level of intracellular and extracellular methionine increased by 15 and 161, respectively, for the Δ MetABJ-Y-CM, and by 10 and 127, respectively, for the Δ MetABJ-Y-DG, relative to wild-type (**Figure 4**).

EXAMPLE 4

Validation of the bioavailability of extracellular methionine secreted from the engineered DG and CM strains

[0219] To confirm via an orthogonal approach the level and bioavailability of the extracellular methionine secreted from each engineered strain, the inventors supplemented fresh minimal media with medium at the end of the bacterial growth. Since no methionine exist in the fresh solution, the presence of methionine in the reused medium should reflect on the ability of the methionine-auxotroph *E. coli* Δ metAB bacteria to grow. To this end, the used media was sterilized by passing it through a 0.22 μ m membrane filter and added into a fresh minimal media in a 1:1 ratio. **Figure 5** shows the growth curves of the auxotroph Δ metAB cells in each of the conditions (i.e., Δ metABJ-Y-DG legend - growth curve of auxotroph Δ metAB cells in reused medium mixture after the Δ metABJ-Y-DG growth). The highest cell density is observed when the methionine-auxotroph cells were cultured when supplemented with the used medium of the Δ metABJ-Y-CM/DG, suggesting that the highest methionine level was in it. Owing to the absence of methionine in the medium, no growth was observed in the used medium of the WT or the auxotroph Δ metABJ. Both results are incongruent with the methionine levels that were measured for these strains in the GC-MS (**Figure 4**).

EXAMPLE 5

metX and metY of DG complements methionine secretion in various mutants

[0220] In the presence of exogenous metY and metX, methionine secretion decreases following the deletion of metABJ, compared to the deletion of metJ alone (**Fig. 6A**). This trend is maintained following overexpression of yjeH (16 vs 11 μ g/ml and 24 vs 16 μ g/ml respectively; **Fig. 6B**)

[0221] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

CLAIMS

What is claimed is:

1. A method of producing an amino acid, the method comprising:
 - (a) providing a bacterial cell comprising an exogenous nucleic acid molecule or a plurality thereof encoding metX and metY; and
 - (b) culturing said bacterial cell from step (a) such that said metX and metY encoded by said exogenous nucleic acid molecule are expressed, thereby, producing the amino acid.
2. The method of claim 1, wherein said bacterial cell further comprises at least one inactive: metA gene, metB gene, metJ gene, and any combination thereof.
3. The method of claim 1 or claim 2, wherein said exogenous nucleic acid molecule or plurality thereof further encodes an amino acid exporter protein, and optionally wherein said exporter protein is expressed.
4. The method of claim 3, wherein said exporter protein is L-methionine and BCAA exporter.
5. The method of any one of claims 1 to 4, wherein said amino acid is an essential amino acid.
6. The method of claim 5, wherein said essential amino acid is selected from the group consisting of: methionine, alanine, valine, leucine, threonine, isoleucine, glutamate, and any combination thereof.
7. The method of claim 6, wherein said methionine is L-methionine.
8. The method of any one of claims 1 to 7, wherein said culturing comprises supplementing said bacterial cell with a culture medium comprising an effective amount of a sulfur compound.
9. The method of claim 7 or 8, wherein the produced levels of said L-methionine being produced by said bacterial cell are at least 5-fold greater than levels of L-methionine being produced by a control bacterial cell.
10. The method of any one of claim 1 to 9, wherein said produced levels of said amino acid comprises intracellular levels, extracellular levels, or both, of said amino acid.
11. A bacterial cell comprising: (i) an exogenous nucleic acid molecule or a plurality thereof, encoding homoserine O-acetyltransferase (metX) and O-acetylhomoserine

sulfhydrylase (metY), and any one of: (ii) inactive homoserine O-succinyltransferase (metA) and cystathionine gamma-synthase (metB) genes; (iii) an inactive metJ methionine repressor gene; or both (ii) and (iii).

12. The bacterial cell of claim 11, wherein any one of: said inactive genes is knocked out, knocked down, mutated, or chemically inhibited.

13. The bacterial cell of claim 11 or claim 12, further comprising one or more inhibitory nucleic acid sequences being complementary to any one of: said metA, metB, and metJ genes.

14. The bacterial cell of claim 12 or claim 13, wherein said inactive gene being mutated comprises a nucleic acid sequence comprising a premature stop codon compared to a wildtype form of said gene.

15. The bacterial cell of any one of claims 11 to 14, wherein said encoded metX, comprises the amino acid sequence set forth in any one of SEQ ID Nos: 1-6, and 50.

16. The bacterial cell of any one of claims 11 to 15, wherein said encoded metX, comprises the amino acid sequence set forth in any one of SEQ ID Nos: 3 and 5.

17. The bacterial cell of any one of claims 11 to 16, wherein said encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 7-12.

18. The bacterial cell of any one of claims 11 to 17, wherein said encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 9 and 11.

19. The bacterial cell of any one of claims 11 to 18, wherein said exogenous nucleic acid molecule or a plurality thereof, comprises a nucleic acid sequence being codon optimized for expression in said bacterial cell.

20. The bacterial cell of any one of claims 11 to 19, being an *Escherichia coli* cell.

21. The bacterial cell of any one of claims 11 to 20, wherein said exogenous nucleic acid molecule encoding said metX comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 13-18.

22. The bacterial cell of any one of claims 11 to 21, wherein said exogenous nucleic acid molecule encoding said metY comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 19-24.

23. The bacterial cell of any one of claims 11 to 22, wherein said encoded metX, comprises the amino acid sequence set forth in any one of SEQ ID Nos: 3 and 5, and said encoded metY comprises the amino acid sequence set forth in any one of SEQ ID Nos: 9 and 11.

24. The bacterial cell of any one of claims 11 to 23, wherein said exogenous nucleic acid molecule encoding said metX comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 15 and 16 and said exogenous nucleic acid molecule encoding said metY comprises the nucleotide sequence set forth in any one of SEQ ID Nos: 21 and 22.

25. The bacterial cell of any one of claims 11 to 24, wherein said exogenous nucleic acid molecule or plurality thereof encoding said metX and said metY are operably linked.

26. The bacterial cell of any one of claims 11 to 25, wherein said exogenous nucleic acid molecule or plurality thereof, is: (i) integrated into the genome of said bacterial cell; or is comprised within a plasmid or an expression vector.

27. The bacterial cell of any one of claims 11 to 26, wherein said exogenous nucleic acid molecule or a plurality thereof comprises the nucleic acid sequence set forth in any one of SEQ ID Nos: 25-30.

28. The bacterial cell of any one of claims 11 to 27, further characterized by expression of the yjeH gene.

29. The bacterial cell of claim 28, comprising an L-methionine and branched chain amino acid (BCAA) exporter protein.

30. A composition comprising the bacterial cell of any one of claims 11 to 29, and a biologically accepted carrier.

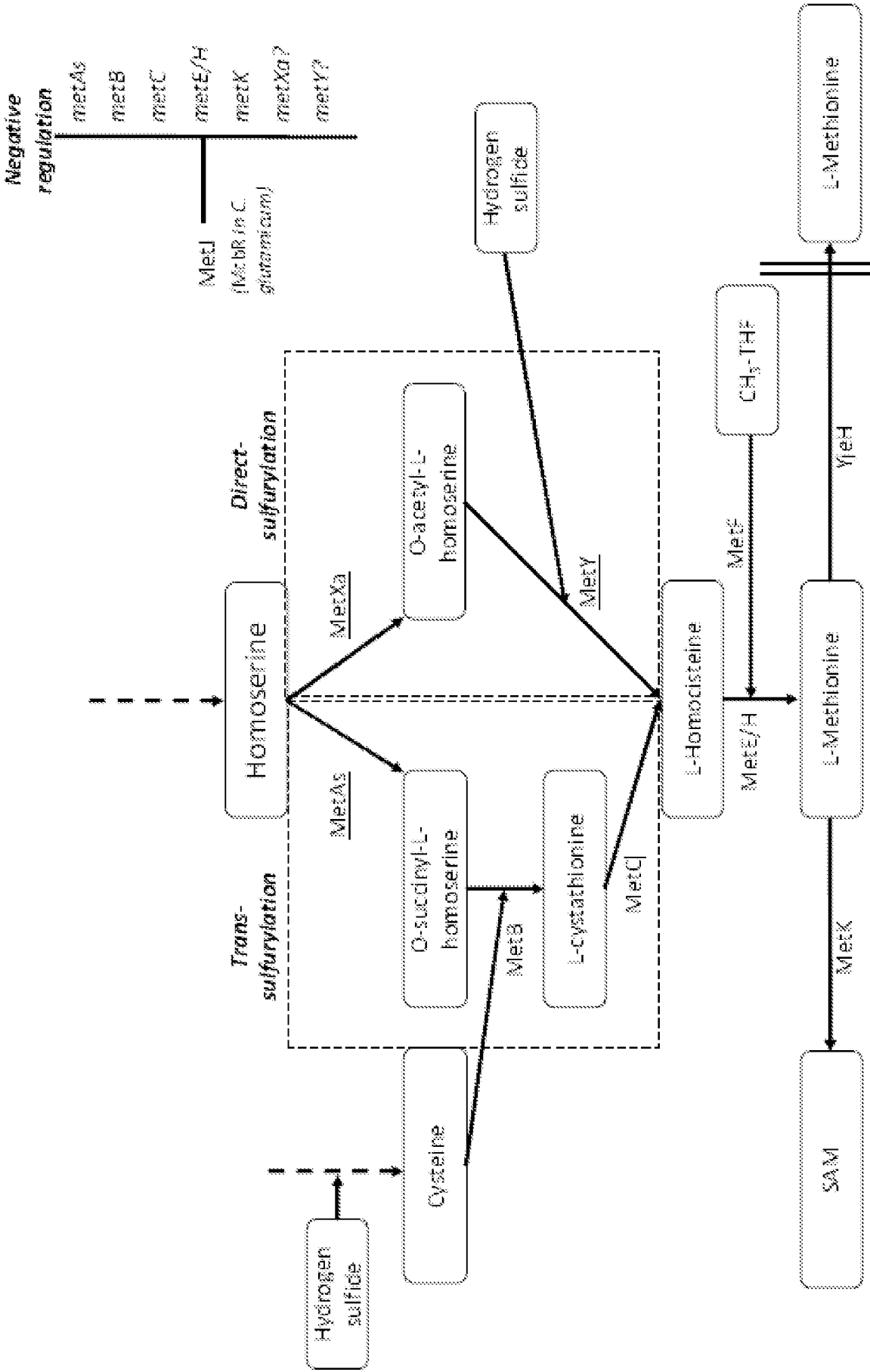


FIGURE 1

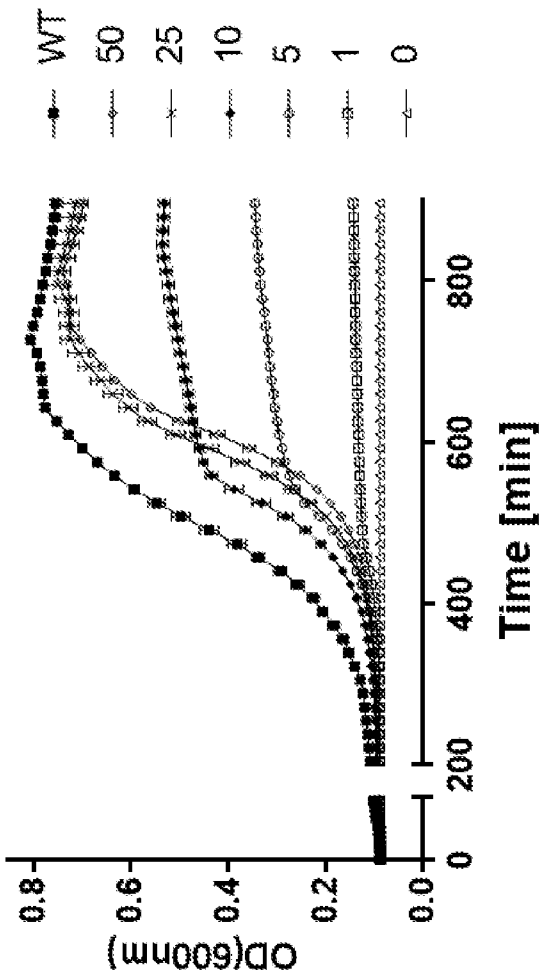


FIGURE 2

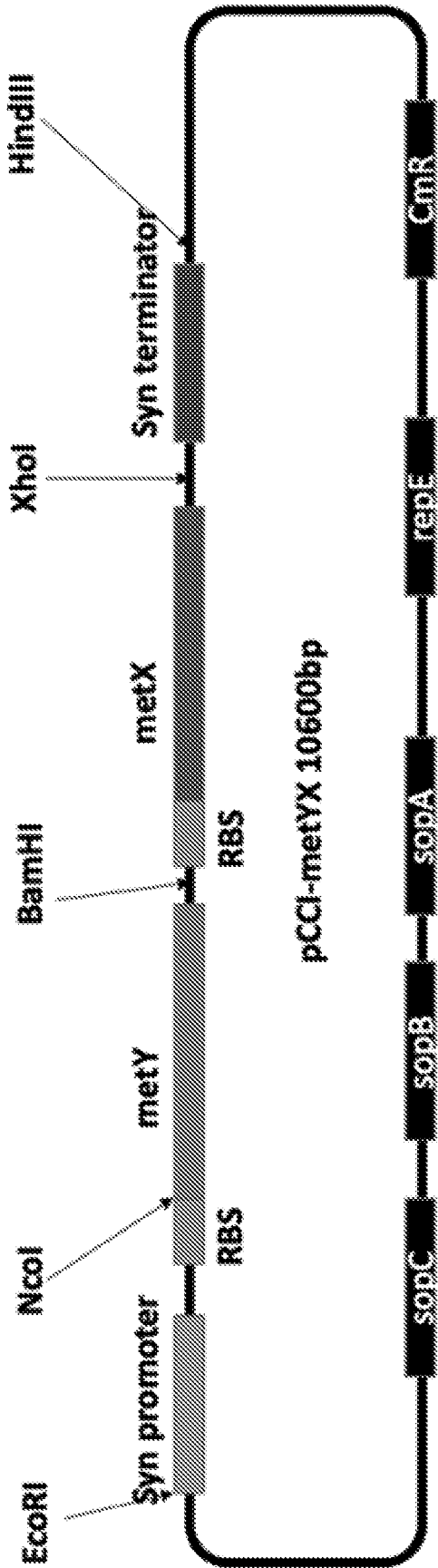


FIGURE 3A

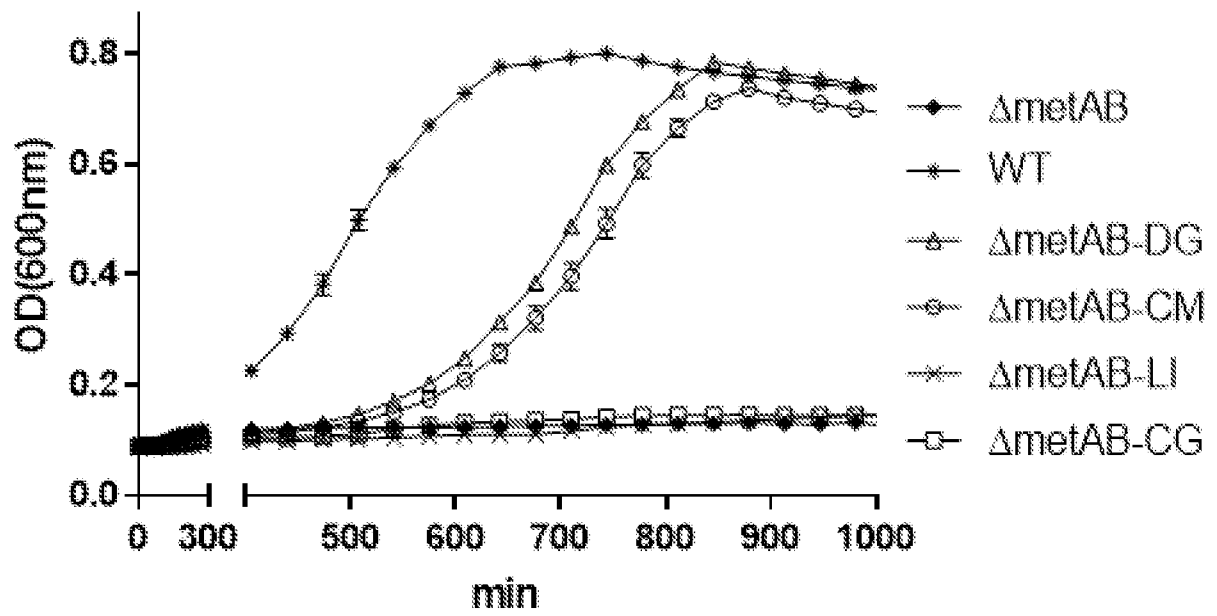


FIGURE 3B

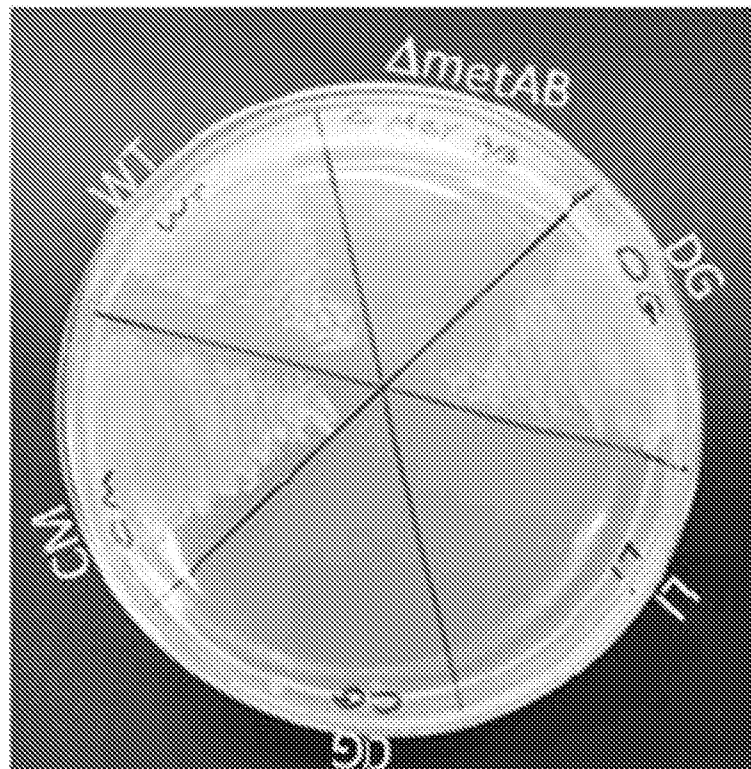


FIGURE 3C

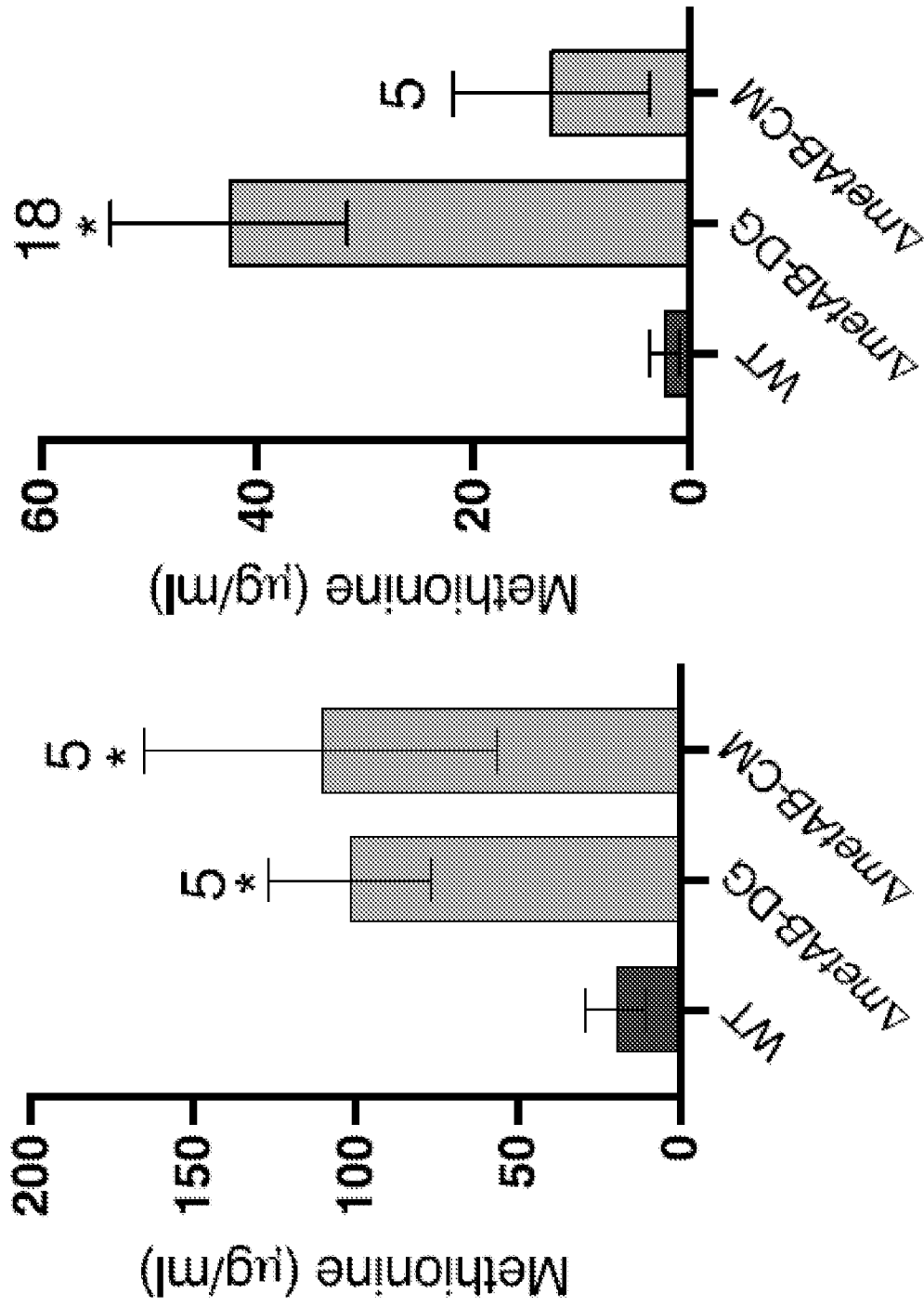


FIGURE 3D

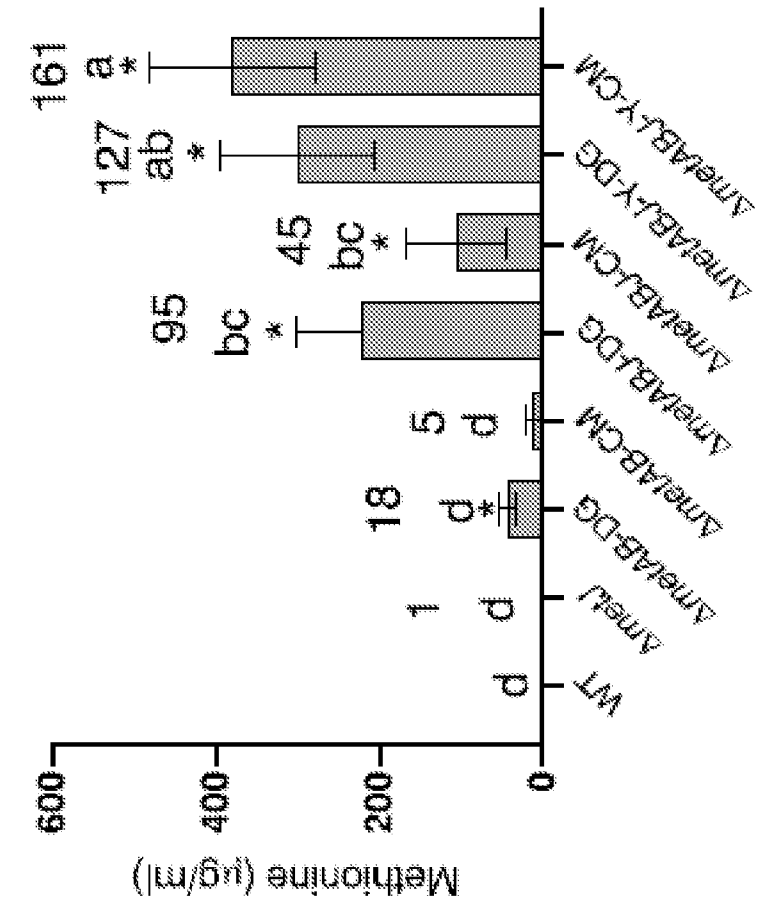


FIGURE 4B

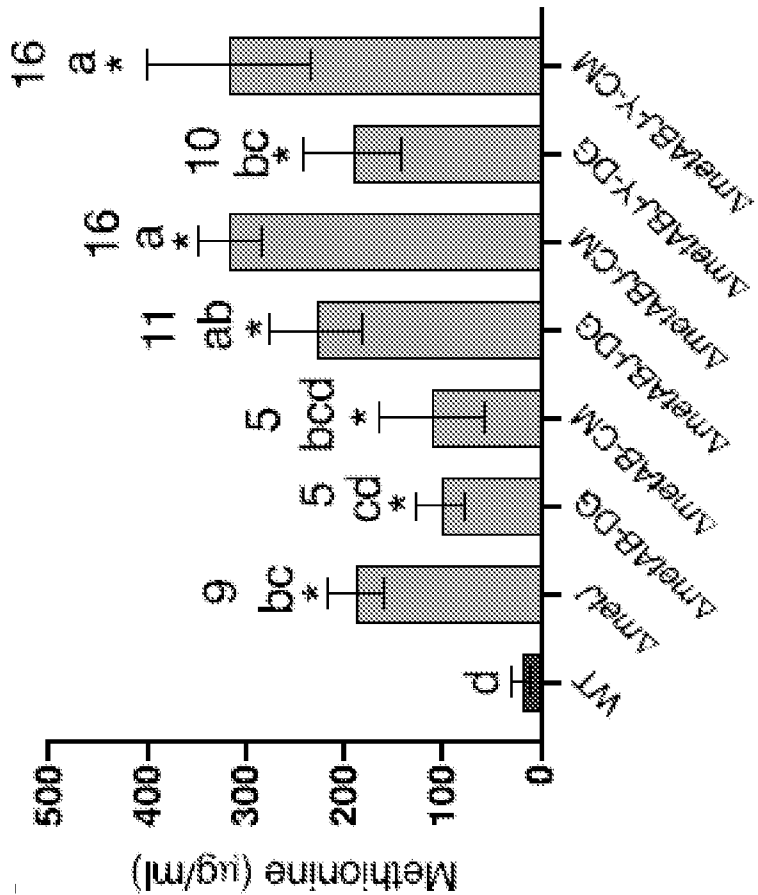


FIGURE 4A

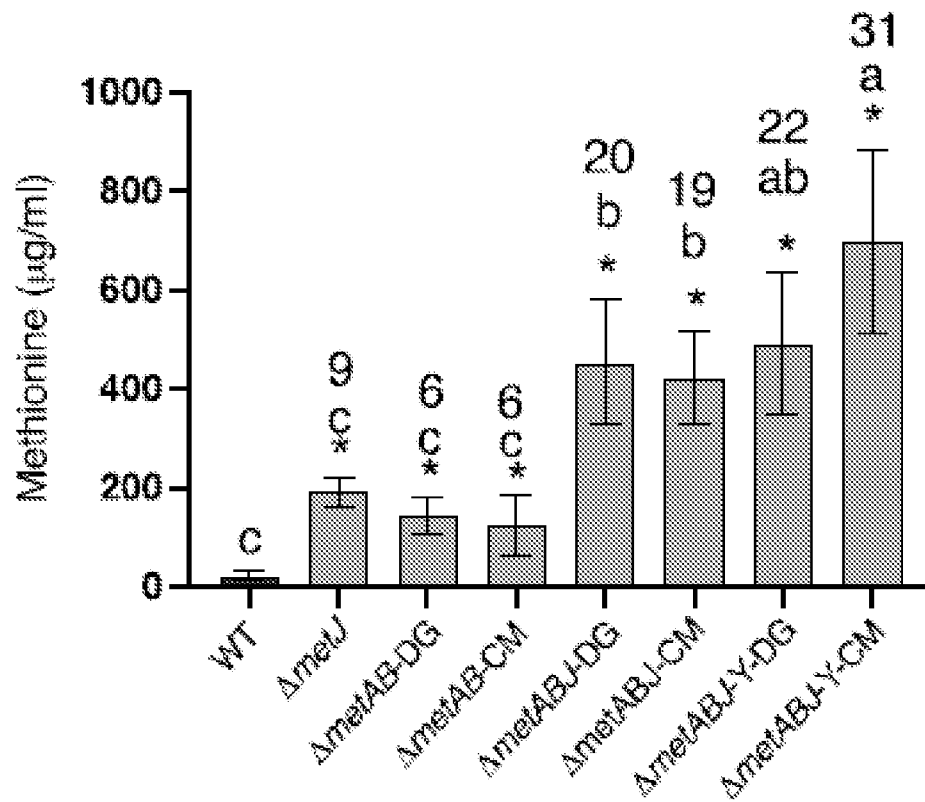


FIGURE 4C

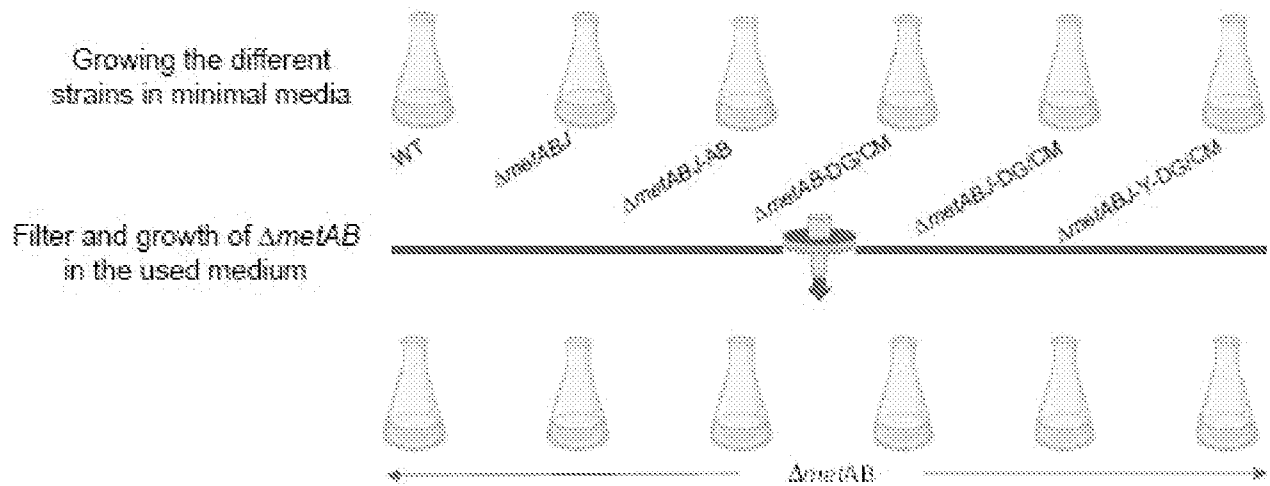


FIGURE 5A

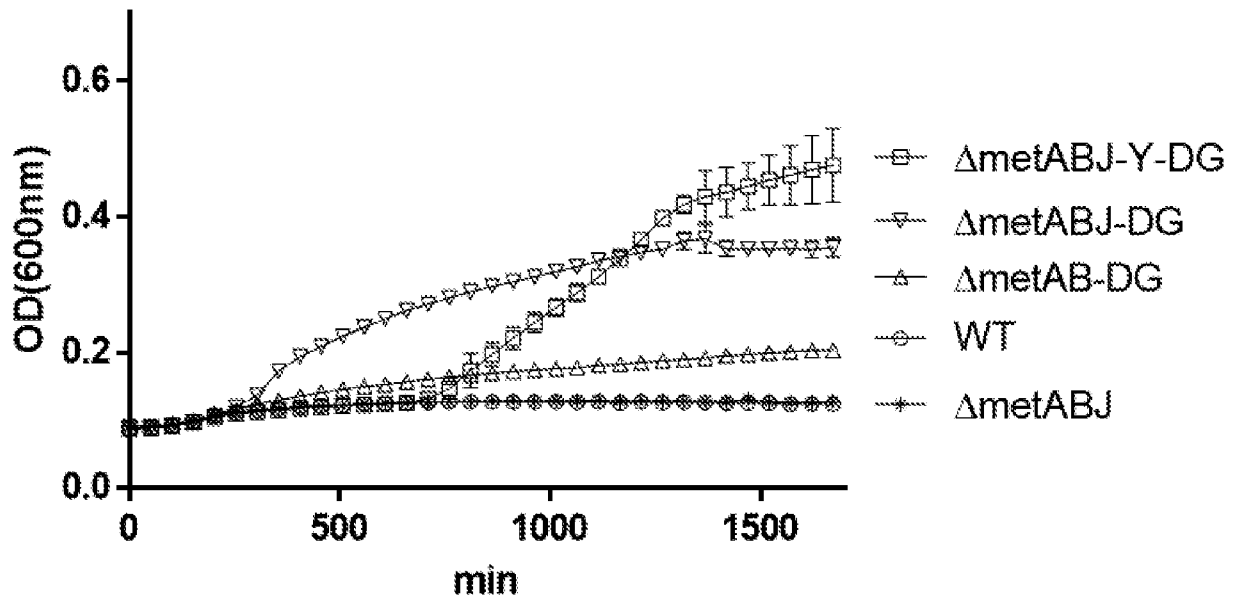


FIGURE 5B

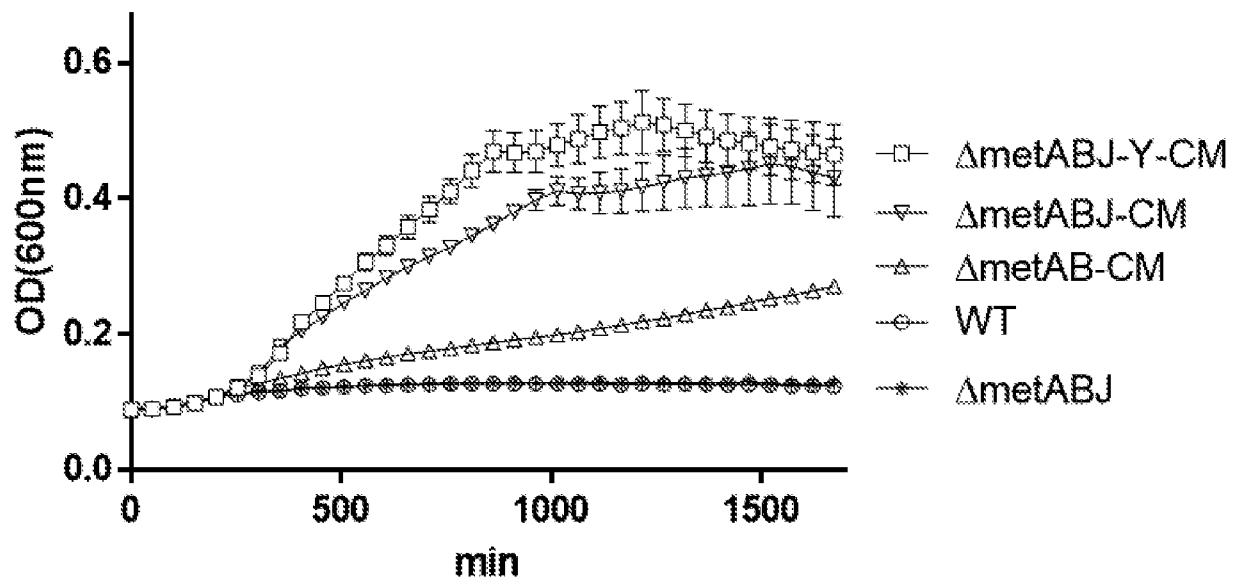
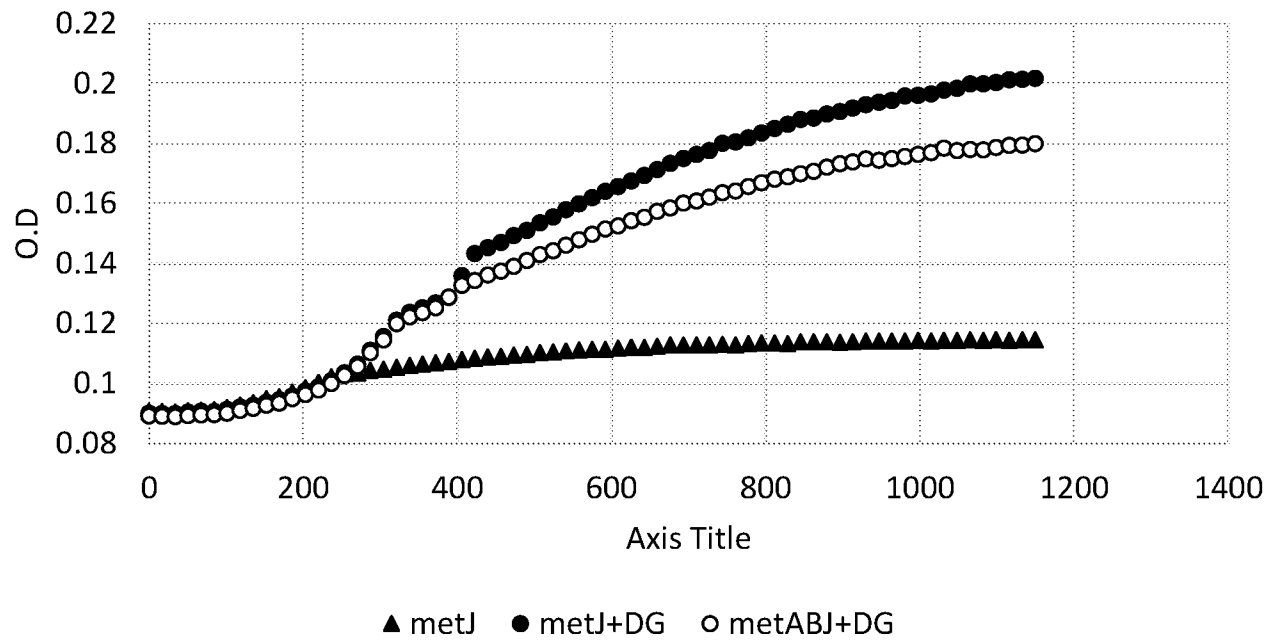
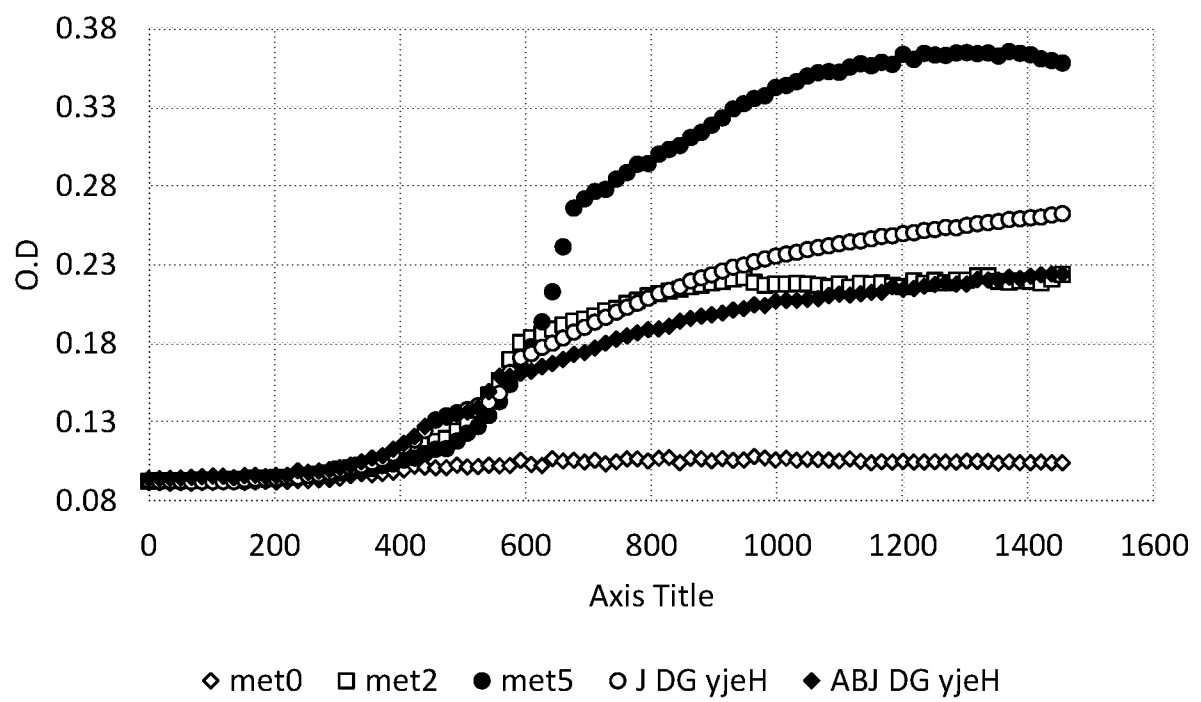


FIGURE 5C

**FIGURE 6A****FIGURE 6B**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2023/051295

A. CLASSIFICATION OF SUBJECT MATTER

C12P 13/12(2024.01)i; **C12N 1/20**(2024.01)i; **C12N 9/10**(2024.01)i; **C12P 13/06**(2024.01)i; **C12N 15/52**(2024.01)i;
C12N 15/63(2024.01)i; **C12N 15/70**(2024.01)i
 CPC:C12P 13/12; C12N 1/20; C12N 9/10; C12N 9/1029; C12P 13/06; C12N 15/52; C12N 15/63; C12N 15/70; C12Y
 203/01046

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)

C12P 13/12; C12N 1/20; C12N 9/10; C12P 13/06; C12N 15/52; C12N 15/63; C12N 15/70
 CPC:C12P 13/12; C12N 1/20; C12N 9/10; C12N 9/1029; C12P 13/06; C12N 15/52; C12N 15/63; C12N 15/70; C12Y
 203/01046

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: BLAST, Esp@cenet, Google Patents, PubMed, Google Scholar, Orbit Search terms used: L-methionine over-production DeltaMetA DeltaMetB DeltaMetJ deltaMetABJ knock out MetA MetB MetJ MetABJ MetXY MetX or MetY methionine knock-out of metx methionine Inventors

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RODRIGUES, Luisa. Designing an optimized met X/Y enzymes for an efficient methionine production in E. coli. A dissertation, University of Skövde, School of Bioscience 2022. RODRIGUES, Luisa (2022/07/12) page 1 para 3; page 2 para 2; page 3 para 2; fig. 2; page 3 para 3, 4; page 4 para 1-2; page 4	3,4,8,28-30
X	page 1 para 3; page 2 para 2; page 3 para 2; fig. 2; page 3 para 3, 4; page 4, para 1-2; page 4	1,2,5-7,9-27
Y	LIU, Qian, et al. YjeH is a novel exporter of L-methionine and branched-chain amino acids in Escherichia coli. Applied and Environmental Microbiology, 2015, 81.22: 7753-7766. LIU, Qian, et al. (2015/08/28) abstract and the whole paper	3,4,28,29

☒ 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
 “D” document cited by the applicant in the international application
 “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

13 March 2024

Date of mailing of the international search report

17 March 2024

Name and mailing address of the ISA/IL

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Authorized officer

COHEN Shmuel

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2023/051295

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013260424 A1 (Cheiljedang Corporation, Seoul [KR]) 03 October 2013 (2013-10-03) para 5; para 107, 108; para 124; para 126; para 127; para 145, para 1	8,30

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2023/051295

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☒ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/IL2023/051295

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2013260424	A1	03 October 2013	US	2013260424	A1	03 October 2013
				US	9150893	B2	06 October 2015
				CN	101688190	A	31 March 2010
				CN	101688190	B	04 June 2014
				EP	2132306	A1	16 December 2009
				EP	2132306	B1	19 December 2012
				EP	2520645	A2	07 November 2012
				EP	2520645	A3	20 February 2013
				EP	2520645	B1	14 January 2015
				EP	2808381	A1	03 December 2014
				EP	2808381	B1	09 March 2016
				EP	2808382	A1	03 December 2014
				EP	2808382	B1	16 March 2016
				ES	2400261	T3	08 April 2013
				ES	2534375	T3	22 April 2015
				ES	2575082	T3	24 June 2016
				ES	2575906	T3	04 July 2016
				JP	2010523145	A	15 July 2010
				JP	5497628	B2	21 May 2014
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				KR	101140906	B1	13 July 2012
				PL	2132306	T3	31 May 2013
				PL	2520645	T3	29 May 2015
				PL	2808381	T3	30 September 2016
				PL	2808382	T3	30 September 2016
				US	2011183383	A1	28 July 2011
				US	8551742	B2	08 October 2013
				US	2016024541	A1	28 January 2016
				US	9493801	B2	15 November 2016
				WO	2008127240	A1	23 October 2008