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(71) Applicant: **ZYMERGEN INC.** [US/US]; 5980 Horton Street, Suite 105, Emeryville, CA 94608 (US).

(72) Inventor: **MANCHESTER, Shawn**; 278 38th Street, Oakland, CA 94611 (US).

(74) Agent: **POMERANZ, Marcelo, C.** et al.; Cooley LLP, 1299 Pennsylvania Avenue, N.W., Suite 700, Washington, DC 20004 (US).

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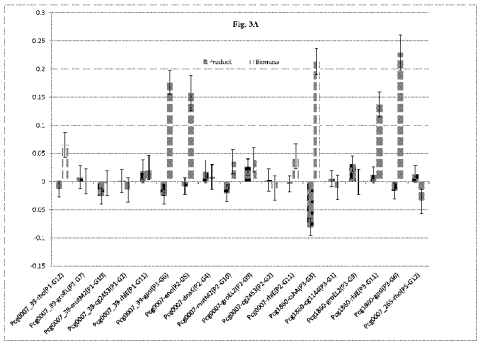
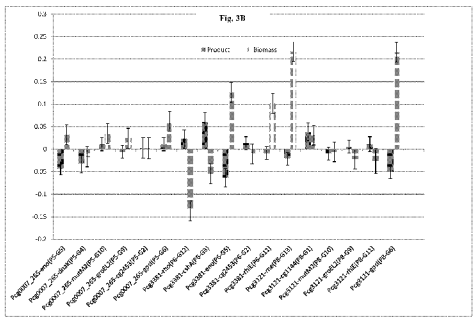
(54) **Title:** GENETIC PERTURBATION OF THE RNA DEGRADOSOME PROTEIN COMPLEX





(57) **Abstract:** The present disclosure provides novel bacterial strains with altered expression or start codon modification of one or more RNA degradation/processing genes. The RNA degradation genes of the present disclosure are controlled by heterologous promoters. The present disclosure further describes methods for generating microbial strains comprising heterologous promoter sequences operably linked to RNA degradation/processing genes.

IN THE UNITED STATES PATENT & TRADEMARK OFFICE PCT PATENT APPLICATION

GENETIC PERTURBATION OF THE RNA DEGRADOSOME PROTEIN COMPLEX

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. provisional application No. 62/358,201 filed on July, 5 2016, which is hereby incorporated by reference in its entirety, including all descriptions, references, figures, and claims for all purposes.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: ZYMR_007_01WO_SeqList_ST25.txt, date recorded: June 21, 2017, file size 113 kilobytes).

FIELD

[0003] The present disclosure is directed to microbial genomic engineering. The disclosed methods, compositions, and kits for RNA degradosome perturbations facilitate steady state modulation of selected mRNAs, and assist researchers in improving bacterial strain production efficiencies.

BACKGROUND

[0004] The regulation of bacterial gene expression occurs at many levels, including transcriptional control, or control of the synthesis of mRNA from a given gene; translational control, or the regulation of the efficiency by which the mRNA is translated into polypeptide sequence by the ribosome; and mRNA stability, or the efficiency at which a given mRNA population within the cell is processed and rendered inactive.

[0005] While methods for altering the transcription levels of mRNA have been widely studied, other techniques for optimizing the post-translational steady state of mRNA have remained more elusive.

[0006] In some embodiments, the present disclosure teaches methods for improving industrial strain efficiency, for example by modulating RNA stability through altering genetic sequences and reaction conditions.

SUMMARY OF THE DISCLOSURE

[0007] The present disclosure provides novel methods for improving the performance of industrial host organisms. In some embodiments, the methods of the present disclosure modulate mRNA steady states, and lead to improvements in biomass or product performance of the host strain.

[0008] In some embodiments, the present disclosure teaches a genetically engineered host cell with enhanced industrial performance, said host cell comprising: a) heterologous promoter polynucleotide, and b) a polynucleotide encoding an RNA degradation gene; wherein the heterologous promoter polynucleotide is operably linked to the polynucleotide.

[0009] In some embodiments, the RNA degradation gene of the present disclosure is an endogenous gene. In other embodiments, of the present disclosure, the host cell comprises a heterologous promoter polynucleotide operably linked to an exogenous RNA degradation enzyme.

[0010] In some embodiments, the exogenous coding polynucleotide is a gene derived from a different species. In other embodiments, the exogenous coding polynucleotide is an endogenous gene that has been mutated.

[0011] In some embodiments, the present disclosure teaches mutating the start codons of one or more of the host cell's RNA degradation enzymes, wherein the non-mutated start codon of the RNA degradation gene is changed from 'ATG' or 'GTG' to 'TTG.' For example, in some embodiments, the present disclosure teaches replacing ATG start codons with TTG. In some embodiments, the present disclosure teaches replacing ATG start codons with GTG. In some embodiments, the present disclosure teaches replacing GTG start codons with ATG. In some embodiments, the present disclosure teaches replacing GTG start codons with TTG. In some embodiments, the present disclosure teaches replacing TTG start codons with ATG. In some embodiments, the present disclosure teaches replacing TTG start codons with GTG.

[0012] In some embodiments the present disclosure teaches a genetically engineered host cell comprising a heterologous promoter operably linked to an RNA degradation gene, wherein the heterologous promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2,

SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

[0013] In some embodiments, the heterologous promoter of the present disclosure comprises a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

[0014] In some embodiments the heterologous promoter polynucleotide sequence is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 6. In some embodiments the heterologous promoter polynucleotide sequence is SEQ ID NO: 1. In some embodiments the heterologous promoter polynucleotide sequence is SEQ ID NO: 2. In some embodiments the heterologous promoter polynucleotide sequence is SEQ ID NO: 3. In some embodiments the heterologous promoter polynucleotide sequence is SEQ ID NO: 6.

[0015] In some embodiments the present disclosure teaches a genetically engineered host cell comprising a heterologous promoter operably linked to a polynucleotide encoding an RNA degradation gene, wherein the RNA degradation gene is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

[0016] In some embodiments the present disclosure teaches a genetically engineered host cell comprising a heterologous promoter operably linked to a polynucleotide encoding an RNA degradation gene, wherein the polynucleotide encodes for an RNA degradation protein is selected from the group consisting of SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47.

[0017] In some embodiments, the polynucleotide encoding the RNA degradation gene is selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 17, and SEQ ID NO: 20.

[0018] In some embodiments the polynucleotide encodes for an RNA degradation gene is selected from the group consisting of SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 45.

[0019] In some embodiments the present disclosure teaches a genetically engineered host cell comprising wherein the genetically engineered host cell comprises a combination of a heterologous promoter operably linked to a polynucleotide encoding an RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 10), **b-** (SEQ ID NO: 1:: SEQ ID NO: 14), **c-** (SEQ ID NO: 1:: SEQ ID NO: 18), **d-** (SEQ ID NO: 1:: SEQ ID NO: 20), **e-** (SEQ ID NO: 2:: SEQ ID NO: 11), **f-** (SEQ ID NO: 2:: SEQ ID NO: 18), **g-** (SEQ ID NO: 2:: SEQ ID NO: 13), **h-** (SEQ ID NO: 2:: SEQ ID NO: 18), **i-** (SEQ ID NO: 2:: SEQ ID NO: 17), **j-** (SEQ ID NO: 2:: SEQ ID NO: 19), **k-** (SEQ ID NO: 3:: SEQ ID NO: 11), **l-** (SEQ ID NO: 3:: SEQ ID NO: 14), **m-** (SEQ ID NO: 3:: SEQ ID NO: 12), **n-** (SEQ ID NO: 3:: SEQ ID NO: 15), **o-** (SEQ ID NO: 3:: SEQ ID NO: 17), **p-** (SEQ ID NO: 3:: SEQ ID NO: 19), **q-** (SEQ ID NO: 5:: SEQ ID NO: 14), **r-** (SEQ ID NO: 5:: SEQ ID NO: 11) (SEQ ID NO: 6:: SEQ ID NO: 13), **s-** (SEQ ID NO: 6:: SEQ ID NO: 19), **t-** (SEQ ID NO: 8:: SEQ ID NO: 21), **u-** (SEQ ID NO: 8:: SEQ ID NO: 14), **v-** (SEQ ID NO: 6:: SEQ ID NO: 20), **w-** (SEQ ID NO: 6:: SEQ ID NO: 11), **x-** (SEQ ID NO: 8:: SEQ ID NO: 9), and **y-** (SEQ ID NO: 8:: SEQ ID NO: 18).

[0020] In some embodiments, the RNA degradation gene is SEQ ID NO: 11. In some embodiments, the RNA degradation gene is SEQ ID NO: 12. In some embodiments, the RNA degradation gene is SEQ ID NO: 13. In some embodiments, the RNA degradation gene is SEQ ID NO: 14. In some embodiments, the RNA degradation gene is SEQ ID NO: 17. In some embodiments, the RNA degradation gene is SEQ ID NO: 20.

[0021] In some embodiments, the methods and compositions of the present disclosure are compatible with any species of host cell organism. In some embodiments, the methods of the present disclosure are applied to prokaryotic host cell. In some embodiments, the methods of the present disclosure are applied to bacteria host cells. In some embodiments, the methods of the present disclosure are applied to eukaryotic host cells.

[0022] In some embodiments, the methods of the present disclosure teach engineered host cell belongs to genus *Corynebacterium*. In some embodiments, the methods of the present disclosure teach genetically engineered host cell is *Corynebacterium glutamicum*.

[0023] In some embodiments, the present disclosure further teaches methods of producing a biomolecule comprising culturing a host cell of the disclosure under conditions suitable for producing the biomolecule.

[0024] In some embodiments, the biomolecule of the present disclosure is an amino acid, an organic acid, or an alcohol.

[0025] In some embodiments, the present disclosure teaches methods of producing amino acids selected from the group consisting of tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, and lysine.

[0026] In some embodiments, the present disclosure teaches methods of producing an organic acid selected from the group consisting of succinate, lactate and pyruvate.

[0027] In some embodiments, the present disclosure teaches methods of producing an alcohol, such as ethanol or isobutanol.

[0028] In some embodiments, the genetically engineered host cells of the present disclosure are capable of producing at least a 2% higher titer of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured under identical conditions.

[0029] In some embodiments, the genetically engineered host cells of the present disclosure are capable of producing at least a 3% higher titer of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured under identical conditions.

[0030] In some embodiments, the genetically engineered host cells of the present disclosure are capable of producing at least a 6% higher titer of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured under identical conditions.

[0031] In some embodiments, the present disclosure measures titer at carbon exhaustion to determine yield for a genetically engineered host. Thus in some embodiments, the genetically engineered host strains of the present disclosure produces at least about 2%-10% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

[0032] In some embodiments, the genetically engineered host cells of the present disclosure exhibit at least about 5% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions. In some embodiments, the genetically engineered host cells of the present disclosure exhibit at least about 10% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

[0033] In some embodiments, the genetically engineered host cells of the present disclosure exhibit at least about 20% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

[0034] In some embodiments, the present disclosure teaches a method for generating a host cell capable of increased production of a biomolecule , the method comprising: introducing a heterologous promoter polynucleotide into the genome of the host cell, wherein the heterologous promoter polynucleotide is operably linked to a polynucleotide encoding an RNA degradation gene, thereby creating a genetically engineered host cell; wherein the genetically engineered host cell produces a higher biomolecule yield compared to the biomolecule yield of a control host cell cultured under identical conditions, wherein the control host cell does not comprise the heterologous promoter polynucleotide.

[0035] In some embodiments, the present disclosure teaches a method for generating a host cell capable of increased saturation biomass, the method comprising: introducing a heterologous promoter polynucleotide into the genome of the host cell, wherein the heterologous promoter polynucleotide is operably linked to a polynucleotide encoding an RNA degradation gene, thereby creating a genetically engineered host cell; wherein the genetically engineered host cell exhibits increased saturation biomass compared to the saturation biomass of a control host cell cultured under identical conditions, wherein the control host cell does not comprise the heterologous promoter polynucleotide.

[0036] In some embodiments, the RNA degradation gene of the present disclosure is an endogenous gene. In other embodiments, of the present disclosure, the host cell comprises a heterologous promoter polynucleotide operably linked to an exogenous coding polynucleotide encoding and RNA degradation enzyme.

[0037] In yet other embodiments, the present disclosure teaches a genetically engineered prokaryotic host cell with enhanced industrial performance, said host cell comprising: a) a coding polynucleotide encoding an RNA degradation gene; and b) a mutation in the start codon of the coding polynucleotide of (a); wherein the mutation results in the replacement of the start codon of the coding polynucleotide with a different start codon.

[0038] Thus, in some embodiments, the present disclosure teaches a method for generating a host cell capable of increased production of a biomolecule, the method comprising: genetically modifying the host cell, wherein the modifying comprises mutating the start codon of an endogenous RNA degradation gene, wherein the modification generates a genetically engineered host cell expressing RNA degradation gene; wherein the genetically engineered host cell produces an increased amount of a biomolecule as compared to the amount of the biomolecule produced from a control host cell, wherein the control host cell does not comprise the mutated start codon.

[0039] In some embodiments, the present disclosure teaches a method for generating a host cell capable of increased yield of a biomolecule or increased saturation biomass, the method comprising: genetically modifying the host cell, wherein the modifying comprises mutating the start codon of an endogenous RNA degradation gene, wherein the modification generates a genetically engineered host cell; wherein the genetically engineered host cell has increased biomolecule yield as compared to the biomolecule yield of a control host cell, or wherein the genetically engineered host cell achieves higher saturation biomass as compared to the saturation biomass of the control host cell, wherein the control host cell does not comprise the start codon mutation of the genetically engineered host cell, and wherein the genetically engineered host cell and the control host cell are cultured under identical conditions.

[0040] In some embodiments, the present disclosure teaches mutating an RNA degradation gene selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22. In some embodiments, the present disclosure mutates SEQ ID NO: 19.

[0041] In some embodiments, the present disclosure teaches particular heterologous promoter::RNA degradation gene combinations designed to improve the biomass of host cell cultures. For example, in some embodiments, the present disclosure teaches a heterologous promoter::RNA degradation gene combination selected from the group consisting of: SEQ ID No: 1:: SEQ ID No: 20, SEQ ID No: 1:: SEQ ID No: 14, SEQ ID No: 2:: SEQ ID No: 13, SEQ ID No: 2:: SEQ ID No: 18, SEQ ID No: 2:: SEQ ID No: 17, SEQ ID No: 2:: SEQ ID No: 19, SEQ ID No: 3:: SEQ ID No: 11, SEQ ID No: 3:: SEQ ID No: 19, SEQ ID No: 3:: SEQ ID No: 14, SEQ ID No:

5:: SEQ ID No: 18, SEQ ID No: 5:: SEQ ID No: 14, SEQ ID No: 6:: SEQ ID No: 13, SEQ ID No: 6:: SEQ ID No: 19, SEQ ID No: 8:: SEQ ID No: 21, and SEQ ID No: 8:: SEQ ID No: 14.

[0042] In some embodiments, the present disclosure teaches particular heterologous promoter::RNA degradation gene combinations designed to improve the product production in host cell cultures. For example, in some embodiments, the present disclosure teaches a heterologous promoter::RNA degradation gene combination selected from the group consisting of: SEQ ID No: 2:: SEQ ID No: 17, SEQ ID No: 3:: SEQ ID No: 17, SEQ ID No: 6:: SEQ ID No: 20, SEQ ID No: 6:: SEQ ID No: 11, and SEQ ID No: 8:: SEQ ID No: 9.

[0043] In some embodiments, the present disclosure teaches particular start codon replacements of RNA degradation genes designed to improve the biomass of host cell cultures. For example, in some embodiments, the present disclosure teaches a start codon replacement of an RNA degradation gene selected from the group consisting of: GTG to TTG of SEQ ID No: 21, ATG to TTG of SEQ ID No: 14, GTG to TTG of SEQ ID No: 19, and ATG to TTG of SEQ ID No: 17.

[0044] In some embodiments, the present disclosure teaches particular start codon replacements of RNA degradation genes designed to improve product production of host cell cultures. For example, in some embodiments, the present disclosure teaches a start codon replacement of an RNA degradation gene selected from the group consisting of: GTG to TTG of SEQ ID No: 21, ATG to TTG of SEQ ID No: 10.

BRIEF DESCRIPTION OF THE FIGURES

[0045] Figure 1 depicts assembly of transformation plasmids of the present disclosure, and their integration into host organisms. The insert DNA is generated by combining one or more synthesized oligos in an assembly reaction. DNA inserts containing the desired sequence are flanked by regions of DNA homologous to the targeted region of the genome. These homologous regions facilitate genomic integration, and, once integrated, form direct repeat regions designed for looping out vector backbone DNA in subsequent steps. Assembled plasmids contain the insert DNA, and optionally, one or more selection markers.

[0046] Figure 2 depicts procedure for looping-out selected regions of DNA from host strains. Direct repeat regions of the inserted DNA and host genome can “loop out” in a recombination

event. Cells counter selected for the selection marker contain deletions of the loop DNA flanked by the direct repeat regions.

[0047] Figures 3A and 3B depict the results of several RNA degradation gene perturbation experiments, according to the methods of the present disclosure. The performance of genetically engineered treatment host cells comprising a heterologous promoter operably linked to a selected RNA degradation enzyme gene was determined. Bars on the graph represent a percent change in saturation biomass or product yield of the genetically engineered host cells over a control culture. Control cultures were genetically identical to the genetically modified cultures, except for lacking the operably linked heterologous promoters of the present disclosure. Error bars represent two standard deviations. Results are spread across Figures 3A and 3B to accommodate for the size of the data.

[0048] Figure 4 depicts the results of several RNA degradation gene perturbation experiments, according to the methods of the present disclosure. The performance of genetically engineered treatment host cells comprising a mutated start codon on a selected RNA degradation enzyme was determined. Bars on the graph represent a percent change in saturation biomass or product yield of the genetically engineered host cells over a control culture. Control cultures were genetically identical to the genetically modified cultures, except for lacking the mutated start codon of the present disclosure. Error bars represent two standard deviations.

[0049] Figure 5 depicts the product yield results of several RNA degradation gene perturbation experiments, according to the methods of the present disclosure. The performance of genetically engineered treatment host cells comprising a heterologous promoter operably linked to a selected RNA degradation enzyme was determined. Bars on the graph represent a percent change in product yield of the genetically engineered host cells over a control culture. Control cultures were genetically identical to the genetically modified cultures, except for lacking the operably linked heterologous promoters of the present disclosure. Error bars represent two standard deviations.

DETAILED DESCRIPTION

Definitions

[0050] While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

[0051] The term "a" or "an" refers to one or more of that entity, *i.e.*, can refer to a plural referents. As such, the terms "a" or "an", "one or more" and "at least one" are used interchangeably herein. In addition, reference to "an element" by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there is one and only one of the elements.

[0052] As used herein the terms "cellular organism" "microorganism" or "microbe" should be taken broadly. These terms are used interchangeably and include, but are not limited to, the two prokaryotic domains, Bacteria and Archaea. In some embodiments, the disclosure refers to the "microorganisms" or "cellular organisms" or "microbes" of lists/tables and figures present in the disclosure. This characterization can refer to not only the identified taxonomic genera of the tables and figures, but also the identified taxonomic species, as well as the various novel and newly identified or designed strains of any organism in said tables or figures. The same characterization holds true for the recitation of these terms in other parts of the Specification, such as in the Examples.

[0053] The term "prokaryotes" is art recognized and refers to cells which contain no nucleus or other cell organelles. The prokaryotes are generally classified in one of two domains, the Bacteria and the Archaea. The definitive difference between organisms of the Archaea and Bacteria domains is based on fundamental differences in the nucleotide base sequence in the 16S ribosomal RNA.

[0054] A "eukaryote" is any organism whose cells contain a nucleus and other organelles enclosed within membranes. Eukaryotes belong to the taxon Eukarya or Eukaryota. The defining feature that sets eukaryotic cells apart from prokaryotic cells (the aforementioned Bacteria and Archaea) is that they have membrane-bound organelles, especially the nucleus, which contains the genetic material, and is enclosed by the nuclear envelope.

[0055] The term "Archaea" refers to a categorization of organisms of the division Mendosicutes, typically found in unusual environments and distinguished from the rest of the prokaryotes by several criteria, including the number of ribosomal proteins and the lack of muramic acid in cell

walls. On the basis of ssrRNA analysis, the Archaea consist of two phylogenetically-distinct groups: Crenarchaeota and Euryarchaeota. On the basis of their physiology, the Archaea can be organized into three types: methanogens (prokaryotes that produce methane); extreme halophiles (prokaryotes that live at very high concentrations of salt (NaCl); and extreme (hyper) thermophilus (prokaryotes that live at very high temperatures). Besides the unifying archaeal features that distinguish them from Bacteria (i.e., no murein in cell wall, ester-linked membrane lipids, etc.), these prokaryotes exhibit unique structural or biochemical attributes which adapt them to their particular habitats. The Crenarchaeota consists mainly of hyperthermophilic sulfur-dependent prokaryotes and the Euryarchaeota contains the methanogens and extreme halophiles.

[0056] "Bacteria" or "eubacteria" refers to a domain of prokaryotic organisms. Bacteria include at least 11 distinct groups as follows: (1) Gram-positive (gram+) bacteria, of which there are two major subdivisions: (1) high G+C group (Actinomycetes, Mycobacteria, Micrococcus, others) (2) low G+C group (Bacillus, Clostridia, Lactobacillus, Staphylococci, Streptococci, Mycoplasmas); (2) Proteobacteria, e.g., Purple photosynthetic + non-photosynthetic Gram-negative bacteria (includes most "common" Gram-negative bacteria); (3) Cyanobacteria, e.g., oxygenic phototrophs; (4) Spirochetes and related species; (5) Planctomyces; (6) Bacteroides, Flavobacteria; (7) Chlamydia; (8) Green sulfur bacteria; (9) Green non-sulfur bacteria (also anaerobic phototrophs); (10) Radioresistant micrococci and relatives; (11) Thermotoga and Thermosipho thermophiles.

[0057] The terms "genetically modified host cell," "recombinant host cell," and "recombinant strain" are used interchangeably herein and refer to microorganisms that have been genetically modified by the cloning and transformation methods of the present disclosure. Thus, the terms include a host cell (*e.g.,* bacteria, yeast cell, fungal cell, CHO, human cell, etc.) that has been genetically altered, modified, or engineered, such that it exhibits an altered, modified, or different genotype and/or phenotype (*e.g.,* when the genetic modification affects coding nucleic acid sequences of the microorganism), as compared to the naturally-occurring microorganism from which it was derived. It is understood that the terms refer not only to the particular recombinant microorganism in question, but also to the progeny or potential progeny of such a microorganism.

[0058] The term "genetically engineered" may refer to any manipulation of a host cell's genome (e.g. by insertion or deletion of nucleic acids).

[0059] The term "strain improvement program" refers to any methods for improving host cell cultures. For example, the present disclosure teaches methods for genetically engineering host cells to exhibit improved performance.

[0060] The term "wild-type" describes a cell or multicellular organism that occurs in nature, i.e. a cell that has not been genetically modified.

[0061] The term "control" or "control host cell" refers to an appropriate comparator host cell for determining the effect of a genetic modification or experimental treatment. In some embodiments, the control host cell is a wild type cell. In other embodiments, a control host cell is genetically identical to the genetically modified host cell, save for the genetic modification differentiating the treatment host cell. Thus, the control for a genetically modified organism of the present disclosure, comprising a heterologous promoter sequence operably linked to an RNA degradation gene could be a genetically identical organism without the heterologous promoter.

[0062] The term "RNA degradation gene" or "RNA degradosome" should be taken broadly. These terms are used interchangeably and include, but are not limited to, any prokaryotic or eukaryotic gene associated with in-vivo RNA degradation, mRNA transcript processing, or other functions resulting in modulation of mRNA stability. In some embodiments, the RNA degradation genes of the present disclosure comprise any polynucleotide encoding for the polypeptide sequences encoded by the protein sequences disclosed in Table 1.

[0063] In some embodiments, the disclosure refers to the "RNA degradosome gene" or "RNA degradation genes" of lists/tables and figures present in the disclosure. This characterization can refer to not only the specific gene of the tables and figures, but also to homologs, orthologs, paralogs, or variants thereof. The same characterization holds true for the recitation of these terms in other parts of the Specification, such as in the Examples. For example, in some embodiments the present disclosure also encompasses RNA degradation polynucleotides and polypeptides disclosed in Table 12.

[0064] As used herein, the term "phenotype" refers to the observable characteristics of an individual cell, cell culture, organism, or group of organisms which results from the interaction between that individual's genetic makeup (i.e., genotype) and the environment.

[0065] As used herein, the term "chimeric" or "recombinant" when describing a nucleic acid sequence or a protein sequence refers to a nucleic acid, or a protein sequence, that links at least two heterologous polynucleotides, or two heterologous polypeptides, into a single macromolecule, or that re-arranges one or more elements of at least one natural nucleic acid or protein sequence. For example, the term "recombinant" can refer to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

[0066] As used herein, a "synthetic nucleotide sequence" or "synthetic polynucleotide sequence" is a nucleotide sequence that is not known to occur in nature or that is not naturally occurring. Generally, such a synthetic nucleotide sequence will comprise at least one nucleotide difference when compared to any other naturally occurring nucleotide sequence.

[0067] As used herein, the term "nucleic acid" refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, or analogs thereof. This term refers to the primary structure of the molecule, and thus includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modified nucleic acids such as methylated and/or capped nucleic acids, nucleic acids containing modified bases, backbone modifications, and the like. The terms "nucleic acid" and "nucleotide sequence" are used interchangeably.

[0068] As used herein, the term "gene" refers to any segment of DNA associated with a biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include non-expressed DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

[0069] As used herein, the term "homologous" or "homologue" or "ortholog" is known in the art and refers to related sequences that share a common ancestor or family member and are determined based on the degree of sequence identity. The terms "homology," "homologous," "substantially similar" and "corresponding substantially" are used interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the instant disclosure such as deletion or

insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the disclosure encompasses more than the specific exemplary sequences. These terms describe the relationship between a gene found in one species, subspecies, variety, cultivar or strain and the corresponding or equivalent gene in another species, subspecies, variety, cultivar or strain. For purposes of this disclosure, homologous sequences are compared. "Homologous sequences" or "homologues" or "orthologs" are thought, believed, or known to be functionally related. A functional relationship may be indicated in any one of a number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the same or similar biological function. Preferably, both (a) and (b) are indicated. Homology can be determined using software programs readily available in the art, such as those discussed in Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.), ALIGN Plus (Scientific and Educational Software, Pennsylvania) and AlignX (Vector NTI, Invitrogen, Carlsbad, CA). Another alignment program is Sequencher (Gene Codes, Ann Arbor, Michigan), using default parameters.

[0070] As used herein, the term "nucleotide change" refers to, e.g., nucleotide substitution, deletion, and/or insertion, as is well understood in the art. For example, mutations contain alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded protein or how the proteins are made.

[0071] As used herein, the term "protein modification" refers to, e.g., amino acid substitution, amino acid modification, deletion, and/or insertion, as is well understood in the art.

[0072] As used herein, the term "at least a portion" or "fragment" of a nucleic acid or polypeptide means a portion having the minimal size characteristics of such sequences, or any larger fragment of the full length molecule, up to and including the full length molecule. A fragment of a polynucleotide of the disclosure may encode a biologically active portion of a genetic regulatory element. A biologically active portion of a genetic regulatory element can be prepared by isolating a portion of one of the polynucleotides of the disclosure that comprises the genetic regulatory element and assessing activity as described herein. Similarly, a portion of a polypeptide may be 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, and so on, going up to the full length

polypeptide. The length of the portion to be used will depend on the particular application. A portion of a nucleic acid useful as a hybridization probe may be as short as 12 nucleotides; in some embodiments, it is 20 nucleotides. A portion of a polypeptide useful as an epitope may be as short as 4 amino acids. A portion of a polypeptide that performs the function of the full-length polypeptide would generally be longer than 4 amino acids.

[0073] For PCR amplifications of the polynucleotides disclosed herein, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any organism of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook et al.(2001) Molecular Cloning: A Laboratory Manual (3rd ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis et al., eds. (1990) PCR Protocols: A Guide to Methods and Applications (Academic Press, New York); Innis and Gelfand, eds. (1995) PCR Strategies (Academic Press, New York); and Innis and Gelfand, eds. (1999) PCR Methods Manual (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

[0074] The term "primer" as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach, thereby serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and composition (A/T vs. G/C content) of primer. A pair of bi-directional primers consists of one forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification.

[0075] The terms "stringency" or "stringent hybridization conditions" refer to hybridization conditions that affect the stability of hybrids, e.g., temperature, salt concentration, pH, formamide concentration and the like. These conditions are empirically optimized to maximize specific

binding and minimize non-specific binding of primer or probe to its target nucleic acid sequence. The terms as used include reference to conditions under which a probe or primer will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g. at least 2-fold over background). Stringent conditions are sequence dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe or primer. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na+ ion, typically about 0.01 to 1.0 M Na + ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for short probes or primers (e.g. 10 to 50 nucleotides) and at least about 60° C for long probes or primers (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringent conditions or "conditions of reduced stringency" include hybridization with a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37° C and a wash in 2×SSC at 40° C. Exemplary high stringency conditions include hybridization in 50% formamide, 1M NaCl, 1% SDS at 37° C, and a wash in 0.1×SSC at 60° C. Hybridization procedures are well known in the art and are described by e.g. Ausubel et al., 1998 and Sambrook et al., 2001. In some embodiments, stringent conditions are hybridization in 0.25 M Na2HPO4 buffer (pH 7.2) containing 1 mM Na2EDTA, 0.5-20% sodium dodecyl sulfate at 45°C, such as 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20%, followed by a wash in 5×SSC, containing 0.1% (w/v) sodium dodecyl sulfate, at 55°C to 65°C.

[0076] As used herein, "promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In some embodiments, the promoter sequence may consist of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence that can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. In some embodiments, the disclosure refers to the

promoter of lists/tables and figures present in the disclosure. This characterization can refer to not only the specific promoter, but also to variants thereof. The same characterization holds true for the recitation of these terms in other parts of the Specification, such as in the Examples. In some embodiments, the present disclosure teaches use of specific promoter::gene combinations.

[0077] As used herein, the term "heterologous" or "heterologous promoter" refers to a nucleic acid sequence which is not naturally found in the particular organism. A sequence may also be heterologous if it is placed outside of its normal sequence context. Thus, a "heterologous promoter" as used in the present disclosure may be a promoter sourced from a different species or strain, or may also be a promoter sequence sourced from the same species, but inserted into a different locus within the genetically engineered host cell.

[0078] As used herein, the term "endogenous," "endogenous gene," or "endogenous RNA degradation gene" refers to the naturally occurring copy of a gene, in the location in which it is found within the host cell genome. In the context of the present disclosure, operably linking a heterologous promoter to an endogenous RNA degradation gene means genetically inserting a heterologous promoter sequence in front of the RNA degradation gene, in the location where that gene is naturally present.

[0079] As used herein, the term "exogenous" is used interchangeably with the term "heterologous," and refers to a substance coming from some source other than its native source. For example, the terms "exogenous protein," or "exogenous gene" refer to a protein or gene from a non-native source or location, and that have been artificially supplied to a biological system. Artificially mutated variants of endogenous genes are considered "exogenous" for the purposes of this disclosure.

[0080] As used herein, the phrases "recombinant construct", "expression construct", "chimeric construct", "construct", and "recombinant DNA construct" are used interchangeably herein. A recombinant construct comprises an artificial combination of nucleic acid fragments, e.g., regulatory and coding sequences that are not found together in nature. For example, a chimeric construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. Such construct may be used by itself or may be used in conjunction with a vector. If a vector is used then the choice of vector is dependent upon

the method that will be used to transform host cells as is well known to those skilled in the art. For example, a plasmid vector can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells comprising any of the isolated nucleic acid fragments of the disclosure. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) EMBO J. 4:2411-2418; De Almeida et al., (1989) Mol. Gen. Genetics 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, immunoblotting analysis of protein expression, or phenotypic analysis, among others. Vectors can be plasmids, viruses, bacteriophages, pro-viruses, phagemids, transposons, artificial chromosomes, and the like, that replicate autonomously or can integrate into a chromosome of a host cell. A vector can also be a naked RNA polynucleotide, a naked DNA polynucleotide, a polynucleotide composed of both DNA and RNA within the same strand, a poly-lysine-conjugated DNA or RNA, a peptide-conjugated DNA or RNA, a liposome-conjugated DNA, or the like, that is not autonomously replicating. As used herein, the term "expression" refers to the production of a functional end-product e.g., an mRNA or a protein (precursor or mature).

[0081] The term "operably linked" means in this context, the sequential arrangement of the promoter polynucleotide according to the disclosure with a further oligo- or polynucleotide, resulting in transcription of said further polynucleotide. In some embodiments, the promoter sequences of the present disclosure are inserted just prior to a gene's 5'UTR, or open reading frame. In other embodiments, the operably linked promoter sequences and gene sequences of the present disclosure are separated by one or more linker nucleotides.

[0082] The term "carbon source" generally refers to a substance suitable to be used as a source of carbon for cell growth. Carbon sources include, but are not limited to, biomass hydrolysates, starch, sucrose, cellulose, hemicellulose, xylose, and lignin, as well as monomeric components of these substrates. Carbon sources can comprise various organic compounds in various forms, including, but not limited to polymers, carbohydrates, acids, alcohols, aldehydes, ketones, amino acids, peptides, etc. These include, for example, various monosaccharides such as glucose, dextrose (D-glucose), maltose, oligosaccharides, polysaccharides, saturated or unsaturated fatty acids, succinate, lactate, acetate, ethanol, etc., or mixtures thereof. Photosynthetic organisms can

additionally produce a carbon source as a product of photosynthesis. In some embodiments, carbon sources may be selected from biomass hydrolysates and glucose.

[0083] The term “feedstock” is defined as a raw material or mixture of raw materials supplied to a microorganism or fermentation process from which other products can be made. For example, a carbon source, such as biomass or the carbon compounds derived from biomass are a feedstock for a microorganism that produces a product of interest (e.g. small molecule, peptide, synthetic compound, fuel, alcohol, etc.) in a fermentation process. However, a feedstock may contain nutrients other than a carbon source.

[0084] The term "product of interest" or "biomolecule" as used herein refers to any product produced by microbes from feedstock. In some cases, the product of interest may be a small molecule, enzyme, peptide, amino acid, organic acid, synthetic compound, fuel, alcohol, etc. For example, the product of interest or biomolecule may be any primary or secondary extracellular metabolite. The primary metabolite may be, inter alia, ethanol, citric acid, lactic acid, glutamic acid, glutamate, lysine, threonine, tryptophan and other amino acids, vitamins, polysaccharides, etc. The secondary metabolite may be, inter alia, an antibiotic compound like penicillin, or an immunosuppressant like cyclosporin A, a plant hormone like gibberellin, a statin drug like lovastatin, a fungicide like griseofulvin, etc. The product of interest or biomolecule may also be any intracellular component produced by a microbe, such as: a microbial enzyme, including: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, and many others. The intracellular component may also include recombinant proteins, such as: insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase and others.

[0085] The term “volumetric productivity” or “production rate” is defined as the amount of product formed per volume of medium per unit of time. Volumetric productivity can be reported in gram per liter per hour (g/L/h).

[0086] The term “specific productivity” is defined as the rate of formation of the product. To describe productivity as an inherent parameter of the microorganism and not of the fermentation process, productivity is herein further defined as the specific productivity in gram product per gram of cell dry weight (CDW) per hour (g/g CDW/h). Using the relation of CDW to OD600 for the

given microorganism specific productivity can also be expressed as gram product per liter culture medium per optical density of the culture broth at 600 nm (OD) per hour (g/L/h/OD).

[0087] The term "yield" is defined as the amount of product obtained per unit weight of raw material and may be expressed as g product per g substrate (g/g) ("Product Yield"). Yield may be expressed as a percentage of actual yield over theoretical yield ("Percent Yield"). "Theoretical yield" is defined as the maximum amount of product that can be generated per a given amount of substrate as dictated by the stoichiometry of the metabolic pathway used to make the product.

[0088] The term "titre" or "titer" is defined as the strength of a solution or the concentration of a substance in solution. For example, the titre of a product of interest (e.g. small molecule, peptide, synthetic compound, fuel, alcohol, etc.) in a fermentation broth is described as g of product of interest in solution per liter of fermentation broth (g/L). In some embodiments, the present disclosure teaches that biomass of a culture can be measured at any time (*e.g.*, titer measurements after a predetermined culture time, or at carbon exhaustion). Titer measured at carbon exhaustion is also considered reflective of the yield of the culture in those conditions.

[0089] The term "total titer" is defined as the sum of all product of interest produced in a process, including but not limited to the product of interest in solution, the product of interest in gas phase if applicable, and any product of interest removed from the process and recovered relative to the initial volume in the process or the operating volume in the process.

[0090] The term "biomass" refers to the cell density of a culture. In some embodiments the biomass the of the cultures of the present disclosure are determined by the optical density of their cultures at 600 nm spectrum minus the optical density of a corresponding control media without the organism. In other embodiments, "biomass" can be measured by other metrics, such as cell density (*e.g.*, via a hemocytometer) or by culture weight after separating cells from their culture media. In some embodiments, the present disclosure teaches that biomass of a culture can be measured at any time.

[0091] The term "saturation biomass" refers to the peak biomass achieved by the host cell under a given set of conditions. For example, in some embodiments, saturation mass can refer to the maximum biomass achieved by a culture in a specific growth media, at a specific temperature and light conditions. In some embodiments, the present disclosure compares the saturation biomass of

various modified microbes under identical growth conditions, such that any differences in saturation biomass are due to the underlying genetic differences between the tested microbes.

Optimizing Gene Expression

[0092] In some embodiments, the present disclosure teaches methods of improving host cell productivity through modulation of cellular gene expression. Gene expression is the measurable output of a multi-level regulatory process comprising transcriptional control of RNA synthesis, mRNA stability, mRNA translation into protein, and protein stability. Although much attention has been devoted to the control of gene expression through the transcriptional modulation of genes (*e.g.,* by changing promoters, or inducing regulatory transcription factors), comparatively few efforts have been made towards understanding the post-transcriptional regulation of desirable genes.

[0093] In some embodiments, the present disclosure teaches methods of improving cellular expression of desirable genes by perturbing an organism's RNA degradation genes (*e.g.,* the RNA degradosome). The RNA degradosome is responsible for the degradation of intracellular RNA, including mRNA, and as such plays an important role in the steady state concentration of RNA in the cell.

[0094] In some embodiments, the present invention is based, in part, on the inventor's recognition that increasing the transcription rate of desirable genes does not always lead to increased expression of the desired protein. In prokaryotes, the tight coupling between transcription and translation is important for mRNA stability. For example, when a prokaryotic mRNA is over expressed through increased transcription by a fast T7 RNAP, long stretches of ribosome-free message occur, rendering the untranslated mRNAs unstable. (Makarova, O. V. et al. (1995) "Transcribing of Escherichia coli genes with mutant T7 RNA polymerases: stability of lacZ mRNA inversely correlates with polymerase speed" Proc Natl Acad Sci USA 92: 12250-12254).

[0095] Without wishing to be bound to any one theory, the present inventors believe that modulation of a cell's RNA degradation genes can improve cellular efficiency by optimizing the mRNA stability of selected genes of interest. Steady state RNA concentrations affect a number of cellular phenotypes through a variety of mechanisms, including serving as the template for protein production in the case of mRNA or regulation of mRNA production through non-coding (nc) RNA. Because the degradosome acts as a hub for RNA degradation, and is composed of a variety of

different proteins which operate on different types of RNA to various extents, systematic perturbation of the genes in the degradosome can have complex transcriptome wide effects, and thus complex effects on phenotype. Coupled with a method to determine the effect of each perturbation on the phenotype of interest in high throughput, this invention allows for the discovery and stacking of perturbations that lead to improved microbial performance across a variety of phenotypes.

RNA Degradosome and RNA Degradation Genes

[0096] In some embodiments, the present disclosure teaches RNA degradation and processing genes. In some embodiments, the RNA degradation genes of the present disclosure are part of the RNA degradosome. The RNA degradosome of *Escherichia coli* is a multienzyme complex that was discovered during efforts to purify and characterize RNase E (Carpousis, A. J. et al. (1994) "Copurification of E. coli RNAase E and PNPase: evidence for a specific association between two enzymes important in RNA processing and degradation" Cell 76: 889-900; Carpousis, A. J. et al. (1999) "mRNA degradation A tale of poly(A) and multiprotein machines" Trends Genet 15: 24-28; Miczak, A. et al. (1996) "Proteins associated with RNase E in a multicomponent ribonucleolytic complex" Proc Natl Acad Sci USA 93: 3865-3869).

[0097] RNase E is a single-strand-specific endonuclease, and is believed to be the principal endonuclease in *E. coli* messenger RNA decay (Regnier, P. and Arraiano, C. M. (2000) "Degradation of mRNA in bacteria: emergence of ubiquitous features" Bioessays 22: 235-244). RNase E is a large, 1061 residue protein, with its nucleolytic activity resides in the N-terminal half of the protein. The C-terminal half of the protein contains a proline rich linker, an arginine rich RNA binding domain (RBD) and a region that is the scaffold for protein-protein interactions with the other components of the degradosome (Carpousis, A.J. 2007 "The RNA Degradosome of Escherichia coli: An mRNA-Degrading Machine Assembled on RNase E" Annu. Rev. Microbiol. 61:71-87).

[0098] Proteins related to RNase E are found throughout the eubacterial kingdom and in some plants (Condon, C. et al. (2001) "Identification of the gene encoding the 5S ribosomal RNA maturase in Bacillus subtilis: mature 5S rRNA is dispensable for ribosome function" RNA 7: 242-253). The plant homologues are presumably in the chloroplast, which is an organelle of eubacterial origin. An RNase E-based degradosome was recently identified in Rhodobacter capsulatus (Jager,

S. et al. (2001) "An mRNA degrading complex in Rhodobacter capsulatus" Nucleic Acids Res 29: 4581-4588).

[0099] The RNAse E complex is believed to contain two DEAD proteins and the transcription termination factor Rho, and PNPase and enolase (Carpousis, A.J. 2007 "The RNA Degradosome of Escherichia coli: An mRNA-Degrading Machine Assembled on RNase E" Annu. Rev. Microbiol. 61:71-87). *E. coli* also encodes a paralogue of RNase E now known as RNase G (Li, Z. et al. (1999) "RNase G (CafA protein) and RNase E are both required for the 5′ maturation of 16S ribosomal RNA" Embo J 18: 2878-2885.). It has significant homology to the N-terminal catalytic domain of RNase E but is smaller because it lacks a c-terminal half. The 'RNase E/G' family of proteins can thus be divided into two groups: the large RNase E-like enzymes that can form degradosomes and the small RNase G-like enzymes that apparently act alone.

[0100] The other integral components of the degradosome are enolase, an RNA helicase (RhlB) and polynucleotide phosphorylase (PNPase). RhlB is a member of the DEAD-box family of RNA helicases (Schmid, S. R., and Linder, P. (1992) "D-E-A-D protein family of putative RNA helicases Mol Microbiol 6: 283-291). PNPase, a single-strand-specific exonuclease, is a member of the RNase PH family of 3′→5′ RNA degrading enzymes (Deutscher, M. P., and Li, Z. (2001) "Exoribonucleases and their multiple roles in RNA metabolism" Prog Nucleic Acid Res Mol Biol 66: 67-105; Symmons, M., Williams et al. (2002) "Running rings around RNA: a superfamily of phosphate-dependent RNases" Trends Biochem. Sci., 27: 11-18). Members of both families are found in a wide range of prokaryotic and eukaryotic organisms.

[0101] Experiments in vitro demonstrated that RhlB in the degradosome facilitates the degradation of structured RNA by PNPase (Coburn, G. A. et al. (1999) "Reconstitution of a minimal RNA degradosome demonstrates functional coordination between a 3′ exonuclease and a DEAD-box RNA helicase" Genes Dev 13: 2594-2603; Py, B. et al. (1996) "A DEAD-box RNA helicase in the Escherichia coli RNA degradosome" Nature 381: 169-172).

[0102] The Rho I enzyme (Rho factor) is another important regulator of mRNA expression, and Rho-dependent transcriptional termination. Thus in some embodiments, the present disclosure teaches methods of perturbing Rho I. Rho-dependent transcriptional termination is responsible for regulating about half of all of *E.coli*'s transcribed genes. Other termination factors discovered in *E. coli* include Tau and nusA (See Sandy B. Primrose and Richard Twyman (2006) "Principles of

Gene Manipulation and Genomics" John Wiley & Sons ISBN 1-4051-3544-1). Rho is a member of the family of ATP-dependent hexameric helicases that function by wrapping nucleic acids around a single cleft extending around the entire hexamer. Rho binds to RNA and then uses its ATPase activity to provide the energy to translocate along the RNA until it reaches the RNA–DNA helical region, where it unwinds the hybrid duplex structure, and leads to transcriptional termination.

[0103] In some embodiments, the present disclosure also teaches use of other DEAD-box helicases, such as CshA, which has been associated with gene expression in stress conditions (Oun, S. et al. "The CshA DEAD-box RNA helicase is important in quorum sensing control in Staphylococcus aureus. RNA Biol. 2013 10(1): 157-165; Hunger, K. et al. "Cold-Induced Putative DEAD Box RNA Helicases CshA and CshB Are Essential for Cold Adaptation and Interact with Cold Shock Protein B in Bacillus subtilis" J Bacteriol. 2006 188(1): 240-248).

[0104] Other helicases with regulatory effect on RNA steady state are also within the scope of the present disclosure. For example, the present disclosure teaches perturbation of the rhlE gene. In E. coli RhlE RNA helicase regulates the function of related RNA helicases during ribosome assembly (Jain, C. "The E. coli RhlE RNA helicase regulates the function of related RNA helicases during ribosome assembly" RNA. 2008 14(2) 381-389).

[0105] In some embodiments, the RNA degradation enzymes of the present disclosure comprise selected protein chaperone genes. For example, in some embodiments, the present disclosure teaches groEL and groEL2 genes. GroEL is a member of the hsp60 family of heat shock proteins. GroEL is a tetradecamer wherein each monomeric subunit has a molecular weight of approximately 57 kD. GroEL facilitates the folding of a number of proteins by two mechanisms; (1) it prevents aggregation by binding to partly folded proteins (Goloubinoff P et al (1989) Nature 342: 884-889; Zahn R and Pluickthun A (1992) Biochemistry 21: 3249-3255), which then refold on GroEL to a native-like state (Zahn R and Plückthun A (1992) Biochemistry 21: 3249-3255; Gray T E and Fersht A R (1993) J Mol Biol L: 1197-1207); and (2) it continuously anneals misfolded proteins by unfolding them to a state from which refolding can start again (Zahn R et al (1996) Science 271: 642-645). Some mutations in the apical domain led to a decrease in polypeptide binding (Fenton W A et al (1994) Nature 371: 614-619), suggesting that this domain is involved in the binding of polypeptides.

[0106] In other embodiments, the present disclosure teaches perturbation of DnaK. DnaK has been demonstrated to be the central protein in a multiprotein bacterial chaperone system including the chaperone protein DnaK and a variety of co-chaperone proteins such as DnaJ and GrpE. The co-chaperone proteins are essential to the efficient physiological processing of both natural and unnatural substrates. One role for this chaperone system is to catalyze the refolding of either unfolded or misfolded bacterial proteins, as is evident from the role of this system in the heat-shock response. An additional role of the DnaK chaperone system is the regulation of gene expression through the processing of specific RNA polymerase subunits.

[0107] In some embodiments, other genes involved in gene expression are within the scope of the present disclosure. For example. In some embodiments, the present disclosure teaches gpsI. GpsI is a putative multifunctional enzyme involved in guanosine pentaphosphate synthesis and polyribonucleotide nucleotidyltransferase. E. coli polynucleotide phosphorylase, purified GPSI was shown to catalyze the polymerization of ADP and the phosphorolysis of poly(A) (Jones, G. and Bibb, M. "Guanosine Pentaphosphate Synthetase from Streptomyces antibioticus Is Also a Polynucleotide Phosphorylase" J. of Bact. 1996 July 4281-4288.).

[0108] In other embodiments, the present disclosure teaches Enolase (eno). Enolase, also known as phosphopyruvate hydratase, is a metalloenzyme responsible for the catalysis of the conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP), the ninth and penultimate step of glycolysis. (See for example, U.S. Patent No. 7,118,904).

[0109] In some embodiments, the present invention teaches modulating gene expression of a host cell by perturbing one or more RNA degradation genes. Persons having skill in the art will recognize that RNA degradation gene perturbations of the present disclosure can, in some embodiments, comprise any method for the modification of proteins that are members of the RNA degradosome for the purpose of optimizing a particular cellular phenotype. In some embodiments, perturbation of an RNA degradation gene can comprise directed or random genetic mutation of the gene sequence itself. In other embodiments, perturbation of an RNA degradation gene can comprise modulating expression with mutated endogenous or exogenous promoters.

[0110] A non-exhaustive list of the RNA degradation genes of the present disclosure is provided in Table 1 below.

Table 1. Selected RNA degradation genes of the present disclosure

Species	Gene name	SEQ ID (cDNA)	SEQ ID (Protein)	Gene "short name"
Corynebacterium glutamicum	cg1144	9	34	G1
Corynebacterium glutamicum	cg2453	10	35	G2
Corynebacterium glutamicum	cshA	11	36	G3
Corynebacterium glutamicum	dnaK	12	37	G4
Corynebacterium glutamicum	eno	13	38	G5
Corynebacterium glutamicum	gpsI	14	39	G6
Corynebacterium glutamicum	groEL	15	40	G7
Corynebacterium glutamicum	groEL homolog	16	41	G8
Corynebacterium glutamicum	groEL2	17	42	G9
Corynebacterium glutamicum	mutM2	18	43	G10
Corynebacterium glutamicum	rhlE	19	44	G11
Corynebacterium glutamicum	rho	20	45	G12
Corynebacterium glutamicum	rne (RNAse E)	21	46	G13
Corynebacterium glutamicum	cg2160/ RNAse J	22	47	G14

[0111] In some embodiments, the RNA degradation genes of the present invention exhibit at least 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, or 75% sequence identity with a gene (either cDNA, or protein) from Table 1.

Promoters

[0112] In some embodiments, the present disclosure teaches methods of selecting promoters with optimal expression properties to modulate RNA degradation function and produce beneficial effects on overall-host strain productivity.

[0113] Promoters regulate the rate at which genes are transcribed and can influence transcription in a variety of ways. For example, in some embodiments, the present disclosure teaches methods of identifying one or more promoters and/or generating variants of one or more promoters within a host cell, which exhibit a range of expression strengths (e.g. promoter ladders discussed infra), or superior regulatory properties (i.e., tighter regulatory control for selected genes).

[0114] Constitutive promoters, for example, direct the transcription of their associated genes at a constant rate regardless of the internal or external cellular conditions, while regulatable promoters increase or decrease the rate at which a gene is transcribed depending on the internal and/or the external cellular conditions, *e.g.* growth rate, temperature, responses to specific environmental chemicals, and the like. Promoters can be isolated from their normal cellular contexts and engineered to regulate the expression of virtually any gene, enabling the effective modification of cellular growth, product yield and/or other phenotypes of interest.

[0115] In some embodiments, the present disclosure teaches methods of identifying one or more promoters and/or generating variants of one or more promoters within a host cell, which exhibit a range of expression strengths (e.g. promoter ladders discussed infra), or superior regulatory properties (*i.e.*, tighter regulatory control for selected genes, or responsiveness to particular conditions). A particular combination of these identified and/or generated promoters can be grouped together as a promoter ladder for use in the RNA degradation perturbation experiments explained in more detail below.

[0116] In some embodiments, promoter ladders are created by identifying natural, native, or wild-type promoters associated with a target gene of interest that have a range of expression strengths. These identified promoters can be grouped together as a promoter ladder.

[0117] In some embodiments, promoter ladders are created by: identifying natural, native, or wild type promoters associated with a target gene of interest and then mutating said promoter to derive multiple mutated promoter sequences. Each of these mutated promoters is tested for effect on target gene expression. In some embodiments, the edited promoters are tested for expression activity across a variety of conditions, such that each promoter variant's activity is documented/characterized/annotated and stored in a database. The resulting edited promoter variants are subsequently organized into promoter ladders arranged based on the strength of their expression (*e.g.*, with highly expressing variants near the top, and attenuated expression near the bottom, therefore leading to the term "ladder").

[0118] In some embodiments, the present disclosure teaches promoter ladders that are a combination of identified naturally occurring promoters and mutated variant promoters.

[0119] In some embodiments, the present disclosure teaches methods of identifying natural, native, or wild type promoters that satisfied both of the following criteria: 1) represented a ladder of constitutive promoters; and 2) could be encoded by short DNA sequences, ideally less than 100 base pairs. In some embodiments, constitutive promoters of the present disclosure exhibit constant gene expression across two selected growth conditions (typically compared among conditions experienced during industrial cultivation). In some embodiments, the promoters of the present disclosure will consist of a ~60 base pair core promoter, and a 5' UTR between 26- and 40 base pairs in length.

[0120] In some embodiments, one or more of the aforementioned identified naturally occurring promoter sequences are chosen for gene editing. In some embodiments, the natural promoters are edited *via* any known genetic mutation methods. In other embodiments, the promoters of the present disclosure are edited by synthesizing new promoter variants with the desired sequence.

[0121] The entire disclosures of U.S. Patent Application No. 62/264,232, filed on December 07, 2015, and PCT Publication No. WO2017/100376, are each hereby incorporated by reference in its entirety for all purposes.

[0122] A non-exhaustive list of the promoters of the present disclosure is provided in Table 2 below.

Table 2. Selected promoter sequences of the present disclosure.

SEQ ID No.	Promoter Short Name
1	P1
2	P2
3	P3
4	P4
5	P5
6	P6
7	P7
8	P8

[0123] In some embodiments, the promoters of the present invention exhibit at least 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, or 75% sequence identity with a promoter sequences from Table 2.

RNA Degradation Gene Expression Perturbation

[0124] In some embodiments, the present disclosure teaches optimizing the expression of one or more RNA degradosome genes by operably linking said gene with a promoter of the present invention. Thus, if one has promoters P1-P8 (representing eight promoters that have been identified and/or generated to exhibit a range of expression strengths) and associates the promoter ladder with a single RNA degradation gene in a microbe (*i.e.*, genetically engineer a microbe with a given promoter operably linked to the native, or exogenous coding region of the RNA degradation gene), then the effect of each of the eight promoters can be ascertained by characterizing each of the eight engineered strains, given that the engineered microbes have an otherwise identical genetic background except the particular promoter associated with the target gene.

[0125] Modulating the steady state levels of genes in an organism are a key point of control for effecting organism behavior. Cells express thousands of different types of proteins, and these proteins interact in numerous complex ways to create function. By varying the steady state levels of selected RNA degradosome genes, the present invention achieves improved host performance. Some alterations to the RNA degradosome may increase performance, and so, coupled to a mechanism for assessing performance, this technique allows for the generation of organisms with improved function.

[0126] Thus, in particular embodiments, the RNA degradation gene perturbation is a multi-step process comprising: i) selecting a promoter from the promoter ladder (*e.g.,* from the promoters listed in Table 2 of this disclosure or a variant thereof), ii) selecting a RNA degradosome gene to target (*e.g.,* from the genes listed in Table 1 of this disclosure or a variant thereof), and iii) operably linking the selected promoter to the selected gene in the genome of a selected host organism. In some embodiments, operably linking the selected promoter to the selected gene is performed as follows: When a native promoter exists in front of selected RNA degradosome gene and its sequence is known, replace the native promoter with the selected promoter. When the native promoter does not exist, or its sequence is unknown, insert the selected promoter in front of the RNA degradosome gene.

[0127] In some embodiments, the present disclosure will refer to a specific combination of an operably linked promoter to a selected RNA degradation gene, by reciting the promoter and gene sequences or names separated by a "::". Thus, the symbol "::" as used in this disclosure is used in lieu of "operably linked." Thus a recitation of SEQ ID NO: 1 :: SEQ ID NO: 22, refers to the promoter from SEQ ID NO:1 operably linked to the RNA degradation gene of SEQ ID NO: 22. Similarly, P1:: G10 refers to promoter 1 from Table 2, operably linked to the mutM2 RNA degradation sequence disclosed in Table 1. In some portions of the specification, the symbol "-" is used interchangeably with "::".

[0128] In some embodiments, the present disclosure also teaches methods of validating genetically engineered host organisms comprising the operably linked promoter and RNA degradation gene by comparing the engineered host against a genetically identical host lacking the operably linked promoter, against one or more metrics is indicative of the performance that is being optimized.

[0129] In some embodiments, the present disclosure teaches specific combinations of selected promoters operably linked to selected RNA degradosome genes. A non-exclusive list of promoter::gene combinations contemplated by this disclosure are summarized in Table 3 below. In consideration of the limited space, these combinations are represented by their promoter and gene short names as defined in earlier portions of the specification.

Table 3. Promoter: RNA Degradosome Gene combinations of the present disclosure.

P1::G1	P2::G1	P3::G1	P4::G1	P5::G1	P6::G1	P7::G1	P8::G1
P1::G2	P2::G2	P3::G2	P4::G2	P5::G2	P6::G2	P7::G2	P8::G2
P1::G3	P2::G3	P3::G3	P4::G3	P5::G3	P6::G3	P7::G3	P8::G3
P1::G4	P2::G4	P3::G4	P4::G4	P5::G4	P6::G4	P7::G4	P8::G4
P1::G5	P2::G5	P3::G5	P4::G5	P5::G5	P6::G5	P7::G5	P8::G5
P1::G6	P2::G6	P3::G6	P4::G6	P5::G6	P6::G6	P7::G6	P8::G6
P1::G7	P2::G7	P3::G7	P4::G7	P5::G7	P6::G7	P7::G7	P8::G7
P1::G8	P2::G8	P3::G8	P4::G8	P5::G8	P6::G8	P7::G8	P8::G8
P1::G9	P2::G9	P3::G9	P4::G9	P5::G9	P6::G9	P7::G9	P8::G9
P1::G10	P2::G10	P3::G10	P4::G10	P5::G10	P6::G10	P7::G10	P8::G10
P1::G11	P2::G11	P3::G11	P4::G11	P5::G11	P6::G11	P7::G11	P8::G11
P1::G12	P2::G12	P3::G12	P4::G12	P5::G12	P6::G12	P7::G12	P8::G12
P1::G13	P2::G13	P3::G13	P4::G13	P5::G13	P6::G13	P7::G13	P8::G13
P1::G14	P2::G14	P3::G14	P4::G14	P5::G14	P6::G14	P7::G14	P8::G14

Start Codon Optimization

[0130] In some embodiments, the present disclosure teaches methods of swapping start and stop codon variants. For example, typical stop codons for *S. cerevisiae* and mammals are UAA and UGA, respectively. The typical stop codon for monocotyledonous plants is UGA, whereas insects and *E. coli* commonly use UAA as the stop codon (Dalphin *et al.* (1996) Nucl. Acids Res. 24: 216-218).

[0131] In other embodiments, the present invention teaches replacing ATG start codons with TTG. In some embodiments, the present invention teaches replacing ATG start codons with GTG. In some embodiments, the present invention teaches replacing GTG start codons with ATG. In some

embodiments, the present invention teaches replacing GTG start codons with TTG. In some embodiments, the present invention teaches replacing TTG start codons with ATG. In some embodiments, the present invention teaches replacing TTG start codons with GTG.

Organisms Amenable to Genetic Design

[0132] The disclosed genomic engineering methods are exemplified with industrial microbial cell cultures, but are applicable to any organism.

[0133] Thus, as used herein, the term "microorganism" should be taken broadly. It includes, but is not limited to, the two prokaryotic domains, Bacteria and Archaea, as well as certain eukaryotic fungi and protists. However, in certain aspects, "higher" eukaryotic organisms such as insects, plants, and animals can be utilized in the methods taught herein.

[0134] Suitable host cells include, but are not limited to: bacterial cells, algal cells, plant cells, fungal cells, insect cells, and mammalian cells. In one illustrative embodiment, suitable host cells include *E. coli* (*e.g.*, SHuffle™ competent *E. coli* available from New England BioLabs in Ipswich, Mass.).

[0135] In one illustrative embodiment, suitable host cells include *E. coli*. Suitable host strains of the *E. coli* species comprise: Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC), Uropathogenic *E. coli* (UPEC), Verotoxin-producing *E. coli*, *E. coli* O157:H7, *E. coli* O104:H4, *Escherichia coli* O121, *Escherichia coli* O104:H21, *Escherichia coli* K1, and Escherichia coli NC101. In some embodiments, the present disclosure teaches genomic engineering of *E. coli* K12, *E. coli* B, and *E. coli* C.

[0136] In some embodiments, the present disclosure teaches genomic engineering of *E. coli* strains NCTC 12757, NCTC 12779, NCTC 12790, NCTC 12796, NCTC 12811, ATCC 11229, ATCC 25922, ATCC 8739, DSM 30083, BC 5849, BC 8265, BC 8267, BC 8268, BC 8270, BC 8271, BC 8272, BC 8273, BC 8276, BC 8277, BC 8278, BC 8279, BC 8312, BC 8317, BC 8319, BC 8320, BC 8321, BC 8322, BC 8326, BC 8327, BC 8331, BC 8335, BC 8338, BC 8341, BC 8344, BC 8345, BC 8346, BC 8347, BC 8348, BC 8863, and BC 8864.

[0137] In some embodiments, the present disclosure teaches verocytotoxigenic *E. coli* (VTEC), such as strains BC 4734 (O26:H11), BC 4735 (O157:H-), BC 4736 , BC 4737 (n.d.), BC 4738

(O157:H7), BC 4945 (O26:H-), BC 4946 (O157:H7), BC 4947 (O111:H-), BC 4948 (O157:H), BC 4949 (O5), BC 5579 (O157:H7), BC 5580 (O157:H7), BC 5582 (O3:H), BC 5643 (O2:H5), BC 5644 (O128), BC 5645 (O55:H-), BC 5646 (O69:H-), BC 5647 (O101:H9), BC 5648 (O103:H2), BC 5850 (O22:H8), BC 5851 (O55:H-), BC 5852 (O48:H21), BC 5853 (O26:H11), BC 5854 (O157:H7), BC 5855 (O157:H-), BC 5856 (O26:H-), BC 5857 (O103:H2), BC 5858 (O26:H11), BC 7832, BC 7833 (O raw form:H-), BC 7834 (ONT:H-), BC 7835 (O103:H2), BC 7836 (O57:H-), BC 7837 (ONT:H-), BC 7838, BC 7839 (O128:H2), BC 7840 (O157:H-), BC 7841 (O23:H-), BC 7842 (O157:H-), BC 7843, BC 7844 (O157:H-), BC 7845 (O103:H2), BC 7846 (O26:H11), BC 7847 (O145:H-), BC 7848 (O157:H-), BC 7849 (O156:H47), BC 7850, BC 7851 (O157:H-), BC 7852 (O157:H-), BC 7853 (O5:H-), BC 7854 (O157:H7), BC 7855 (O157:H7), BC 7856 (O26:H-), BC 7857, BC 7858, BC 7859 (ONT:H-), BC 7860 (O129:H-), BC 7861, BC 7862 (O103:H2), BC 7863, BC 7864 (O raw form:H-), BC 7865, BC 7866 (O26:H-), BC 7867 (O raw form:H-), BC 7868, BC 7869 (ONT:H-), BC 7870 (O113:H-), BC 7871 (ONT:H-), BC 7872 (ONT:H-), BC 7873, BC 7874 (O raw form:H-), BC 7875 (O157:H-), BC 7876 (O111:H-), BC 7877 (O146:H21), BC 7878 (O145:H-), BC 7879 (O22:H8), BC 7880 (O raw form:H-), BC 7881 (O145:H-), BC 8275 (O157:H7), BC 8318 (O55:K-:H-), BC 8325 (O157:H7), and BC 8332 (ONT), BC 8333.

[0138] In some embodiments, the present disclosure teaches enteroinvasive *E. coli* (EIEC), such as strains BC 8246 (O152:K-:H-), BC 8247 (O124:K(72):H3), BC 8248 (O124), BC 8249 (O112), BC 8250 (O136:K(78):H-), BC 8251 (O124:H-), BC 8252 (O144:K-:H-), BC 8253 (O143:K:H-), BC 8254 (O143), BC 8255 (O112), BC 8256 (O28a.e), BC 8257 (O124:H-), BC 8258 (O143), BC 8259 (O167:K-:H5), BC 8260 (O128a.c.:H35), BC 8261 (O164), BC 8262 (O164:K-:H-), BC 8263 (O164), and BC 8264 (O124).

[0139] In some embodiments, the present disclosure teaches enterotoxigenic *E. coli (ETEC),* such as strains BC 5581 (O78:H11), BC 5583 (O2:K1), BC 8221 (O118), BC 8222 (O148:H-), BC 8223 (O111), BC 8224 (O110:H-), BC 8225 (O148), BC 8226 (O118), BC 8227 (O25:H42), BC 8229 (O6), BC 8231 (O153:H45), BC 8232 (O9), BC 8233 (O148), BC 8234 (O128), BC 8235 (O118), BC 8237 (O111), BC 8238 (O110:H17), BC 8240 (O148), BC 8241 (O6H16), BC 8243 (O153), BC 8244 (O15:H-), BC 8245 (O20), BC 8269 (O125a.c:H-), BC 8313 (O6:H6), BC 8315 (O153:H-), BC 8329, BC 8334 (O118:H12), and BC 8339.

[0140] In some embodiments, the present disclosure teaches enteropathogenic *E. coli* (EPEC), such as strains BC 7567 (O86), BC 7568 (O128), BC 7571 (O114), BC 7572 (O119), BC 7573 (O125), BC 7574 (O124), BC 7576 (O127a), BC 7577 (O126), BC 7578 (O142), BC 7579 (O26), BC 7580 (OK26), BC 7581 (O142), BC 7582 (O55), BC 7583 (O158), BC 7584 (O-), BC 7585 (O-), BC 7586 (O-), BC 8330, BC 8550 (O26), BC 8551 (O55), BC 8552 (O158), BC 8553 (O26), BC 8554 (O158), BC 8555 (O86), BC 8556 (O128), BC 8557 (OK26), BC 8558 (O55), BC 8560 (O158), BC 8561 (O158), BC 8562 (O114), BC 8563 (O86), BC 8564 (O128), BC 8565 (O158), BC 8566 (O158), BC 8567 (O158), BC 8568 (O111), BC 8569 (O128), BC 8570 (O114), BC 8571 (O128), BC 8572 (O128), BC 8573 (O158), BC 8574 (O158), BC 8575 (O158), BC 8576 (O158), BC 8577 (O158), BC 8578 (O158), BC 8581 (O158), BC 8583 (O128), BC 8584 (O158), BC 8585 (O128), BC 8586 (O158), BC 8588 (O26), BC 8589 (O86), BC 8590 (O127), BC 8591 (O128), BC 8592 (O114), BC 8593 (O114), BC 8594 (O114), BC 8595 (O125), BC 8596 (O158), BC 8597 (O26), BC 8598 (O26), BC 8599 (O158), BC 8605 (O158), BC 8606 (O158), BC 8607 (O158), BC 8608 (O128), BC 8609 (O55), BC 8610 (O114), BC 8615 (O158), BC 8616 (O128), BC 8617 (O26), BC 8618 (O86), BC 8619, BC 8620, BC 8621, BC 8622, BC 8623, BC 8624 (O158), and BC 8625 (O158).

[0141] Other suitable host organisms of the present disclosure include microorganisms of the genus *Corynebacterium*. In some embodiments, preferred *Corynebacterium* strains/species include: *C. efficiens*, with the deposited type strain being DSM44549, *C. glutamicum*, with the deposited type strain being ATCC13032, and *C. ammoniagenes*, with the deposited type strain being ATCC6871. In some embodiments the preferred host of the present disclosure is *C. glutamicum*.

[0142] Suitable host strains of the genus *Corynebacterium*, in particular of the species *Corynebacterium glutamicum*, are in particular the known wild-type strains: *Corynebacterium glutamicum* ATCC13032, *Corynebacterium acetoglutamicum* ATCC15806, *Corynebacterium acetoacidophilum* ATCC13870, *Corynebacterium melassecola* ATCC17965, *Corynebacterium thermoaminogenes* FERM BP-1539, *Brevibacterium flavum* ATCC14067, *Brevibacterium lactofermentum* ATCC13869, and *Brevibacterium divaricatum* ATCC14020; and L-amino acid-producing mutants, or strains, prepared therefrom, such as, for example, the L-lysine-producing strains: *Corynebacterium glutamicum* FERM-P 1709, *Brevibacterium flavum* FERM-P 1708, *Brevibacterium lactofermentum* FERM-P 1712, *Corynebacterium glutamicum* FERM-P 6463,

Corynebacterium glutamicum FERM-P 6464, *Corynebacterium glutamicum* DM58-1, *Corynebacterium glutamicum* DG52-5, *Corynebacterium glutamicum* DSM5714, and *Corynebacterium glutamicum* DSM12866.

[0143] The term "*Micrococcus glutamicus*" has also been in use for *C. glutamicum*. Some representatives of the species *C. efficiens* have also been referred to as *C. thermoaminogenes* in the prior art, such as the strain FERM BP-1539, for example.

[0144] In other embodiments, the host cell is a prokaryotic cell. Suitable prokaryotic cells include gram positive, gram negative, and gram-variable bacterial cells. The host cell may be a species of, but not limited to: *Agrobacterium, Alicyclobacillus, Anabaena, Anacystis, Acinetobacter, Acidothermus, Arthrobacter, Azobacter, Bacillus, Bifidobacterium, Brevibacterium, Butyrivibrio, Buchnera, Campestris, Camplyobacter, Clostridium, Corynebacterium, Chromatium, Coprococcus, Escherichia, Enterococcus, Enterobacter, Erwinia, Fusobacterium, Faecalibacterium, Francisella, Flavobacterium, Geobacillus, Haemophilus, Helicobacter, Klebsiella, Lactobacillus, Lactococcus, Ilyobacter, Micrococcus, Microbacterium, Mesorhizobium, Methylobacterium, Methylobacterium, Mycobacterium, Neisseria, Pantoea, Pseudomonas, Prochlorococcus, Rhodobacter, Rhodopseudomonas, Rhodopseudomonas, Roseburia, Rhodospirillum, Rhodococcus, Scenedesmus, Streptomyces, Streptococcus, Synecoccus, Saccharopolyspora, Staphylococcus, Serratia, Salmonella, Shigella, Thermoanaerobacterium, Tropheryma, Tularensis, Temecula, Thermosynechococcus, Thermococcus, Ureaplasma, Xanthomonas, Xylella, Yersinia,* and *Zymomonas.* In some embodiments, the host cell is *Corynebacterium glutamicum*. In some embodiments, the bacterial host strain is an industrial strain. Numerous bacterial industrial strains are known and suitable in the methods and compositions described herein.

[0145] In some embodiments, the bacterial host cell is of the Agrobacterium species (e.g., A. radiobacter, A. rhizogenes, A. rubi), the Arthrobacterspecies (e.g., A. aurescens, A. citreus, A. globformis, A. hydrocarboglutamicus, A. mysorens, A. nicotianae, A. paraffineus, A. protophonniae, A. roseoparaffinus, A. sulfureus, A. ureafaciens), the Bacillus species (e.g., B. thuringiensis, B. anthracis, B. megaterium, B. subtilis, B. lentus, B. circulars, B. pumilus, B. lautus, B. coagulans, B. brevis, B. firmus, B. alkaophius, B. licheniformis, B. clausii, B. stearothermophilus, B. halodurans and B. amyloliquefaciens. In particular embodiments, the host

cell will be an industrial Bacillus strain including but not limited to B. subtilis, B. pumilus, B. licheniformis, B. megaterium, B. clausii, B. stearothermophilus and B. amyloliquefaciens. In some embodiments, the host cell will be an industrial Clostridium species (e.g., C. acetobutylicum, C. tetani E88, C. lituseburense, C. saccharobutylicum, C. perfringens, C. beijerinckii). In some embodiments, the host cell will be an industrial Corynebacterium species (e.g., C. glutamicum, C. acetoacidophilum). In some embodiments, the host cell will be an industrial Escherichia species (e.g., E. coli). In some embodiments, the host cell will be an industrial Erwinia species (e.g., E. uredovora, E. carotovora, E. ananas, E. herbicola, E. punctata, E. terreus). In some embodiments, the host cell will be an industrial Pantoea species (e.g., P. citrea, P. agglomerans). In some embodiments, the host cell will be an industrial Pseudomonas species, (e.g., P. putida, P. aeruginosa, P. mevalonii). In some embodiments, the host cell will be an industrial Streptococcus species (e.g., S. equisimiles, S. pyogenes, S. uberis). In some embodiments, the host cell will be an industrial Streptomyces species (e.g., S. ambofaciens, S. achromogenes, S. avermitilis, S. coelicolor, S. aureofaciens, S. aureus, S. fungicidicus, S. griseus, S. lividans). In some embodiments, the host cell will be an industrial Zymomonas species (e.g., Z. mobilis, Z. lipolytica), and the like. In some embodiments, the host cell of the present disclosure is a eukaryotic cell. Suitable eukaryotic host cells include, but are not limited to: fungal cells, algal cells, insect cells, animal cells, and plant cells. Suitable fungal host cells include, but are not limited to: Ascomycota, Basidiomycota, Deuteromycota, Zygomycota, Fungi imperfecti. Certain preferred fungal host cells include yeast cells and filamentous fungal cells. Suitable filamentous fungi host cells include, for example, any filamentous forms of the subdivision Eumycotina and Oomycota. (see, e.g., Hawksworth et al., In Ainsworth and Bisby's Dictionary of The Fungi, 8th edition, 1995, CAB International, University Press, Cambridge, UK, which is incorporated herein by reference). Filamentous fungi are characterized by a vegetative mycelium with a cell wall composed of chitin, cellulose and other complex polysaccharides. The filamentous fungi host cells are morphologically distinct from yeast.

[0146] In certain illustrative, but non-limiting embodiments, the filamentous fungal host cell may be a cell of a species of: Achlya, Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Cephalosporium, Chrysosporium, Cochliobolus, Corynascus, Cryphonectria, Cryptococcus, Coprinus, Coriolus, Diplodia, Endothis, Fusarium, Gibberella, Gliocladium, Humicola, Hypocrea, Myceliophthora (e.g., Myceliophthora thermophila), Mucor, Neurospora,

Penicillium, Podospora, Phlebia, Piromyces, Pyricularia, Rhizomucor, Rhizopus, Schizophyllum, Scytalidium, Sporotrichum, Talaromyces, Thermoascus, Thielavia, Tramates, Tolypocladium, Trichoderma, Verticillium, Volvariella, or teleomorphs, or anamorphs, and synonyms or taxonomic equivalents thereof.

[0147] Suitable yeast host cells include, but are not limited to: Candida, Hansenula, Saccharomyces, Schizosaccharomyces, Pichia, Kluyveromyces, and Yarrowia. In some embodiments, the yeast cell is Hansenula polymorpha, Saccharomyces cerevisiae, Saccaromyces carlsbergensis, Saccharomyces diastaticus, Saccharomyces norbensis, Saccharomyces kluyveri, Schizosaccharomyces pombe, Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia kodamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia quercuum, Pichia pijperi, Pichia stipitis, Pichia methanolica, Pichia angusta, Kluyveromyces lactis, Candida albicans, or Yarrowia lipolytica.

[0148] In certain embodiments, the host cell is an algal cell such as, *Chlamydomonas* (*e.g.*, *C. Reinhardtii*) and *Phormidium* (*P.* sp. ATCC29409).

[0149] In some embodiments, the methods of the present disclosure are also applicable to multi-cellular organisms. For example, the platform could be used for improving the performance of crops. The organisms can comprise a plurality of plants such as *Gramineae, Fetucoideae, Poacoideae, Agrostis, Phleum, Dactylis, Sorgum, Setaria, Zea, Oryza, Triticum, Secale, Avena, Hordeum, Saccharum, Poa, Festuca, Stenotaphrum, Cynodon, Coix, Olyreae, Phareae, Compositae* or *Leguminosae*. For example, the plants can be corn, rice, soybean, cotton, wheat, rye, oats, barley, pea, beans, lentil, peanut, yam bean, cowpeas, velvet beans, clover, alfalfa, lupine, vetch, lotus, sweet clover, wisteria, sweet pea, sorghum, millet, sunflower, canola or the like. Similarly, the organisms can include a plurality of animals such as non-human mammals, fish, insects, or the like.

[0150] The present disclosure is also suitable for use with a variety of animal cell types, including mammalian cells, for example, human (including 293, WI38, PER.C6 and Bowes melanoma cells), mouse (including 3T3, NS0, NS1, Sp2/0), hamster (CHO, BHK), monkey (COS, FRhL, Vero), and hybridoma cell lines.

[0151] In various embodiments, strains that may be used in the practice of the disclosure are readily accessible to the public from a number of culture collections such as American Type

Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM), Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

Assembling/Cloning Custom Plasmids

[0152] In some embodiments, the present disclosure teaches methods for constructing vectors capable of inserting desired target DNA sections (*e.g.* containing a particular promoter, or promoter::gene combination) into the genome of host organisms. In some embodiments, the present disclosure teaches methods of cloning vectors comprising the target DNA, homology arms, and at least one selection marker (*see* Figure 1).

[0153] In some embodiments, the present disclosure is compatible with any vector suited for transformation into the host organism. In some embodiments, the present disclosure teaches use of shuttle vectors compatible with a host cell. In one embodiment, a shuttle vector for use in the methods provided herein is a shuttle vector capable of propagating in at least two different species (e.g., compatible with an *E. coli* for initial cloning/amplification and *Corynebacterium* for integration). In some embodiments, vectors for use in the methods provided herein can comprise markers for selection and/or counter-selection as described herein. The markers can be any markers known in the art and/or provided herein. The shuttle vectors can further comprise any regulatory sequence(s) and/or sequences useful in the assembly of said shuttle vectors as known in the art. The shuttle vectors can further comprise any origins of replication that may be needed for propagation in a host cell as provided herein such as, for example, *E. coli* or *C. glutamicum*. In some embodiments, the vectors of the present disclosure comprise at least one regulatory sequence. In some embodiments, the regulatory sequence can be any regulatory sequence known in the art or provided herein such as, for example, a promoter, start, stop, signal, secretion and/or termination sequence used by the genetic machinery of the host cell. In certain instances, the target DNA can be inserted into vectors, constructs or plasmids obtainable from any repository or catalogue product, such as a commercial vector (*see e.g.,* DNA2.0 custom or GATEWAY® vectors).

[0154] In some embodiments, the assembly/cloning methods of the present disclosure may employ at least one of the following assembly strategies: **i)** type II conventional cloning, **ii)** type II S-mediated or "Golden Gate" cloning (*see, e.g.*, Engler, C., R. Kandzia, and S. Marillonnet. 2008 "A one pot, one step, precision cloning method with high-throughput capability". PLos One 3:e3647;

Kotera, I., and T. Nagai. 2008 "A high-throughput and single-tube recombination of crude PCR products using a DNA polymerase inhibitor and type IIS restriction enzyme." J Biotechnol 137:1-7.; Weber, E., R. Gruetzner, S. Werner, C. Engler, and S. Marillonnet. 2011 Assembly of Designer TAL Effectors by Golden Gate Cloning. PloS One 6:e19722), **iii)** GATEWAY® recombination, **iv)** TOPO® cloning, exonuclease-mediated assembly (Aslanidis and de Jong 1990. "Ligation-independent cloning of PCR products (LIC-PCR)." Nucleic Acids Research, Vol. 18, No. 20 6069), **v)** homologous recombination, **vi)** non-homologous end joining, or a combination thereof. Modular type IIS based assembly strategies are disclosed in PCT Publication WO 2011/154147, the disclosure of which is incorporated herein by reference.

[0155] In some embodiments, the present disclosure teaches cloning vectors with at least one selection marker. Various selection marker genes are known in the art often encoding antibiotic resistance function for selection in prokaryotic (e.g., against ampicillin, kanamycin, tetracycline, chloramphenycol, zeocin, spectinomycin/streptomycin) or eukaryotic cells (e.g. geneticin, neomycin, hygromycin, puromycin, blasticidin, zeocin) under selective pressure. Other marker systems allow for screening and identification of wanted or unwanted cells such as the well-known blue/white screening system used in bacteria to select positive clones in the presence of X-gal or fluorescent reporters such as green or red fluorescent proteins expressed in successfully transduced host cells. Another class of selection markers most of which are only functional in prokaryotic systems relates to counter selectable marker genes often also referred to as "death genes" which express toxic gene products that kill producer cells. Examples of such genes include sacB, rpsL(strA), tetAR, pheS, thyA, gata-1, or ccdB, the function of which is described in (Reyrat et al. 1998 "Counterselectable Markers: Untapped Tools for Bacterial Genetics and Pathogenesis." Infect Immun. 66(9): 4011-4017).

Transformation of Host Cells

[0156] In some embodiments, the vectors of the present disclosure may be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation (Davis, L., Dibner, M., Battey, I., 1986 "Basic Methods in Molecular Biology"). Other methods of transformation include for example, lithium acetate transformation and electroporation See, *e.g.*,

Gietz *et al.*, Nucleic Acids Res. 27:69-74 (1992); Ito *et al.*, J. Bacterol. 153:163-168 (1983); and Becker and Guarente, Methods in Enzymology 194:182-187 (1991). In some embodiments, transformed host cells are referred to as recombinant host strains.

[0157] In some embodiments, the insert DNA of the present disclosure is incorporated into the target genomic DNA region by single-crossover or double crossover recombination (*see* Nakashima et al., 2014 "Bacterial Cellular Engineering by Genome Editing and Gene Silencing" Int. J. Mol Sci. 15(2), 2773-2793).

[0158] In some embodiments, the present disclosure teaches screening transformed cells with one or more selection markers as described above. In one such embodiment, cells transformed with a vector comprising a kanamycin resistance marker (KanR) are plated on media containing effective amounts of the kanamycin antibiotic. Colony forming units visible on kanamycin-laced media are presumed to have incorporated the vector cassette into their genome. Insertion of the desired sequences can be confirmed via PCR, restriction enzyme analysis, and/or sequencing of the relevant insertion site.

Looping Out of Selected Sequences

[0159] In some embodiments, the present disclosure teaches methods of looping out selected regions of DNA from the host organisms. In some embodiments, the present disclosure teaches looping out selection markers from positive transformants. Looping out deletion techniques are known in the art, and are described in (Tear *et al.* 2014 "Excision of Unstable Artificial Gene-Specific inverted Repeats Mediates Scar-Free Gene Deletions in Escheria coli." Appl. Biochem. Biotech. 175:1858-1867).

[0160] The looping out methods used in the methods provided herein can be performed using single-crossover homologous recombination or double-crossover homologous recombination. In one embodiment, looping out of selected regions as described herein is performed using single-crossover homologous recombination.

[0161] First, loop out vectors are inserted into selected target regions within the genome of the host organism (*e.g.,* via homologous recombination, CRISPR, or other gene editing technique). The inserted vector is designed with a sequence which is a direct of an existing or introduced nearby host sequence, such that the direct repeats flank the region of DNA slated for looping and

deletion. Once inserted, cells containing the loop out vector can be counter selected for deletion of the selection region (*e.g.*, lack of resistance to the selection gene). In one such embodiment, a SNP is inserted in a loopout vector in which the selection marker is flanked by the direct repeat sequences. Insertion of the vector is confirmed through its selection marker. Once confirmed, the selection marker is then removed by selecting for a looping out of the DNA slated for deletion (*see* Figure 2).

Cell Culture and Fermentation

[0162] Cells of the present disclosure can be cultured in conventional nutrient media modified as appropriate for any desired biosynthetic reactions or selections. In some embodiments, the present disclosure teaches culture in inducing media for activating promoters. In some embodiments, the present disclosure teaches media with selection agents, including selection agents of transformants (*e.g.*, antibiotics), or selection of organisms suited to grow under inhibiting conditions (*e.g.*, high ethanol conditions). In some embodiments, the present disclosure teaches growing cell cultures in media optimized for cell growth. In other embodiments, the present disclosure teaches growing cell cultures in media optimized for product yield. In some embodiments, the present disclosure teaches growing cultures in media capable of inducing cell growth and also contains the necessary precursors for final product production (*e.g.*, high levels of sugars for ethanol production).

[0163] Culture conditions, such as temperature, pH and the like, are those suitable for use with the host cell selected for expression, and will be apparent to those skilled in the art. As noted, many references are available for the culture and production of many cells, including cells of bacterial, plant, animal (including mammalian) and archebacterial origin. *See e.g.*, Sambrook, Ausubel (all supra), as well as Berger, *Guide to Molecular Cloning Techniques, Methods in Enzymology* volume 152 Academic Press, Inc., San Diego, CA; and Freshney (1994) *Culture of Animal Cells, a Manual of Basic Technique*, third edition, Wiley-Liss, New York and the references cited therein; Doyle and Griffiths (1997) *Mammalian Cell Culture: Essential Techniques* John Wiley and Sons, NY; Humason (1979) *Animal Tissue Techniques*, fourth edition W.H. Freeman and Company; and Ricciardelle *et al.*, (1989) *In Vitro Cell Dev. Biol.* 25:1016-1024, all of which are incorporated herein by reference. For plant cell culture and regeneration, Payne *et al.* (1992) *Plant Cell and Tissue Culture in Liquid Systems* John Wiley & Sons, Inc. New York, N.Y.; Gamborg and Phillips (eds) (1995) *Plant Cell, Tissue and Organ Culture*;

Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg N.Y.); Jones, ed. (1984) *Plant Gene Transfer and Expression Protocols*, Humana Press, Totowa, N.J. and *Plant Molecular Biology* (1993) R. R. D. Croy, Ed. Bios Scientific Publishers, Oxford, U.K. ISBN 0 12 198370 6, all of which are incorporated herein by reference. Cell culture media in general are set forth in Atlas and Parks (eds.) *The Handbook of Microbiological Media* (1993) CRC Press, Boca Raton, Fla., which is incorporated herein by reference. Additional information for cell culture is found in available commercial literature such as the *Life Science Research Cell Culture Catalogue* from Sigma-Aldrich, Inc. (St Louis, Mo.) ("Sigma-LSRCCC") and, for example, *The Plant Culture Catalogue* and supplement also from Sigma-Aldrich, Inc. (St Louis, Mo.) ("Sigma-PCCS"), all of which are incorporated herein by reference.

[0164] The culture medium to be used must in a suitable manner satisfy the demands of the respective strains. Descriptions of culture media for various microorganisms are present in the "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

[0165] The present disclosure furthermore provides a process for fermentative preparation of a product of interest, comprising the steps of: a) culturing a microorganism according to the present disclosure in a suitable medium, resulting in a fermentation broth; and b) concentrating the product of interest in the fermentation broth of a) and/or in the cells of the microorganism.

[0166] In some embodiments, the present disclosure teaches that the microorganisms produced may be cultured continuously—as described, for example, in WO 05/021772—or discontinuously in a batch process (batch cultivation) or in a fed-batch or repeated fed-batch process for the purpose of producing the desired organic-chemical compound. A summary of a general nature about known cultivation methods is available in the textbook by Chmiel (Bioprozesstechnik. 1: Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren and periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

[0167] In some embodiments, the cells of the present disclosure are grown under batch or continuous fermentations conditions.

[0168] Classical batch fermentation is a closed system, wherein the compositions of the medium is set at the beginning of the fermentation and is not subject to artificial alternations during the fermentation. A variation of the batch system is a fed-batch fermentation which also finds use in

the present disclosure. In this variation, the substrate is added in increments as the fermentation progresses. Fed-batch systems are useful when catabolite repression is likely to inhibit the metabolism of the cells and where it is desirable to have limited amounts of substrate in the medium. Batch and fed-batch fermentations are common and well known in the art.

[0169] Continuous fermentation is a system where a defined fermentation medium is added continuously to a bioreactor and an equal amount of conditioned medium is removed simultaneously for processing and harvesting of desired proteins. In some embodiments, continuous fermentation generally maintains the cultures at a constant high density where cells are primarily in log phase growth. In some embodiments, continuous fermentation generally maintains the cultures at a stationary or late log/stationary, phase growth. Continuous fermentation systems strive to maintain steady state growth conditions.

[0170] Methods for modulating nutrients and growth factors for continuous fermentation processes as well as techniques for maximizing the rate of product formation are well known in the art of industrial microbiology.

[0171] For example, a non-limiting list of carbon sources for the cultures of the present disclosure include, sugars and carbohydrates such as, for example, glucose, sucrose, lactose, fructose, maltose, molasses, sucrose-containing solutions from sugar beet or sugar cane processing, starch, starch hydrolysate, and cellulose; oils and fats such as, for example, soybean oil, sunflower oil, groundnut oil and coconut fat; fatty acids such as, for example, palmitic acid, stearic acid, and linoleic acid; alcohols such as, for example, glycerol, methanol, and ethanol; and organic acids such as, for example, acetic acid or lactic acid.

[0172] A non-limiting list of the nitrogen sources for the cultures of the present disclosure include, organic nitrogen-containing compounds such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soybean flour, and urea; or inorganic compounds such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, and ammonium nitrate. The nitrogen sources can be used individually or as a mixture.

[0173] A non-limiting list of the possible phosphorus sources for the cultures of the present disclosure include phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts.

[0174] The culture medium may additionally comprise salts, for example in the form of chlorides or sulfates of metals such as, for example, sodium, potassium, magnesium, calcium and iron, such as, for example, magnesium sulfate or iron sulfate, which are necessary for growth.

[0175] Finally, essential growth factors such as amino acids, for example homoserine and vitamins, for example thiamine, biotin or pantothenic acid, may be employed in addition to the abovementioned substances.

[0176] In some embodiments, the pH of the culture can be controlled by any acid or base, or buffer salt, including, but not limited to sodium hydroxide, potassium hydroxide, ammonia, or aqueous ammonia; or acidic compounds such as phosphoric acid or sulfuric acid in a suitable manner. In some embodiments, the pH is generally adjusted to a value of from 6.0 to 8.5, preferably 6.5 to 8.

[0177] In some embodiments, the cultures of the present disclosure may include an anti-foaming agent such as, for example, fatty acid polyglycol esters. In some embodiments the cultures of the present disclosure are modified to stabilize the plasmids of the cultures by adding suitable selective substances such as, for example, antibiotics.

[0178] In some embodiments, the culture is carried out under aerobic conditions. In order to maintain these conditions, oxygen or oxygen-containing gas mixtures such as, for example, air are introduced into the culture. It is likewise possible to use liquids enriched with hydrogen peroxide. The fermentation is carried out, where appropriate, at elevated pressure, for example at an elevated pressure of from 0.03 to 0.2 MPa. The temperature of the culture is normally from 20°C to 45°C and preferably from 25°C to 40°C, particularly preferably from 30°C to 37°C. In batch or fed-batch processes, the cultivation is preferably continued until an amount of the desired product of interest (*e.g.* an organic-chemical compound) sufficient for being recovered has formed. This aim can normally be achieved within 10 hours to 160 hours. In continuous processes, longer cultivation times are possible. The activity of the microorganisms results in a concentration (accumulation) of the product of interest in the fermentation medium and/or in the cells of said microorganisms.

[0179] In some embodiments, the culture is carried out under anaerobic conditions.

Validating Genetically Engineered Strains

[0180] In some embodiments, the present disclosure teaches methods of validating genetically engineered strains comprising the selected promoter operably linked to the selected RNA

degradation gene. In some embodiments, validation comprises comparing the genetically engineered strain to a control strain. In some embodiments, the control strain is a genetically identical strain, which lacks the promoter::RNA degradation gene modification of the genetically engineered strain. Thus in some embodiments, the present disclosure teaches methods of comparing the host performance of the genetically engineered strain to those of the control strain. In some embodiments, enhanced host performance will be measured against a specific selection goal, as described below.

Selection Criteria and Goals

[0181] The testing criteria applied to the methods of the present disclosure will vary with the specific goals of the strain improvement program. The present disclosure may be adapted to meet any program goals. For example, in some embodiments, the program goal may be to maximize single batch yields of reactions with no immediate time limits. In other embodiments, the program goal may be to rebalance biosynthetic yields to produce a specific product, or to produce a particular ratio of products. In other embodiments, the program goal may be to modify the chemical structure of a product, such as lengthening the carbon chain of a polymer. In some embodiments, the program goal may be to improve performance characteristics such as yield, titer, productivity, by-product elimination, tolerance to process excursions, optimal growth temperature and growth rate. In some embodiments, the program goal is improved host performance as measured by volumetric productivity, specific productivity, yield or titer, of a product of interest produced by a host cell.

[0182] In other embodiments, the program goal may be to optimize synthesis efficiency of a commercial strain in terms of final product yield per quantity of inputs (*e.g.,* total amount of ethanol produced per pound of sucrose). In some embodiments, the program goal may be to optimize percent yield. In other embodiments, the program goal may be to optimize synthesis speed, as measured for example in terms of batch completion rates, or yield rates in continuous culturing systems. In other embodiments, the program goal may be to increase strain resistance to a particular phage, or otherwise increase strain vigor/robustness under culture conditions. In yet other embodiments, the program goal may be to improve the strain growth rate (e.g., saturation biomass).

[0183] In some embodiments, strain improvement projects may be subject to more than one goal. In some embodiments, the goal of the strain project may hinge on quality, reliability, or overall profitability. In some embodiments, the present disclosure teaches methods of associated selected mutations or groups of mutations with one or more of the strain properties described above.

[0184] Persons having ordinary skill in the art will recognize how to tailor strain testing criteria to meet the particular project goal. For example, selections of a strain's single batch max yield at reaction saturation may be appropriate for identifying strains with high single batch yields. Selection based on consistency in yield across a range of temperatures and conditions may be appropriate for identifying strains with increased robustness and reliability.

[0185] In some embodiments, the selection criteria for the initial small-batch phase and the tank-based validation will be identical. In other embodiments, tank-based selection may operate under additional and/or different selection criteria. For example, in some embodiments, high-throughput strain selection might be based on single batch reaction completion yields, while tank-based selection may be expanded to include selections based on yields for reaction speed.

[0186] In some embodiments, the genetically engineered host cells of the present disclosure exhibit moderate performance increases over control cells. In some embodiments, the genetically engineered host cells of the present disclosure comprising a selected heterologous promoter polynucleotide operably linked to a selected RNA degradation gene will exhibit at least a 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, 4%, 4.1%, 4.2%, 4.3%, 4.4%, 4.5%, 4.6%, 4.7%, 4.8%, 4.9%, 5%, 5.1%, 5.2%, 5.3%, 5.4%, 5.5%, 5.6%, 5.7%, 5.8%, 5.9%, 6%, 6.1%, 6.2%, 6.3%, 6.4%, 6.5%, 6.6%, 6.7%, 6.8%, 6.9%, 7%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, 8%, 8.1%, 8.2%, 8.3%, 8.4%, 8.5%, 8.6%, 8.7%, 8.8%, 8.9%, 9%, 9.1%, 9.2%, 9.3%, 9.4%, 9.5%, 9.6%, 9.7%, 9.8%, 9.9%, or 10.0% increase in performance when compared to a genetically identical host cell lacking the heterologous promoter polynucleotide. In some embodiments, the present disclosure teaches various ways of measuring performance, including—but not limited to—a microbe's yield, percent yield, productivity, or saturation biomass. A person skilled in the art would thus understand that the presently disclosed percentages or fold increases of microbe performance may refer to any performance increase disclosed herein. In some embodiments, increased performance percentages refer directly to

increases in yield compared to a genetically identical host cell lacking the heterologous promoter polynucleotide.

[0187] In some embodiments, the genetically engineered host cells of the present disclosure exhibit good performance increases over control cells. In some embodiments, the genetically engineered host cells of the present disclosure comprising a selected heterologous promoter polynucleotide operably linked to a selected RNA degradation gene will exhibit at least a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 101%, 102%, 103%, 104%, 105%, 106%, 107%, 108%, 109%, 110%, 111%, 112%, 113%, 114%, 115%, 116%, 117%, 118%, 119%, 120%, 121%, 122%, 123%, 124%, 125%, 126%, 127%, 128%, 129%, 130%, 131%, 132%, 133%, 134%, 135%, 136%, 137%, 138%, 139%, 140%, 141%, 142%, 143%, 144%, 145%, 146%, 147%, 148%, 149%, 150%, 151%, 152%, 153%, 154%, 155%, 156%, 157%, 158%, 159%, 160%, 161%, 162%, 163%, 164%, 165%, 166%, 167%, 168%, 169%, 170%, 171%, 172%, 173%, 174%, 175%, 176%, 177%, 178%, 179%, 180%, 181%, 182%, 183%, 184%, 185%, 186%, 187%, 188%, 189%, 190%, 191%, 192%, 193%, 194%, 195%, 196%, 197%, 198%, 199%, or 200% increase in performance when compared to a genetically identical host cell lacking the heterologous promoter polynucleotide.

[0188] In some embodiments, the genetically engineered host cells of the present disclosure exhibit stellar performance increases over control cells. In some embodiments, the genetically engineered host cells of the present disclosure comprising a selected heterologous promoter polynucleotide operably linked to a selected RNA degradation gene will exhibit at least a 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold or more, increase in performance when compared to a genetically identical host cell lacking the heterologous promoter polynucleotide. In some embodiments, the increased performance is assessed by a productivity measure selected from the group consisting of volumetric productivity, specific productivity, yield, titer, and total titer. In some embodiments, the increased performance is host-cell yield of the desired product. In some

embodiments, performance is determined by saturation biomass. In some embodiments, the present disclosure teaches that genetically engineered host cells with other perturbations of one or more RNA degradation genes can also exhibit moderate, good, and stellar performance improvements as described above. For example, in some embodiments, host cells with a mutated RNA degradation gene (*e.g.*, a mutated start codon), can exhibit moderate (1-10%), good (1-200%), or stellar (2-20-fold) increase in performance over a control cells.

Product Recovery and Quantification

[0189] Methods for analyzing the production of products of interest are known to those of skill in the art and are discussed throughout the present specification. Such methods may be employed when screening the strains of the disclosure.

[0190] In some embodiments, the present disclosure teaches methods of improving strains designed to produce non-secreted intracellular products. For example, the present disclosure teaches methods of improving the robustness, yield, efficiency, or overall desirability of cell cultures producing intracellular enzymes, oils, pharmaceuticals, or other valuable small molecules or peptides. The recovery or isolation of non-secreted intracellular products can be achieved by lysis and recovery techniques that are well known in the art, including those described herein.

[0191] For example, in some embodiments, cells of the present disclosure can be harvested by centrifugation, filtration, settling, or other method. Harvested cells are then disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well known to those skilled in the art.

[0192] The resulting product of interest, *e.g.* a polypeptide, may be recovered/isolated and optionally purified by any of a number of methods known in the art. For example, a product polypeptide may be isolated from the nutrient medium by conventional procedures including, but not limited to: centrifugation, filtration, extraction, spray-drying, evaporation, chromatography (*e.g.*, ion exchange, affinity, hydrophobic interaction, chromatofocusing, and size exclusion), or precipitation. Finally, high performance liquid chromatography (HPLC) can be employed in the final purification steps. (*See* for example Purification of intracellular protein as described in Parry *et al.*, 2001, *Biochem. J.*353:117, and Hong *et al.*, 2007, *Appl. Microbiol. Biotechnol.* 73:1331, both incorporated herein by reference).

[0193] In addition to the references noted *supra*, a variety of purification methods are well known in the art, including, for example, those set forth in: Sandana (1997) *Bioseparation of Proteins*, Academic Press, Inc.; Bollag *et al.* (1996) *Protein Methods,* 2nd Edition, Wiley-Liss, NY; Walker (1996) *The Protein Protocols Handbook* Humana Press, NJ; Harris and Angal (1990) *Protein Purification Applications: A Practical Approach*, IRL Press at Oxford, Oxford, England; Harris and Angal *Protein Purification Methods: A Practical Approach*, IRL Press at Oxford, Oxford, England; Scopes (1993) *Protein Purification: Principles and Practice* 3rd Edition, Springer Verlag, NY; Janson and Ryden (1998) *Protein Purification: Principles, High Resolution Methods and Applications*, Second Edition, Wiley-VCH, NY; and Walker (1998) *Protein Protocols on CD-ROM*, Humana Press, NJ, all of which are incorporated herein by reference.

[0194] In some embodiments, the present disclosure teaches the methods of improving strains designed to produce secreted products. For example, the present disclosure teaches methods of improving the robustness, yield, efficiency, or overall desirability of cell cultures producing valuable small molecules or peptides.

[0195] In some embodiments, immunological methods may be used to detect and/or purify secreted or non-secreted products produced by the cells of the present disclosure. In one example approach, antibody raised against a product molecule (*e.g.*, against an insulin polypeptide or an immunogenic fragment thereof) using conventional methods is immobilized on beads, mixed with cell culture media under conditions in which the endoglucanase is bound, and precipitated. In some embodiments, the present disclosure teaches the use of enzyme-linked immunosorbent assays (ELISA).

[0196] In other related embodiments, immunochromatography is used, as disclosed in U.S. Pat. No. 5,591,645, U.S. Pat. No. 4,855,240, U.S. Pat. No. 4,435,504, U.S. Pat. No. 4,980,298, and Se-Hwan Paek, *et al.*, "Development of rapid One-Step Immunochromatographic assay, Methods", 22, 53-60, 2000), each of which are incorporated by reference herein. A general immunochromatography detects a specimen by using two antibodies. A first antibody exists in a test solution or at a portion at an end of a test piece in an approximately rectangular shape made from a porous membrane, where the test solution is dropped. This antibody is labeled with latex particles or gold colloidal particles (this antibody will be called as a labeled antibody hereinafter). When the dropped test solution includes a specimen to be detected, the labeled

antibody recognizes the specimen so as to be bonded with the specimen. A complex of the specimen and labeled antibody flows by capillarity toward an absorber, which is made from a filter paper and attached to an end opposite to the end having included the labeled antibody. During the flow, the complex of the specimen and labeled antibody is recognized and caught by a second antibody (it will be called as a tapping antibody hereinafter) existing at the middle of the porous membrane and, as a result of this, the complex appears at a detection part on the porous membrane as a visible signal and is detected.

[00100] In some embodiments, the screening methods of the present disclosure are based on photometric detection techniques (absorption, fluorescence). For example, in some embodiments, detection may be based on the presence of a fluorophore detector such as GFP bound to an antibody. In other embodiments, the photometric detection may be based on the accumulation on the desired product from the cell culture. In some embodiments, the product may be detectable via UV of the culture or extracts from said culture.

Example 1- Evaluation of Candidate Promoter Activity.

[0197] To evaluate candidate promoter activity, a set of plasmid based fluorescence reporter constructs was designed. Briefly, each promoter was cloned in front of *eyfp*, a gene encoding yellow fluorescent protein in the shuttle vector pK18rep. These plasmids were transformed into *C. glutamicum* NRRL B-11474 and promoter activity was assessed by measuring the accumulation of YFP protein by spectrometry.

[0198] The shuttle vector pK18rep was constructed by replacing the sacB gene in pK18mobSacB (ATCC 87087) with the pBL1 origin of replication (GenBank: AF092037.1) resulting in a vector able to propagate in both *E. coli* and *C. glutamicum*.

[0199] *C. glutamicum* host cells transformed with eyfp pK18rep plasmids comprising each of the selected promoters from SEQ ID Nos 1-8 were selected on BHI agar plus 25 µg/mL Kanamycin. For each transformation, multiple single colonies were picked and inoculated into individual wells of a 96 mid-well block containing 300 µL of BHI media plus 25 µg/mL Kanamycin. The cells were grown to saturation by incubation for 48 h at 30 °C shaking at 1,000 rpm.

[0200] After incubation, cultures were centrifuged for 5 min at 3,500 rpm and the media was removed by aspiration. Cells were washed once by resuspension in 300 µL of PBS and

centrifugation for 5 min at 3,500 rpm followed by aspiration of the supernatant and a final resuspension in 300 5 µL of PBS. A 20 µL aliquot of this mixture was transferred to a 96-well full area black clear bottom assay plate containing 180µL of PBS. The optical density of the cells at 600 nm was measured with the SpectraMax M5 microplate reader and the fluorescence was measured with the TECAN M1000 microplate leader by exciting at 514 nm and measuring emission at 527 nm. For each well a normalized fluorescence activity was calculated by dividing fluorescence by optical density.

[0201] Host cells transformed with the parent plasmid pK18rep acted as a negative control. Normalized fluorescence activity was compared between reporter constructs and between biological replicates. A numerical summary of promoter activity is presented in Table 4 below.

Table 4. Expression strength of selected promoters.

SEQ ID No.	Promoter Expression Strength (Mean)	Standard Deviation	Standard Error of Mean	No. of Replicates
1	114402	52987.9	15296	12
2	89243	16162.2	3708	19
3	44527	18110.3	4155	19
4	43592	3643	1152	10
5	11286	10459.4	3154	11
6	4723	1854.3	425	19
7	661	731.9	173	18
8	98	537.5	144	14
Control	-45	214.9	48	20

[0202] The entire disclosures of U.S. Patent Application No. 62/264,232, filed on December 07, 2015, and PCT Publication No. WO2017/100376 are each hereby incorporated by reference in its entirety for all purposes.

Example 2- Effect of Promoter Perturbation on Saturation Biomass

[0203] Promoter::gene combinations of the present disclosure were empirically tested to determine their effect on culture saturation biomass.

[0204] Targets for perturbation of the *C. glutamicum* RNA degradosome were selected based on their annotation in the KEGG database (http://www.genome.jp/kegg/kegg1.html), as disclosed in the Table 1 of the present disclosure. The native promoter for each of the targeted genes was determined based on a literature search. A list of the identified native promoters is provided in Table 5 below.

TABLE 5. Identified Native Promoter Sequences

Gene Target	Short Name	Identified Native Promoter Sequence	Number of Base Pairs Replaced (From Start Codon)
cg1144	G1	SEQ ID No: 23	101
cg2453	G2	SEQ ID No: 24	61
cshA	G3	SEQ ID No: 25	62
dnak	G4	SEQ ID No: 26	179
eno	G5	SEQ ID No: 27	129
gpsI	G6	SEQ ID No: 28	154
groEL	G7	SEQ ID No: 29	205
groEL2	G9	None Identified	0

Gene Target	Short Name	Identified Native Promoter Sequence	Number of Base Pairs Replaced (From Start Codon)
mutM2	G10	SEQ ID No: 30	59
rhlE	G11	SEQ ID No: 31	59
rho	G12	SEQ ID No: 32	101
rne	G13	SEQ ID No: 33	140
cg2160/RNAse J	G14	None Identified	0

[0205] If available, the entire native promoter sequence was replaced with each of promoters of the present disclosure as outlined in Table 5 above. If no native promoter could be identified, each of 8 promoters in the promoter ladder was inserted directly 5' of the target's start codon.

[0206] Plasmids to make these changes in the *C. glutamicum* genome were generated using yeast homologous recombination and were then propagated in *E. coli*. Each plasmid was built from a common backbone into which ~2kb homologous regions were inserted that flank the location of the genomic edit. These homologous regions were PCR amplified from *C. glutamicum* genomic DNA. In between these 2kb homologous regions, the new promoter or start codon was encoded in the 5' end of the primer used to PCR the homologous region.

[0207] Plasmids were sequence confirmed and then electroporated into *C. glutamicum*. After selection for genomic integration, plasmid backbone DNA was removed using the loopout counter selection methods of the present disclosure. Correctly built *C. glutamicum* strains were confirmed by PCR and sequencing.

[0208] Correctly built genetically engineered *C. glutamicum* strains were consolidated and then propagated in small scale cultures designed to assess saturation biomass performance. Small-scale cultures conducted using media reflective of media from industrial scale cultures. Saturation biomass was measured by determining OD600 of cultures at 96 hrs. Data from this small-scale test is included in Table 6 below. Promoter::Gene combinations exhibiting the best improvements in

biomass are highlighted. A visual representation of the results from this experiment is provided in Figures 3A and 3B.

[0209] The results as outlined in Figures 3A and 3B demonstrated that promoter Pcg1860 (P3, SEQ ID NO: 3) was particularly effective when operably linked with cshA (G3, SEQ ID NO: 11) and gpsI (G6, SEQ ID NO: 6) at increasing saturation biomass.

[0210] The results further demonstrated that promoter Pcg3121 (P8, SEQ ID NO: 8) was particularly effective when operably linked with gpsI (G6, SEQ ID NO: 6) and rne (G13, SEQ ID NO: 21) at increasing saturation biomass.

TABLE 6. Effect of Various Promoter::Gene combinations on Culture Biomass

Number of replicates	Promoter Short Name	Gene Short name	Mean Saturation Biomass value OD600	Std Error	Lower 95%	Upper 95%		
91	WT	reference control	0.23611	0.00064	0.2348	0.2374		
48	Control	Promoter Parent Strain	0.19739	0.00089	0.1957	0.1991	percent performance change from parent	sigma
8	P1	G12	0.21027	0.00217	0.206	0.2145	6.5%	1.1%
8	P1	G7	0.19747	0.00217	0.1932	0.2017	0.0%	1.1%
8	P1	G10	0.1969	0.00217	0.1926	0.2012	-0.2%	1.1%
8	P1	G2	0.19447	0.00217	0.1902	0.1987	-1.5%	1.1%
8	P1	G11	0.20231	0.00217	0.198	0.2066	2.5%	1.1%
8	P1	G6	0.23219	0.00217	0.2279	0.2364	17.6%	1.1%
4	P2	G5	0.22844	0.00307	0.2224	0.2345	15.7%	1.6%
8	P2	G4	0.19898	0.00217	0.1947	0.2032	0.8%	1.1%
8	P2	G10	0.20436	0.00217	0.2001	0.2086	3.5%	1.1%
8	P2	G9	0.20499	0.00217	0.2007	0.2093	3.9%	1.1%

Number of replicates	Promoter Short Name	Gene Short name	Mean Saturation Biomass value OD600	Std Error	Lower 95%	Upper 95%	percent performance change from parent	sigma
91	WT	reference control	0.23611	0.00064	0.2348	0.2374		
48	Control	Promoter Parent Strain	0.19739	0.00089	0.1957	0.1991		
8	P2	G2	0.19516	0.00217	0.1909	0.1994	-1.1%	1.1%
8	P2	G11	0.20623	0.00217	0.202	0.2105	4.5%	1.1%
7	P3	G3	0.23954	0.00232	0.235	0.2441	21.4%	1.2%
8	P3	G1	0.19539	0.00217	0.1911	0.1997	-1.0%	1.1%
8	P3	G9	0.19734	0.00217	0.1931	0.2016	0.0%	1.1%
8	P3	G11	0.22449	0.00217	0.2202	0.2288	13.7%	1.1%
4	P3	G6	0.24268	0.00307	0.2367	0.2487	22.9%	1.6%
8	P5	G12	0.19038	0.00217	0.1861	0.1946	-3.6%	1.1%
8	P5	G7	0.19095	0.00217	0.1867	0.1952	-3.3%	1.1%
8	P5	G5	0.20366	0.00217	0.1994	0.2079	3.2%	1.1%
8	P5	G4	0.19408	0.00217	0.1898	0.1983	-1.7%	1.1%
8	P5	G10	0.20437	0.00217	0.2001	0.2086	3.5%	1.1%

Number of replicates	Promoter Short Name	Gene Short name	Mean Saturation Biomass value OD600	Std Error	Lower 95%	Upper 95%	percent performance change from parent	sigma
91	WT	reference control	0.23611	0.00064	0.2348	0.2374		
48	Control	Promoter Parent Strain	0.19739	0.00089	0.1957	0.1991		
8	P5	G9	0.2022	0.00217	0.1979	0.2065	2.4%	1.1%
7	P5	G2	0.19791	0.00232	0.1934	0.2025	0.3%	1.2%
8	P5	G6	0.20965	0.00217	0.2054	0.2139	6.2%	1.1%
8	P6	G12	0.17041	0.00217	0.1661	0.1747	-13.7%	1.1%
8	P6	G3	0.18666	0.00217	0.1824	0.1909	-5.4%	1.1%
8	P6	G5	0.22218	0.00217	0.2179	0.2264	12.6%	1.1%
8	P6	G2	0.19527	0.00217	0.191	0.1995	-1.1%	1.1%
8	P6	G11	0.21746	0.00217	0.2132	0.2217	10.2%	1.1%
8	P8	G13	0.24008	0.00217	0.2358	0.2443	21.6%	1.1%
8	P8	G1	0.20357	0.00217	0.1993	0.2078	3.1%	1.1%
8	P8	G10	0.19629	0.00217	0.192	0.2006	-0.6%	1.1%
8	P8	G9	0.19315	0.00217	0.1889	0.1974	-2.1%	1.1%

Number of replicates	Promoter Short Name	Gene Short name	Mean Saturation Biomass value OD600	Std Error	Lower 95%	Upper 95%	percent performance change from parent	sigma
91	WT	reference control	0.23611	0.00064	0.2348	0.2374		
48	Control	Promoter Parent Strain	0.19739	0.00089	0.1957	0.1991		
5	P8	G11	0.19225	0.00274	0.1869	0.1976	-2.6%	1.4%
8	P8	G6	0.23994	0.00217	0.2357	0.2442	21.6%	1.1%

Example 3- Effect of Promoter Perturbation on Product Titer (Yield)

[0211] Genetically engineered cultures with the various promoter::gene combinations from Example 2 were propagated in small-scale cultures designed to assess product titer performance. Small-scale cultures were conducted using media reflective of media from industrial scale cultures. Product titer was optically measured at carbon exhaustion (*i.e.*, yield) with a standard colorimetric assay. Cultures were grown until no further changes in product culture could be measured. Data from this small-scale test is summarized in Table 7 below. Promoter::Gene combinations exhibiting the best improvements in yield are highlighted. A visual representation of the results from this experiment is provided in Figures 3A and 3B.

[0212] Start coding swapping and promoter swapping can affect multiple phenotypes: Pcg3381 (P6, SEQ ID NO: 6) linked to cshA (G3, SEQ ID NO: 11) improves product yield whereas Pcg3381 (P6, SEQ ID NO: 6) linked to eno (G5, SEQ ID NO: 13) improves saturation biomass.

[0213] The results also show that the same RNA degradation gene target can positively affect different phenotypes depending on how it's targeted, Pcg1860 (P3, SEQ ID NO: 3) linked to cshA (G3, SEQ ID NO: 11) improves saturation biomass, whereas Pcg3381 (P6, SEQ ID NO: 6) linked to cshA (G3, SEQ ID NO: 11) improves product yield.

TABLE 7. Effect of Various Promoter::Gene combinations on Product Yield

Number of replicates	Promoter Short Name	Gene Short Name	Mean titer performance value	Std Error	Lower 95%	Upper 95%		
96	WT	reference control	1.02651	0.00198	1.0226	1.0304		
40	WT	Promoter Parent Strain	0.95429	0.00307	0.9483	0.9603	percent performance change from parent	sigma
8	P1	G12	0.94201	0.00687	0.9285	0.9555	-1.3%	0.7%
4	P1	G7	0.9615	0.00972	0.9424	0.9806	0.8%	1.0%
8	P1	G10	0.9302	0.00687	0.9167	0.9437	-2.5%	0.7%
4	P1	G2	0.95563	0.00972	0.9365	0.9747	0.1%	1.0%
4	P1	G11	0.97209	0.00972	0.953	0.9912	1.9%	1.0%
8	P1	G6	0.92957	0.00687	0.9161	0.9431	-2.6%	0.7%
8	P2	G5	0.94668	0.00687	0.9332	0.9602	-0.8%	0.7%
4	P2	G4	0.97126	0.00972	0.9522	0.9904	1.8%	1.0%
8	P2	G10	0.9347	0.00687	0.9212	0.9482	-2.1%	0.7%
8	P2	G9	0.97912	0.00687	0.9656	0.9926	2.6%	0.7%
4	P2	G2	0.95691	0.00972	0.9378	0.976	0.3%	1.0%
8	P2	G11	0.95053	0.00687	0.937	0.964	-0.4%	0.7%
8	P3	G3	0.87718	0.00687	0.8637	0.8907	-8.1%	0.7%

Number of replicates	Promoter Short Name	Gene Short Name	Mean titer performance value	Std Error	Lower 95%	Upper 95%		
96	WT	reference control	1.02651	0.00198	1.0226	1.0304		
40	WT	Promoter Parent Strain	0.95429	0.00307	0.9483	0.9603	percent performance change from parent	sigma
8	P3	G1	0.95909	0.00687	0.9456	0.9726	0.5%	0.7%
8	P3	G9	0.98443	0.00687	0.9709	0.9979	3.2%	0.7%
8	P3	G11	0.96568	0.00687	0.9522	0.9792	1.2%	0.7%
8	P3	G6	0.93892	0.00687	0.9254	0.9524	-1.6%	0.7%
8	P5	G12	0.96723	0.00687	0.9537	0.9807	1.4%	0.7%
6	P5	G7	0.93788	0.00793	0.9223	0.9535	-1.7%	0.8%
8	P5	G5	0.91367	0.00687	0.9002	0.9272	-4.3%	0.7%
4	P5	G4	0.92425	0.00972	0.9051	0.9434	-3.1%	1.0%
8	P5	G10	0.96478	0.00687	0.9513	0.9783	1.1%	0.7%
8	P5	G9	0.94909	0.00687	0.9356	0.9626	-0.5%	0.7%
3	P5	G2	0.95719	0.01122	0.9351	0.9793	0.3%	1.2%
8	P5	G6	0.96477	0.00687	0.9513	0.9783	1.1%	0.7%
4	P6	G12	0.97625	0.00972	0.9571	0.9954	2.3%	1.0%
4	P6	G3	1.01238	0.00972	0.9933	1.0315	6.1%	1.0%
4	P6	G5	0.89369	0.00972	0.8746	0.9128	-6.4%	1.0%
8	P6	G2	0.96711	0.00687	0.9536	0.9806	1.3%	0.7%

Number of replicates	Promoter Short Name	Gene Short Name	Mean titer performance value	Std Error	Lower 95%	Upper 95%		
96	WT	reference control	1.02651	0.00198	1.0226	1.0304		
40	WT	Promoter Parent Strain	0.95429	0.00307	0.9483	0.9603	percent performance change from parent	sigma
8	P6	G11	0.9461	0.00687	0.9326	0.9596	-0.9%	0.7%
8	P8	G13	0.93432	0.00687	0.9208	0.9478	-2.1%	0.7%
4	P8	G1	0.9909	0.00972	0.9718	1.01	3.8%	1.0%
8	P8	G10	0.9453	0.00687	0.9318	0.9588	-0.9%	0.7%
8	P8	G9	0.95958	0.00687	0.9461	0.9731	0.6%	0.7%
6	P8	G11	0.965	0.00793	0.9494	0.9806	1.1%	0.8%
8	P8	G6	0.90574	0.00687	0.8922	0.9193	-5.1%	0.7%

Example 4- Effect of Mutations on Saturation Biomass and Product Titer (Yield)

[0214] The product titer and saturation biomass effects of start codon replacements on selected RNA degradation genes of the present disclosure were tested. The plasmids used to genetically engineer the *C. glutamicum* genome were generated using yeast homologous recombination and were then propagated in *E. coli*. Each plasmid was built from a common backbone into which ~2kb homologous regions were inserted that flank the location of the genomic edit. These homologous regions were PCR amplified from *C. glutamicum* genomic DNA. In between these 2kb homologous regions, the new start codon was encoded in the 5' end of the primer used to PCR the homologous region.

[0215] Plasmids were sequenced to confirm successful cloning, and were then electroporated into *C. glutamicum*. After selection for genomic integration, plasmid backbone DNA was removed using the loopout counter selection methods of the present disclosure. Correctly engineered *C. glutamicum* strains were confirmed by PCR and sequencing. In this case, all start codons were changed to TTG, though other changes to any start codons selected from the group consisting of ATG, GTG, and TTG, are within the scope of this invention.

[0216] Genetically engineered cultures with the various start codon replacements were propagated in small scale cultures designed to assess product performance and biomass of the culture. Culture conditions and biomass and titer measurements were conducted as described in Examples 2 and 3. Measurements of product titer at carbon exhaustion therefore represented product yield values for the host cell culture. Cultures were grown until no further changes in product culture could be measured. Biomass cultures were grown for 96 hrs. Data from this small-scale test is included in Tables 8 and 9 below. Start Codon replacements exhibiting the best improvements in product yield and saturation biomass are highlighted. A visual representation of the results from this experiment is also provided in Figure 4.

[0217] Interestingly, promoter replacement of the gpsI gene increases saturation biomass of genetically engineered cultures, while switching the start codon to a less expressed codon decreases it.

[0218] The results further suggest that rhlE (G11, SEQ ID NO: 19) GTG>TTG codon change improves product yield whereas rne (G13, SEQ ID NO: 21) GTG>TTG improves saturation biomass.

[0219] In some embodiments, a single target perturbation can improve both product yield and saturation biomass, as in the case of rne (G13, SEQ ID NO: 21) GTG>TTG.

TABLE 8. Effect of Start Codon Changes on Saturation Biomass

Number of replicates	Start Codon Change	Gene	Mean saturation biomass value OD600	Std Error	Lower 95%	Upper 95%		
12	WT	reference control	0.23128	0.00122	0.2289	0.2337		
47	WT	parent for start codon swap strains	0.23464	0.00062	0.2334	0.2359	percent performance change from parent	sigma
4	GTG>TTG	G13	0.24833	0.00211	0.2442	0.2525	5.8%	0.9%
8	ATG>TTG	G2	0.24097	0.00149	0.238	0.2439	2.7%	0.6%
8	ATG>TTG	G6	0.22117	0.00149	0.2182	0.2241	-5.7%	0.6%
4	GTG>TTG	G11	0.23333	0.00211	0.2292	0.2375	-0.6%	0.9%
6	ATG>TTG	G4	0.23264	0.00173	0.2292	0.236	-0.9%	0.7%
8	ATG>TTG	G9	0.22951	0.00149	0.2266	0.2325	-2.2%	0.6%

TABLE 9. Effect of Start Codon Changes on Product Yield

Number of replicates	Start Codon Change	Gene	Mean titer performance value	Std Error	Lower 95%	Upper 95%		
16	WT	reference control	1.01491	0.0047	1.0057	1.0242		
62	WT	parent for start codon swap strains	1.01782	0.00239	1.0131	1.0225	**percent performance change from parent**	**sigma**
12	GTG>TTG	G13	1.03308	0.00543	1.0224	1.0438	1.5%	0.5%
12	ATG>TTG	G2	1.027	0.00543	1.0163	1.0377	0.9%	0.5%
12	ATG>TTG	G6	1.06301	0.00543	1.0523	1.0737	4.4%	0.5%
4	GTG>TTG	G11	1.06204	0.0094	1.0435	1.0805	4.3%	0.9%
12	ATG>TTG	G4	1.02328	0.00543	1.0126	1.034	0.5%	0.5%
12	ATG>TTG	G9	1.0478	0.00543	1.0371	1.0585	2.9%	0.5%

Example 5- Validation of Additional Promoter::Gene combinations Improving Product Titer

[0220] Promoter::gene combinations of the present disclosure were empirically tested to determine their effect on titers of a product of interest.

[0221] Targets for perturbation of the *C. glutamicum* RNA degradosome were selected as per Example 2. If available, the entire native promoter sequence was replaced with each of promoters of the present disclosure as outlined in Table 5 above. If no native promoter could be identified, each of 8 promoters in the promoter ladder was inserted directly 5' of the target's start codon.

[0222] Plasmids to make these changes in the *C. glutamicum* genome were generated using yeast homologous recombination and were then propagated in *E. coli*. Each plasmid was built from a common backbone into which ~2kb homologous regions were inserted that flank the location of the genomic edit. These homologous regions were PCR amplified from *C. glutamicum* genomic DNA. In between these 2kb homologous regions, the new promoter or start codon was encoded in the 5' end of the primer used to PCR the homologous region.

[0223] Plasmids were sequence confirmed and then electroporated into *C. glutamicum*. After selection for genomic integration, plasmid backbone DNA was removed using the loopout counter selection methods of the present disclosure. Correctly built *C. glutamicum* strains were confirmed by PCR and sequencing.

[0224] Correctly built genetically engineered *C. glutamicum* strains were consolidated and then propagated in small scale cultures designed to assess product titers. The product of interest is generated by methylation of substrate included in the fermentation medium. This methylation reaction is catalyzed by a heterologous S-adenosyl methionine-dependent o-methyltransferase expressed from a replicating plasmid.

[0225] After a separate biomass propagation step in 96-well microwell plates, cell mass was added to fermentation media containing substrate in 96-well microwell plates and bioconversion was allowed to proceed for 24 hrs. Titers of product were determined for each strain using high-performance liquid chromatography from samples taken at 24 hrs, in order to measure expected yield. Cultures were grown until no further changes in product culture could be measured. Data from this test is included in Table 10 below. Promoter::gene combinations exhibiting the best

improvements in product yield are highlighted. A visual representation of the results from this experiment is provided in Figure 5.

Table 10. Effect of various promoter::gene combinations on product yield in small scale cultivation

Number of replicates	Promoter Short Name	Gene Short name	Mean performance value	Std Error	Lower 95%	Upper 95%		
4	Control	Parent Strain	347.065	37.749	272.37	421.76	percent performance change from parent	sigma
4	P5	G9	367.928	37.749	293.23	442.63	6	10
4	P8	G9	393.817	37.749	319.12	468.51	13	10
4	P6	G9	375.547	37.749	300.85	450.24	8	10
4	P3	G11	144.664	37.749	69.97	219.36	-58	26
4	P5	G11	297.898	37.749	223.2	372.6	-14	13
4	P1	G11	377.716	37.749	303.02	452.41	9	10
4	P8	G11	407.026	37.749	332.33	481.72	17	9
4	P6	G11	387.75	37.749	313.05	462.45	12	10
4	P5	G3	417.166	37.749	342.47	491.86	20	9
4	P2	G3	413.269	37.749	338.57	487.97	19	9
4	P6	G3	394.046	37.749	319.35	468.74	14	10
4	P1	G12	435.684	37.749	360.99	510.38	26	9
4	P2	G12	401.982	37.749	327.28	476.68	16	9
4	P6	G12	365.794	37.749	291.1	440.49	5	10
4	P3	G6	417.801	37.749	343.1	492.5	20	9
4	P5	G6	375.335	37.749	300.64	450.03	8	10
4	P2	G6	283.543	37.749	208.84	358.24	-18	13

Number of replicates	Promoter Short Name	Gene Short name	Mean performance value	Std Error	Lower 95%	Upper 95%		
4	Control	Parent Strain	347.065	37.749	272.37	421.76	percent performance change from parent	sigma
4	P8	G6	264.055	37.749	189.36	338.75	-24	14
3	P6	G6	400.818	43.588	314.56	487.07	15	11
4	P3	G2	181.222	37.749	106.52	255.92	-48	21
4	P5	G2	298.603	37.749	223.91	373.3	-14	13
4	P6	G2	290.191	37.749	215.49	364.89	-16	13
4	P3	G13	204.889	37.749	130.19	279.59	-41	18
4	P5	G13	403.111	37.749	328.41	477.81	16	9
4	P8	G13	363.237	37.749	288.54	437.94	5	10
4	P3	G7	422.051	37.749	347.35	496.75	22	9
4	P5	G7	267.106	37.749	192.41	341.8	-23	14
4	P1	G7	291.39	37.749	216.69	366.09	-16	13
4	P3	G4	425.878	37.749	351.18	500.58	23	9
4	P3	G10	350.593	37.749	275.89	425.29	1	11
4	P5	G10	320.718	37.749	246.02	395.42	-8	12
4	P1	G10	410.906	37.749	336.21	485.6	18	9
4	P2	G10	413.604	37.749	338.91	488.3	19	9
4	P8	G10	416.955	37.749	342.26	491.65	20	9
4	P6	G10	345.231	37.749	270.53	419.93	-1	11

Example 6- Validation of High Throughput Results in Larger Cultures.

[0226] The beneficial promoter::gene combinations identified by high throughput analysis of Example 5 were evaluated in a larger volume shake flask system. Cell mass was generated by cultivation in 250mL baffled Erlenmeyer flasks, and transferred to flasks containing fermentation medium and substrate. Bioconversion of substrate to product was allowed to proceed for 24 hrs and product titers evaluated by high-performance liquid chromatography as above. Data from this validation of the host cell strain comprising (P1::G12) is summarized in Table 11.

Table 11. Effect of P1::G12 promoter gene combination on product titer in Erlenmeyer flask cultivation

Number of replicates	Promoter Short Name	Gene Short name	Mean performance value	Lower 95%	Upper 95%	Percent performance change from parent
4	Control	Parent Strain	622.752	605.055	640.449	0
4	P1	G12	749.943	736.374	763.512	20

[0227] Host cell cultures comprising the P1 promoter operably linked to the G12 RNA degradation gene exhibited 20% higher titer at carbon exhaustion, demonstrating significantly higher yields than the control parent host cell cultures lacking the rhoI (G12) promoter modification.

Example 7- Identification of RNA Degradation Gene Homologs in Other Species

[0228] The RNA degradation gene sequences from the *Corynebacteria* disclosed in Table 1 were used to identify homologous gene variants from organisms in the same genus, as well as orthologous genes from other eukaryotic and prokaryotic organisms.

[0229] Briefly, amino acid sequences for the RNA degradation genes disclosed in Table 1 were used as search strings in the NCBI BLASTP® database to identify related sequences with high homology to the search gene. Initial searches were conducted with default search parameters in order to identify highly related bacterial homologs for each searched gene. Secondary searches limited to specific *Saccharomyces cerevisiae* were also conducted to identify orthologous sequences in selected genus/species.

[0230] The following Table 12 provides the NCBI Reference Sequence Name of the polypeptide sequences of genes identified during this search. Additional homologs and orthologs are

identifiable by additional sequence searches based on the RNA degradation gene sequences of the present disclosure.

Table 12. RNA Degradation Gene Homologs Identified Through BLASTP® Homology Search Engine

Gene	*Corynebacterium*	*Saccharomyces cerevisiae*
cg1144	WP_004568112.1 BAB98402.1 WP_011897001.1 WP_044029870.1 WP_003856809.1 BAU95388.1 WP_053544501.1 ANE03625.1 WP_011075272.1	None Identified
cg2453	WP_011014974.1 WP_020948617.1 WP_011897604.1 CAF20576.1 WP_006283992.1 WP_040967649.1 ANE04470.1 BAU96563.1 BAB99627.1	None Identified
cshA	WP_011014161.1 WP_040072671.1 WP_060564360.1 WP_011265695.1	AJU31713.1 NP_011932.2 GAA23780.1 AJU22152.1

Gene	*Corynebacterium*	*Saccharomyces cerevisiae*
	WP_034983681.1	AJU20676.1
	WP_063967450.1	CAY80069.1
	WP_040967279.1	AJU31455.1
	WP_038583556.1	AJU18404.1
	WP_003854929.1	AJU16618.1
		AJU22408.1
dnaK	WP_003862798.1	P0CS91.1
	WP_003862798.1	NP_012579.1
	WP_003862798.1	NP_011029.3
	WP_011015390.1	AJU42857.1
	WP_003853569.1	NP_009478.1
	BAU97148.1	NP_010884.1
	ANE04953.1	EDN63079.1
	WP_053545750.1	AJU50999.1
	WP_047253930.1	CAY79287.1
		AJV34706.1
eno	WP_003856756.1	AJU27263.1
	WP_053544480.1	AJR76839.1
	WP_015650797.1	AJU32945.1
	WP_011075256.1	AJU25070.1
	WP_018019189.1	AJR81784.1
	WP_018119032.1	AJU34506.1
	WP_055122813.1	AJU33817.1
	WP_055178258.1	AJP39022.1
	WP_010187392.1	AHY7959.1
		AJU19515.1
gpsI	WP_038584450.1	None Identified
	WP_040967544.1	
	WP_044030042.1	
	WP_003861678.1	

Gene	*Corynebacterium*	*Saccharomyces cerevisiae*
	WP_003857481.1 WP_011014796.1 WP_063967578.1 WP_006284228.1 WP_011897394.1	
groEL	WP_038585947.1 WP_003862917.1 WP_063967760.1 WP_040967902.1 WP_060565225.1 WP_003853751.1 BAU97072.1 ANE04893.1 WP_053545701.1 WP_006769076.1	AJV50345.1 AJV51242.1 AJP40402.1 CAY81488.1 AJV60668.1 NP_013360.1 EGA73773.1 AJV59776.1 AJV70941.1 EGA57589.1
groEL2	WP_003854561.1 WP_011013754.1 WP_015439426.1 WP_006284375.1 WP_011896815.1 BAU94999.1 WP_053544202.1 ANE03290.1 WP_015650463.1 WP_006769721.1	AJV51242.1 AJP40402.1 AJV50345.1 EGA57589.1 AJV60668.1 AJV74435.1 AJV59776.1 NP_013360.1 EGA73773.1 CAY81488.1
mutM2	WP_060565392.1 WP_038586460.1 WP_011015556.1 WP_011266054.1	None Identified

Gene	*Corynebacterium*	*Saccharomyces cerevisiae*
	WP_059290038.1 WP_003861221.1 WP_003855116.1 WP_040073075.1 WP_006286827.1 BAU97354.1	
rhlE	WP_060564204.1 WP_003863544.1 WP_003858152.1 WP_006283683.1 WP_011013876.1 WP_038583081.1 BAU95157.1 ANE03421.1 WP_015650586.1 WP_053544338.1	EGA60267.1 NP_014287.3 AJT17782.1 A6ZRX0.1 AJT33306.1 AJT08370.1 KZV08510.1 EGA84542.1 AJT14054.1 EWG85578.1
rho	WP_060564388.1 WP_038583630.1 WP_011897097.1 WP_059289111.1 WP_003854867.1 WP_031511799.1 WP_003861319.1 WP_040967300.1 WP_063967458.1 ANE03769.1	None Identified
rne	WP_060564901.1 WP_038585170.1 WP_003859300.1 WP_011897695.1	None Identified

Gene	*Corynebacterium*	*Saccharomyces cerevisiae*
	WP_034983859.1 WP_011015068.1 WP_040072884.1 WP_004567676.1 WP_040967734.1 WP_059289673.1	
cg2160/RNAse J	WP_011014791.1 WP_003857476.1 WP_044030039.1 WP_040967540.1 WP_063967576.1 WP_059289432.1 WP_011897391.1 BAU96303.1 WP_015651527.1 ANE04245.1	None Identified

Further Embodiments of the Invention

[0231] Other subject matter contemplated by the present disclosure is set out in the following numbered embodiments:

1. A genetically engineered host cell with enhanced industrial performance, said host cell comprising:
    a. a heterologous promoter polynucleotide, and
    b. a polynucleotide encoding an RNA degradation gene;
    wherein the heterologous promoter polynucleotide is operably linked to the polynucleotide encoding the RNA degradation gene.

2. The genetically engineered host cell of embodiment 1, wherein the RNA degradation gene is an endogenous gene.

3. The genetically engineered host cell of embodiment 1, wherein the heterologous promoter is a promoter comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

4. The genetically engineered host cell of embodiment 3, wherein the polynucleotide encoding the RNA degradation gene is a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

4.1 The genetically engineered host cell of embodiment 3, wherein the polynucleotide encoding the RNA degradation gene encodes for an amino acid sequence selected from the group consisting of SEQ ID NO: 42, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 34, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47.

5. The genetically engineered host cell of embodiment 1, wherein the heterologous promoter is a promoter comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 5, and SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

5.1 The genetically engineered host cell of embodiment 1, wherein the heterologous promoter is a promoter comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 5, and SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene encodes for SEQ ID NO: 45.

6. The genetically engineered host cell of embodiment 1, wherein the genetically engineered host cell comprises a combination of the heterologous promoter operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 10), **b-** (SEQ ID NO: 1:: SEQ ID NO: 14), **c-** (SEQ ID NO: 1:: SEQ ID NO: 18), **d-** (SEQ ID NO: 1:: SEQ ID NO: 20), **e-** (SEQ ID NO: 2:: SEQ ID NO: 11), **f-** (SEQ ID NO: 2:: SEQ ID NO: 18), **g-** (SEQ ID NO: 2:: SEQ ID NO: 13), **h-** (SEQ ID NO: 2:: SEQ ID NO: 18), **i-** (SEQ ID NO: 2:: SEQ ID NO: 17), **j-** (SEQ ID NO: 2:: SEQ ID NO: 19), **k-** (SEQ ID NO: 3:: SEQ ID NO: 11), **l-** (SEQ ID NO: 3:: SEQ ID NO: 14), **m-** (SEQ ID NO: 3:: SEQ ID NO: 12), **n-** (SEQ ID NO: 3:: SEQ ID NO: 15), **o-** (SEQ ID NO: 3:: SEQ ID NO: 17), **p-** (SEQ ID NO: 3:: SEQ ID NO: 19), **q-** (SEQ ID NO: 5:: SEQ ID NO: 14), **r-** (SEQ ID NO: 5:: SEQ ID NO: 11) (SEQ ID NO: 6:: SEQ ID NO: 13), **s-** (SEQ ID NO: 6:: SEQ ID NO: 19), **t-** (SEQ ID NO: 8:: SEQ ID NO: 21), **u-** (SEQ ID NO: 8:: SEQ ID NO: 14), **v-** (SEQ ID NO: 6:: SEQ ID NO: 20), **w-** (SEQ ID NO: 6:: SEQ ID NO: 11), **x-** (SEQ ID NO: 8:: SEQ ID NO: 9), and **y-** (SEQ ID NO: 8:: SEQ ID NO: 18).

6.1 The genetically engineered host cell of embodiment 1, wherein the genetically engineered host cell comprises a combination of the heterologous promoter operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 35), **b-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 39), **c-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 43), **d-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 45), **e-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 36), **f-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 43), **g-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 38), **h-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 43), **i-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 42), **j-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 44), **k-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 36), **l-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 39), **m-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 37), **n-** (SEQ ID NO: 3:: a

polynucleotide encoding for SEQ ID NO: 40), **o-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 42), **p-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 44), **q-** (SEQ ID NO: 5:: a polynucleotide encoding for SEQ ID NO: 39), **r-** (SEQ ID NO: 5:: a polynucleotide encoding for SEQ ID NO: 36) (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 38), **s-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 44), **t-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 46), **u-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 39), **v-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 45), **w-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 36), **x-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 34), and **y-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 43).

7. The genetically engineered host cell of embodiment 1, wherein the genetically engineered host cell comprises a combination of the heterologous promoter operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 14), **b-** (SEQ ID NO: 2:: SEQ ID NO: 13), **c-** (SEQ ID NO: 3:: SEQ ID NO: 11), **d-** (SEQ ID NO: 3:: SEQ ID NO: 14), **e-** (SEQ ID NO: 6:: SEQ ID NO: 11), and **f-** (SEQ ID NO: 8:: SEQ ID NO: 9).

7.1 The genetically engineered host cell of embodiment 1, wherein the genetically engineered host cell comprises a combination of the heterologous promoter operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 39), **b-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 38), **c-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 36), **d-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 39), **e-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 36), and **f-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 34).

8. The genetically engineered host cell of embodiment 1, wherein the heterologous promoter is a promoter comprising the nucleotide sequence of SEQ ID NO: 1, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

9. The genetically engineered host cell of embodiment 1, wherein the heterologous promoter is a promoter comprising the nucleotide sequence of SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

9.1 The genetically engineered host cell of embodiment 1, wherein the heterologous promoter is a promoter comprising the nucleotide sequence of SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene encodes for SEQ ID NO: 45.

10. The genetically engineered host cell of any one of embodiments 1-9.1, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

11. The genetically engineered host cell of any one of embodiments 1-10, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

12. The genetically engineered host cell of any one of embodiments 1-11, wherein the enhanced industrial performance is saturation biomass, and wherein the genetically engineered host cell exhibits at least about 5% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

13. The genetically engineered host cell of any one of embodiments 1-11, wherein the enhanced industrial performance is saturation biomass, and wherein the genetically engineered host cell exhibits at least about 10% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

14. The genetically engineered host cell of any one of embodiments 1-11, wherein the enhanced industrial performance is saturation biomass, and wherein the genetically engineered host cell exhibits at least about 20% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

15. The genetically engineered host cell of any one of embodiments 1-14, wherein the genetically engineered host cell produces a biomolecule selected from the group consisting of an amino acid, an organic acid, and an alcohol.

16. The genetically engineered host cell of any embodiment 15, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

17. The genetically engineered host cell of embodiment 15, wherein the organic acid is succinate, lactate or pyruvate.

18. The genetically engineered host cell of embodiment 15, wherein the alcohol is ethanol or isobutanol.

19. The genetically engineered host cell of embodiment 15, wherein the enhanced industrial performance is product yield, and wherein the genetically engineered host cell produces at least about 2% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

20. The genetically engineered host cell of embodiment 15, wherein the enhanced industrial performance is product yield, and wherein the genetically engineered host cell produces at least about 3% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

21. The genetically engineered host cell of embodiment 15, wherein the enhanced industrial performance is product yield, and wherein the genetically engineered host cell produces at least about 6% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

22. A method of producing a biomolecule comprising culturing a host cell of any one of embodiments 1-21 under conditions suitable for producing the biomolecule.

23. A method for generating a host cell capable of increased biomolecule yield, the method comprising:

    a. introducing a heterologous promoter polynucleotide into the genome of the host cell, wherein the heterologous promoter polynucleotide is operably linked to a polynucleotide encoding an RNA degradation gene, thereby creating a genetically engineered host cell;

wherein the genetically engineered host cell produces a higher biomolecule yield compared to the biomolecule yield of a control host cell cultured under identical conditions, wherein the control host cell does not comprise the heterologous promoter polynucleotide.

24. A method for generating a host cell capable of increased saturation biomass, the method comprising:

    a. introducing a heterologous promoter polynucleotide into the genome of the host cell, wherein the heterologous promoter polynucleotide is operably linked to a polynucleotide encoding an RNA degradation gene, thereby creating a genetically engineered host cell;

wherein the genetically engineered host cell exhibits increased saturation biomass compared to the saturation biomass of a control host cell cultured under identical conditions, wherein the control host cell does not comprise the heterologous promoter polynucleotide.

25. The method of any one of embodiments 23-24, wherein the RNA degradation gene is an endogenous gene.

26. The method of any one of embodiments 23-25, wherein the heterologous promoter polynucleotide is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

27. The method of any one of embodiments 23-26, wherein the polynucleotide encoding the RNA degradation gene is selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

27.1 The method of any one of embodiments 23-26, wherein the polynucleotide encoding the RNA degradation gene encodes for an amino acid sequence selected from the group consisting of SEQ ID NO: 42, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 34, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47.

28. The method of any one of embodiments 23-26, wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

28.1 The method of any one of embodiments 23-26, wherein the polynucleotide encoding the RNA degradation gene encodes for SEQ ID NO: 45.

29. The method of any one of embodiments 23 or 25-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 2:: SEQ ID NO: 17), **b-** (SEQ ID NO: 3:: SEQ ID NO: 17), **c-** (SEQ ID NO: 6:: SEQ ID NO: 20), **d-** (SEQ ID NO: 6:: SEQ ID NO: 11), and **f-** (SEQ ID NO: 8:: SEQ ID NO: 9).

29.1 The method of any one of embodiments 23 or 25-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 42), **b-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 42), **c-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 45), **d-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 36), and **f-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 34).

30. The method of any one of embodiments 23 or 25-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-**(SEQ ID NO: 6:: SEQ ID NO: 11), and **f-** (SEQ ID NO: 8:: SEQ ID NO: 9).

30.1 The method of any one of embodiments 23 or 25-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-**(SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 36), and **f-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 34).

31. The method of any one of embodiments 23 or 25-26, wherein the heterologous promoter polynucleotide is SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

31.1 The method of any one of embodiments 23 or 25-26, wherein the heterologous promoter polynucleotide is SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene encodes for SEQ ID NO: 45.

32. The method of any one of embodiments 24-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 10), **b-** (SEQ ID NO: 1:: SEQ ID NO: 14), **c-** (SEQ ID NO: 2:: SEQ ID NO: 13), **d-** (SEQ ID NO: 2:: SEQ ID NO: 18), **e-** (SEQ ID NO: 2:: SEQ ID NO: 17), **f-** (SEQ ID NO: 2:: SEQ ID NO: 19), **g-** (SEQ ID NO: 3:: SEQ ID NO: 11), **h-** (SEQ ID NO: 3:: SEQ ID NO: 14), **i-** (SEQ ID NO: 3:: SEQ ID NO: 19), **j-** (SEQ ID NO:

5:: SEQ ID NO: 14), **k-** (SEQ ID NO: 6:: SEQ ID NO: 13), **l-** (SEQ ID NO: 6:: SEQ ID NO: 19), **m-** (SEQ ID NO: 8:: SEQ ID NO: 21), and **n-** (SEQ ID NO: 8:: SEQ ID NO: 14).

32.1 The method of any one of embodiments 24-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 35), **b-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 39), **c-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 38), **d-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 43), **e-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 42), **f-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 44), **g-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 36), **h-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 39), **i-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 44), **j-** (SEQ ID NO: 5:: a polynucleotide encoding for SEQ ID NO: 39), **k-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 38), **l-** (SEQ ID NO: 6:: a polynucleotide encoding for SEQ ID NO: 44), **m-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 46), and **n-** (SEQ ID NO: 8:: a polynucleotide encoding for SEQ ID NO: 39).

33. The method of any one of embodiments 24-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 14), **b-** (SEQ ID NO: 2:: SEQ ID NO: 13), **c-** (SEQ ID NO: 3:: SEQ ID NO: 11), and **d-** (SEQ ID NO: 3:: SEQ ID NO: 14).

33.1 The method of any one of embodiments 24-26, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: a polynucleotide encoding for SEQ ID NO: 39), **b-** (SEQ ID NO: 2:: a polynucleotide encoding for SEQ ID NO: 38), **c-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 36), and **d-** (SEQ ID NO: 3:: a polynucleotide encoding for SEQ ID NO: 39).

34. The method of any one of embodiments 24-26, wherein the heterologous promoter polynucleotide is SEQ ID NO: 1, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

34.1 The method of any one of embodiments 24-26, wherein the heterologous promoter polynucleotide is SEQ ID NO: 1, and wherein the polynucleotide encoding the RNA degradation gene encodes for SEQ ID NO: 45.

35. The method of any one of embodiments 23-34.1, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

36. The method of any one of embodiments 23-35, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

37. The method of any one of embodiments 23, and 25-31, wherein the biomolecule is selected from the group consisting of an amino acid, an organic acid, and an alcohol.

38. The method of embodiment 37, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

39. The method of embodiment 37, wherein the organic acid is succinate, lactate or pyruvate.

40. The method of embodiment 37, wherein the alcohol is ethanol or isobutanol.

41. A genetically engineered host cell with enhanced industrial performance, said host cell comprising:

    a. a polynucleotide encoding an RNA degradation gene;

    b. a mutation in the start codon of the polynucleotide of (a);

    wherein the mutation results in the replacement of the endogenous start codon of the polynucleotide with a different start codon.

42. The genetically engineered host cell of embodiment 41, wherein the polynucleotide encoding the RNA degradation gene is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

42.1 The genetically engineered host cell of embodiment 41, wherein the polynucleotide encoding the RNA degradation gene encodes for an amino acid sequence selected from the group

consisting of SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47.

43. The genetically engineered host cell of embodiment 41, wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 19.

43.1 The genetically engineered host cell of embodiment 41, wherein the polynucleotide encoding the RNA degradation gene encodes for SEQ ID NO: 44.

44. The genetically engineered host cell of any one of embodiments 41-43.1, wherein the start codon of the endogenous RNA degradation gene is changed from 'ATG' or 'GTG,' to 'TTG'

45. The genetically engineered host cell of any one of embodiments 41-44, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

46. The genetically engineered host cell of any one of embodiments 41-45, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

47. The genetically engineered host cell of any one of embodiments 41-46, wherein the genetically engineered host produces a biomolecule selected from the group consisting of an amino acid, an organic acid, and an alcohol.

48. The genetically engineered host cell of embodiment 47, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

49. The genetically engineered host cell of embodiment 47, wherein the organic acid is succinate, lactate or pyruvate.

50. The genetically engineered host cell of embodiment 47, wherein the alcohol is ethanol or isobutanol.

51. The genetically engineered host cell of embodiment 47, wherein the genetically engineered host cell produces at least a 2% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, under identical culture conditions.

52. A method for generating a host cell capable of increased yield of a biomolecule or increased saturation biomass, the method comprising:

a. genetically modifying the host cell, wherein the modifying comprises mutating the start codon of an endogenous RNA degradation gene, wherein the modification generates a genetically engineered host cell;

wherein the genetically engineered host cell has increased biomolecule yield as compared to the biomolecule yield of a control host cell, or wherein the genetically engineered host cell achieves higher saturation biomass as compared to the saturation biomass of the control host cell, wherein the control host cell does not comprise the start codon mutation of the genetically engineered host cell, and wherein the genetically engineered host cell and the control host cell are cultured under identical conditions.

53. The method of embodiment 52, wherein the endogenous RNA degradation gene is a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

53.1 The method of embodiment 52, wherein the endogenous RNA degradation gene encodes for an amino acid sequence selected from the group consisting of SEQ ID NO: 42, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 34, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 47.

54. The method of embodiment 52, wherein the RNA degradation gene is a gene comprising the nucleotide sequence of SEQ ID NO: 19.

54.1 The method of embodiment 52, wherein the RNA degradation gene encodes for SEQ ID NO: 44.

55. The method of any one of embodiments 52-54.1, wherein the start codon of the endogenous RNA degradation gene is changed from 'ATG' or 'GTG,' to 'TTG'

56. The method of any one of embodiments 52-55, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

57. The method of any one of embodiments 52-56, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

58. The method of any one of embodiments 52-55, wherein the biomolecule is selected from the group consisting of an amino acid, an organic acid, and an alcohol.

59. The method of embodiment 58, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

60. The method of embodiment 58, wherein the organic acid is succinate, lactate or pyruvate.

61. The method of embodiment 58, wherein the alcohol is ethanol or isobutanol.

62. The method of embodiment 58, wherein the genetically engineered host cell produces at least a 2% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured under identical conditions.

*****

INCORPORATION BY REFERENCE

[0232] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes.

[0233] However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

CLAIMS

1. A genetically engineered host cell with enhanced industrial performance, said host cell comprising:
    a. a heterologous promoter polynucleotide, and
    b. a polynucleotide encoding an RNA degradation gene;
    wherein the heterologous promoter polynucleotide is operably linked to the polynucleotide encoding the RNA degradation gene.

2. The genetically engineered host cell of claim 1, wherein the RNA degradation gene is an endogenous gene.

3. The genetically engineered host cell of claim 1, wherein the heterologous promoter is a promoter comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

4. The genetically engineered host cell of claim 3, wherein the polynucleotide encoding the RNA degradation gene is a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

5. The genetically engineered host cell of claim 1, wherein the heterologous promoter is a promoter comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 5, and SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

6. The genetically engineered host cell of claim 1, wherein the genetically engineered host cell comprises a combination of the heterologous promoter operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 10), **b-** (SEQ ID NO: 1:: SEQ ID NO: 14), **c-** (SEQ ID NO: 1:: SEQ ID NO: 18), **d-** (SEQ ID NO: 1:: SEQ ID NO: 20), **e-** (SEQ ID NO: 2:: SEQ ID NO: 11), **f-** (SEQ ID NO: 2:: SEQ ID NO: 18), **g-** (SEQ ID NO: 2:: SEQ ID NO: 13), **h-** (SEQ ID NO: 2:: SEQ ID NO: 18), **i-** (SEQ ID NO: 2:: SEQ ID NO: 17), **j-** (SEQ ID NO: 2:: SEQ ID NO: 19), **k-** (SEQ ID NO: 3:: SEQ ID NO: 11), **l-** (SEQ ID NO: 3:: SEQ ID NO: 14), **m-** (SEQ

ID NO: 3:: SEQ ID NO: 12), **n-** (SEQ ID NO: 3:: SEQ ID NO: 15), **o-** (SEQ ID NO: 3:: SEQ ID NO: 17), **p-** (SEQ ID NO: 3:: SEQ ID NO: 19), **q-** (SEQ ID NO: 5:: SEQ ID NO: 14), **r-** (SEQ ID NO: 5:: SEQ ID NO: 11) (SEQ ID NO: 6:: SEQ ID NO: 13), **s-** (SEQ ID NO: 6:: SEQ ID NO: 19), **t-** (SEQ ID NO: 8:: SEQ ID NO: 21), **u-** (SEQ ID NO: 8:: SEQ ID NO: 14), **v-** (SEQ ID NO: 6:: SEQ ID NO: 20), **w-** (SEQ ID NO: 6:: SEQ ID NO: 11), **x-** (SEQ ID NO: 8:: SEQ ID NO: 9), and **y-** (SEQ ID NO: 8:: SEQ ID NO: 18).

7. The genetically engineered host cell of claim 1, wherein the genetically engineered host cell comprises a combination of the heterologous promoter operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 14), **b-** (SEQ ID NO: 2:: SEQ ID NO: 13), **c-** (SEQ ID NO: 3:: SEQ ID NO: 11), **d-** (SEQ ID NO: 3:: SEQ ID NO: 14), **e-** (SEQ ID NO: 6:: SEQ ID NO: 11), and **f-** (SEQ ID NO: 8:: SEQ ID NO: 9).

8. The genetically engineered host cell of claim 1, wherein the heterologous promoter is a promoter comprising the nucleotide sequence of SEQ ID NO: 1, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

9. The genetically engineered host cell of claim 1, wherein the heterologous promoter is a promoter comprising the nucleotide sequence of SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

10. The genetically engineered host cell of any one of claims 1-9, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

11. The genetically engineered host cell of any one of claims 1-10, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

12. The genetically engineered host cell of claim 1, wherein the enhanced industrial performance is saturation biomass, and wherein the genetically engineered host cell exhibits at least about 5% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

13. The genetically engineered host cell of claim 1, wherein the enhanced industrial performance is saturation biomass, and wherein the genetically engineered host cell exhibits at least about

10% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

14. The genetically engineered host cell of claim 1, wherein the enhanced industrial performance is saturation biomass, and wherein the genetically engineered host cell exhibits at least about 20% higher saturation biomass than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

15. The genetically engineered host cell of claim 1, wherein the genetically engineered host cell produces a biomolecule selected from the group consisting of an amino acid, an organic acid, and an alcohol.

16. The genetically engineered host cell of claim 15, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

17. The genetically engineered host cell of claim 15, wherein the organic acid is succinate, lactate or pyruvate.

18. The genetically engineered host cell of claim 15, wherein the alcohol is ethanol or isobutanol.

19. The genetically engineered host cell of claim 15, wherein the enhanced industrial performance is product yield, and wherein the genetically engineered host cell produces at least about 2% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

20. The genetically engineered host cell of claim 15, wherein the enhanced industrial performance is product yield, and wherein the genetically engineered host cell produces at least about 3% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

21. The genetically engineered host cell of claim 15, wherein the enhanced industrial performance is product yield, and wherein the genetically engineered host cell produces at least about 6% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured in identical conditions.

22. A method of producing a biomolecule comprising culturing a host cell of any one of claims 1-21 under conditions suitable for producing the biomolecule.

23. A method for generating a host cell capable of increased biomolecule yield, the method comprising:

    a. introducing a heterologous promoter polynucleotide into the genome of the host cell, wherein the heterologous promoter polynucleotide is operably linked to a polynucleotide encoding an RNA degradation gene, thereby creating a genetically engineered host cell;

    wherein the genetically engineered host cell produces a higher biomolecule yield compared to the biomolecule yield of a control host cell cultured under identical conditions, wherein the control host cell does not comprise the heterologous promoter polynucleotide.

24. A method for generating a host cell capable of increased saturation biomass, the method comprising:

    a. introducing a heterologous promoter polynucleotide into the genome of the host cell, wherein the heterologous promoter polynucleotide is operably linked to a polynucleotide encoding an RNA degradation gene, thereby creating a genetically engineered host cell;

    wherein the genetically engineered host cell exhibits increased saturation biomass compared to the saturation biomass of a control host cell cultured under identical conditions, wherein the control host cell does not comprise the heterologous promoter polynucleotide.

25. The method of claim 23 or 24, wherein the RNA degradation gene is an endogenous gene.

26. The method of claim 23 or 24, wherein the heterologous promoter polynucleotide is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

27. The method of claim 23 or 24, wherein the polynucleotide encoding the RNA degradation gene is selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

28. The method of claim 23 or 24, wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

29. The method of claim 23, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 2:: SEQ ID NO: 17), **b-** (SEQ ID NO: 3:: SEQ ID NO: 17), **c-** (SEQ ID NO: 6:: SEQ ID NO: 20), **d-** (SEQ ID NO: 6:: SEQ ID NO: 11), and **f-** (SEQ ID NO: 8:: SEQ ID NO: 9).

30. The method of claim 23, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-**(SEQ ID NO: 6:: SEQ ID NO: 11), and **f-** (SEQ ID NO: 8:: SEQ ID NO: 9).

31. The method of claim 23, wherein the heterologous promoter polynucleotide is SEQ ID NO: 6, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

32. The method of claim 24, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 10), **b-** (SEQ ID NO: 1:: SEQ ID NO: 14), **c-** (SEQ ID NO: 2:: SEQ ID NO: 13), **d-** (SEQ ID NO: 2:: SEQ ID NO: 18), **e-** (SEQ ID NO: 2:: SEQ ID NO: 17), **f-** (SEQ ID NO: 2:: SEQ ID NO: 19), **g-** (SEQ ID NO: 3:: SEQ ID NO: 11), **h-** (SEQ ID NO: 3:: SEQ ID NO: 14), **i-** (SEQ ID NO: 3:: SEQ ID NO: 19), **j-** (SEQ ID NO: 5:: SEQ ID NO: 14), **k-** (SEQ ID NO: 6:: SEQ ID NO: 13), **l-** (SEQ ID NO: 6:: SEQ ID NO: 19), **m-** (SEQ ID NO: 8:: SEQ ID NO: 21), and **n-** (SEQ ID NO: 8:: SEQ ID NO: 14).

33. The method of claim 24, wherein the genetically engineered host cell comprises a combination of the heterologous promoter polynucleotide operably linked to the polynucleotide encoding the RNA degradation gene, said combination selected from the group consisting of: **a-** (SEQ ID NO: 1:: SEQ ID NO: 14), **b-** (SEQ ID NO: 2:: SEQ ID NO: 13), **c-** (SEQ ID NO: 3:: SEQ ID NO: 11), and **d-** (SEQ ID NO: 3:: SEQ ID NO: 14).

34. The method of claim 24, wherein the heterologous promoter polynucleotide is SEQ ID NO: 1, and wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 20.

35. The method of any one of claims 23-34, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

36. The method of any one of claims 23-35, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

37. The method of any one of claims 23, and 25-31, wherein the biomolecule is selected from the group consisting of an amino acid, an organic acid, and an alcohol.

38. The method of claim 37, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

39. The method of claim 37, wherein the organic acid is succinate, lactate or pyruvate.

40. The method of claim 37, wherein the alcohol is ethanol or isobutanol.

41. A genetically engineered host cell with enhanced industrial performance, said host cell comprising:
    a. a polynucleotide encoding an RNA degradation gene;
    b. a mutation in the start codon of the polynucleotide of (a);
    wherein the mutation results in the replacement of the endogenous start codon of the polynucleotide with a different start codon.

42. The genetically engineered host cell of claim 41, wherein the polynucleotide encoding the RNA degradation gene is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

43. The genetically engineered host cell of claim 41, wherein the polynucleotide encoding the RNA degradation gene is SEQ ID NO: 19.

44. The genetically engineered host cell of claim 42, wherein the start codon of the endogenous RNA degradation gene is changed from 'ATG' or 'GTG,' to 'TTG'

45. The genetically engineered host cell of any one of claims 41-44, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

46. The genetically engineered host cell of any one of claims 41-45, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

47. The genetically engineered host cell of claim 41, wherein the genetically engineered host produces a biomolecule selected from the group consisting of an amino acid, an organic acid, and an alcohol.

48. The genetically engineered host cell of claim 47, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

49. The genetically engineered host cell of claim 47, wherein the organic acid is succinate, lactate or pyruvate.

50. The genetically engineered host cell of claim 47, wherein the alcohol is ethanol or isobutanol.

51. The genetically engineered host cell of claim 47, wherein the genetically engineered host cell produces at least a 2% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, under identical culture conditions.

52. A method for generating a host cell capable of increased yield of a biomolecule or increased saturation biomass, the method comprising:

    a. genetically modifying the host cell, wherein the modifying comprises mutating the start codon of an endogenous RNA degradation gene, wherein the modification generates a genetically engineered host cell;

wherein the genetically engineered host cell has increased biomolecule yield as compared to the biomolecule yield of a control host cell, or wherein the genetically engineered host cell achieves higher saturation biomass as compared to the saturation biomass of the control host cell, wherein the control host cell does not comprise the start codon mutation of the genetically engineered host cell, and wherein the genetically engineered host cell and the control host cell are cultured under identical conditions.

53. The method of claim 52, wherein the endogenous RNA degradation gene is a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

54. The method of claim 52, wherein the RNA degradation gene is a gene comprising the nucleotide sequence of SEQ ID NO: 19.

55. The method of claim 53, wherein the start codon of the endogenous RNA degradation gene is changed from 'ATG' or 'GTG,' to 'TTG'

56. The method of any one of claims 52-55, wherein the genetically engineered host cell belongs to the genus *Corynebacterium*.

57. The method of any one of claims 52-56, wherein the genetically engineered host cell is *Corynebacterium glutamicum*.

58. The method of any one of claims 52-55, wherein the biomolecule is selected from the group consisting of an amino acid, an organic acid, and an alcohol.

59. The method of claim 58, wherein the amino acid is tyrosine, phenylalanine, tryptophan, aspartic acid, asparagine, threonine, isoleucine, methionine, or lysine.

60. The method of claim 58, wherein the organic acid is succinate, lactate or pyruvate.

61. The method of claim 58, wherein the alcohol is ethanol or isobutanol.

62. The method of claim 58, wherein the genetically engineered host cell produces at least a 2% higher yield of the biomolecule than a genetically identical host cell lacking said heterologous promoter polynucleotide, when cultured under identical conditions.

Fig. 1

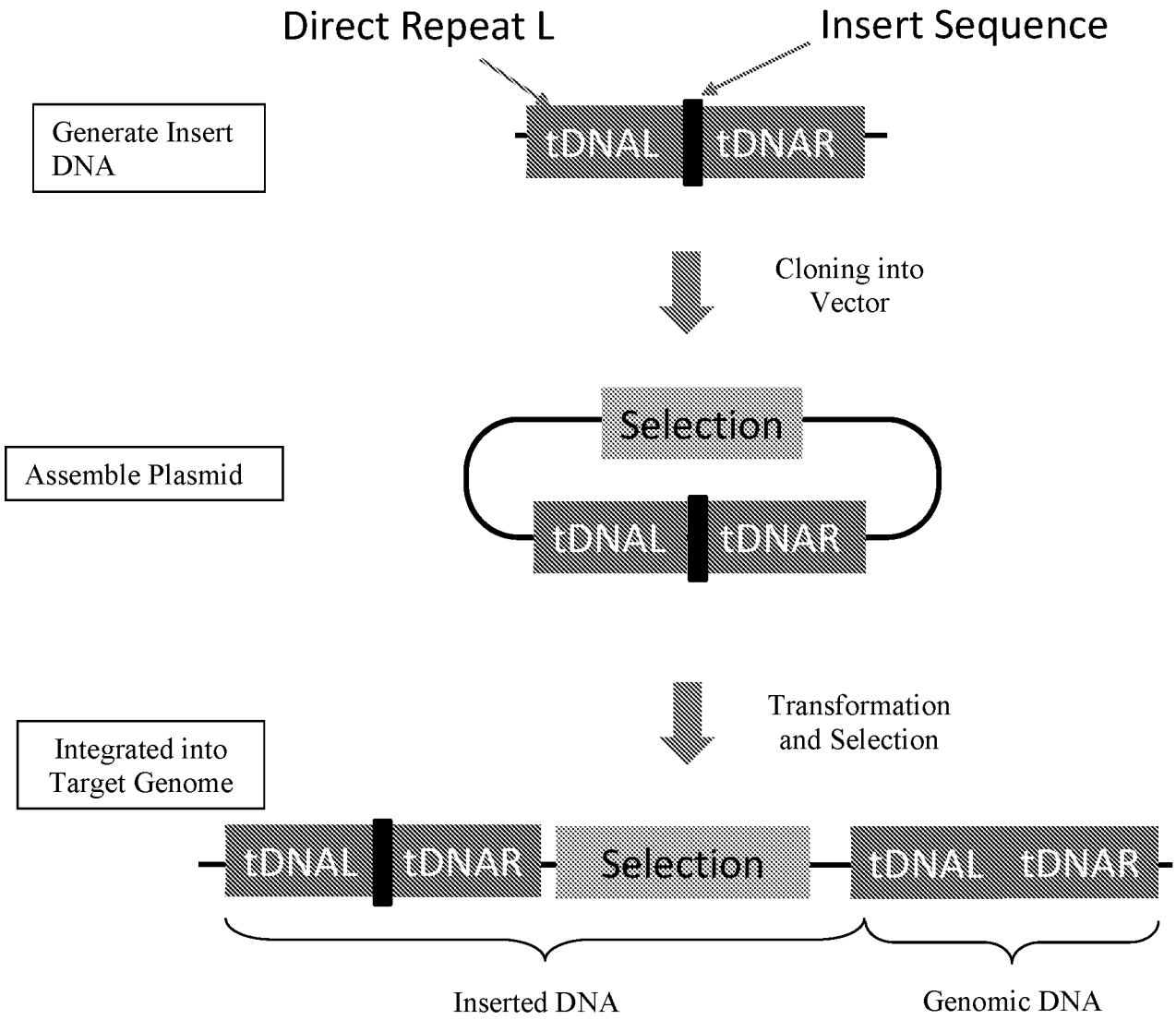

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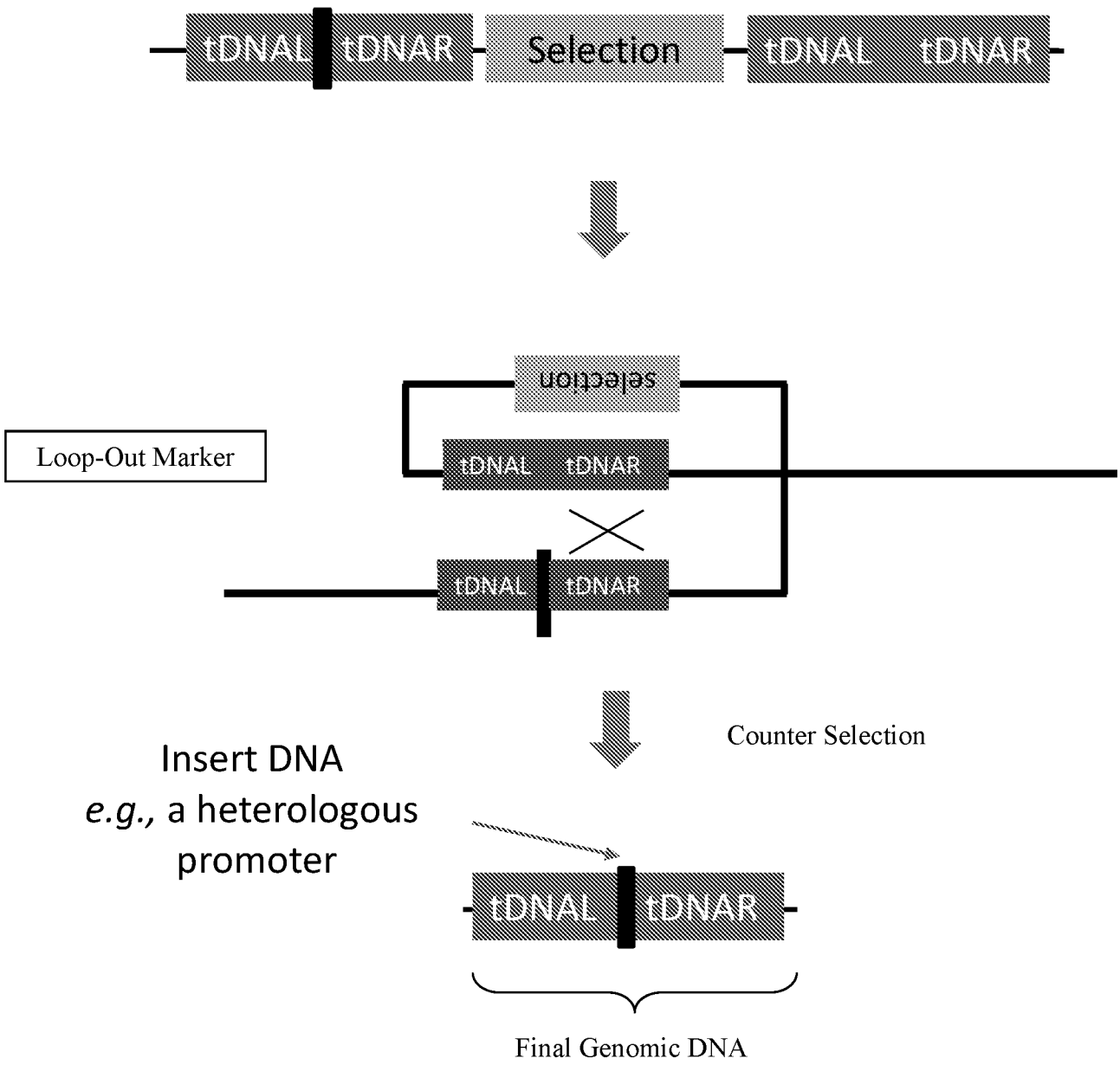
Fig. 2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Final Genomic DNA

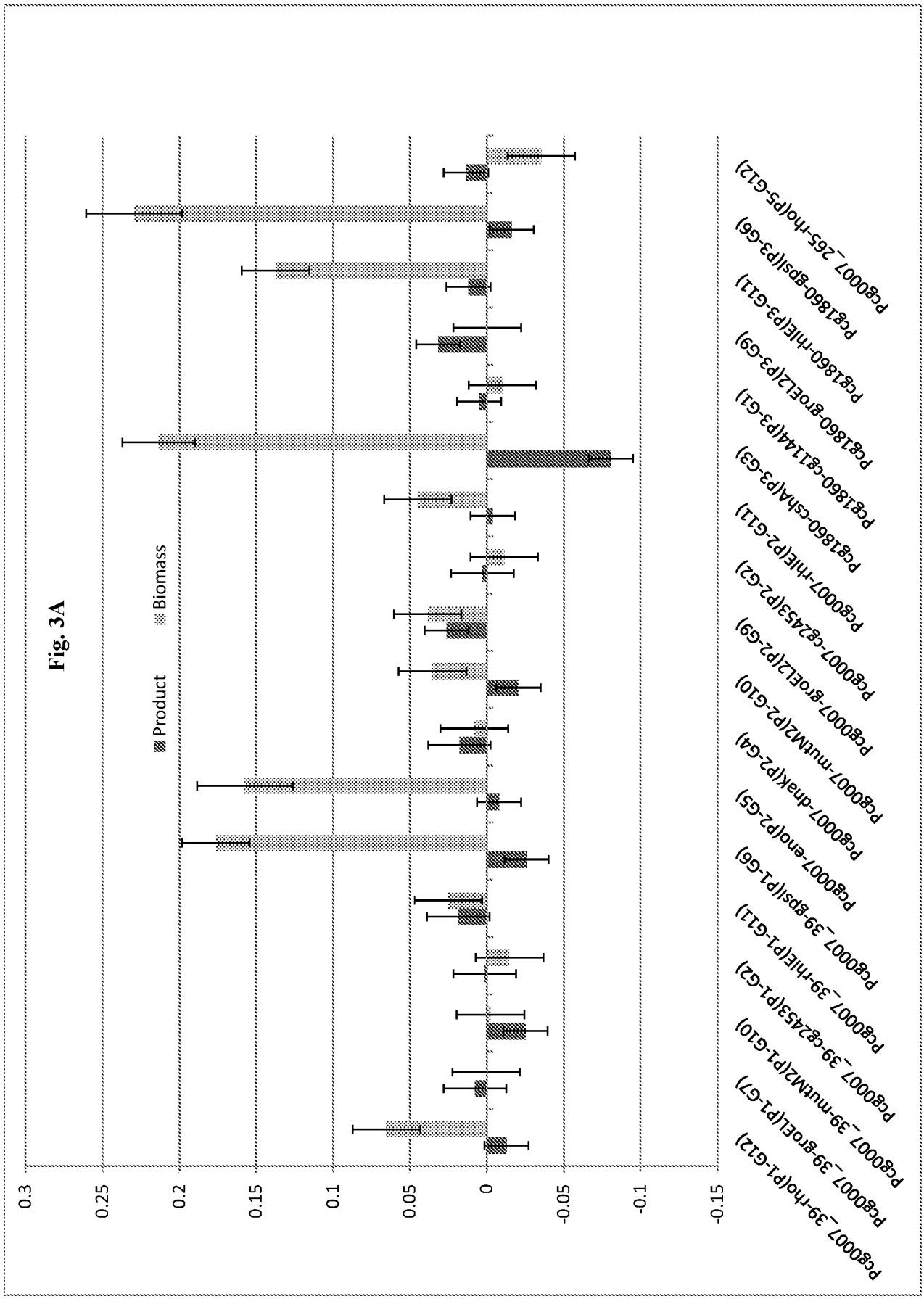
Fig. 3A
Product
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0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
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PcG0007_39-groEL1P1-G7
PcG0007_39-nusM2P1-G10
PcG0007_39-cg2453P1-G2
PcG0007_39-rhiEP1-G11
PcG0007_39-gpsIP1-G6
PcG0007-enoP2-G5
PcG0007-dnaKP2-G4
PcG0007-nusM2P2-G10
PcG0007-groEL2P2-G9
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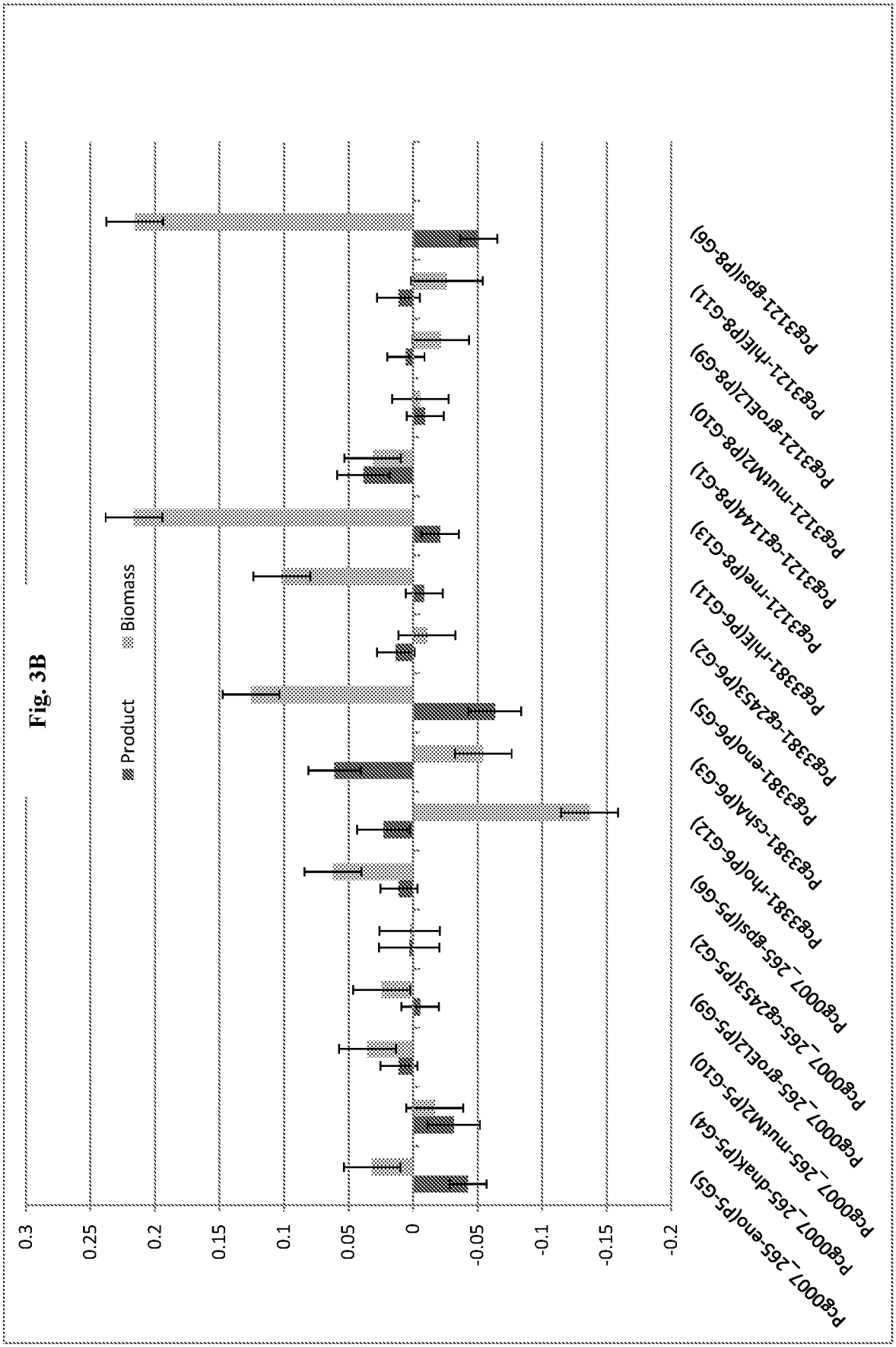
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pCg3381-cshA(P6-G3)
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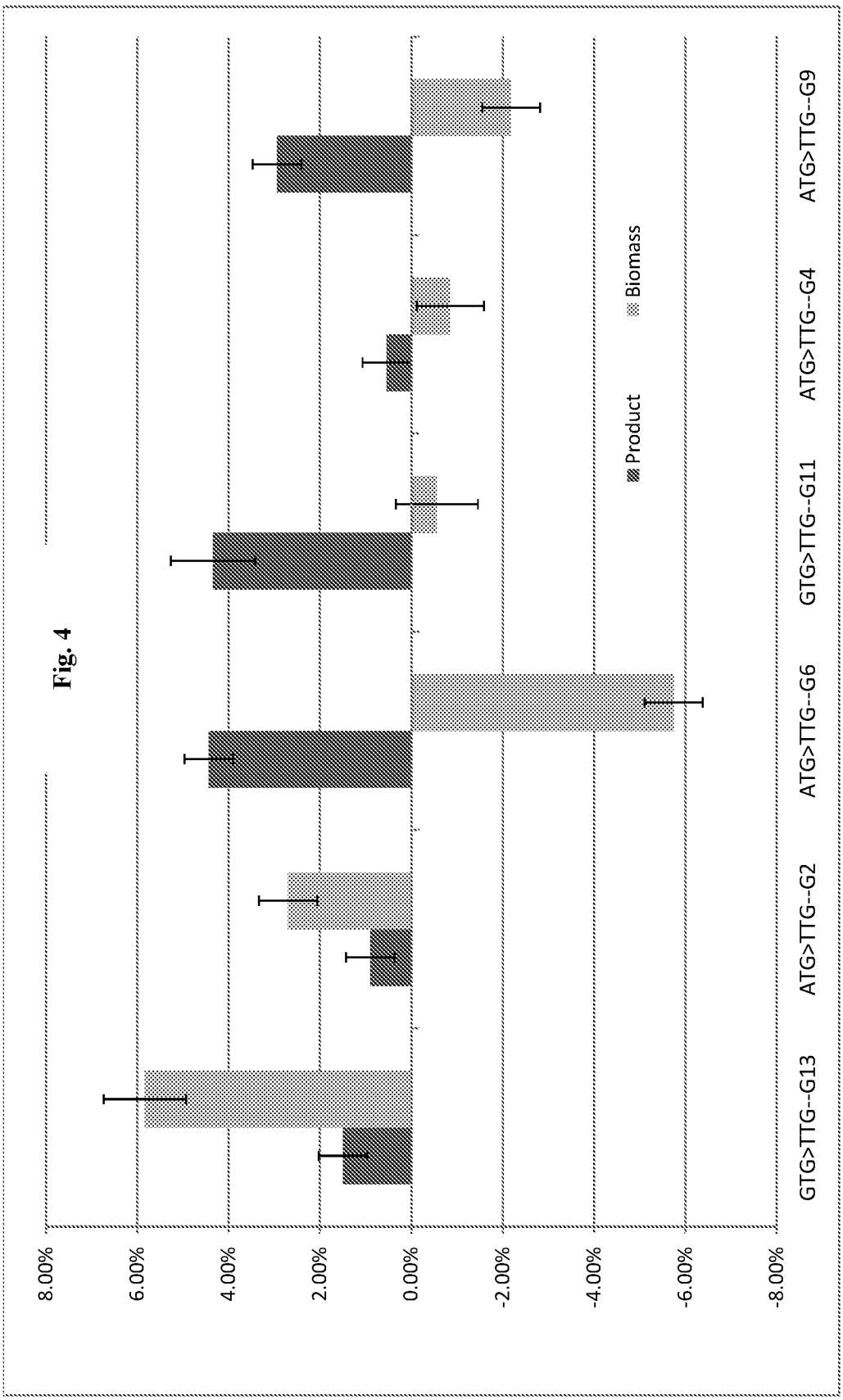
Fig. 3B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Product
Biomass
GTG>TTG--G13
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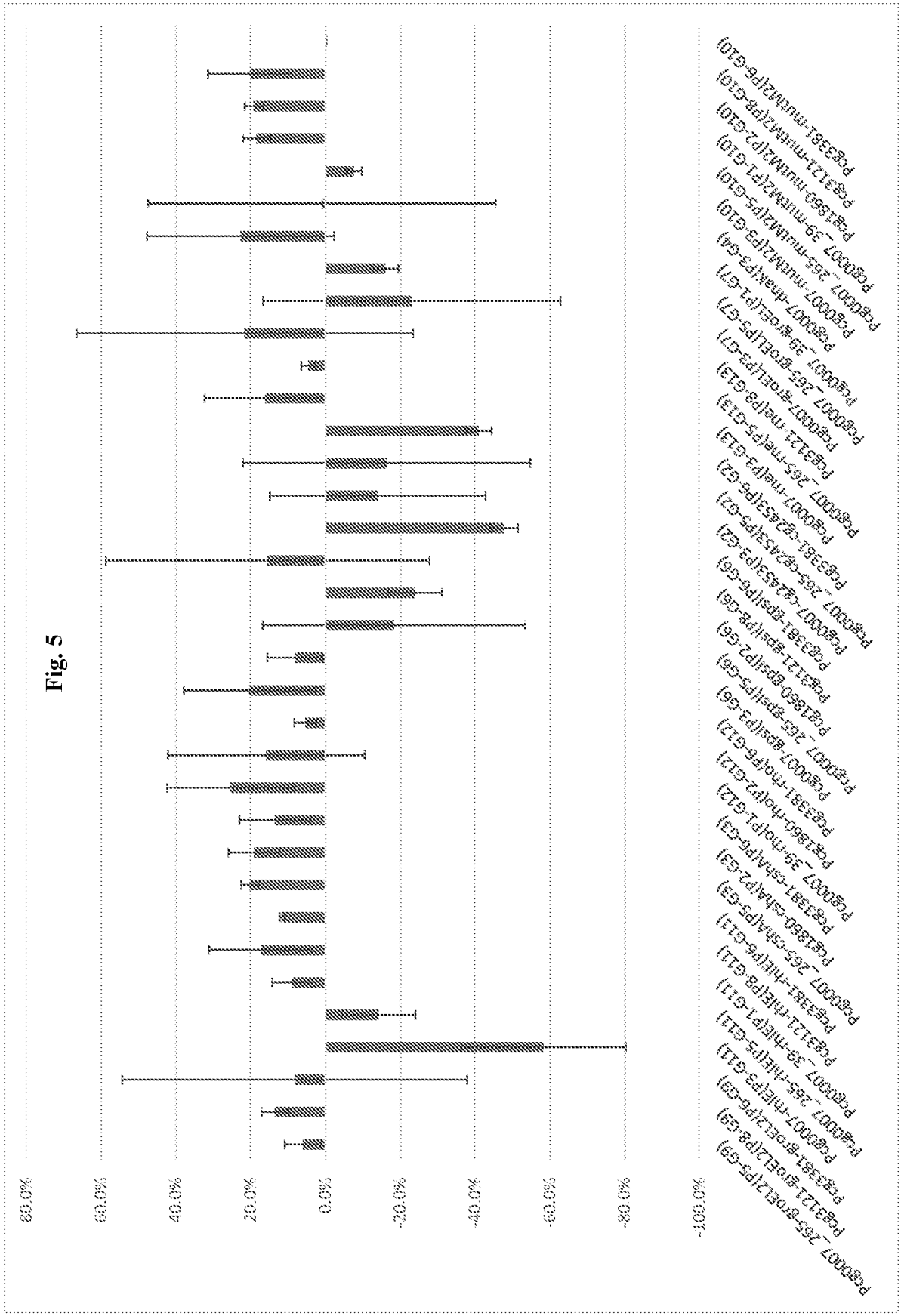
Fig. 4

Fig. 5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PcGS007_39-rhlE/P3-G11)
PcGS121-rhlE/P3-G11)
PcGS121-rhlE/P1-G11)
PcGS381-rhlE/P8-G11)
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PcGS007-dnaK/P3-G4)
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PcGS007_265-rnuM2/P6-G10)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/39452

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/34, 14/195; C12N 1/21; C12R 1/15 (2017.01)

CPC - C07K 14/34, 14/195; C12N 1/20; C12R 1/15

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2007/0274972 A1 (MULLER et al.) November 29, 2007; abstract; paragraphs [0027], [0030], [0035], [0060], [0061], [0124]-[0126]	1 --- 2, 10/1-2, 12-21, 23-24, 25/23-24, 41, 45/41, 47-52, 56/52, 58/52, 59/58/52, 60/58/52, 61/58/52, 62/58/52
Y	WO 2007/141580 A2 (MEDICAL RESEARCH COUNCIL et al.) December 13, 2007; page 29, lines 9-11; page 40, first paragraph; Table 2; page 49, lines 15-18; figure 12	2, 10/2, 25/23-24, 41, 45/41, 45/45, 47-52, 56/52, 58/52, 59/58/52, 60/58/52, 61/58/52, 62/58/52
Y	US 2002/0197605 A1 (NAKAGAWA et al.) December 26, 2002; paragraph [0389]	10/1-2, 45/41, 56/52
	WO 2015/175793 A1 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC.) November 19, 2015; page 21, lines 1-10; page 22, lines 3-11; figure 3	12-21, 23-24, 25/23-24, 47-52, 56/52, 58/52, 59/58/52, 60/58/52, 61/58/52, 62/58/52
Y	US 2015/0140626 A1 (SAMSUNG ELECTRONICS CO., LTD.) May 21, 2015; abstract; paragraphs [0035], [0090]	17, 49, 60/58/52

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2017 (15.09.2017)	29 SEP 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/39452

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0356921 A1 DARTMOUTH COLLEGE et al.) December 4, 2014; abstract; paragraph [0045]	18, 50, 61/58/52
A	US 2006/0269975 A1 (POMPEJUS et al.) November 30, 2006; Table 1	3-8, 10/3-8, 26/23-24, 27/23-24, 29-30, 32-33, 42, 53, 55, 58/53, 58/55, 59/58/53, 59/58/55, 60/58/53, 60/58/55, 61/58/53, 61/58/55, 62/58/53, 62/58/55
A	US 2007/0042474 A1 (POMPEJUS et al.) February 22, 2007; Table 1	9, 10/9, 31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/39452

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

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

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

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

3. ☒ Claims Nos.: 11, 22, 35-40, 46, 57
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.