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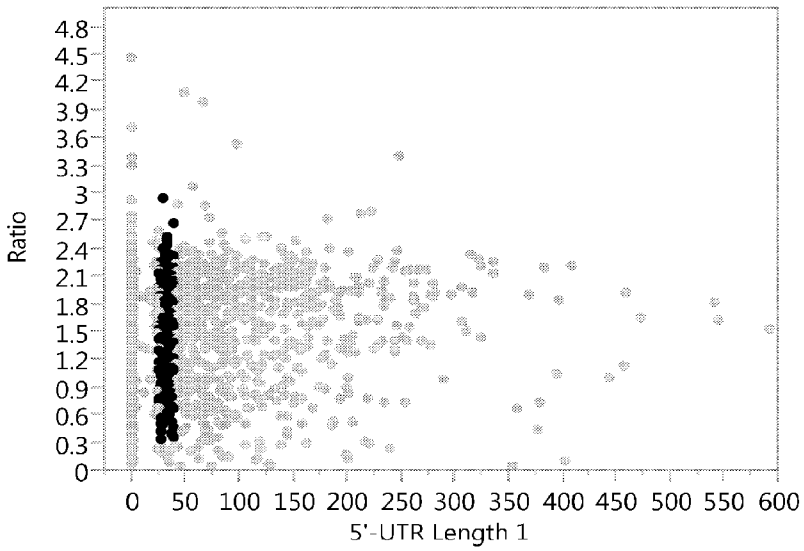

FIG. 1

(57) Abstract: Provided are native promoters comprising polynucleotides isolated from *Corynebacterium glutamicum*, and mutant promoters derived therefrom, which may be used to regulate, i.e., either increase or decrease, gene expression. Also provided are promoter ladders comprising a plurality of the promoters having incrementally increasing promoter activity. Also provided are host cells and recombinant vectors comprising the promoters, and methods of expressing genes of interest and producing biomolecules using the host cells.

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PROMOTERS FROM CORYNEBACTERIUM GLUTAMICUM

CROSS REFERENCE

This application claims the benefit of U.S. Provisional Patent Application No. 62/264,232, filed December 7, 2015, and U.S. Provisional Patent Application No. 62/431,409 filed December 7, 2016, which applications are incorporated herein by reference in their their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

A Sequence Listing is provided herewith as a text file, "AMG-001-PCT_SL.txt" created on November 30, 2016 and having a size of 4,575 bytes. The contents of the text file are incorporated by reference herein in their entirety.

BACKGROUND

Field

The invention relates to native promoters comprising polynucleotides isolated from *Corynebacterium glutamicum*, and mutant promoters derived therefrom, host cells and recombinant vectors comprising the promoters, and methods of modifying the expression of target genes and producing biomolecules comprising culturing the host cells.

Description of the Related Art

Strains of coryneform bacteria, in particular *Corynebacterium glutamicum*, play a significant role in the production of biomolecules such as amino acids, organic acids, vitamins, nucleosides and nucleotides, and continuous efforts are being made to improve production processes. Said processes may be improved with respect to fermentation related measures such as, for example, stirring and oxygen supply, or the composition of nutrient media, such as, for example, sugar concentration during fermentation, or the work-up into the product form, for example by means of ion exchange chromatography, or the intrinsic performance characteristics of the microorganism itself.

Performance characteristics can include, for example, yield, titer, productivity, by-product elimination, tolerance to process excursions, optimal growth temperature and growth rate. One way to improve performance of a microbial strain is to increase the expression of genes that control the production of a metabolite. Increasing expression of a gene can increase the activity of an enzyme that is encoded by that gene. Increasing enzyme activity can increase the rate of synthesis of the metabolic products made by the pathway to which that enzyme belongs. In some instances, increasing the rate of production of a metabolite can unbalance other cellular processes and inhibit growth of a microbial

culture. Sometimes, down regulating activity is important to improve performance of a strain. For example, re-directing flux away from by-products can improve yield. Accordingly, fine-tuning of expression levels of the various components simultaneously within a metabolic pathway is often necessary.

Promoters regulate the rate at which genes are transcribed and can influence transcription in a variety of ways. 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.

There is clearly a need for a broader assortment of well-defined *Corynebacterium* species promoters than has been heretofore described. Such promoters would be useful in the coordinated expression of genes in coryneform cells. For example, a collection of *C. glutamicum* promoters would facilitate the industrial-scale production of biomolecules in *C. glutamicum* cells by enhancing the expression of genes that encode components of the biosynthetic pathways for the desired biomolecules. The promoters described herein help meet these and other needs.

BRIEF SUMMARY

In brief, the present disclosure is directed to native promoters comprising polynucleotides isolated from *Corynebacterium glutamicum*, and mutant promoters derived therefrom, which can each be encoded by short DNA sequences, ideally less than 100 base pairs, and which together represent a ladder of constitutive promoters having incrementally increasing expression levels. It is possible for various genes to be expressed advantageously under the control of said promoters.

One embodiment of the present invention relates to a first promoter polynucleotide comprising a sequence selected from: SEQ ID NO:1, SEQ ID NO:5, or SEQ ID NO:7. In some embodiments, the first promoter polynucleotide consists of a sequence selected from: SEQ ID NO:1, SEQ ID NO:5, or SEQ ID NO:7. One embodiment of the present invention relates to combinations of promoter polynucleotides comprising at least two first promoter polynucleotides described herein. One embodiment of the present invention relates to combinations of promoter polynucleotides comprising at least one first promoter polynucleotide described herein, and at least one second promoter polynucleotide comprising a sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8. One embodiment of the present invention relates to combinations of promoter

polynucleotides comprising at least one first promoter polynucleotide described herein, and at least one second promoter polynucleotide consisting of a sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

One embodiment of the present invention relates to host cells comprising the first promoter polynucleotide described herein. One embodiment of the present invention relates to recombinant vectors comprising the first promoter polynucleotide described herein. In some embodiments, the first promoter polynucleotide is functionally linked to a first target gene. One embodiment of the present invention relates to host cells comprising the combinations of promoter polynucleotides described herein. One embodiment of the present invention relates to recombinant vectors comprising the combinations of promoter polynucleotides described herein. In some embodiments, each promoter polynucleotide is functionally linked to a different target gene. In some embodiments, the target genes are part of the same metabolic pathway. In some embodiments, the target genes are not part of the same metabolic pathway. One embodiment of the present invention relates to host cells transformed with the recombinant vectors described herein.

One embodiment of the present invention relates to host cells comprising at least one promoter polynucleotide functionally linked to a target gene; wherein the promoter polynucleotide comprises a sequence selected from: 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; wherein when the promoter polynucleotide comprises a sequence selected from: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, the target gene is other than the promoter polynucleotide's endogenous gene. In some embodiments, the host cell comprises at least two promoter polynucleotides, wherein each promoter polynucleotide is functionally linked to a different target gene. One embodiment of the present invention relates to recombinant vectors comprising at least one promoter polynucleotide functionally linked to a target gene; wherein the promoter polynucleotide comprises a sequence selected from: 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; wherein when the promoter polynucleotide comprises a sequence selected from: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, the target gene is other than the promoter polynucleotide's endogenous gene. In some embodiments, the recombinant vector comprises at least two promoter polynucleotides, wherein each promoter polynucleotide is functionally linked to a different target gene. In some embodiments, the target genes are part of the same metabolic pathway. In some embodiments, the target genes are not part of the same metabolic pathway. One embodiment of the present invention relates to host cells transformed with the recombinant vectors described herein.

One embodiment of the present invention relates to methods of modifying the expression of one or more target genes, comprising culturing a host cell described herein, wherein the modification of each

target gene is independently selected from: up-regulating and down-regulating. The target gene preferably codes for one or more polypeptides or proteins of the biosynthetic pathway of biomolecules including, *e.g.*, amino acids, organic acids, nucleic acids, proteins, and polymers.

Another embodiment of the present invention relates to methods of producing a biomolecule comprising culturing a host cell described herein, under conditions suitable for producing the biomolecule. In some embodiments the target gene is associated with a biosynthetic pathway producing a biomolecule selected from: amino acids, organic acids, flavors and fragrances, biofuels, proteins and enzymes, polymers/monomers and other biomaterials, lipids, nucleic acids, small molecule therapeutics, protein or peptide therapeutics, fine chemicals, and nutraceuticals. In preferred embodiments, the biomolecule is an L-amino acid. In specific embodiments, the L-amino acid is lysine.

In some embodiments, the host cell belongs to genus *Corynebacterium*. In some embodiments, the host cell is *Corynebacterium glutamicum*.

BRIEF DESCRIPTION OF THE DRAWINGS

Error! Reference source not found. shows a graph of 5' UTR length (x axis) versus expression ratio across two growth conditions (y axis) for each gene in the *C. glutamicum* ATCC 13032 genome. Genes having both an expression ratio across the two growth conditions of between 0.33 and 3, and a 5' UTR length of between 26 and 40 base pairs are represented by black circles. Genes that failed to match both criteria are represented by grey circles.

Error! Reference source not found. shows a graph of normalized activity (x axis) of eight candidate promoters (y axis) in a yellow fluorescent protein-based assay. Each biological replicate of each candidate promoter is represented by a black circle. The parent plasmid pK18rep acted as a negative control.

Error! Reference source not found. presents a diagram of the genetic and biochemical pathway for the biosynthesis of the amino acid L-lysine. Genes that divert intermediates in the biosynthetic pathway (*e.g.*, *pck*, *odx*, *icd*, and *hom*) are underlined.

Error! Reference source not found. presents a graph of the results of exemplary embodiments according to the present specification of changes to L-lysine production in host cells of *C. glutamicum* transformed with recombinant nucleic acid molecules having promoter polynucleotide sequences selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to the heterologous target genes *fbp*, *dapB*, *ptsG*, *lysA*, *pgi*, and *ppc*, from *C. glutamicum*.

Error! Reference source not found. presents a graph of the results of exemplary embodiments according to the present specification of changes to L-lysine production in host cells of *C. glutamicum* transformed with recombinant nucleic acid molecules having promoter polynucleotide sequences selected

from the group consisting of SEQ ID NOs:1 to 8 functionally linked to the heterologous target genes *dapS*, cg0931, DapB, and *lysA*, from *C. glutamicum*.

DETAILED DESCRIPTION

In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments of the disclosure. However, one skilled in the art will understand that the disclosure may be practiced without these details.

Unless the context requires otherwise, throughout the present specification and claims, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is as "including, but not limited to".

As used herein, the term "recombinant nucleic acid molecule" refers to a recombinant DNA molecule or a recombinant RNA molecule. A recombinant nucleic acid molecule is any nucleic acid molecule containing joined nucleic acid molecules from different original sources and not naturally attached together. Recombinant RNA molecules include RNA molecules transcribed from recombinant DNA molecules. In particular, a recombinant nucleic acid molecule includes a nucleic acid molecule comprising a promoter of SEQ ID NOs:1 to 8 functionally linked to a heterologous target gene.

As used herein, the term "heterologous target gene" refers to any gene or coding sequence that is not controlled in its natural state (*e.g.*, within a non-genetically modified cell) by the promoter to which it is operably linked in a particular genome. As provided herein, all target genes functionally linked to non-naturally occurring promoters are considered "heterologous target genes". More specifically, as promoter polynucleotide sequences of SEQ ID NOs:1, 5, and 7 do not occur in nature, all functionally linked target gene sequences are "heterologous target gene" sequences. As used herein, a heterologous target gene can include one or more target genes that are part of an operon. That is, the endogenous promoter of an operon is replaced with a promoter polynucleotide sequence having a nucleic sequence of SEQ ID NOs:1 to 8. As used herein, the term "promoter polynucleotide sequence" refers to nucleic acids having a sequence as recited in the associated SEQ ID NO.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Polynucleotides Having Promoter Activity

Native *C. glutamicum* promoters were identified that satisfy both of the following criteria: 1) represent a ladder of constitutive promoters, *i.e.*, a plurality of promoters with incrementally increasing levels of promoter activity; and 2) encoded by short DNA sequences, ideally less than 100 base pairs. A published data set describing global gene expression levels in *C. glutamicum* ATCC 13032 (Lee *et al.*, Biotechnol Lett (2013) 35:709-717) was examined to identify genes that were constitutively expressed across different growth conditions. Genes whose expression level remained constant (defined as a ratio of expression between 0.33 and 3) across two growth conditions, namely chemostat growth in minimal media with and without the addition of hydrogen peroxide satisfied the first criterion. A published data set describing the *C. glutamicum* ATCC 13032 transcriptome (Pfeifer-Sancar *et al.*, BMC Genomics 2013, 14:888) was examined to find genes with compact promoters, *i.e.* those consisting of the 60 base pair core promoter region and a 5' untranslated region between 26 and 40 base pairs in length. The two data sets were cross-referenced to identify promoters that satisfied both criteria. See **Error! Reference source not found.**. The following five wild-type promoters were identified (Table 1).

Table 1: Promoters of *C. glutamicum* Having Increasing Levels of Expression and Constituent Expression Under Different Growth Conditions

Strain	SEQ ID NO	Mean Activity
Pcg1860-eyfp	2	89243
Pcg0007-eyfp	3	44527
Pcg0755-eyfp	4	43592
Pcg3381-eyfp	6	4723
Pcg3121-eyfp	8	98

The wild-type promoters cg1860, and cg3121 are not described in the literature. The wild-type promoter cg0007-*gyrB* is also not described in the literature, however, Neumann and Quiñones, (J Basic Microbiol. 1997;37(1):53-69) describes regulation of gyrB gene expression in *E. coli*. The wild-type promoter cg0755 is a known part of the methionine biosynthesis pathway (Suda *et al.*, Appl Microbiol Biotechnol (2008) 81:505-513; and Rey *et al.*, Journal of Biotechnology 103 (2003) 51-65). The wild-type promoter cg3381 is a *tatA* homolog. The tatA pathway in *Corynebacterium* is described by Kikuchi *et al.*, Applied and Environmental Microbiology, Nov. 2006, p. 7183-7192. The strong constitutive promoter Pcg0007 was chosen for mutagenesis. Four out of six positions in the predicted –10 element (TAAGAT) of Pcg0007 were randomized to generate both stronger and attenuated promoter variants (SEQ ID NOs 1, 5, and 7).

Accordingly, one embodiment of the present invention relates to native promoters comprising polynucleotides isolated from *C. glutamicum*, and mutant promoters derived therefrom that together represent a ladder of constitutive promoters with incrementally increasing levels of promoter activity. In some embodiments a *C. glutamicum* promoter can be encoded by a short DNA sequence. In some embodiments a *C. glutamicum* promoter can be encoded by a DNA sequence of less than 100 base pairs.

One embodiment of the present invention relates to a promoter polynucleotide comprising a sequence selected from: SEQ ID NO:1 (Pcg0007_lib_39), SEQ ID NO:2 (Pcg1860), SEQ ID NO:3 (Pcg0007), SEQ ID NO:4 (Pcg0755), SEQ ID NO:5 (Pcg0007_lib_265), SEQ ID NO:6 (Pcg3381), SEQ ID NO:7 (Pcg0007_lib_119), or SEQ ID NO:8 (Pcg3121). In another embodiment, the present specification provides for, and includes, a promoter polynucleotide comprising of SEQ ID NO:1 functionally linked to at least one heterologous target gene. In an embodiment, the present specification provides for, and includes, a promoter polynucleotide of SEQ ID NO:2 functionally linked to at least one heterologous target gene. In another embodiment, the present specification provides for, and includes, a promoter polynucleotide of SEQ ID NO:3 functionally linked to at least one heterologous target gene. In another embodiment, the present specification provides for, and includes, a promoter polynucleotide of SEQ ID NO:4 functionally linked to at least one heterologous target gene. In another embodiment, the present specification provides for, and includes, a promoter polynucleotide of SEQ ID NO:5 functionally linked to at least one heterologous target gene. In another embodiment, the present specification provides for, and includes, a promoter polynucleotide comprising of SEQ ID NO:5 functionally linked to at least one heterologous target gene. In another embodiment, the present specification provides for, and includes, a promoter polynucleotide of SEQ ID NO:7 functionally linked to at least one heterologous target gene. In another embodiment, the present specification provides for, and includes, a promoter polynucleotide of SEQ ID NO:8 functionally linked to at least one heterologous target gene.

As used herein, a "promoter cassette" refers to the polynucleotide sequences comprising a promoter polynucleotide of SEQ ID NOs:1 to 8 functionally linked to at least one heterologous target gene. In certain embodiments of the present disclosure, a "promoter cassette" may further include one or more of a linker polynucleotide, a transcription terminator following the heterologous gene, a ribosome binding site upstream of the start codon of the heterologous gene, and combinations of each.One embodiment of the present invention relates to a promoter polynucleotide consisting of a sequence selected from: 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, or SEQ ID NO:8. In an embodiment, the present specification provides for, and includes a promoter polynucleotide sequence of SEQ ID NO:1. In an embodiment, the present specification provides for, and includes a promoter polynucleotide sequence of SEQ ID NO:5. In an embodiment, the present specification provides for, and includes a promoter polynucleotide sequence of

SEQ ID NO:7. As used hereing a promoter cassette may described by reference the promoter name followed by the name of the heterologous target gene that is functionally linked to it. For example, the promoter of SEQ ID NO: 1, entitled Pcg1860, functionally linke to the gene zwf encoding the glucose-6-phosphate 1-dehydrogenase gene is referenced as Pcg1860-zwf. Similarly, Pcg0007_39-lysA is the 0007_39 promoter of SEQ ID NO:1 functionally linked to target gene lysA encoding the polypeptide diaminopimelate decarboxylase.

One embodiment of the present invention relates to combinations of the promoter polynucleotides described herein. In this context the term "combinations of promoter polynucleotides" refers to two or more polynucleotides that may be present as separate isolated sequences, as components of separate polynucleotide molecules, or as components of the same polynucleotide molecule, and combinations thereof. Examples of polynucleotide molecules include chromosomes and plasmids.

The invention also relates to an isolated promoter polynucleotide, which essentially consists of a polynucleotide having the nucleotide sequence depicted in 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, or SEQ ID NO:8. In an embodiment, the present specification provides for, and includes an isolated promoter polynucleotide of SEQ ID NO:1. In an embodiment, the present specification provides for, and includes an isolated promoter polynucleotide of SEQ ID NO:5. In an embodiment, the present specification provides for, and includes an isolated promoter polynucleotide of SEQ ID NO:7.

The term "essentially" in this context means that a polynucleotide of no more than 1,000, no more than 800, no more than 700, no more than 600, no more than 500 or no more than 400 nucleotides in length; and a polynucleotide of no more than 15,000, no more than 10,000, no more than 7,500, no more than 5,000, no more than 2,500, no more than 1,000, no more than 800, no more than 700, no more than 600, no more than 500, or no more than 400 nucleotides in length have been added to the 5' end and 3' end, respectively, of the polynucleotides 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, or SEQ ID NO:8.

Any useful combination of the features from the preceding two lists of polynucleotides added to the 5' end and 3' end, respectively, of the polynucleotides 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, or SEQ ID NO:8, is in accordance with the invention here. "Useful combination" means, for example, a combination of features which results in an efficient recombination being carried out. The use of additions of the same length flanking a DNA region to be replaced facilitates the transfer of the region by homologous recombination in the experimental procedure. Relatively long flanking homologous regions are advantageous for efficient recombination between circular DNA molecules but cloning of the replacement vector is made more difficult with increasing length of the flanks (Wang *et al.*, Molecular Biotechnology, 432:43-53 (2006)).

The specification provides for, and includes, homologous regions flanking a promoter polynucleotide sequence of SEQ ID NOs:1 to 8 functionally linked to at least on heterologous target gene (e.g., the "promoter cassette") to direct homologous recombination and replacement of a target gene sequence. In an embodiment, the homologus regions are direct repeat regions. In an embodiment, the homologous regions comprises between 500 base pairs (bp) and 5000 bp each of the target gene sequence flanking the promoter cassette. In an embodiment, the homologous regions comprises at least 500 bp each of the target gene sequence flanking the promoter cassette. In an embodiment, the homologous regions comprises at least 1000 bp (1 Kb) each of the target gene sequence flanking the promoter cassette. In an embodiment, the homologous regions comprises at least 2 Kb each of the target gene sequence flanking the promoter cassette. In an embodiment, the homologous regions comprises at least 5 Kb each of the target gene sequence flanking the promoter cassette.

The invention furthermore relates to an isolated promoter polynucleotide, which consists of the nucleotide sequence depicted in 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, or SEQ ID NO:8. In an embodiment, the isolate promoter polynucleotide consists of the polynucleotide sequence of SEQ ID NO:1. In an embodiment, the isolate promoter polynucleotide consists of the polynucleotide sequence of SEQ ID NO:5. In an embodiment, the isolate promoter polynucleotide consists of the polynucleotide sequence of SEQ ID NO:7.

Details regarding the biochemistry and chemical structure of polynucleotides as present in living things such as microorganisms, for example, can be found *inter alia* in the text book "Biochemie" [Biochemistry] by Berg *et al.* (Spektrum Akademischer Verlag Heidelberg Berlin, Germany, 2003; ISBN 3-8274-1303-6).

Polynucleotides consisting of deoxyribonucleotide monomers containing the nucleobases or bases adenine (A), guanine (G), cytosine (C) and thymine (T) are referred to as deoxyribo-polynucleotides or deoxyribonucleic acid (DNA). Polynucleotides consisting of ribonucleotide monomers containing the nucleobases or bases adenine (A), guanine (G), cytosine (C) and uracil (U) are referred to as ribopolynucleotides or ribonucleic acid (RNA). The monomers in said polynucleotides are covalently linked to one another by a 3',5'-phosphodiester bond.

A "promoter polynucleotide" or a "promoter" or a "polynucleotide having promoter activity" means a polynucleotide, preferably deoxyribopolynucleotide, or a nucleic acid, preferably deoxyribonucleic acid (DNA), which when functionally linked to a polynucleotide to be transcribed determines the point and frequency of initiation of transcription of the coding polynucleotide, thereby enabling the strength of expression of the controlled polynucleotide to be influenced. The term "promoter ladder" as used herein refers to a plurality of promoters with incrementally increasing levels of promoter activity. The term "promoter activity" as used herein refers to the ability of the promoter to initiate

transcription of an polynucleotide sequence into mRNA. Methods of assessing promoter activity are well known to those of skill in the art and include, for example the methods described in Example 2 below. The term "constitutive promoter" as used herein refers to a promoter that directs the transcription of tits associated genes at a constant rate regardless of the internal or external cellular conditions.

Owing to the double-stranded structure of DNA, the strand complementary to the strand in 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, or SEQ ID NO:8 of the sequence listing is likewise a subject of the invention.

Kits

One embodiment of the present invention relates to kits comprising a first promoter polynucleotide comprising a sequence selected from: SEQ ID NO:1, SEQ ID NO:5, and SEQ ID NO:7, and a suitable storage means for the polynucleotide. In some embodiments, the first promoter polynucleotide consists of a sequence selected from: SEQ ID NO:1, SEQ ID NO:5, and SEQ ID NO:7. In some embodiments, the kits comprise combinations of promoter polynucleotides comprising at least two first promoter polynucleotides described herein. In some embodiments, the kits comprise combinations of promoter polynucleotides comprising at least one first promoter polynucleotide described herein, and at least one second promoter polynucleotide comprising a sequence selected from: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8. In some embodiments, the kits comprise combinations of promoter polynucleotides comprising at least one first promoter polynucleotide described herein, and at least one second promoter polynucleotide consisting of a sequence selected from: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

Target Genes

One embodiment of the present invention relates to methods of expressing a target gene, comprising culturing a host cell transformed with a recombinant vector comprising a promoter polynucleotide as described herein. Target genes are polynucleotides the expression of which are controlled by the promoters described herein. The target genes may be coding polynucleotides which code for one or more polypeptide(s) or non-coding polynucleotides such as non-coding RNAs. A polynucleotide coding for a protein/polypeptide essentially consists of a start codon selected from the group consisting of ATG, GTG and TTG, preferably ATG or GTG, particularly preferably ATG, a protein-encoding sequence and one or more stop codon(s) selected from the group consisting of TAA, TAG and TGA.

"Transcription" means the process by which a complementary RNA molecule is produced starting from a DNA template. This process involves proteins such as RNA polymerase, "sigma factors"

and transcriptional regulatory proteins. Where the target gene is a coding polynucleotide, the synthesized RNA (messenger RNA, mRNA) then serves as a template in the process of translation which subsequently yields the polypeptide or protein.

"Functionally linked" means in this context the sequential arrangement of the promoter polynucleotide according to the invention with a further oligo- or polynucleotide, resulting in transcription of said further polynucleotide to produce a sense RNA transcript.

If the further polynucleotide is a target gene which codes for a polypeptide/protein and consists of the coding region for a polypeptide, starting with a start codon, including the stop codon and, where appropriate, including a transcription termination sequence, "functionally linked" then means the sequential arrangement of the promoter polynucleotide according to the invention with the target gene, resulting in transcription of said target gene and translation of the synthesized RNA.

If the target gene codes for a plurality of proteins/polypeptides, each gene may be preceded by a ribosome-binding site. Where appropriate, a termination sequence is located downstream of the last gene.

The target gene preferably codes for one or more polypeptides or proteins of the biosynthetic pathway of biomolecules, preferably selected from the group of proteinogenic amino acids, non-proteinogenic amino acids, vitamins, nucleosides, nucleotides and organic acids. The target gene preferably consists of one or more of the polynucleotides listed in Table 1 of EP 1 108 790 A2 which is hereby incorporated by reference.

The present specification provides for, and includes, recombinant nucleic acid molecules comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to any one of the heterologous target genes identifiable in the Kyoto Encyclopedia of Genes and Genomes (KEGG) as genes involved in metabolic and biosynthetic pathways. The KEGG database is available on the internet at genome.jp/kegg. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the lysine biosynthesis pathway as represented in KEGG map number 00300. In an embodiment, the one or more target genes are selected from the Lysine succinyl-DAP biosynthesis pathway, M00016. In an embodiment, the one or more target genes are selected from the lysine acetyl-DAP biosynthesis pathway, M00525. In an embodiment, the one or more target genes are selected from the lysine DAP dehydrogenase biosynthesis pathway, M00526. In an embodiment, the one or more target genes are selected from the lysine DAP aminotransferase biosynthesis pathway, M00527. In an embodiment, the one or more target genes are selected from the AAA pathway biosynthesis pathway, M00030. In an embodiment, the one or more target genes are selected from the lysine biosynthesis pathway from 2-oxoglutarate, M00433 or the lysine biosynthesis pathway mediated by LysW, M00031.

The present disclosure provides for, and includes, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the the serine biosynthesis pathway comprising genes of entry M00020. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the threonine biosynthesis pathway comprising genes of KEGG entry M00018. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the cysteine biosynthesis pathway comprising genes of KEGG entry M00021. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the cysteine biosynthesis pathway comprising genes of KEGG entry M00338. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the cysteine biosynthesis pathway comprising genes of KEGG entry M00609. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the methionine biosynthesis pathway comprising genes of KEGG entry M00017. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the valine/isoleucine biosynthesis pathway comprising genes of KEGG entry M00019. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the isoleucine biosynthesis pathway comprising genes of KEGG entry M00535. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the isoleucine biosynthesis pathway comprising genes of KEGG entry M00570. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the leucine biosynthesis pathway comprising genes of KEGG entry M00432. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the proline biosynthesis pathway comprising genes of KEGG entry M00015. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the ornithine biosynthesis pathway comprising genes of KEGG entry M00028. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the ornithine biosynthesis pathway comprising genes of KEGG entry M00763. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the histidine biosynthesis pathway comprising genes of KEGG entry M00026. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the shikimate biosynthesis pathway comprising genes of KEGG entry M00022. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the tryptophan biosynthesis pathway comprising genes of entry M00023. In

an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the phenylalanine biosynthesis pathway comprising genes of KEGG entry M00024. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the tyrosine biosynthesis pathway comprising genes of KEGG entry M00025. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1 to 8 are functionally linked to one or more target genes of the tyrosine biosynthesis pathway comprising genes of KEGG entry M00040.

The present disclosure provides for, and includes, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the the serine biosynthesis pathway comprising genes of entry M00020. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the threonine biosynthesis pathway comprising genes of KEGG entry M00018. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the cysteine biosynthesis pathway comprising genes of KEGG entry M00021. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the cysteine biosynthesis pathway comprising genes of KEGG entry M00338. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the cysteine biosynthesis pathway comprising genes of KEGG entry M00609. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the methionine biosynthesis pathway comprising genes of KEGG entry M00017. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the valine/isoleucine biosynthesis pathway comprising genes of KEGG entry M00019. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the isoleucine biosynthesis pathway comprising genes of KEGG entry M00535. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the isoleucine biosynthesis pathway comprising genes of KEGG entry M00570. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the leucine biosynthesis pathway comprising genes of KEGG entry M00432. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the proline biosynthesis pathway comprising genes of KEGG entry M00015. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the ornithine biosynthesis pathway comprising genes of KEGG entry M00028. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to

one or more target genes of the ornithine biosynthesis pathway comprising genes of KEGG entry M00763. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the histidine biosynthesis pathway comprising genes of KEGG entry M00026. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the shikimate biosynthesis pathway comprising genes of KEGG entry M00022. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the tryptophan biosynthesis pathway comprising genes of entry M00023. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the phenylalanine biosynthesis pathway comprising genes of KEGG entry M00024. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the tyrosine biosynthesis pathway comprising genes of KEGG entry M00025. In an embodiment, the promoter polynucleotide sequences of SEQ ID NOs:1, 5 or 7 are functionally linked to one or more target genes of the tyrosine biosynthesis pathway comprising genes of KEGG entry M00040.

The present specification provides for, and includes, recombinant nucleic acid molecules comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to any one of the heterologous target genes from *Corynebacterium glutamicum* ATCC 13032 provided in Table 2 or any *Corynebacterium glutamicum* equivalent thereof. Sequence start and end positions correspond to genomic nucleotide accession NC_003450.3. It will be understood by those of ordinary skill in the art that corresponding genes exist in other strains of *C. glutamicum* and may be readily identified from Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a

heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant nucleic acid molecule comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 2.

Table 2: Target genes from *Corynebacterium glutamicum* according to the present specification

Gene ID	Symbol	Aliases	description	start	end	orientation
1021315	NCgl0248	NCgl0248, Cgl0252	aspartate-semialdehyde dehydrogenase	270660	271694	plus
1021300	NCgl0223	NCgl0223, Cgl0226	prephenate dehydrogenase	241880	242902	minus
1021294	NCgl0247	NCgl0247, Cgl0251	aspartate kinase	269371	270636	plus
1021282	NCgl0215	NCgl0215, Cgl0218	aminotransferase	232257	233282	minus
1021250	NCgl0181	NCgl0181, Cgl0184	glutamine 2-oxoglutarate aminotransferase large subunit	195240	199772	plus
1021247	gltD	NCgl0182, Cgl0185	glutamate synthase	199772	201292	plus
1021203	aroE	NCgl0409, Cgl0424	quinate/shikimate dehydrogenase	446538	447389	plus
1021149	NCgl0245	NCgl0245, Cgl0248	2-isopropylmalate synthase	266151	267896	minus
1021136	gpmA	NCgl0390, Cgl0402	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	425177	425923	plus
1021131	NCgl0408	NCgl0408, Cgl0423	3-dehydroquinate dehydratase	446087	446524	plus
1021078	NCgl0398	NCgl0398, Cgl0410	pyrroline-5-carboxylate reductase	434877	435698	plus
1020978	trpA	NCgl2932, Cgl3035	tryptophan synthase subunit alpha	3239333	3240175	plus
1020976	NCgl2931	NCgl2931, Cgl3034	tryptophan synthase subunit beta	3238083	3239336	plus
1020975	NCgl2930	NCgl2930, trpC, trpF	bifunctional indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase	3236642	3238066	plus
1020974	trpD	NCgl2929, Cgl3032	anthranilate phosphoribosyltransferase	3235603	3236649	plus

1020973	NCgl2928	NCgl2928, Cgl3031	anthranilate synthase II	3234957	3235583	plus
1020972	NCgl2927	NCgl2927, Cgl3029	anthranilate synthase I	3233404	3234960	plus
1020852	NCgl2809	NCgl2809, Cgl2910	pyruvate kinase	3110462	3112321	minus
1020842	NCgl2799	NCgl2799, Cgl2899	prephenate dehydratase	3098576	3099523	minus
1020841	NCgl2798	NCgl2798, Cgl2898	phosphoglycerate mutase	3097902	3098573	minus
1020788	NCgl2747	NCgl2747, Cgl2844	aminotransferase	3030670	3031983	plus
1020745	NCgl2704	NCgl2704, Cgl2802	nucleosidase	2988212	2988772	minus
1020729	NCgl2688	NCgl2688, Cgl2786	cystathionine gamma-synthase	2972058	2973206	minus
1020714	NCgl2673	NCgl2673, Cgl2770	fructose-bisphosphate aldolase	2954239	2955273	minus
1020594	NCgl2557	NCgl2557, Cgl2646	dihydrodipicolinate synthase	2815459	2816397	plus
1020564	NCgl2528	NCgl2528, Cgl2617	D-2-hydroxyisocaproate dehydrogenase	2786754	2787716	minus
1020509	NCgl2474	NCgl2474, Cgl2563	serine acetyltransferase	2723065	2723613	plus
1020508	NCgl2473	NCgl2473, Cgl2562	cysteine synthase	2721905	2722861	plus
1020471	NCgl2436	NCgl2436, Cgl2522	phosphoserine phosphatase	2669555	2670856	minus
1020393	NCgl2360	NCgl2360, Cgl2446	cystathionine gamma-synthase	2590310	2591470	minus
1020370	NCgl2337	NCgl2337, Cgl2423	ribose-5-phosphate isomerase B	2563930	2564403	minus
1020307	NCgl2274	NCgl2274, Cgl2356	gamma-glutamyl kinase	2496668	2497777	minus
1020305	proA	NCgl2272, Cgl2354	gamma-glutamyl phosphate reductase	2494337	2495635	minus
1020301	NCgl2268	NCgl2268, Cgl2350	fructose-2,6-bisphosphatase	2491149	2491859	minus
1020260	NCgl2227	NCgl2227, Cgl2309	PLP-dependent aminotransferase	2444607	2445713	plus
1020188	NCgl2155	NCgl2155, Cgl2236	bifunctional RNase H/acid phosphatase	2371410	2372558	minus
1020181	NCgl2148	NCgl2148, Cgl2229	glutamine synthase	2362816	2364156	minus
1020172	NCgl2139	NCgl2139, Cgl2220	threonine synthase	2353598	2355043	minus

1020166	NCgl2133	NCgl2133, Cgl2214	glutamine synthase	2348830	2350263	plus
1020155	NCgl2123	NCgl2123, Cgl2204	branched-chain amino acid aminotransferase	2335913	2337016	minus
1020130	NCgl2098	NCgl2098, Cgl2178	3-deoxy-7-phosphoheptulonate synthase	2307695	2309095	minus
1020087	NCgl2055	NCgl2055, Cgl2136	cysteine synthase	2258360	2259313	minus
1020086	NCgl2054	NCgl2054, Cgl2135	diaminopimelate decarboxylase	2255736	2257025	minus
1020080	NCgl2048	NCgl2048, Cgl2129	methionine synthase II	2247004	2248209	minus
1020078	NCgl2046	NCgl2046, Cgl2127	threonine dehydratase	2244862	2246172	minus
1020053	hisD	NCgl2021, Cgl2102	histidinol dehydrogenase	2217597	2218925	minus
1020052	NCgl2020	NCgl2020, Cgl2101	histidinol-phosphate aminotransferase	2216491	2217591	minus
1020051	hisB	NCgl2019, Cgl2100	imidazoleglycerol-phosphate dehydratase	2215866	2216474	minus
1020048	hisH	NCgl2016, Cgl2097	imidazole glycerol phosphate synthase subunit HisH	2212638	2213273	minus
1020047	NCgl2015	NCgl2015, Cgl2096	phosphoribosyl isomerase A	2211879	2212619	minus
1020045	hisF	NCgl2013, Cgl2094	imidazole glycerol phosphate synthase subunit HisF	2210270	2211046	minus
1020044	hisI	NCgl2012, Cgl2093	phosphoribosyl-AMP cyclohydrolase	2209917	2210273	minus
1020042	NCgl2010	NCgl2010, Cgl2091	indole-3-glycerol phosphate synthase	2208364	2209149	minus
1020040	NCgl2008	NCgl2008, Cgl2089	pyruvate kinase	2205665	2207092	minus
1019930	NCgl1898	NCgl1898, Cgl1973	4-hydroxy-tetrahydrodipicolinate reductase	2081188	2081934	minus
1019928	dapA	NCgl1896, Cgl1971	4-hydroxy-tetrahydrodipicolinate synthase	2079278	2080183	minus
1019900	dapF	NCgl1868, Cgl1943	diaminopimelate epimerase	2051842	2052675	minus
1019614	NCgl1583	NCgl1583, Cgl1645	L-serine deaminase	1744884	1746233	plus

1019598	aroE	NCgl1567, Cgl1629	shikimate 5-dehydrogenase	1724609	1725439	minus
1019592	NCgl1561	NCgl1561, Cgl1623	chorismate synthase	1719666	1720898	minus
1019591	aroK	NCgl1560, Cgl1622	shikimate kinase	1719104	1719676	minus
1019590	aroB	NCgl1559, Cgl1621	3-dehydroquinate synthase	1717935	1719032	minus
1019571	NCgl1541	NCgl1541, Cgl1603	methionine adenosyltransferase	1699174	1700397	minus
1019566	NCgl1536	NCgl1536, Cgl1598	ribulose-phosphate 3-epimerase	1693259	1693918	minus
1019556	NCgl1526	NCgl1526, Cgl1588	glyceraldehyde-3-phosphate dehydrogenase	1682621	1683625	minus
1019555	pgk	NCgl1525, Cgl1587	phosphoglycerate kinase	1681187	1682404	minus
1019554	tpiA	NCgl1524, Cgl1586	triosephosphate isomerase	1680329	1681108	minus
1019550	NCgl1520	NCgl1520, Cgl1582	ornithine cyclodeaminase	1674120	1675268	minus
1019543	NCgl1513	NCgl1513, Cgl1575	transaldolase	1666673	1667755	plus
1019542	NCgl1512	NCgl1512, Cgl1574	transketolase	1664403	1666505	plus
1019512	NCgl1482	NCgl1482, Cgl1540	aconitate hydratase	1626279	1629110	plus
1019480	NCgl1450	NCgl1450, Cgl1507	methionine synthase I cobalamin-binding subunit	1587570	1591235	minus
1019478	hisE	NCgl1448, Cgl1505	phosphoribosyl-ATP pyrophosphatase	1586462	1586725	minus
1019477	hisG	NCgl1447, Cgl1504	ATP phosphoribosyltransferase	1585600	1586445	minus
1019377	NCgl1347	NCgl1347, Cgl1401	argininosuccinate lyase	1471477	1472910	plus
1019376	NCgl1346	NCgl1346, Cgl1400	argininosuccinate synthase	1470211	1471416	plus
1019374	NCgl1344	NCgl1344, Cgl1398	ornithine carbamoyltransferase	1468565	1469524	plus
1019373	argD	NCgl1343, Cgl1397	acetylornithine aminotransferase	1467376	1468551	plus
1019372	NCgl1342	NCgl1342, Cgl1396	acetylglutamate kinase	1466422	1467375	plus
1019371	argJ	NCgl1341, Cgl1395	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase	1465210	1466376	plus

1019370	argC	NCgl1340, Cgl1394	N-acetyl-gamma-glutamyl-phosphate reductase	1464053	1465126	plus
1019293	leuD	NCgl1263, Cgl1316	3-isopropylmalate dehydratase small subunit	1381902	1382495	plus
1019292	NCgl1262	NCgl1262, Cgl1315	3-isopropylmalate dehydratase large subunit	1380440	1381885	plus
1019267	NCgl1237	NCgl1237, Cgl1286	3-isopropylmalate dehydrogenase	1353489	1354511	plus
1019265	NCgl1235	NCgl1235, Cgl1284	D-3-phosphoglycerate dehydrogenase	1350855	1352447	plus
1019254	NCgl1224	NCgl1224, Cgl1273	ketol-acid reductoisomerase	1340724	1341740	plus
1019253	ilvH	NCgl1223, Cgl1272	acetolactate synthase small subunit	1340025	1340543	plus
1019252	NCgl1222	NCgl1222, Cgl1271	acetolactate synthase large subunit	1338131	1340011	plus
1019249	NCgl1219	NCgl1219, Cgl1268	dihydroxy-acid dehydratase	1333439	1335280	minus
1019232	NCgl1202	NCgl1202, Cgl1250	6-phosphofructokinase	1315046	1316086	plus
1019167	NCgl1137	NCgl1137, Cgl1184	homoserine kinase	1243855	1244784	plus
1019166	NCgl1136	NCgl1136, Cgl1183	homoserine dehydrogenase	1242507	1243844	plus
1019163	NCgl1133	NCgl1133, Cgl1180	diaminopimelate decarboxylase	1239929	1241266	plus
1019124	NCgl1094	NCgl1094, Cgl1139	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase	1188385	1190622	minus
1019117	aroE	NCgl1087, Cgl1132	shikimate 5-dehydrogenase	1180869	1181675	minus
1019094	NCgl1064	NCgl1064, Cgl1109	succinyl-diaminopimelate desuccinylase	1155731	1156840	plus
1019093	NCgl1063	NCgl1063, Cgl1108	tetrahydrodipicolinate N-succinyltransferase	1154726	1155676	minus
1019091	NCgl1061	NCgl1061, Cgl1106	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	1152370	1153263	minus
1019042	NCgl1013	NCgl1013, Cgl1058	phosphoglycerate mutase	1107503	1108204	plus
1018983	glyA	NCgl0954, Cgl0996	serine hydroxymethyltransferase	1050624	1051928	plus
1018979	NCgl0950	NCgl0950, Cgl0990	phospho-2-dehydro-3-deoxyheptonate aldolase	1046610	1047710	plus

1018968	NCgl0939	NCgl0939, Cgl0978	threonine dehydratase	1038718	1039650	minus
1018964	eno	NCgl0935, Cgl0974	phosphopyruvate hydratase	1034949	1036226	plus
1018934	NCgl0905	NCgl0905, Cgl0942	ribose-phosphate pyrophosphokinase	997463	998440	minus
1018929	NCgl0900	NCgl0900, Cgl0937	glyceraldehyde-3-phosphate dehydrogenase	993174	994616	plus
1018848	NCgl0819	NCgl0819, Cgl0853	hypothetical protein	910852	911157	minus
1018824	gltA	NCgl0795, Cgl0829	type II citrate synthase	877838	879151	plus
1018823	NCgl0794	NCgl0794, Cgl0828	phosphoserine aminotransferase	875982	877112	minus
1018809	NCgl0780	NCgl0780, Cgl0814	aminotransferase	861592	862755	plus
1018794	NCgl0765	NCgl0765, Cgl0799	fructose-1,6-bisphosphatase	841514	842296	minus
1018759	NCgl0730	NCgl0730, Cgl0764	3-phosphoshikimate 1-carboxyvinyltransferase	801187	802479	minus
1018688	NCgl0659	NCgl0659, Cgl0689	pyruvate carboxylase	705211	708633	plus
1018663	NCgl0634	NCgl0634, Cgl0664	monomeric isocitrate dehydrogenase (NADP+)	677828	680044	minus

In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7

functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 2.

In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 2. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 2. As used herein, a host cell refers to an organisms described below in the section entitled 'Expression' that have been transformed with one or more of the promoter cassettes. As will be apparent to one of ordinary skill in the art, a host cell may comprise one or more promoter cassettes as described herein.

In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally

linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 3. As used herein, a host cell refers to an organisms described below in the section entitled 'Expression' that have been transformed with one or more of the promoter cassettes. As will be apparent to one of ordinary skill in the art, a host cell may comprise one or more promoter cassettes as described herein.

Table 3: *C. glutamican* L-lysine Biosynthetic Pathway

Symbol	Gene Name (EC #)	*C. Glutamicum* Gene	Position	Expression
asd	aspartate-semialdehyde dehydrogenase (EC:1.2.1.11)	asd	270660..271694	+
dapA	4-hydroxy-tetrahydrodipicolinate synthase (EC:4.3.3.7)	dapA	Complement (2079278..2080183)	+
dapB	dihydrodipicolinate reductase (EC:1.17.1.8)	Cgl1973	complement(2081188..2081934)	+
dapD	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117)	dapD	complement(1153838..1154731)	+
dapD	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117)	dapD2	complement(1156194..1157144)	
cg0931	N-succinyldiaminopimelate aminotransferase (EC:2.6.1.17)	cg0931	863063..864226	+
dapE	succinyl-diaminopimelate	dapE	1157199..1158308	+

	desuccinylase (EC:3.5.1.18)			
dapF	diaminopimelate epimerase (EC:5.1.1.7)	dapF	complement(2021891..2022724)	+
lysA	diaminopimelate decarboxylase (EC:4.1.1.20)	lysA	1241397..1242734	+
ddh	diaminopimelate dehydrogenase (EC:1.4.1.16)	ddh	complement(2760062..2761024)	+
ask (lysC)	Aspartokinase Lysc Alpha And Beta Subunits (EC:2.7.2.4)	lysC	269371..270636	+
aspB	Aspartate Aminotransferase (EC:2.6.1.1)	aspB	256618..257898	+
PTS	Phosphotransferase System (PTS); Glucose-Specific Enzyme II BC Component Of PTS (EC:2.7.1.69)	ptsG	1424684..1426735	+
zwf	glucose-6-phosphate 1-dehydrogenase (EC:1.1.1.49 1.1.1.363)	zwf	1669327..1670871	+
pgi	glucose-6-phosphate isomerase (EC:5.3.1.9)	pgi	complement(909227..910849)	+
tkt	transketolase (EC:2.2.1.1)	tkt	1665870..1667972	+
fbp	6-phosphofructokinase 1 (EC:2.7.1.11)	Cgl1250	1315046..1316086	+
ppc	phosphoenolpyruvate carboxylase (EC:4.1.1.31)	ppc	complement(1678851..1681610)	+
pyc	pyruvate carboxylase (EC:6.4.1.1)	pyc	706684..710106	+
icd	isocitrate dehydrogenase (EC:1.1.1.42)	icd	complement(679301..681517)	-
pck	phosphoenolpyruvate carboxykinase (GTP) (EC:4.1.1.32)	pck	complement(3025365..3027197)	-
odx	Oxaloacetate decarboxylase (EC 4.1.1.3)	odx	AP017369.1:1508967..1509782 (from C. glutamicum N24)	-
hom	homoserine kinase (EC:2.7.1.39)	Cgl1184	1243855..1244784	-
	homoserine dehydrogenase (EC:1.1.1.3);	Cgl1183	1242507..1243844	-
	threonine synthase (EC:4.2.3.1)	Cgl2220	complement(2353598..2355043)	-

In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous

target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 3.

In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 3. In an embodiment, the present specification

provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 3. As used herein, a host cell refers to an organisms described below in the section entitled 'Expression' that have been transformed with one or more of the promoter cassettes. As will be apparent to one of ordinary skill in the art, a host cell may comprise one or more promoter cassettes as described herein.

The present specification provides for a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to any one of the heterologous target genes from *Corynebacterium glutamicum* ATCC 13032 provided in Table 4 or their *Corynebacterium glutamicum* equivalent therof. Sequence start and end positions correspond to genomic nucleotide accession NC_003450.3. It will be understood by those of ordinary skill in the art that corresponding genes exist in other strains of *C. glutamicum* and may be readily identified from Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 4. As used herein, a host cell refers to an organisms described below in the section entitled 'Expression' that have been transformed with one or more of the promoter cassettes. As

will be apparent to one of ordinary skill in the art, a host cell may comprise one or more promoter cassettes as described herein.

Table 4: *C. glutamican* L-methionine Biosynthetic Pathway

Symbol	Gene Name (EC #)	*C. Glutamicum* Gene	Position
lysC	aspartate kinase [EC:2.7.2.4]	Cgl0251	269371..270636
	aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	Cgl0252	270660..271694
dapA	4-hydroxy-tetrahydrodipicolinate synthase [EC:4.3.3.7]	dapA	complement(2079278..2080183)
dapA	4-hydroxy-tetrahydrodipicolinate synthase [EC:4.3.3.7]	Cgl2646	2815459..2816397
dapB	4-hydroxy-tetrahydrodipicolinate reductase [EC:1.17.1.8]	Cgl1973	complement(2081188..2081934)
dapD	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase [EC:2.3.1.117]	Cgl1106	complement(1152370..1153263)
dapC	N-succinyldiaminopimelate aminotransferase [EC:2.6.1.17]	Cgl0814	861592..862755
dapE	succinyl-diaminopimelate desuccinylase [EC:3.5.1.18]	Cgl1109	1155731..1156840
dapF	diaminopimelate epimerase [EC:5.1.1.7]	dapF	complement(2051842..2052675)
lysA	diaminopimelate decarboxylase [EC:4.1.1.20]	Cgl1180	1239929..1241266

In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for,

and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 4.

In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:1 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:2 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:3 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:4 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:5 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:6 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:7 functionally linked to a heterologous target gene recited in Table 4. In an embodiment, the present specification provides for, and includes, a host cell transformed with a recombinant vector comprising a promoter polynucleotide sequence of SEQ ID NO:8 functionally linked to a heterologous target gene recited in Table 4.

In some embodiments the target gene is associated with a biosynthetic pathway producing a biomolecule selected from: amino acids, organic acids, flavors and fragrances, biofuels, proteins and enzymes, polymers/monomers and other biomaterials, lipids, nucleic acids, small molecule therapeutics, protein therapeutics, fine chemicals, and nutraceuticals.

In some embodiments the target gene is associated with a biosynthetic pathway producing a secondary metabolite selected from: antibiotics, alkaloids, terpenoids, and polyketides. In some embodiments the target gene is associated with a metabolic pathway producing a primary metabolite selected from: alcohols, amino acids, nucleotides, antioxidants, organic acids, polyols, vitamins, and lipids/fatty acids. In some embodiments the target gene is associated with a biosynthetic pathway producing a macromolecule selected from: proteins, nucleic acids, and polymers

In addition it may be advantageous for the production of L-amino acids to enhance, in particular to overexpress one or more enzymes of the respective biosynthesis pathway, glycolysis, anaplerosis, citric acid cycle, pentose phosphate cycle, amino acid export and optionally regulatory proteins.

Thus for example, for the production of L-amino acids, it may be advantageous for one or more genes selected from the following group to be enhanced, in particular overexpressed: the gene *dapA* coding for dihydrodipicolinate synthase (EP-B 0 197 335); the gene *eno* coding for enolase (DE: 19947791.4); the gene *gap* coding for glyceraldehyde-3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086); the gene *tpi* coding for triosephosphate isomerase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086); the gene *pgk* coding for 3-phosphoglycerate kinase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086); the gene *zwf* coding for glucose-6-phosphate dehydrogenase (JP-A-09224661); the gene *pyc* coding for pyruvate carboxylase (DE-A-198 31 609; Eikmanns (1992), Journal of Bacteriology 174:6076–6086); the gene *mqo* coding for malate-quinone-oxidoreductase (Molenaar *et al.*, European Journal of Biochemistry 254, 395-403 (1998)); the gene *lysC* coding for a feedback-resistant aspartate kinase (Accession No. P26512); the gene *lysE* coding for lysine export (DE-A-195 48 222); the gene *hom* coding for homoserine dehydrogenase (EP-A 0131171); the gene *ilvA* coding for threonine dehydratase (Möckel *et al.*, Journal of Bacteriology (1992) 8065-8072)) or the allele *ilvA* (*Fbr*) coding for a feedback-resistant threonine dehydratase (Möckel *et al.*, (1994) Molecular Microbiology 13: 833-842); the gene *ilvBN* coding for acetohydroxy acid synthase (EP-B 0356739); the gene *ilvD* coding for dihydroxy acid dehydratase (Sahm and Eggeling (1999) Applied and Environmental Microbiology 65: 1973-1979); and the gene *zwa1* coding for the Zwa1 protein (DE: 19959328.0, DSM 13115).

Furthermore it may be advantageous for the production of L-amino acids also to attenuate, in particular to reduce, the expression of one or more genes selected from the group: the gene pck coding for phosphoenol pyruvate carboxykinase (DE 199 50 409.1; DSM 13047); the gene pgi coding for glucose-6-phosphate isomerase (U.S. Pat. No. 6,586,214; DSM 12969); the gene poxB coding for pyruvate oxidase (DE: 1995 1975.7; DSM 13114); and the gene zwa2 coding for the Zwa2 protein (DE: 19959327.2, DSM 13113).

In addition, it may furthermore be advantageous, for the production of amino acids, in particular L-lysine, to eliminate undesirable side reactions, (Nakayama: "Breeding of Amino Acid Producing Micro-organisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The promoter according to the invention can thus be used in each case for overexpressing or underexpressing the target gene in *C. glutamicum*.

Linkers

The target gene is positioned downstream of the promoter polynucleotide according to the invention, *i.e.* at the 3' end, such that both polynucleotides are functionally linked to one another either directly or by means of a linker oligonucleotide or linker polynucleotide. Preference is given to the promoter and the target gene being functionally linked to one another by means of a linker oligonucleotide or linker polynucleotide. Said linker oligonucleotide or linker polynucleotide consists of deoxyribonucleotides.

In this context, the expression "functionally linked to one another directly" means that the nucleotide at the 3' end 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, or SEQ ID NO:8 is linked directly to the first nucleotide of the start codon of a target gene. This results in "leaderless" mRNAs which start immediately with the 5'-terminal AUG start codon and therefore do not have any other translation initiation signals.

In this context, the expression "functionally linked to one another by means of a linker oligonucleotide" means that the nucleotide at the 3' end 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, or SEQ ID NO:8 is linked by an oligonucleotide of 1, 2, 3, 4 or 5 nucleotides in length to the first nucleotide of the start codon of a target gene.

In this context, the expression "functionally linked to one another by means of a linker polynucleotide" means that the nucleotide at the 3' end 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, or SEQ ID NO:8 is linked by a polynucleotide of from 6 to no more than 600 nucleotides in length to the first nucleotide of the start codon of a target gene.

In this context, the expression "functionally linked to one another" means that the target gene is bound to the promoter polynucleotide according to the invention in such a way that transcription of the target gene and translation of the synthesized RNA are ensured.

Depending on the technical requirement, the linker polynucleotide is:

6 - 600, 6 - 500, 6 - 400, 6 - 300, 6 - 200, 6 - 180, 6 -160, 6 - 140, 6 - 120, 6 - 100, 6 - 80, 6 - 60, 6 - 50, 6 - 40, 6 - 30, 6 - 28, 6 - 27, 6 - 26, 6 - 25; or

8 - 600, 8 - 500, 8 - 400, 8 - 300, 8 - 200, 8 - 180, 8 -160, 8 - 140, 8 - 120, 8 - 100, 8 - 80, 8 - 60, 8 - 50, 8 - 40, 8 - 30, 8 - 28, 8 - 27, 8 - 26, 8 - 25; or

10 - 600, 10 - 500, 10 - 400, 10 - 300, 10 - 200, 10 - 180, 10 - 160, 10 - 140, 10 - 120, 10 - 100, 10 - 80, 10 - 60, 10 - 50, 10 - 40, 10 - 30, 10 - 28, 10 - 27, 10 - 26, 10 -25; or

12 - 600, 12 - 500, 12 - 400, 12 - 300, 12 - 200, 12 - 180, 12 - 160, 12 - 140, 12 - 120, 12 - 100, 12 - 80, 12 - 60, 12 - 50, 12 - 40, 12 - 30, 12 - 28, 12 - 27, 12 - 26, 12 -25; or

14 - 600, 14 - 500, 14 - 400, 14 - 300, 14 - 200, 14 - 180, 14 - 160, 14 - 140, 14 - 120, 14 - 100, 14 - 80, 14 - 60, 14 - 50, 14 - 40, 14 - 30, 14 - 28, 14 - 27, 14 - 26, 14 -20; or

16 - 600, 16 - 500, 16 - 400, 16 - 300, 16 - 200, 16 - 180, 16 - 160, 16 - 140, 16 - 120, 16 - 100, 16 - 80, 16 - 60, 16 - 50, 16 - 40, 16 - 30, 16 - 28, 16 - 27, 16 - 26, 16 -25; or

18 - 600, 18 - 500, 18 - 400, 18 - 300, 18 - 200, 18 - 180, 18 - 160, 18 - 140, 18 - 120, 18 - 100, 18 - 80, 18 - 60, 18 - 50, 18 - 40, 18 - 30, 18 - 28, 18 - 27, 18 - 26, 18 -25; or

20 - 600, 20 - 500, 20 - 400, 20 - 300, 20 - 200, 20 - 180, 20 - 160, 20 - 140, 20 - 120, 20 - 100, 20 - 80, 20 - 60, 20 - 50, 20 - 40, 20 - 30, 20 - 28, 20 - 27, 20 - 26, 20 -25 nucleotides in length.

In particularly preferred embodiments, the linker polynucleotide is 20, 21, 22, 23, 24, or 25 nucleotides in length because this produces preferably functional constructs.

The invention further relates accordingly to an isolated promoter polynucleotide, essentially consisting of a polynucleotide 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, or SEQ ID NO:8, which, via the nucleotide at its 3' end, is functionally linked, directly or by means of a linker polynucleotide which ensures translation of RNA, to a target gene which contains at its 5' end an ATG or GTG start codon and codes for one or more polypeptide(s). Preference is given to the promoter and target gene being functionally linked to one another by means of a linker polynucleotide.

The invention furthermore also relates to an isolated polynucleotide, essentially consisting of a polynucleotide 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, or SEQ ID NO:8, which, via the nucleotide at its 3' end, is functionally linked to a linker oligonucleotide.

In addition, the invention furthermore relates to an isolated polynucleotide, essentially consisting of a polynucleotide 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, or SEQ ID NO:8, which, via the nucleotide at its 3' end, is functionally linked to a linker polynucleotide which ensures translation of RNA.

In this context, the term "essentially" means that a polynucleotide of no more than 1,000, no more than 800, no more than 700, no more than 600, no more than 500, or no more than 400 nucleotides in length has been added to the 5' end of the polynucleotide 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, or SEQ ID NO:8 and a polynucleotide of no more than 1,000, no more than 800, no more than 700, no more than 600, no more than 500, or no more than 400 nucleotides in length has been added to the 3' of the target gene, or a polynucleotide of no more than 15,000, no more than 10,000, no more than 7,500, no more than 5,000, no more than 2,500, no more than 1,000, no more than 800, no more than 700, no more than 600, no more than 500, or no more than 400 nucleotides in length has been added to the 3' end of the linker oligo- or polynucleotide.

Any useful combination of the features from the preceding three lists of polynucleotides is in accordance with the invention here. "Useful combination" means, for example, a combination of features which results in an efficient recombination being carried out. The use of additions of the same length flanking a DNA region to be replaced facilitates the transfer of the region by homologous recombination in the experimental procedure. Relatively long flanking homologous regions are advantageous for efficient recombination between circular DNA molecules but cloning of the replacement vector is made more difficult with increasing length of the flanks (Wang *et al.*, Molecular Biotechnology 32:43-53 (2006)).

In addition, the flank at the 3' end of the linker oligo- or polynucleotide increases in length to no more than 15,000 nucleotides when the 3' end is functionally linked to a target gene which contains at its 5' end an ATG or GTG start codon and codes for one or more polypeptide(s).

These particularly preferred embodiments of the linker polynucleotide ensure translation of RNA in an advantageous manner.

To facilitate chemical linking between the polynucleotide according to the invention having the nucleotide sequence 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, or SEQ ID NO:8, the linker polynucleotide which ensures translation of RNA, and the target gene coding for one or more polypeptide(s), which has an ATG or GTG start codon at its 5' end, functional nucleotide sequences required for cloning may be incorporated into said polynucleotides at their 5' and 3' ends and are at least partially retained even after said cloning.

The term "functional nucleotide sequence required for cloning" here represents any REII (type II restriction endonuclease) cleavage site present, whose sequence normally consists of from 4 to 8 nucleotides.

In addition, it should be mentioned here that site-specific mutagenesis by means of mutagenesis primers or a *de novo* gene synthesis (*e.g.* by GENEART AG (Regensburg, Germany)) of the nucleotide

sequences to remove cleavage sites for restriction endonucleases may introduce silent mutations into the sequence in order to enable said cleavage sites to be used advantageously for subsequent cloning steps.

The polynucleotide resulting from the promoter according to the invention being functionally linked to the linker polynucleotide which ensures translation of RNA is also referred to as expression unit herein below.

Expression

The invention furthermore relates to the use of the promoter according to the invention or of the expression unit according to the invention for expressing target genes or polynucleotides in microorganisms. The promoter according to the invention or the expression unit according to the invention ensures transcription and translation of the synthesized RNA, preferably mRNA, into a polypeptide. As used herein, the term "host cell" refers to a transformed cell of a microorganism.

The present disclosure, provides for, and includes, transformed host cells comprising the recombinant nucleic acids and recombinant vectors described in detail above. The present disclosure further provides for, and includes, host cells transformed with two recombinant nucleic acids. In an embodiment, the host cells are transformed with three recombinant nucleic acids. As provided above, the nucleic acids may be selected from biosynthetic pathways based on the overall effect on the yield of the desired product. There is no practical limit the the number of recombinant nucleic acids that may be incorporated into the host cells of the present specification. Expression is preferably carried out in microorganisms of the genus *Corynebacterium*. Preference is given to strains within the genus *Corynebacterium* which are based on the following species: *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. Very particular preference is given to the species *C. glutamicum*. In this way it is possible to express polynucleotides that code for polypeptides having a property, preferably enzyme activity, which are not present or detectable in the corresponding host. Thus, for example, Yukawa *et al.* describe expression of *Escherichia coli* genes for utilizing D-xylose in *C. glutamicum* R under the control of the constitutive Ptrc promoter (Applied Microbiology and Biotechnology 81, 691-699 (2008)).

The present specification provides for, and includes *C. glutamicum* having two or more genes of a biosynthetic pathway under the control of the promoter polynucleotide sequences described above. In various embodiments, one or more target genes are placed under the control of a promoter polynucleotide sequence having as sequence of SEQ ID NOs:1 to 8 as described above. In other embodiments, one or more target genes are placed under the control of a promoter polynucleotide sequence having as sequence of SEQ ID NOs:1, 5 or 7 as described above.

In certain embodiments according to the present specification, *C. glutamicum* host cells have two target genes under the control of the promoters having sequences of SEQ ID NOs:1 to 8. In certain other embodiments according to the present specification, *C. glutamicum* host cells have two target genes under the control of the promoters having sequences of SEQ ID NOs:1, 5 or 7. Using homologous recombination, the promoters of the present disclosure replace the endogenous promoter and endogenous sequence to prepare a promoter functionally linked to a heterologous gene. One of ordinary skill in the art would recognize that the recombination results in a replacement of the endogenous promoter while retaining the gene in its native locus. Specific non-limiting examples are illustrated below in Table 8. Multiple promoter-heterologous target pairs (e.g., promoter cassettes) can be readily incorporated into the genome of a host cell. In an embodiment, the promoter cassettes can be incorporated into host cells sequentially. In certain embodiments, the recombinant vectors of the present disclosure provide for two or more different promoter cassettes in a single construct. The present specification provides no practical limit to the number of promoter replacements that can be developed using the described methods.

In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-lysA and Pcg3121-pgi. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-pyc and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-lysA and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pck and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-ppc and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pck and Pcg3121-pgi. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-ddh and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_265-dapB and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-zwf and Pcg3121-pgi. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-ddh and Pcg3121-pgi. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pgi and Pcg1860-pyc. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-pyc and Pcg0007_265-dapB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-pyc and Pcg0007-lysA. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-asd and Pcg0007-zwf. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_265-dapB and Pcg3121-pgi. In an embodiment the host cell

is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-pyc and Pcg1860-asd. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-aspB and Pcg1860-pyc. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-fbp and Pcg1860-pyc. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-ddh and Pcg3381-fbp. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0755-ptsG and Pcg3121-pgi. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-pyc and Pcg3121-pck. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-asd and Pcg3121-pgi. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-asd and Pcg3381-fbp. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-lysE and Pcg3381-fbp. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-fbp and Pcg0007-lysA. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-lysE and Pcg1860-pyc. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pgi and Pcg3381-fbp. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pck and Pcg0007-lysA. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-lysA and Pcg0007_265-dapB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_265-dapB and Pcg1860-asd. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pgi and Pcg0007_265-dapD. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-lysA and Pcg3381-ddh. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pck and Pcg1860-asd. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-lysA and Pcg1860-asd. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3121-pck and Pcg0007_265-dapB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-ddh and Pcg1860-asd. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-ppc and Pcg1860-asd. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-ppc and Pcg0007-lysA. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-ddh and Pcg0007_265-dapB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_265-dapB and Pcg3381-fbp. In an embodiment the host cell is a

transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-ppc and Pcg0007_265-dapB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-aspB and Pcg3121-pck. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_265-dapB and Pcg0007_265-dapD. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-lysE and Pcg3381-aspB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007_39-lysE and Pcg0007_265-dapD. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-aspB and Pcg0007_265-dapB. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg1860-asd and Pcg0007_265-dapD. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-aspB and Pcg0007-lysA. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg3381-aspB and Pcg3381-ddh. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0755-ptsG and Pcg1860-pyc. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0755-ptsG and Pcg3381-fbp. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0007-zwf and Pcg3381-fbp. In an embodiment the host cell is a transgenic *C. glutamicum* host cell comprising the promoter cassettes Pcg0755-ptsG and Pcg0007_265-dapD.

The present disclosure provides for, and includes, host cells having three or more promoter cassettes as described above. In an embodment, the host cell includes the Pcg0007_39-zwf, Pcg0007_39-lysA and Pcg1860-pyc promoter cassettes. In an embodiment, the host cell is a *C. glutamicum* host cell.

The promoter according to the invention or the expression unit according to the invention is furthermore used for improving the performance characteristics of microorganisms, which can include, for example, yield, titer, productivity, by-product elimination, tolerance to process excursions, optimal growth temperature and growth rate. In some embodiments, the promoter according to the invention or the expression unit according to the invention is used for up-regulating a target gene in a microorganism (overexpression). Overexpression generally means an increase in the intracellular concentration or activity of a ribonucleic acid, a protein (polypeptide) or an enzyme in comparison with the starting strain (parent strain) or wild-type strain, if the latter is the starting strain. In some embodiments, the promoter according to the invention or the expression unit according to the invention is used for down-regulating a target gene in a microorganism (underexpression). Underexpression generally means an decrease in the intracellular concentration or activity of a ribonucleic acid, a protein (polypeptide) or an enzyme in comparison with the starting strain (parent strain) or wild-type strain, if the latter is the starting strain. In some embodiments, a combination of promoters and/or expression units according to the invention are

used for regulating expression of more than one target gene in a microorganism, wherein each target gene is either up-regulated or down-regulated. In some embodiments the target genes up- or down-regulated by the combination of promoters and/or expression units are part of the same metabolic pathway. In some embodiments the target genes up- or down-regulated by the combination of promoters and/or expression units are not part of the same metabolic pathway.

The promoters described herein can be used in combination with other methods very well-known in the art for attenuating (reducing or eliminating) the intracellular activity of one or more enzymes (proteins) in a microorganism which are coded by the corresponding DNA, for example by using a weak promoter or using a gene, or allele, which codes for a corresponding enzyme with a low activity, or inactivates the corresponding gene or enzyme (protein), and optionally combining these measures.

The reduction in gene expression can take place by suitable culturing or by genetic modification (mutation) of the signal structures of gene expression. Signal structures of gene expression are, for example, repressor genes, activator genes, operators, promoters, attenuators, ribosome binding sites, the start codon and terminators. The expert can find information on this *e.g.* in the patent application WO 96/15246, in Boyd and Murphy (Journal of Bacteriology 170: 5949 (1988)), in Voskuil and Chambliss (Nucleic Acids Research 26: 3548 (1998), in Jensen and Hammer (Biotechnology and Bioengineering 58: 191 (1998)), in Patek *et al.* (Microbiology 142: 1297 (1996)), Vašicová *et al.* (Journal of Bacteriology 181: 6188 (1999)) and in known textbooks of genetics and molecular biology, such as *e.g.* the textbook by Knippers ("Molekulare Genetik [Molecular Genetics]", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995) or that by Winnacker ("Gene und Klone [Genes and Clones]", VCH Verlagsgesellschaft, Weinheim, Germany, 1990).

Mutations which lead to a change or reduction in the catalytic properties of enzyme proteins are known from the prior art; examples which may be mentioned are the works by Qiu and Goodman (Journal of Biological Chemistry 272: 8611–8617 (1997)), Sugimoto *et al.* (Bioscience Biotechnology and Biochemistry 61: 1760-1762 (1997)) and Möckel ("Die Threonindehydratase aus *Corynebacterium glutamicum*: Aufhebung der allosterischen Regulation und Struktur des Enzyms [Threonine dehydratase from *Corynebacterium glutamicum*: Cancelling the allosteric regulation and structure of the enzyme]", Reports from the Jülich Research Centre, Jül-2906, ISSN09442952, Jülich, Germany, 1994). Comprehensive descriptions can be found in known textbooks of genetics and molecular biology, such as *e.g.* that by Hagemann ("Allgemeine Genetik [General Genetics]", Gustav Fischer Verlag, Stuttgart, 1986).

Possible mutations are transitions, transversions, insertions and deletions. Depending on the effect of the amino acid exchange on the enzyme activity, missense mutations or nonsense mutations are referred to. Insertions or deletions of at least one base pair in a gene lead to frame shift mutations, as a

consequence of which incorrect amino acids are incorporated or translation is interrupted prematurely. Deletions of several codons typically lead to a complete loss of the enzyme activity. Instructions on generation of such mutations are prior art and can be found in known textbooks of genetics and molecular biology, such as *e.g.* the textbook by Knippers ("Molekulare Genetik [Molecular Genetics]", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995), that by Winnacker ("Gene und Klone [Genes and Clones]", VCH Verlagsgesellschaft, Weinheim, Germany, 1990) or that by Hagemann ("Allgemeine Genetik [General Genetics]", Gustav Fischer Verlag, Stuttgart, 1986). A common method of mutating genes of *C. glutamicum* is the method of gene disruption and gene replacement described by Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)).

In the method of gene disruption a central part of the coding region of the gene of interest is cloned in a plasmid vector which can replicate in a host (typically *E. coli*), but not in *C. glutamicum*. Possible vectors are, for example, pSUP301 (Simon *et al.*, Bio/Technology 1, 784–791 (1983)), pK18mob or pK19mob (Schäfer *et al.*, Gene 145, 69–73 (1994)), pK18mobsacB or pK19mobsacB (Jäger *et al.*, Journal of Bacteriology 174: 5462–65 (1992)), pGEM-T (Promega corporation, Madison, Wis., USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological Chemistry 269:32678–84; U.S. Pat. No. 5,487,993), pCR®Blunt (Invitrogen, Groningen, Holland; Bernard *et al.*, Journal of Molecular Biology, 234: 534–541 (1993)) or pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510–4516). The plasmid vector which contains the central part of the coding region of the gene is then transferred into the desired strain of *C. glutamicum* by conjugation or transformation. The method of conjugation is described, for example, by Schäfer *et al.* (Applied and Environmental Microbiology 60, 756–759 (1994)). Methods for transformation are described, for example, by Thierbach *et al.* (Applied Microbiology and Biotechnology 29, 356–362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067–1070 (1989)) and Tauch *et al.* (FEMS Microbiological Letters 123, 343–347 (1994)). After homologous recombination by means of a "cross-over" event, the coding region of the gene in question is interrupted by the vector sequence and two incomplete alleles are obtained, one lacking the 3' end and one lacking the 5' end. This method has been used, for example, by Fitzpatrick *et al.* (Applied Microbiology and Biotechnology 42, 575–580 (1994)) to eliminate the recA gene of *C. glutamicum*.

In the method of gene replacement, a mutation, such as *e.g.* a deletion, insertion or base exchange, is established *in vitro* in the gene of interest. The allele prepared is in turn cloned in a vector which is not replicative for *C. glutamicum* and this is then transferred into the desired host of *C. glutamicum* by transformation or conjugation. After homologous recombination by means of a first "cross-over" event which effects integration and a suitable second "cross-over" event which effects excision in the target gene or in the target sequence, the incorporation of the mutation or of the allele is

achieved. This method was used, for example, by Peters-Wendisch (Microbiology 144, 915-927 (1998)) to eliminate the pyc gene of *C. glutamicum* by a deletion.

The promoters described herein can be used in combination with other methods very well-known in the art for raising (enhancing) the intracellular activity of one or more enzymes in a microorganism that are coded by the corresponding DNA, by for example increasing the number of copies of the gene or genes, using a strong promoter, or using a gene that codes for a corresponding enzyme having a high activity, and optionally combining these measures.

In order to achieve an overexpression the number of copies of the corresponding genes can be increased, or alternatively the promoter and regulation region or the ribosome binding site located upstream of the structure gene can be mutated. Expression cassettes that are incorporated upstream of the structure gene act in the same way. By means of inducible promoters it is in addition possible to increase the expression in the course of the enzymatic amino acid production. The expression is similarly improved by measures aimed at prolonging the lifetime of the m-RNA. Furthermore, the enzyme activity is also enhanced by preventing the degradation of the enzyme protein. The genes or gene constructs may either be present in plasmids having different numbers of copies, or may be integrated and amplified in the chromosome. Alternatively, an overexpression of the relevant genes may furthermore be achieved by altering the composition of the media and the culture conditions.

The person skilled in the art can find details on the above in, *inter alia*, Martin *et al.* (Bio/Technology 5, 137-146 (1987)), in Guerrero *et al.* (Gene 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns *et al.* (Gene 102, 93-98 (1991)), in European Patent Specification 0 472 869, in U.S. Pat. No. 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991), in Reinscheid *et al.* (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre *et al.* (Journal of Bacteriology 175, 1001-1007 (1993)), in Patent Application WO 96/15246, in Malumbres *et al.* (Gene 134, 15-24 (1993)), in Japanese laid open Specification JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in known textbooks on genetics and molecular biology.

Genes may be overexpressed for example by means of episomal plasmids. Suitable plasmids are those that are replicated in coryneform bacteria. Numerous known plasmid vectors, such as for example pZ1 (Menkel *et al.*, Applied and Environmental Microbiology (1989) 64: 549-554), pEKEx1 (Eikmanns *et al.*, Gene 102:93-98 (1991)) or pHS2-1 (Sonnen *et al.*, Gene 107:69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, such as for example those based on pCG4 (U.S. Pat. No. 4,489,160), or pNG2 (Serwold-Davis *et al.*, FEMS Microbiology Letters 66, 119-124 (1990)), or pAG1 (U.S. Pat. No. 5,158,891) may be used in a similar way.

Furthermore, also suitable are those plasmid vectors with the aid of which the process of gene amplification by integration in the chromosome can be employed, such as has been described for example by Reinscheid *et al.* (Applied and Environmental Microbiology 60, 126-132 (1994)) for the duplication and amplification of the hom-thrB operon. In this method the complete gene is cloned into a plasmid vector that can replicate in a host (typically *E. coli*) but not in *C. glutamicum*. Suitable vectors are for example pSUP301 (Simon *et al.*, Bio/Technology 1, 784-791 (1983)), pK18mob or pK19 mob (Schäfer *et al.*, Gene 145, 69-73 (1994)), pGEM-T (Promega Corporation, Madison, Wis., USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological Chemistry 269:32678-84; U.S. Pat. No. 5,487,993), pCR®Blunt (Invitrogen, Groningen, Netherlands; Bernard *et al.*, Journal of Molecular Biology, 234: 534-541 (1993)), pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510-4516) or pBGS8 (Spratt *et al.*, 1986, Gene 41: 337-342). The plasmid vector that contains the gene to be amplified is then transferred by conjugation or transformation into the desired strain of *C. glutamicum*. The method of conjugation is described for example in Schäfer *et al.* (Applied and Environmental Microbiology 60, 756-759 (1994)). Transformation methods are described for example in Thierbach *et al.* (Applied Microbiology and Biotechnology 29, 356-362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch *et al.* (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means of a crossover event, the resulting strain contains at least two copies of the relevant gene.

Methods of regulating, *i.e.*, either increasing or decreasing, gene expression include recombinant methods in which a microorganism is produced using a DNA molecule provided *in vitro*. Such DNA molecules comprise, for example, promoters, expression cassettes, genes, alleles, coding regions, *etc.* They are introduced into the desired microorganisms by methods of transformation, conjugation, transduction or similar methods.

In the case of the present invention, the promoters are preferably a polynucleotide 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, or SEQ ID NO:8, and the expression cassettes are preferably a polynucleotide 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, or SEQ ID NO:8 which, via the nucleotide at its 3' end, are functionally linked to a linker polynucleotide which ensures translation of RNA.

The measures of overexpression using the promoter according to the invention or the expression unit according to the invention increase the activity or concentration of the corresponding polypeptide usually by at least 10%, 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400% or 500%, preferably by no more than 1,000%, 2,000%, 4,000%, 10,000% or 20,000%, based on the activity or concentration of said polypeptide in the strain prior to the measure resulting in overexpression.

The extent of expression or overexpression may be established by measuring the amount of mRNA transcribed from the gene, by determining the amount of polypeptide and by determining enzyme activity.

The amount of mRNA may be determined *inter alia* by using the methods of "Northern Blotting" and of quantitative RT-PCR. Quantitative RT-PCR involves reverse transcription which precedes the polymerase chain reaction. For this, the LightCycler™ System from Roche Diagnostics (Boehringer Mannheim GmbH, Roche Molecular Biochemicals, Mannheim, Germany) may be used, as described in Jungwirth *et al.* (FEMS Microbiology Letters 281, 190-197 (2008)), for example. The concentration of the protein may be determined via 1- and 2-dimensional protein gel fractionation and subsequent optical identification of the protein concentration using appropriate evaluation software in the gel. A customary method of preparing protein gels for coryneform bacteria and of identifying said proteins is the procedure described by Hermann *et al.* (Electrophoresis, 22:1712-23 (2001)). The protein concentration may likewise be determined by Western-Blot hybridization using an antibody specific for the protein to be detected (Sambrook *et al.*, Molecular cloning: a laboratory manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) and subsequent optical evaluation using appropriate software for concentration determination (Lohaus and Meyer (1998) Biospektrum 5:32-39; Lottspeich, Angewandte Chemie 321: 2630-2647 (1999)). The statistical significance of the data collected is determined by means of a T test (Gosset, Biometrika 6(1): 1-25 (1908)).

The measure of overexpressing target genes using the promoter according to the invention may be combined in a suitable manner with further overexpression measures. Overexpression is achieved by a multiplicity of methods available in the prior art. These include increasing the copy number in addition to modifying the nucleotide sequences which direct or control expression of the gene. The copy number may be increased by means of plasmids which replicate in the cytoplasm of the microorganism. To this end, an abundance of plasmids are described in the prior art for very different groups of microorganisms, which plasmids can be used for setting the desired increase in the copy number of the gene. Plasmids suitable for the genus *Corynebacterium* are described, for example, in Tauch *et al.* (Journal of Biotechnology 104 (1-3), 27-40, (2003)), and in Stansen *et al.* (Applied and Environmental Microbiology 71, 5920-5928 (2005)).

The copy number may furthermore be increased by at least one (1) copy by introducing further copies into the chromosome of the microorganism. Methods suitable for the genus *Corynebacterium* are described, for example, in the patents WO 03/014330, WO 03/040373 and WO 04/069996.

Gene expression may furthermore be increased by positioning a plurality of promoters upstream of the target gene or functionally linking them to the gene to be expressed and achieving increased expression in this way. Examples of this are described in the patent WO 2006/069711.

Transcription of a gene is controlled, where appropriate, by proteins which suppress (repressor proteins) or promote (activator proteins) transcription. Accordingly, overexpression can likewise be achieved by increasing the expression of activator proteins or reducing or switching off the expression of repressor proteins or else eliminating the binding sites of the repressor proteins. The rate of elongation is influenced by the codon usage, it being possible to enhance translation by utilizing codons for transfer RNAs (tRNAs) which are frequent in the starting strain. Moreover, replacing a start codon with the ATG codon most frequent in many microorganisms (77% in *E. coli*) may considerably improve translation, since, at the RNA level, the AUG codon is two to three times more effective than the codons GUG and UUG, for example (Khudyakov *et al.*, FEBS Letters 232(2):369-71(1988); Reddy *et al.*, Proceedings of the National Academy of Sciences of the USA 82(17):5656-60 (1985)). It is also possible to optimize the sequences surrounding the start codon because synergistic effects between the start codon and the flanking regions have been described (Stenström *et al.*, Gene 273(2):259-65 (2001); Hui *et al.*, EMBO Journal 3(3):623-9 (1984)).

Instructions for handling DNA, digestion and ligation of DNA, transformation and selection of transformants can be found *inter alia* in the known manual by Sambrook *et al.* "Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor Laboratory Press, 1989).

The invention also relates to vectors comprising the polynucleotides according to the invention.

Kirchner and Tauch (Journal of Biotechnology 104:287-299 (2003)) describe a selection of vectors to be used in *C. glutamicum*.

Homologous recombination using the vectors according to the invention allows DNA segments on the chromosome to be replaced with polynucleotides according to the invention which are transported into the cell by the vector. For efficient recombination between the circular DNA molecule of the vector and the target DNA on the chromosome, the DNA region to be replaced with the polynucleotide according to the invention is provided at the ends with nucleotide sequences homologous to the target site which determine the site of integration of the vector and of replacement of the DNA.

Thus the promoter polynucleotide according to the invention may: 1) be replaced with the native promoter at the native gene locus of the target gene in the chromosome; or 2) be integrated with the target gene at the native gene locus of the latter or at another gene locus.

"Replacement of the native promoter at the native gene locus of the target gene" means the fact that the naturally occurring promoter of the gene which usually is naturally present by way of a single copy at its gene locus in the corresponding wild type or corresponding starting organism in the form of its nucleotide sequence is replaced.

"Another gene locus" means a gene locus whose nucleotide sequence is different from the sequence of the target gene. Said other gene locus or the nucleotide sequence at said other gene locus is

preferably located within the chromosome and normally is not essential for growth and for production of the desired chemical compounds. It is furthermore possible to use intergenic regions within the chromosome, *i.e.* nucleotide sequences without coding function.

Expression or overexpression is preferably carried out in microorganisms of the genus *Corynebacterium*. Within the genus *Corynebacterium*, preference is given to strains based on the following species: *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. Very particular preference is given to the species *C. glutamicum*.

Suitable 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*.

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.

The microorganisms or strains (starting strains) employed for the expression or overexpression measures according to the invention preferably already possess the ability to secrete a desired fine chemical into the surrounding nutrient medium and accumulate there. The expression "to produce" is also used for this herein below. More specifically, the strains employed for the overexpression measures possess the ability to accumulate the desired fine chemical in concentrations of at least 0.10 g/L, at least 0.25 g/L, at least 0.5 g/L, at least 1.0 g/L, at least 1.5 g/L, at least 2.0 g/L, at least 4.0 g/L, or at least 10.0 g/L in no more than 120 hours, no more than 96 hours, no more than 48 hours, no more than 36 hours, no more than 24 hours, or no more than 12 hours in the cell or in the nutrient medium. The starting strains are preferably strains prepared by mutagenesis and selection, by recombinant DNA technologies or by a combination of both methods.

A person skilled in the art understands that a microorganism suitable for the measures of the invention may also be obtained by firstly employing the promoter according to the invention 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, or SEQ ID NO:8 for overexpression of the target genes in a wild strain such as, for example, the *C. glutamicum* type strain ATCC 13032 or the strain ATCC 14067, and then, by means of further genetic measures described in the prior art, causing the microorganism to produce the desired fine chemical(s).

The term "biomolecules" means with regard to the measures of the invention amino acids, organic acids, vitamins, nucleosides and nucleotides. Particular preference is given to proteinogenic amino acids, non-proteinogenic amino acids, macromolecules, and organic acids.

"Proteinogenic amino acids" mean the amino acids which occur in natural proteins, *i.e.* in proteins of microorganisms, plants, animals and humans. They serve as structural units for proteins in which they are linked to one another via peptide bonds.

Where L-amino acids or amino acids are mentioned hereinbelow, they are to be understood as meaning one or more amino acids, including their salts, selected from the group L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine. L-lysine is especially preferred. L-Amino acids, in particular lysine, are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and very particularly in animal nutrition. There is therefore a general interest in providing new improved processes for the preparation of amino acids, in particular L-lysine.

The terms protein and polypeptide are interchangeable.

The present invention provides a microorganism which produces a fine chemical, said microorganism having increased expression of one or more genes in comparison to the particular starting strain by using the promoter according to the invention 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, or SEQ ID NO:8.

Fermentative Preparation

The present invention furthermore provides a process for fermentative preparation of a fine chemical, comprising the steps of:

a) culturing the above-described microorganism according to the present invention in a suitable medium, resulting in a fermentation broth; and

b) concentrating the fine chemical in the fermentation broth of a) and/or in the cells of the microorganism.

Preference is given here to obtaining from the fine chemical-containing fermentation broth the fine chemical or a liquid or solid fine chemical-containing product. 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)).

The culture medium or fermentation 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). The terms culture medium and fermentation medium are interchangeable.

It is possible to use, as carbon source, 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.

It is possible to use, as nitrogen source, 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.

It is possible to use, as phosphorus source, phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts.

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. 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.

Said starting materials may be added to the culture in the form of a single batch or be fed in during the cultivation in a suitable manner.

The pH of the culture can be controlled by employing basic compounds such as sodium hydroxide, potassium hydroxide, ammonia, or aqueous ammonia; or acidic compounds such as phosphoric acid or sulfuric acid in a suitable manner. The pH is generally adjusted to a value of from 6.0 to 8.5, preferably 6.5 to 8. To control foaming, it is possible to employ antifoams such as, for example, fatty acid polyglycol esters. To maintain the stability of plasmids, it is possible to add to the medium

suitable selective substances such as, for example, antibiotics. The fermentation is preferably 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 organic-chemical compound sufficient for being recovered has formed. This aim is normally 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 organic-chemical compound in the fermentation medium and/or in the cells of said microorganisms.

Examples of suitable fermentation media can be found *inter alia* in the patents US 5,770,409, US 5,990,350, US 5,275,940, WO 2007/012078, US 5,827,698, WO 2009/043803, US 5,756,345 and US 7,138,266.

Analysis of L-amino acids to determine the concentration at one or more time(s) during the fermentation can take place by separating the L-amino acids by means of ion exchange chromatography, preferably cation exchange chromatography, with subsequent post-column derivatization using ninhydrin, as described in Spackman *et al.* (Analytical Chemistry 30:1190-1206 (1958)). It is also possible to employ *ortho*-phthaldialdehyde rather than ninhydrin for post-column derivatization. An overview article on ion exchange chromatography can be found in Pickering (LC-GC Magazine of Chromatographic Science) 7(6), 484-487 (1989)).

It is likewise possible to carry out a pre-column derivatization, for example using *ortho*-phthaldialdehyde or phenyl isothiocyanate, and to fractionate the resulting amino acid derivates by reversed-phase (RP) chromatography, preferably in the form of high-performance liquid chromatography (HPLC). A method of this type is described, for example, in Lindroth *et al.* (Analytical Chemistry 51:1167-1174 (1979)).

Detection is carried out photometrically (absorption, fluorescence).

A review regarding amino acid analysis can be found *inter alia* in the textbook "Bioanalytik" from Lottspeich and Zorbas (Spektrum Akademischer Verlag, Heidelberg, Germany 1998).

Determination of the concentration of α-ketoacids at one or more time point(s) in the course of the fermentation may be carried out by separating the ketoacids and other secreted products by means of ion exchange chromatography, preferably cation exchange chromatography, on a sulfonated styrene-divinylbenzene polymer in the H+ form, for example by means of 0.025 M sulfuric acid with subsequent UV detection at 215 nm (alternatively also at 230 or 275 nm). Preferably, a REZEK RFQ - Fast Fruit H+

column (Phenomenex) may be employed, but other suppliers for the separating phase (*e.g.* Aminex from BioRad) are feasible. Similar separations are described in application examples by the suppliers.

The performance of the processes or fermentation processes containing the promoter variants according to the invention, in terms of one or more of the parameters selected from the group of concentration (compound formed per unit volume), yield (compound formed per unit carbon source consumed), formation (compound formed per unit volume and time) and specific formation (compound formed per unit dry cell matter or dry biomass and time or compound formed per unit cellular protein and time) or else other process parameters and combinations thereof, is increased by at least 0.5%, at least 1%, at least 1.5%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% based on processes or fermentation processes using microorganisms not containing the promoter variants according to the invention. This is considered to be very worthwhile in terms of a large-scale industrial process.

The fermentation measures result in a fermentation broth which contains the desired fine chemical, preferably amino acids, organic acids, vitamins, nucleosides or nucleotides.

A product containing the fine chemical is then provided or produced or recovered in liquid or solid form.

A fermentation broth means a fermentation medium or nutrient medium in which a microorganism has been cultivated for a certain time and at a certain temperature. The fermentation medium or the media employed during fermentation comprise(s) all the substances or components which ensure production of the desired compound and typically propagation and viability.

When the fermentation is complete, the resulting fermentation broth accordingly comprises:

a) the biomass (cell mass) of the microorganism, said biomass having been produced due to propagation of the cells of said microorganism;

b) the desired fine chemical formed during the fermentation;

c) the organic byproducts possibly formed during the fermentation; and

d) the constituents of the fermentation medium employed or of the starting materials, such as, for example, vitamins such as biotin or salts such as magnesium sulfate, which have not been consumed in the fermentation.

The organic byproducts include substances which are produced by the microorganisms employed in the fermentation in addition to the particular desired compound and are optionally secreted.

The fermentation broth is removed from the culture vessel or fermentation tank, collected where appropriate, and used for providing a product containing the fine chemical in liquid or solid form. The expression “recovering the fine chemical-containing product” is also used for this. In the simplest case,

the fine chemical-containing fermentation broth itself, which has been removed from the fermentation tank, constitutes the recovered product.

One or more of the measures selected from the group consisting of

a) partial (> 0% to < 80%) to complete (100%) or virtually complete (≥ 80%, ≥ 90%, ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98%, or ≥ 99%) removal of the water;

b) partial (> 0% to < 80%) to complete (100%) or virtually complete (≥ 80%, ≥ 90%, ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98%, or ≥ 99%) removal of the biomass, the latter being optionally inactivated before removal;

c) partial (> 0% to < 80%) to complete (100%) or virtually complete (≥ 80%, ≥ 90%, ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98%, ≥ 99%, ≥ 99.3%, or ≥ 99.7%) removal of the organic byproducts formed during fermentation; and

d) partial (> 0%) to complete (100%) or virtually complete (≥ 80%, ≥ 90%, ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98%, ≥ 99%, ≥ 99.3%, or ≥ 99.7%) removal of the constituents of the fermentation medium employed or of the starting materials, which have not been consumed in the fermentation, from the fermentation broth achieves concentration or purification of the desired organic-chemical compound. Products having a desired content of said compound are isolated in this way.

The partial (> 0% to < 80%) to complete (100%) or virtually complete (≥ 80% to < 100%) removal of the water (measure a)) is also referred to as drying.

In one variant of the process, complete or virtually complete removal of the water, of the biomass, of the organic byproducts and of the unconsumed constituents of the fermentation medium employed results in pure (≥ 80% by weight, ≥ 90% by weight) or high-purity (≥ 95% by weight, ≥ 97% by weight, or ≥ 99% by weight) product forms of the desired organic-chemical compound. An abundance of technical instructions for measures a), b), c) and d) are available in the prior art.

Depending on requirements, the biomass can be removed wholly or partly from the fermentation broth by separation methods such as, for example, centrifugation, filtration, decantation or a combination thereof, or be left completely therein. Where appropriate, the biomass or the biomass-containing fermentation broth is inactivated during a suitable process step, for example by thermal treatment (heating) or by addition of acid.

In one procedure, the biomass is completely or virtually completely removed so that no (0%) or at most 30%, at most 20%, at most 10%, at most 5%, at most 1% or at most 0.1% biomass remains in the prepared product. In a further procedure, the biomass is not removed, or is removed only in small proportions, so that all (100%) or more than 70%, 80%, 90%, 95%, 99% or 99.9% biomass remains in the product prepared. In one process according to the invention, accordingly, the biomass is removed in proportions of from ≥ 0% to ≤ 100%.

Finally, the fermentation broth obtained after the fermentation can be adjusted, before or after the complete or partial removal of the biomass, to an acidic pH with an inorganic acid such as, for example, hydrochloric acid, sulfuric acid, or phosphoric acid; or organic acid such as, for example, propionic acid, so as to improve the handling properties of the final product (GB 1,439,728 or EP 1 331220). It is likewise possible to acidify the fermentation broth with the complete content of biomass. Finally, the broth can also be stabilized by adding sodium bisulfite ($NaHCO_3$, GB 1,439,728) or another salt, for example ammonium, alkali metal, or alkaline earth metal salt of sulfurous acid.

During the removal of the biomass, any organic or inorganic solids present in the fermentation broth are partially or completely removed. The organic byproducts dissolved in the fermentation broth, and the dissolved unconsumed constituents of the fermentation medium (starting materials), remain at least partly (> 0%), preferably to an extent of at least 25%, particularly preferably to an extent of at least 50% and very particularly preferably to an extent of at least 75% in the product. Where appropriate, they also remain completely (100%) or virtually completely, meaning > 95% or > 98% or > 99%, in the product. If a product in this sense comprises at least part of the constituents of the fermentation broth, this is also described by the term "product based on fermentation broth".

Subsequently, water is removed from the broth, or said broth is thickened or concentrated, by known methods such as, for example, using a rotary evaporator, thin-film evaporator, falling-film evaporator, by reverse osmosis or by nanofiltration. This concentrated fermentation broth can then be worked up to free-flowing products, in particular to a fine powder or preferably coarse granules, by methods of freeze drying, spray drying, spray granulation or by other processes such as in the circulating fluidized bed, as described for example according to PCT/EP2004/006655. A desired product is isolated where appropriate from the resulting granules by screening or dust removal. It is likewise possible to dry the fermentation broth directly, *i.e.* without previous concentration by spray drying or spray granulation.

"Free-flowing" means powders which, from a series of glass orifice vessels with orifices of different sizes, flow unimpeded at least out of the vessel with a 5 mm orifice (Klein: Seifen, Öle, Fette, Wachse 94, 12 (1968)).

"Fine" means a powder predominantly (> 50%) having a particle size of diameter from 20 to 200 μm.

"Coarse" means a product predominantly (> 50%) of a particle size of diameter from 200 to 2000 μm.

The particle size determination can be carried out by methods of laser diffraction spectrometry. Corresponding methods are described in the textbook "Teilchengrößenmessung in der Laborpraxis" by R. H. Müller and R. Schuhmann, Wissenschaftliche Verlagsgesellschaft Stuttgart (1996) or in the text book "Introduction to Particle Technology" by M. Rhodes, published by Wiley &Sons (1998).

The free-flowing, fine powder can in turn be converted by suitable compaction or granulation processes into a coarse, very free-flowing, storable and substantially dust-free product.

The term "dust-free" means that the product comprises only small proportions (< 5%) of particle sizes below 100 μm in diameter.

"Storable" in the sense of this invention means a product which can be stored for at least one (1) year or longer, preferably at least 1.5 years or longer, particularly preferably two (2) years or longer, in a dry and cool environment without any substantial loss of the respective organic-chemical compound occurring. "Substantial loss" means a loss of >5%.

It is advantageous to employ during the granulation or compaction the usual organic or inorganic auxiliaries or carriers such as starch, gelatin, cellulose derivatives or similar substances, as normally used in the processing of food products or feeds as binders, gelling agents or thickeners, or further substances such as, for example, silicas, silicates (EP0743016A) and stearates.

It is further advantageous to treat the surface of the resulting granules with oils or fats as described in WO04/054381. Oils which can be used are mineral oils, vegetable oils or mixtures of vegetable oils. Examples of such oils are soybean oil, olive oil, soybean oil/lecithin mixtures. In the same way, silicone oils, polyethylene glycols or hydroxyethylcellulose are also suitable. Treatment of the surfaces of the granules with said oils achieves an increased abrasion resistance of the product and a reduction in the dust content. The oil content in the product is 0.02 to 2.0% by weight, preferably 0.02 to 1.0% by weight, and very particularly preferably 0.2 to 1.0% by weight, based on the total amount of the feed additive.

Preferred products have a proportion of ≥ 97% by weight with a particle size of from 100 to 1800 μm or a proportion of ≥ 95% by weight with a particle size of diameter 300 to 1800 μm. The proportion of dust, *i.e.* particles with a particle size < 100 pm, is preferably > 0 to 1% by weight, particularly preferably not exceeding 0.5% by weight.

However, alternatively, the product may also be absorbed on an organic or inorganic carrier known and customary in the processing of feeds, such as, for example, silicas, silicates, meals, brans, flours, starches, sugars or others, and/or be mixed and stabilized with customary thickeners or binders. Examples of use and processes therefor are described in the literature (Die Mühle + Mischfuttertechnik 132 (1995) 49, page 817).

Embodiments:

1. A recombinant nucleic acid molecule comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to at least one heterologous target gene.

2. The recombinant nucleic acid molecule according to claim 1, wherein said promoter polynucleotide sequence is selected from the group consisting of SEQ ID NOs:1, 5 and 7.
3. The recombinant nucleic acid molecule according to embodiments 1 or 2, further comprising a linker oligonucleotide or linker polynucleotide.
4. The recombinant nucleic acid molecule according to embodiment 1, wherein said at least one heterologous target gene is a gene that is a component of a biosynthetic pathway producing a biomolecule selected from the group consisting of amino acids, organic acids, proteins and polymers.
5. The recombinant nucleic acid molecule according to embodiment 4, wherein said at least one heterologous target gene is a gene that is a component of an amino acid biosynthetic pathway selected from the group consisting of:

the serine biosynthesis pathway comprising genes of entry M00020;
the threonine biosynthesis pathway comprising genes of KEGG entry M00018;
the cysteine biosynthesis pathway comprising genes of KEGG entry M00021;
the cysteine biosynthesis pathway comprising genes of KEGG entry M00338;
the cysteine biosynthesis pathway comprising genes of KEGG entry M00609;
the methionine biosynthesis pathway comprising genes of KEGG entry M00017;
the valine/isoleucine biosynthesis pathway comprising genes of KEGG entry M00019;
the isoleucine biosynthesis pathway comprising genes of KEGG entry M00535;
the isoleucine biosynthesis pathway comprising genes of KEGG entry M00570;
the leucine biosynthesis pathway comprising genes of KEGG entry M00432;
the lysine biosynthesis pathway comprising genes of KEGG entry M00016;
the lysine biosynthesis pathway comprising genes of KEGG entry M00525;
the lysine biosynthesis pathway comprising genes of KEGG entry M00526;
the lysine biosynthesis pathway comprising genes of KEGG entry M00527;
the lysine biosynthesis pathway comprising genes of KEGG entry M0030;
the lysine biosynthesis pathway comprising genes of KEGG entry M00433;
the lysine biosynthesis pathway comprising genes of KEGG entry M0031;
the proline biosynthesis pathway comprising genes of KEGG entry M00015;
the ornithine biosynthesis pathway comprising genes of KEGG entry M00028;
the ornithine biosynthesis pathway comprising genes of KEGG entry M00763;
the histidine biosynthesis pathway comprising genes of KEGG entry M00026;
the shikimate biosynthesis pathway comprising genes of KEGG entry M00022;
the tryptophan biosynthesis pathway comprising genes of entry M00023;
the phenylalanine biosynthesis pathway comprising genes of KEGG entry M00024;

the tyrosine biosynthesis pathway comprising genes of KEGG entry M00025;
the tyrosine biosynthesis pathway comprising genes of KEGG entry M00040;
and combinations of the genes of any of the biosynthesis pathways thereof.

6. The recombinant nucleic acid molecule according to embodiment 1, further comprising a one or more additional promoter polynucleotide sequences selected from the group consisting of SEQ ID NOs:1 to 8, each promoter functionally linked to at least one additional heterologous gene.
7. The recombinant nucleic acid molecule according to embodiment 1, wherein said recombinant nucleic acid molecule is isolated.
8. A recombinant vector comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8, and combinations thereof, each promoter functionally linked to at least one heterologous target gene.
9. The recombinant vector according to embodiment 8, wherein said promoter polynucleotide sequence is selected from the group consisting of SEQ ID NOs:1, 5 and 7.
10. The recombinant vector according to embodiments 8 or 9, comprising a combination of two or more recombinant nucleic acid molecules comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8, and combinations thereof, each promoter functionally linked to at least one heterologous target gene.
11. The recombinant vector according to embodiment 10, wherein each of said promoter polynucleotide sequences is functionally linked to a different heterologous target gene.
12. The recombinant vector according to embodiment 11, wherein said target genes are part of the same metabolic pathway.
13. The recombinant vector according to embodiment 11, wherein the target genes are not part of the same metabolic pathway.
14. A host cell comprising a recombinant nucleic acid molecule according to any one of embodiments 1 to 6, or a combination thereof according to embodiment 10, or the recombinant vector according to any one of embodiments 8 to 13.
15. The host cell according to embodiment 14, comprising a combination of promoter polynucleotide sequences, wherein each of said promoter polynucleotide sequences is functionally linked to a different heterologous target gene.
16. The host cell according to embodiment 15, wherein each of said different heterologous target genes are part of the same metabolic pathway.
17. The host cell according to embodiment 15, wherein each of said different heterologous target genes are not part of the same metabolic pathway.

18. The host cell according to any one of embodiments 14 to 17, which belongs to genus *Corynebacterium*.
19. The host cell according to embodiment 18, which is *Corynebacterium glutamicum*.
20. A method of modifying the expression of one or more target genes, comprising culturing a host cell according to any one of embodiments 12 to 19, wherein each of said one or more target genes are different heterologous target genes functionally linked to a polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 and wherein the modification of expression of each heterologous target gene is independently selected from: up-regulating or down-regulating.
21. A method of modifying the expression of one or more target genes, comprising culturing a host cell according to any one of embodiments 12 to 19, wherein each of said one or more target genes are different heterologous target genes functionally linked to a polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 and wherein the modification of expression of each heterologous target gene is independently selected from: up-regulating or down-regulating.
22. A method of producing a biomolecule comprising culturing a host cell according to any one of embodiments 12 to 19, under conditions suitable for producing the biomolecule.
23. The method according to embodiment 20, wherein said biomolecule is an L-amino acid.
24. The method according to embodiment 22, wherein said L-amino acid is L-lysine.
25. The method according to embodiment 20, wherein said at least one heterologous target gene is a gene encoding a protein selected from the group consisting of aspartate-semialdehyde dehydrogenase (EC:1.2.1.11); 4-hydroxy-tetrahydrodipicolinate synthase (EC:4.3.3.7); dihydrodipicolinate reductase (EC:1.17.1.8); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); N-succinyldiaminopimelate aminotransferase (EC:2.6.1.17); succinyl-diaminopimelate desuccinylase (EC:3.5.1.18); diaminopimelate epimerase (EC:5.1.1.7); diaminopimelate decarboxylase (EC:4.1.1.20); diaminopimelate dehydrogenase (EC:1.4.1.16); Aspartokinase Lysc Alpha And Beta Subunits (EC:2.7.2.4); Aspartate Aminotransferase (EC:2.6.1.1); Phosphotransferase System (PTS); Glucose-Specific Enzyme II BC Component Of PTS (EC:2.7.1.69); glucose-6-phosphate 1-dehydrogenase (EC:1.1.1.49 1.1.1.363); glucose-6-phosphate isomerase (EC:5.3.1.9); transketolase (EC:2.2.1.1); 6-phosphofructokinase 1 (EC:2.7.1.11); phosphoenolpyruvate carboxylase (EC:4.1.1.31); pyruvate carboxylase (EC:6.4.1.1); isocitrate dehydrogenase

(EC:1.1.1.42); phosphoenolpyruvate carboxykinase (GTP) (EC:4.1.1.32); Oxaloacetate decarboxylase (EC 4.1.1.3); homoserine kinase (EC:2.7.1.39); homoserine dehydrogenase (EC:1.1.1.3); threonine synthase (EC:4.2.3.1), and combinations thereof.

26. A host cell comprising at least one promoter polynucleotide functionally linked to a heterologous target gene; wherein the promoter polynucleotide 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.

27. The host cell according to embodiment 25, comprising a combination of two or more promoter polynucleotide sequences functionally linked to a heterologous target gene wherein each promoter polynucleotide is functionally linked to a different heterologous target gene.

28. The host cell according to embodiment 26, wherein said combination comprises two heterologous target genes selected from the group consisting of aspartate-semialdehyde dehydrogenase (EC:1.2.1.11); 4-hydroxy-tetrahydrodipicolinate synthase (EC:4.3.3.7); dihydrodipicolinate reductase (EC:1.17.1.8); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); N-succinyldiaminopimelate aminotransferase (EC:2.6.1.17); succinyl-diaminopimelate desuccinylase (EC:3.5.1.18); diaminopimelate epimerase (EC:5.1.1.7); diaminopimelate decarboxylase (EC:4.1.1.20); diaminopimelate dehydrogenase (EC:1.4.1.16); Aspartokinase Lysc Alpha And Beta Subunits (EC:2.7.2.4); Aspartate Aminotransferase (EC:2.6.1.1); Phosphotransferase System (PTS); Glucose-Specific Enzyme II BC Component Of PTS (EC:2.7.1.69); glucose-6-phosphate 1-dehydrogenase (EC:1.1.1.49 1.1.1.363); glucose-6-phosphate isomerase (EC:5.3.1.9); transketolase (EC:2.2.1.1); 6-phosphofructokinase 1 (EC:2.7.1.11); phosphoenolpyruvate carboxylase (EC:4.1.1.31); pyruvate carboxylase (EC:6.4.1.1); isocitrate dehydrogenase (EC:1.1.1.42); phosphoenolpyruvate carboxykinase (GTP) (EC:4.1.1.32); Oxaloacetate decarboxylase (EC 4.1.1.3); homoserine kinase (EC:2.7.1.39); homoserine dehydrogenase (EC:1.1.1.3); and threonine synthase (EC:4.2.3.1), each functionally linked to a promoter selected from the group consisting of SEQ ID NOs:1 to 8.

29. The host cell according to embodiment 27, wherein said combination comprises a promoter selected from the group consisting of SEQ ID NOs: 1,5 and 7 functionally linked to said heterologous target genes.

30. The host cell according to embodiment 26, wherein said combination comprises three heterologous target genes selected from the group consisting of aspartate-semialdehyde dehydrogenase (EC:1.2.1.11); 4-hydroxy-tetrahydrodipicolinate synthase (EC:4.3.3.7); dihydrodipicolinate reductase (EC:1.17.1.8); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); N-succinyldiaminopimelate aminotransferase (EC:2.6.1.17); succinyl-diaminopimelate desuccinylase (EC:3.5.1.18); diaminopimelate epimerase (EC:5.1.1.7); diaminopimelate decarboxylase (EC:4.1.1.20); diaminopimelate dehydrogenase (EC:1.4.1.16); Aspartokinase Lysc Alpha And Beta Subunits (EC:2.7.2.4); Aspartate Aminotransferase (EC:2.6.1.1); Phosphotransferase System (PTS); Glucose-Specific Enzyme II BC Component Of PTS (EC:2.7.1.69); glucose-6-phosphate 1-dehydrogenase (EC:1.1.1.49 1.1.1.363); glucose-6-phosphate isomerase (EC:5.3.1.9); transketolase (EC:2.2.1.1); 6-phosphofructokinase 1 (EC:2.7.1.11); phosphoenolpyruvate carboxylase (EC:4.1.1.31); pyruvate carboxylase (EC:6.4.1.1); isocitrate dehydrogenase (EC:1.1.1.42); phosphoenolpyruvate carboxykinase (GTP) (EC:4.1.1.32); Oxaloacetate decarboxylase (EC 4.1.1.3); homoserine kinase (EC:2.7.1.39); homoserine dehydrogenase (EC:1.1.1.3); and threonine synthase (EC:4.2.3.1), each functionally linked to a promoter selected from the group consisting of SEQ ID NOs:1 to 8.

31. The host cell according to embodiment 29, wherein said combination comprises a promoter selected from the group consisting of SEQ ID NOs: 1,5 and 7 functionally linked to said heterologous target genes.

32. The host cell according to embodiment 26, wherein said heterologous target genes are part of the same metabolic pathway.

33. The host cell according to embodiment 26, wherein said heterologous target genes are not part of the same metabolic pathway.

34. The host cell according to any one of embodiments 25 to 32, which belongs to genus *Corynebacterium*.

35. The host cell according to any one of embodiments 25 to 33, which is *Corynebacterium glutamicum*.

36. A method of modifying the expression of one or more target genes, comprising culturing a host cell according to any one of embodiments 25 to 34, wherein the modification of each

heterologous target gene is independently selected from: up-regulating or down-regulating, wherein said up-regulating or down-regulating is relative to the level of expression of said target gene under the control of the endogenous promoter.

37. A method of producing a biomolecule comprising culturing a host cell according to any one of embodiments 25 to 35, under conditions suitable for producing the biomolecule.
38. The method according to embodiment 36, wherein said biomolecule is an L-amino acid.
39. The method according to embodiment 37, wherein said L-amino acid is L-lysine.
40. The method according to embodiment 38, wherein said at least one heterologous target gene is selected from the group consisting of aspartate-semialdehyde dehydrogenase (EC:1.2.1.11); 4-hydroxy-tetrahydrodipicolinate synthase (EC:4.3.3.7); dihydrodipicolinate reductase (EC:1.17.1.8); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (EC:2.3.1.117); N-succinyldiaminopimelate aminotransferase (EC:2.6.1.17); succinyl-diaminopimelate desuccinylase (EC:3.5.1.18); diaminopimelate epimerase (EC:5.1.1.7); diaminopimelate decarboxylase (EC:4.1.1.20); diaminopimelate dehydrogenase (EC:1.4.1.16); Aspartokinase Lysc Alpha And Beta Subunits (EC:2.7.2.4); Aspartate Aminotransferase (EC:2.6.1.1); Phosphotransferase System (PTS); Glucose-Specific Enzyme II BC Component Of PTS (EC:2.7.1.69); glucose-6-phosphate 1-dehydrogenase (EC:1.1.1.49 1.1.1.363); glucose-6-phosphate isomerase (EC:5.3.1.9); transketolase (EC:2.2.1.1); 6-phosphofructokinase 1 (EC:2.7.1.11); phosphoenolpyruvate carboxylase (EC:4.1.1.31); pyruvate carboxylase (EC:6.4.1.1); isocitrate dehydrogenase (EC:1.1.1.42); phosphoenolpyruvate carboxykinase (GTP) (EC:4.1.1.32); Oxaloacetate decarboxylase (EC 4.1.1.3); homoserine kinase (EC:2.7.1.39); homoserine dehydrogenase (EC:1.1.1.3); threonine synthase (EC:4.2.3.1), and combinations thereof.
41. A recombinant vector comprising at least one promoter polynucleotide functionally linked to a heterologous target gene; wherein the promoter polynucleotide comprises a sequence selected from: 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; wherein when the promoter polynucleotide comprises a sequence selected from: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, the target gene is other than the promoter polynucleotide's endogenous gene.

42. The recombinant vector according to embodiment 40, comprising at least two promoter polynucleotides, wherein each promoter polynucleotide is functionally linked to a different target gene.
43. The recombinant vector according to embodiment 41, wherein the target genes are part of the same metabolic pathway.
44. The recombinant vector according to embodiment 42, wherein the target genes are not part of the same metabolic pathway.
45. A host cell transformed with the recombinant vector according to any one of embodiments 40 to 43.
46. The host cell according to embodiment 44, which belongs to genus Corynebacterium.
47. The host cell according to embodiment 46, which is Corynebacterium glutamicum.
48. A method of modifying the expression of one or more target genes, comprising culturing a host cell according to any one of embodiments 44 to 46, wherein the modification of each target gene is independently selected from: up-regulating and down-regulating.
49. A method of producing a biomolecule comprising culturing a host cell according to any one of embodiments 44 to 47, under conditions suitable for producing the biomolecule.
50. The method according to embodiment 48, wherein said biomolecule is an L-amino acid.

The following examples are provided for purposes of illustration, not limitation.

EXAMPLES

Example 1: Identification of Candidate Promoters.

The following procedure was used to identify native *C. glutamicum* 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. A published data set describing global gene expression levels in *C. glutamicum* ATCC 13032 (Lee *et al.*, Biotechnology Letters, 2013) was examined to identify genes that were constitutively expressed across different growth conditions. Genes whose expression level remained constant (defined as a ratio of expression between 0.33 and 3) across two growth conditions, namely chemostat growth in minimal media with and without the addition of hydrogen peroxide satisfied the first criterion. A published data set describing the *C. glutamicum* ATCC 13032 transcriptome (Pfeifer-Sancar *et al.*, BMC Genomics 2013, 14:888) was examined to find genes with compact promoters, *i.e.* those consisting of a 60 base pair core promoter region and a 5 prime untranslated region between 26 and 40 base pairs in length. The two data sets were cross-referenced to identify

promoters that satisfied both criteria. See **Error! Reference source not found.**. Five candidate promoters (SEQ ID NOs: 2, 3, 4, 6, and 8) were selected for further evaluation.

Example 2: Evaluation of Candidate Promoter Activity

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.

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*. Briefly, we PCR amplified a portion of pK18mobSacB containing the *E. coli* origin of replication and the Kanamycin resistance gene nptII using the primers pK18F (TCATGACCAAAATCCCTTAACGTG (SEQ ID NO:9)) and pK18R (GCGTACTCTTCGATGGTGAAAACATCTC (SEQ ID NO:10)) and PCR amplified synthetic DNA encoding the pBL1 origin of replication with the primers pBL1F (GACCTAAAATGTGTAAAGGGCAAAGTGTATACaacaacaagacccatcatagtttgc (SEQ ID NO:11)) and pBL1R (CACGTTAAGGGATTTTGGTCATGAcacatgcagtcatgtcgtgc (SEQ ID NO:12)). The PCR products were treated with DpnI (New England Biolabs) when appropriate, purified with DNA Clean & Concentrate-5 (Zymo Research), and assembled using the Gibson Assembly method with Gibson Assembly Master Mix (NEB) according to manufactures instructions. The Gibson Assembly reaction was transformed into NEB Turbo competent cells (New England Biolabs) according to the manufactures instructions. Transformants were selected on LB agar plus 25 μg/mL Kanamycin and verified by Sanger sequencing.

The reporter construct pK18rep-Psod-eyfp was constructed by restriction digestion and ligation of pK18rep and a synthetic DNA construct consisting of 191 base pair DNA sequence that encodes the superoxide disumutase (GenBank: BA000036.3) promoter from *C. glutamicum* ATCC 13032 upstream of eyfp gene followed by a 77 base pair DNA sequence encoding the sod terminator from *C. glutamicum* ATCC 13032 flanked by EcoRI and SalI restriction sites. The parent vector and synthetic DNA insert were digested with EcoRI-HF and SalI-HF (New England Biolabs) and the resulting products were run on an agarose gel. The DNA was extracted from the gel and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research) and ligated with T4 DNA ligase (New England Biolabs) according to the manufactures instructions. The ligation reaction was transformed into NEB Turbo competent cells (New

England Biolabs) according to the manufactures instructions. Transformants were selected on LB agar plus 25 μg/mL Kanamycin and verified by Sanger sequencing.

Additional promoter reporter constructs were constructed by replacing the sod promoter in pK18rep-Psod-eyfp. We PCR amplified pK18rep-Psod-eyfp excluding the sod promoter with primers pK18repR (gcttgcatgcctgcaggtcga (SEQ ID NO:13)) and yfpF (ATGGTGAGCAAGGGCGAGGAGC (SEQ ID NO:14)). The PCR product was treated with DpnI (New England Biolabs) and purified with DNA Clean & Concentrate-5 (Zymo Research) and assembled with synthetic DNA constructs encoding the promoter of interest plus 25 base pair homology sequence to the destination vector using the Gibson Assembly method with Gibson Assembly Master Mix (NEB) according to manufactures instructions. The Gibson Assembly reaction was transformed into NEB Turbo competent cells (New England Biolabs) according to the manufacturer's instructions. Transformants were selected on LB agar plus 25 μg/mL Kanamycin and verified by Sanger sequencing.

Additionally, the strong constitutive promoter Pcg0007 (SEQ ID NO:2) was chosen for mutagenesis. In *C. glutamicum*, the -10 element is thought to play a key role in determining promoter activity (Pfeifer-Sancar *et al.*, BMC Genomics 2013, 14:888) therefore four out of six positions in predicted -10 element (TAAGAT) of Pc0007 were randomized in order to generate both stronger and attenuated promoter variants (SEQ ID NOs 1, 5, and 7). This library was generated by PCR amplifying pK18rep-Pc0007-eyfp with Pcg0007Fwd (GGAAACGTCTGTATCGGATAAGTAG (SEQ ID NO:15)) and Pc0007Rev (CTACTTATCCGATACAGACGTTTCCANNNNACACGCTTAGGTCCCCACGTAGTACCA (SEQ ID NO:16)), treated with DpnI (New England Biolabs) and assembled using the Gibson Assembly method with Gibson Assembly Master Mix (NEB) according to manufactures instructions. The Gibson Assembly reaction was transformed into NEB Turbo competent cells (New England Biolabs) according to the manufacturer's instructions. Transformants were selected on LB agar plus 25 μg/mL Kanamycin and individual colonies were characterized by Sanger sequencing. Colonies were pooled by scraping the agar and purified plasmid DNA was isolated using the Zyppy minipred kit (Zymo Research).

Purified reporter construct plasmids were transformed into *C. glutamicum* NRRL B-11474 by electroporation (Haynes *et al.*, Journal of General Microbiology, 1990). Transformants 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. 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 μL of PBS. A 20 μL

aliquot of this mixture was transferred to a 96-well full area black clear bottom assay plate containing 180 µL 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. The parent plasmid pK18rep acted as a negative control. Normalized fluorescence activity was compared between reporter constructs and between biological replicates (**Error! Reference source not found.**). A numerical summary of promoter activity is presented in Table 5 below.

Table 5: Recombinant *C. glutamicum* Expressing Yellow Fluorescent Protein Under the Control of Promoters

Strain	SEQ ID NO	No. of Replicates	Mean Activity	Standard Deviation	Standard Error of Mean	95% Confidence Interval	Relative Expression
0007_lib_39	1	12	114402	52987.9	15296	80735-148069	1167
Pcg1860-eyfp	2	19	89243	16162.2	3708	81453-97033	911
Pcg0007-eyfp	3	19	44527	18110.3	4155	35798-53256	454
Pcg0755-eyfp	4	10	43592	3643	1152	40986-46198	445
0007_lib_265	5	11	11286	10459.4	3154	4260-18313	115
Pcg3381-eyfp	6	19	4723	1854.3	425	3829-5617	48
0007_lib_119	7	18	661	731.9	173	297-1025	7
Pcg3121-eyfp	8	14	98	537.5	144	−212-409	1
pK18rep	-	20	-45	214.9	48	−145-56	

Example 3: Application of Candidate Promoters to the L-lysine Biosynthetic Pathway

The promoters of the present disclosure are useful for improved processes for the production of biomolecules in host cells. An example of the application and use of the promotor of the present disclosure is directed to the production of the amino acid L-lysine. **Error! Reference source not found.Error! Reference source not found.** presents the biosynthetic pathway for the production of L-lysine and includes the genes *pck*, *odx*, *icd*, and *hom (e.g.*, the homoserine/threonine synthase pathway), that divert intermediates from the pathway leading to reductions in overall L-lysine yield. The symbols, gene names, Enzyme Commission number (EC number), and map position in *C. glutamicum* strain ATCC 13032 are provided below in Table 3.

Recombinant vectors comprising a promoter of SEQ ID NOs: 1 to 8 functionally linked to a target gene as provided in Table 3 are cloned into *Corynebacterium* cloning vectors using yeast

homologous recombination cloning techniques to assemble a vector in which each promoter was flanked by direct repeat regions to provide for homologous recombination in *Corynebacterium glutamican* at the target gene locus. Upon recombination, the endogenous promoter is replaced by the promoter of SEQ ID NOs: 1 to 8 functionally linked to the respective target gene in the endogenous *C. glutamican* locus. A variety of targeting vectors comprising the promoter and functionally linked target gene included a range of homology direct repeat arm lengths ranging from 0.5Kb, 1Kb, 2Kb, and 5Kb. Each DNA insert was produced by PCR amplification of homologous regions using commercially sourced oligos and the host strain genomic DNA described above as template. The promoter to be introduced into the genome was encoded in the oligo tails. PCR fragments were assembled into the vector backbone using homologous recombination in yeast.

Vectors are initially transformed into *E.coli* using standard heat shock transformation techniques and correctly assembled clones are identified and validated. Transformed *E.coli* bacteria are tested for assembly success. Four colonies from each *E. coli* transformation plate are cultured and tested for correct assembly via PCR. Vectors are amplified in the *E. coli* hosts to provide vector DNA for Corynebacterium transformation.

Validated clones are transformed into *Corynebacterium glutamicum* host cells via electroporation. For each transformation, the number of Colony Forming Units (CFUs) per µg of DNA is determined as a function of the insert size. Coryne genome integration is analyzed as a function of homology arm length. Shorter arms had a lower efficiency.

Cultures of *Corynebacterium* identified as having successful integrations of the insert cassette are cultured on media containing 5% sucrose to counter select for loop outs of the *sacb* selection gene. Sucrose resistance frequency for various homology direct repeat arms do not vary significantly with arm length. These results suggest that loopout efficiencies remain steady across homology arm lengths of 0.5 kb to 5kb.

In order to further validate loop out events, colonies exhibiting sucrose resistance are cultured and analyzed via sequencing. The results for the sequencing of the insert genomic regions are summarized below in Table 6.

Table 6: Loop-out Validation Frequency

Outcome	**Frequency (sampling error 95% confidence)**
Successful Loop out	13% (9%/20%)

Loop Still present	42% (34%/50%)
Mixed read	44% (36%/52%)

Sequencing results show a 10-20% efficiency in loop outs. Not to be limited by any particular theory, loop-out may be dependent on insert sequence. Even if correct, picking 10-20 sucrose-resistant colonies leads to high success rates.

Upon integration, the recombinant vectors replace the endogenous promoter sequences with a promoter selected from the group consisting of Pcg1860 (SEQ ID NO:2), Pcg0007 (SEQ ID NO:3), Pcg0755 (SEQ ID NO:4), Pcg0007_lib_265 (SEQ ID NO:5), Pcg3381 (SEQ ID NO:6), Pcg007_lib_119 (SEQ ID NO:7), and Pcg3121 (SEQ ID NO:8). A list of the resulting recombinant strains is provided below in Table 7.

Multiple single colonies (N in Table 7) are picked, inoculated and grown as a small scale culture. Each newly created strain comprising a test promoter is tested for lysine yield in small scale cultures designed to assess product titer performance. Small scale cultures are conducted using media from industrial scale cultures. Product titer is optically measured at carbon exhaustion (*i.e.*, representative of single batch yield) with a standard colorimetric assay. Briefly, a concentrated assay mixture is prepared and is added to fermentation samples such that final concentrations of reagents are 160 mM sodium phosphate buffer, 0.2 mM Amplex Red, 0.2 U/mL Horseradish Peroxidase and 0.005 U/mL of lysine oxidase. Reactions proceed to completion and optical density is measured using a Tecan M1000 plate spectrophotometer at a 560nm wavelength.

As shown in Table 7, the yield of L-lysine is increased by over 24% (*e.g.*, recombinant strain 7000007840) over the non-engineered strain. In other embodiments, the yield of L-lysine is decreased by nearly 90% (e.g., recombinant strain 700000773). As provided in Table 7, replacement of the promoter for the pgi and zwf results in greater than 10% improvements to L-lysine production.

Notably, the production of L-lysine is not a simple dependence on incorporating the most active promoters. As illustrated in **Error! Reference source not found.**, lysine yield is maximized by a relatively weak promoter (*e.g.*, pgi having relative promoter expression of 1, 7x, or 48x, or dapB at a relative promoter strength of 7x) or maximized by intermediate expression (*e.g.*, lysA at having a relative promoter expression of 454x). In certain cases, expression is maximal when the relative promoter strength is maximized (*e.g.*, ppc). As exemplified **Error! Reference source not found.**, the location of the gene in the genetic pathway (**Error! Reference source not found.**) does not reliably predict the relative increase or decrease in L-lysine yield or the optimal promoter strength. For example, high level

expression of cg0931 results in improved yield while higher levels of dapD result in no improvement or decreased yield.

Table 7: Recombinant strains of *C. glutamicum* having modified expression of L-lysine Biosynthetic Genes

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007713	Pcg1860-asd	8	0.84595	0.00689	3.927615
7000007736	Pcg0755-asd	4	0.84036	0.00974	3.240866
7000007805	Pcg0007_119-asd	8	0.82493	0.00689	1.345242
7000007828	Pcg3121-asd	8	0.8246	0.00689	1.3047
7000007759	Pcg0007_265-asd	8	0.81155	0.00689	-0.29853
7000007782	Pcg3381-asd	8	0.8102	0.00689	-0.46438
7000007712	Pcg1860-ask	8	0.83958	0.00689	3.14504
7000007735	Pcg0755-ask	8	0.81673	0.00689	0.337846
7000007827	Pcg3121-ask	8	0.81498	0.00689	0.122853
7000007804	Pcg0007_119-ask	8	0.81492	0.00689	0.115482
7000007758	Pcg0007_265-ask	8	0.80381	0.00689	-1.24942
7000007781	Pcg3381-ask	8	0.80343	0.00689	-1.2961
7000007780	Pcg3381-aspB	8	0.84072	0.00689	3.285093
7000007803	Pcg0007_119-aspB	8	0.82106	0.00689	0.8698
7000007809	Pcg0007_119-cg0931	8	0.83446	0.00689	2.516032
7000007717	Pcg1860-cg0931	4	0.83129	0.00974	2.126588
7000007763	Pcg0007_265-cg0931	4	0.82628	0.00974	1.511094
7000007671	Pcg0007_39-cg0931	8	0.82554	0.00689	1.420182
7000007740	Pcg0755-cg0931	8	0.81921	0.00689	0.642522
7000007694	Pcg0007-cg0931	8	0.80444	0.00689	-1.17202
7000007691	Pcg0007-dapA	8	0.8299	0.00689	1.955822

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007783	Pcg3381-dapA	8	0.80951	0.00689	-0.54915
7000007760	Pcg0007_265-dapA	8	0.76147	0.00689	-6.45102
7000007806	Pcg0007_119-dapA	8	0.35394	0.00689	-56.5174
7000007761	Pcg0007_265-dapB	8	0.84157	0.00689	3.389518
7000007738	Pcg0755-dapB	4	0.84082	0.00974	3.297378
7000007692	Pcg0007-dapB	8	0.83088	0.00689	2.076218
7000007784	Pcg3381-dapB	8	0.82474	0.00689	1.3219
7000007715	Pcg1860-dapB	8	0.82232	0.00689	1.024595
7000007830	Pcg3121-dapB	8	0.81236	0.00689	-0.19902
7000007807	Pcg0007_119-dapB	4	0.69622	0.00974	-14.4672
7000007762	Pcg0007_265-dapD	8	0.84468	0.00689	3.771591
7000007808	Pcg0007_119-dapD	8	0.83869	0.00689	3.035701
7000007785	Pcg3381-dapD	8	0.83397	0.00689	2.455834
7000007670	Pcg0007_39-dapD	8	0.81698	0.00689	0.368559
7000007831	Pcg3121-dapD	4	0.8155	0.00974	0.186737
7000007693	Pcg0007-dapD	8	0.8117	0.00689	-0.28011
7000007716	Pcg1860-dapD	8	0.79044	0.00689	-2.89196
7000007739	Pcg0755-dapD	8	0.78694	0.00689	-3.32195
7000007787	Pcg3381-dapE	8	0.83814	0.00689	2.968132
7000007833	Pcg3121-dapE	8	0.83721	0.00689	2.853878
7000007741	Pcg0755-dapE	8	0.83263	0.00689	2.291211
7000007810	Pcg0007_119-dapE	8	0.83169	0.00689	2.175729
7000007718	Pcg1860-dapE	8	0.81855	0.00689	0.561439

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007672	Pcg0007_39-dapE	8	0.80932	0.00689	-0.5725
7000007765	Pcg0007_265-dapF	8	0.8327	0.00689	2.299811
7000007788	Pcg3381-dapF	8	0.82942	0.00689	1.896853
7000007811	Pcg0007_119-dapF	8	0.82926	0.00689	1.877196
7000007696	Pcg0007-dapF	8	0.82099	0.00689	0.861201
7000007719	Pcg1860-dapF	8	0.82067	0.00689	0.821888
7000007673	Pcg0007_39-dapF	8	0.82062	0.00689	0.815745
7000007789	Pcg3381-ddh	8	0.84817	0.00689	4.200349
7000007835	Pcg3121-ddh	8	0.82141	0.00689	0.912799
7000007812	Pcg0007_119-ddh	8	0.82093	0.00689	0.853829
7000007674	Pcg0007_39-ddh	8	0.81494	0.00689	0.117939
7000007720	Pcg1860-ddh	8	0.81473	0.00689	0.09214
7000007766	Pcg0007_265-ddh	8	0.81427	0.00689	0.035627
7000007743	Pcg0755-ddh	8	0.80655	0.00689	-0.9128
7000007697	Pcg0007-ddh	8	0.80621	0.00689	-0.95457
7000007779	Pcg3381-fbp	8	0.85321	0.00689	4.819529
7000007802	Pcg0007_119-fbp	4	0.81425	0.00974	0.03317
7000007710	Pcg1860-fbp	4	0.40253	0.00974	-50.5479
7000007687	Pcg0007-fbp	8	0.14881	0.00689	-81.7182
7000007825	Pcg3121-fbp	4	0.12471	0.00974	-84.679
7000007733	Pcg0755-fbp	4	0.08217	0.00974	-89.9052
7000007746	Pcg0755-hom	8	0.81925	0.00689	0.647436
7000007792	Pcg3381-hom	4	0.77674	0.00974	-4.57505

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007723	Pcg1860-hom	8	0.71034	0.00689	-12.7325
7000007838	Pcg3121-hom	8	0.559	0.00689	-31.3251
7000007800	Pcg0007_119-icd	8	0.83236	0.00689	2.258041
7000007823	Pcg3121-icd	8	0.83155	0.00689	2.15853
7000007777	Pcg3381-icd	8	0.82844	0.00689	1.776456
7000007708	Pcg1860-icd	8	0.82384	0.00689	1.211332
7000007662	Pcg0007_39-icd	12	0.82008	0.00562	0.749404
7000007685	Pcg0007-icd	8	0.81257	0.00689	-0.17322
7000007754	Pcg0007_265-icd	4	0.81172	0.00974	-0.27765
7000007698	Pcg0007-lysA	4	0.8504	0.00974	4.474311
7000007675	Pcg0007_39-lysA	8	0.84414	0.00689	3.705251
7000007836	Pcg3121-lysA	4	0.83545	0.00974	2.637657
7000007767	Pcg0007_265-lysA	8	0.83249	0.00689	2.274012
7000007813	Pcg0007_119-lysA	8	0.83096	0.00689	2.086046
7000007790	Pcg3381-lysA	8	0.8118	0.00689	-0.26782
7000007676	Pcg0007_39-lysE	8	0.84394	0.00689	3.68068
7000007699	Pcg0007-lysE	4	0.83393	0.00974	2.45092
7000007768	Pcg0007_265-lysE	8	0.83338	0.00689	2.383351
7000007837	Pcg3121-lysE	4	0.83199	0.00974	2.212585
7000007791	Pcg3381-lysE	8	0.81476	0.00689	0.095825
7000007814	Pcg0007_119-lysE	8	0.81315	0.00689	-0.10197
7000007775	Pcg3381-odx	8	0.82237	0.00689	1.030738
7000007752	Pcg0007_265-odx	8	0.81118	0.00689	-0.34399

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007729	Pcg0755-odx	8	0.81103	0.00689	-0.36242
7000007683	Pcg0007-odx	8	0.80507	0.00689	-1.09462
7000007706	Pcg1860-odx	4	0.79332	0.00974	-2.53815
7000007660	Pcg0007_39-odx	8	0.79149	0.00689	-2.76297
7000007798	Pcg0007_119-odx	8	0.77075	0.00689	-5.31094
7000007821	Pcg3121-odx	4	0.74788	0.00974	-8.12059
7000007822	Pcg3121-pck	8	0.85544	0.00689	5.093491
7000007776	Pcg3381-pck	8	0.8419	0.00689	3.43006
7000007799	Pcg0007_119-pck	8	0.83851	0.00689	3.013588
7000007753	Pcg0007_265-pck	8	0.82738	0.00689	1.646232
7000007730	Pcg0755-pck	4	0.81785	0.00974	0.475442
7000007661	Pcg0007_39-pck	8	0.80976	0.00689	-0.51844
7000007684	Pcg0007-pck	8	0.79007	0.00689	-2.93742
7000007707	Pcg1860-pck	8	0.71566	0.00689	-12.0789
7000007840	Pcg3121-pgi	4	1.01046	0.00974	24.13819
7000007817	Pcg0007_119-pgi	7	0.99238	0.00736	21.917
7000007794	Pcg3381-pgi	7	0.99008	0.00736	21.63444
7000007771	Pcg0007_265-pgi	8	0.94665	0.00689	16.29893
7000007725	Pcg1860-pgi	8	0.85515	0.00689	5.057864
7000007702	Pcg0007-pgi	4	0.8056	0.00974	-1.02951
7000007658	Pcg0007_39-ppc	4	0.85221	0.00974	4.696676
7000007750	Pcg0007_265-ppc	8	0.84486	0.00689	3.793705
7000007727	Pcg0755-ppc	8	0.84166	0.00689	3.400575

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007773	Pcg3381-ppc	4	0.82883	0.00974	1.824369
7000007796	Pcg0007_119-ppc	8	0.82433	0.00689	1.27153
7000007704	Pcg1860-ppc	8	0.81736	0.00689	0.415244
7000007819	Pcg3121-ppc	8	0.79898	0.00689	-1.8428
7000007732	Pcg0755-ptsG	8	0.84055	0.00689	3.264208
7000007709	Pcg1860-ptsG	8	0.81075	0.00689	-0.39682
7000007663	Pcg0007_39-ptsG	8	0.80065	0.00689	-1.63763
7000007778	Pcg3381-ptsG	8	0.23419	0.00689	-71.229
7000007801	Pcg0007_119-ptsG	8	0.17295	0.00689	-78.7525
7000007824	Pcg3121-ptsG	8	0.16035	0.00689	-80.3005
7000007705	Pcg1860-pyc	8	0.85143	0.00689	4.60085
7000007728	Pcg0755-pyc	8	0.79803	0.00689	-1.95951
7000007659	Pcg0007_39-pyc	8	0.75539	0.00689	-7.19797
7000007751	Pcg0007_265-pyc	8	0.73664	0.00689	-9.50146
7000007682	Pcg0007-pyc	4	0.73142	0.00974	-10.1428
7000007774	Pcg3381-pyc	4	0.66667	0.00974	-18.0975
7000007797	Pcg0007_119-pyc	4	0.52498	0.00974	-35.5046
7000007820	Pcg3121-pyc	8	0.52235	0.00689	-35.8277
7000007841	Pcg3121-tkt	8	0.82565	0.00689	1.433696
7000007818	Pcg0007_119-tkt	8	0.81674	0.00689	0.339075
7000007749	Pcg0755-tkt	8	0.81496	0.00689	0.120396
7000007703	Pcg0007-tkt	4	0.76763	0.00974	-5.69424
7000007795	Pcg3381-tkt	8	0.72213	0.00689	-11.2841

Strain	promoter-target	N	Mean (A_{560})	Std Error	% Yield Change From Base
7000007772	Pcg0007_265-tkt	8	0.68884	0.00689	-15.3738
7000007701	Pcg0007-zwf	4	0.95061	0.00974	16.78542
7000007747	Pcg0755-zwf	8	0.92595	0.00689	13.75587
7000007770	Pcg0007_265-zwf	8	0.9029	0.00689	10.9241
7000007724	Pcg1860-zwf	8	0.79309	0.00689	-2.5664
7000007839	Pcg3121-zwf	4	0.13379	0.00974	-83.5635

Example 4: Engineering the L-lysine biosynthetic pathway

The yield of L-lysine is modified by swapping pairs of promoters for target genes as provided in Table 8. The constructs of Example 3 are used to prepare recombinant organsims as provided in Table 8. As shown, the combination of Pcg0007-lysA and Pcg3121-pgi provide for the highest yields of L-lysine.

Table 8: Paired Promoter Swapping of Target Genes in the L-lysine biosynthetic pathway

Strain ID	Number	PRO Swap 1	PRO Swap 2	Mean Yield (A_{560})	Std Dev
7000008489	4	Pcg0007-lysA	Pcg3121-pgi	1.17333	0.020121
7000008530	8	Pcg1860-pyc	Pcg0007-zwf	1.13144	0.030023
7000008491	7	Pcg0007-lysA	Pcg0007-zwf	1.09836	0.028609
7000008504	8	Pcg3121-pck	Pcg0007-zwf	1.09832	0.021939
7000008517	8	Pcg0007_39-ppc	Pcg0007-zwf	1.09502	0.030777
7000008502	4	Pcg3121-pck	Pcg3121-pgi	1.09366	0.075854
7000008478	4	Pcg3381-ddh	Pcg0007-zwf	1.08893	0.025505
7000008465	4	Pcg0007_265-dapB	Pcg0007-zwf	1.08617	0.025231
7000008535	8	Pcg0007-zwf	Pcg3121-pgi	1.06261	0.019757

Strain ID	Number	PRO Swap 1	PRO Swap 2	Mean Yield (A_{560})	Std Dev
7000008476	6	Pcg3381-ddh	Pcg3121-pgi	1.04808	0.084307
7000008510	8	Pcg3121-pgi	Pcg1860-pyc	1.04112	0.021087
7000008525	8	Pcg1860-pyc	Pcg0007_265-dapB	1.0319	0.034045
7000008527	8	Pcg1860-pyc	Pcg0007-lysA	1.02278	0.043549
7000008452	5	Pcg1860-asd	Pcg0007-zwf	1.02029	0.051663
7000008463	4	Pcg0007_265-dapB	Pcg3121-pgi	1.00511	0.031604
7000008524	8	Pcg1860-pyc	Pcg1860-asd	1.00092	0.026355
7000008458	4	Pcg3381-aspB	Pcg1860-pyc	1.00043	0.020083
7000008484	8	Pcg3381-fbp	Pcg1860-pyc	0.99686	0.061364
7000008474	8	Pcg3381-ddh	Pcg3381-fbp	0.99628	0.019733
7000008522	8	Pcg0755-ptsG	Pcg3121-pgi	0.99298	0.066021
7000008528	8	Pcg1860-pyc	Pcg3121-pck	0.99129	0.021561
7000008450	4	Pcg1860-asd	Pcg3121-pgi	0.98262	0.003107
7000008448	8	Pcg1860-asd	Pcg3381-fbp	0.97814	0.022285
7000008494	8	Pcg0007_39-lysE	Pcg3381-fbp	0.97407	0.027018
7000008481	8	Pcg3381-fbp	Pcg0007-lysA	0.9694	0.029315
7000008497	8	Pcg0007_39-lysE	Pcg1860-pyc	0.9678	0.028569
7000008507	8	Pcg3121-pgi	Pcg3381-fbp	0.96358	0.035078
7000008501	8	Pcg3121-pck	Pcg0007-lysA	0.96144	0.018665
7000008486	8	Pcg0007-lysA	Pcg0007_265-dapB	0.94523	0.017578
7000008459	8	Pcg0007_265-	Pcg1860-asd	0.94462	0.023847

Strain ID	Number	PRO Swap 1	PRO Swap 2	Mean Yield (A_{560})	Std Dev
		dapB			
7000008506	2	Pcg3121-pgi	Pcg0007_265-dapD	0.94345	0.014014
7000008487	8	Pcg0007-lysA	Pcg3381-ddh	0.94249	0.009684
7000008498	8	Pcg3121-pck	Pcg1860-asd	0.94154	0.016802
7000008485	8	Pcg0007-lysA	Pcg1860-asd	0.94135	0.013578
7000008499	8	Pcg3121-pck	Pcg0007_265-dapB	0.93805	0.013317
7000008472	8	Pcg3381-ddh	Pcg1860-asd	0.93716	0.012472
7000008511	8	Pcg0007_39-ppc	Pcg1860-asd	0.93673	0.015697
7000008514	8	Pcg0007_39-ppc	Pcg0007-lysA	0.93668	0.027204
7000008473	8	Pcg3381-ddh	Pcg0007_265-dapB	0.93582	0.030377
7000008461	7	Pcg0007_265-dapB	Pcg3381-fbp	0.93498	0.037862
7000008512	8	Pcg0007_39-ppc	Pcg0007_265-dapB	0.93033	0.017521
7000008456	8	Pcg3381-aspB	Pcg3121-pck	0.92544	0.020075
7000008460	8	Pcg0007_265-dapB	Pcg0007_265-dapD	0.91723	0.009508
7000008492	8	Pcg0007_39-lysE	Pcg3381-aspB	0.91165	0.012988
7000008493	8	Pcg0007_39-lysE	Pcg0007_265-dapD	0.90609	0.031968
7000008453	8	Pcg3381-aspB	Pcg0007_265-dapB	0.90338	0.013228
7000008447	8	Pcg1860-asd	Pcg0007_265-dapD	0.89886	0.028896

Strain ID	Number	PRO Swap 1	PRO Swap 2	Mean Yield (A_{560})	Std Dev
7000008455	8	Pcg3381-aspB	Pcg0007-lysA	0.89531	0.027108
7000008454	6	Pcg3381-aspB	Pcg3381-ddh	0.87816	0.025807
7000008523	8	Pcg0755-ptsG	Pcg1860-pyc	0.87693	0.030322
7000008520	8	Pcg0755-ptsG	Pcg3381-fbp	0.87656	0.018452
7000008533	4	Pcg0007-zwf	Pcg3381-fbp	0.84584	0.017012
7000008519	8	Pcg0755-ptsG	Pcg0007_265-dapD	0.84196	0.025747

All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification are incorporated herein by reference, in their entirety to the extent not inconsistent with the present description.

From the foregoing it will be appreciated that, although specific embodiments described herein have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope described herein. Accordingly, the disclosure is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. A recombinant nucleic acid molecule comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to at least one heterologous target gene.

2. The recombinant nucleic acid molecule according to claim 1, wherein said promoter polynucleotide sequence is selected from the group consisting of SEQ ID NOs:1, 5 and 7.

3. The recombinant nucleic acid molecule according to claims 1 or 2, further comprising a linker oligonucleotide or linker polynucleotide.

4. The recombinant nucleic acid molecule according to claim 1, wherein said at least one heterologous target gene is a gene that is a component of a biosynthetic pathway producing a biomolecule selected from the group consisting of amino acids, organic acids, proteins and polymers.

5. The recombinant nucleic acid molecule according to claim 4, wherein said at least one heterologous target gene is a gene that is a component of an amino acid biosynthetic pathway selected from the group consisting of:
the lysine biosynthesis pathway comprising genes of entry M00016 of the Kyoto Encyclopedia of Genes and Genomes (KEGG);
the lysine biosynthesis pathway comprising genes of KEGG entry M00525;
the lysine biosynthesis pathway comprising genes of KEGG entry M00526;
the lysine biosynthesis pathway comprising genes of KEGG entry M00527;
the lysine biosynthesis pathway comprising genes of KEGG entry M0030;
the lysine biosynthesis pathway comprising genes of KEGG entry M00433; and
the lysine biosynthesis pathway comprising genes of KEGG entry M0031.

6. The recombinant nucleic acid molecule according to claim 1, further comprising one or more additional promoter polynucleotide sequences selected from the group consisting of

SEQ ID NOs:1 to 8, each promoter functionally linked to at least one additional heterologous gene.

7. The recombinant nucleic acid molecule according to claim 1, wherein said recombinant nucleic acid molecule is isolated.

8. A recombinant vector comprising a promoter polynucleotide sequence selected from the group consisting of SEQ ID NOs:1 to 8 functionally linked to at least one heterologous target gene.

9. The recombinant vector according to claim 8, wherein said promoter polynucleotide sequence is selected from the group consisting of SEQ ID NOs:1, 5 and 7.

10. The recombinant vector according to claim 8 or 9, wherein said target genes are part of the same metabolic pathway.

11. The recombinant vector according to claim 8 or 9, wherein said target genes are not part of the same metabolic pathway.

12. A host cell comprising a recombinant nucleic acid molecule according to any one of claims 1 to 7 or transformed with a recombinant vector according to claims 8 to 11.

13. The host cell according to claim 12, further comprising one or more additional recombinant nucleic acid molecules according to any one of claims 1 to 7 or transformed with one or more additional recombinant vectors according to claims 8 to 11.

14. The host cell according to claim 13, wherein said one or more additional recombinant nucleic acid molecules according to any one of claims 1 to 7 or said one or more additional recombinant vectors according to claims 8 to 11 are functionally linked to a different heterologous target gene.

15. The host cell according to claim 12, comprising a combination of promoter polynucleotide sequences, wherein each of said promoter polynucleotide sequences is functionally linked to a different heterologous target gene.

16. The host cell according to claim 15, wherein each of said different heterologous target genes are part of the same metabolic pathway.

17. The host cell according to claim 15, wherein each of said different heterologous target genes are not part of the same metabolic pathway.

18. The host cell according to any one of claims 12 to 17, which belongs to genus *Corynebacterium*.

19. The host cell according to claim 18, which is *Corynebacterium glutamicum*.

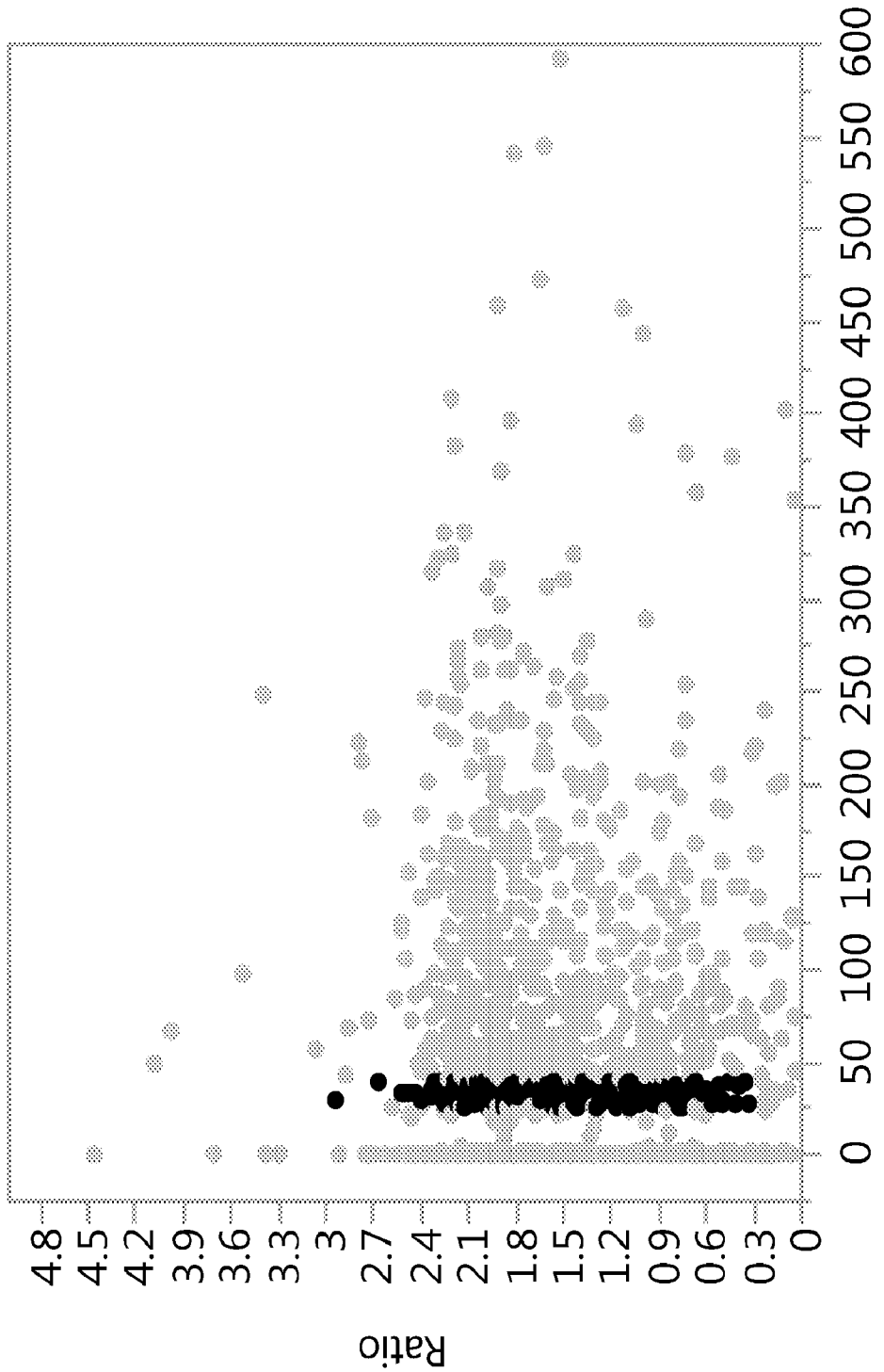
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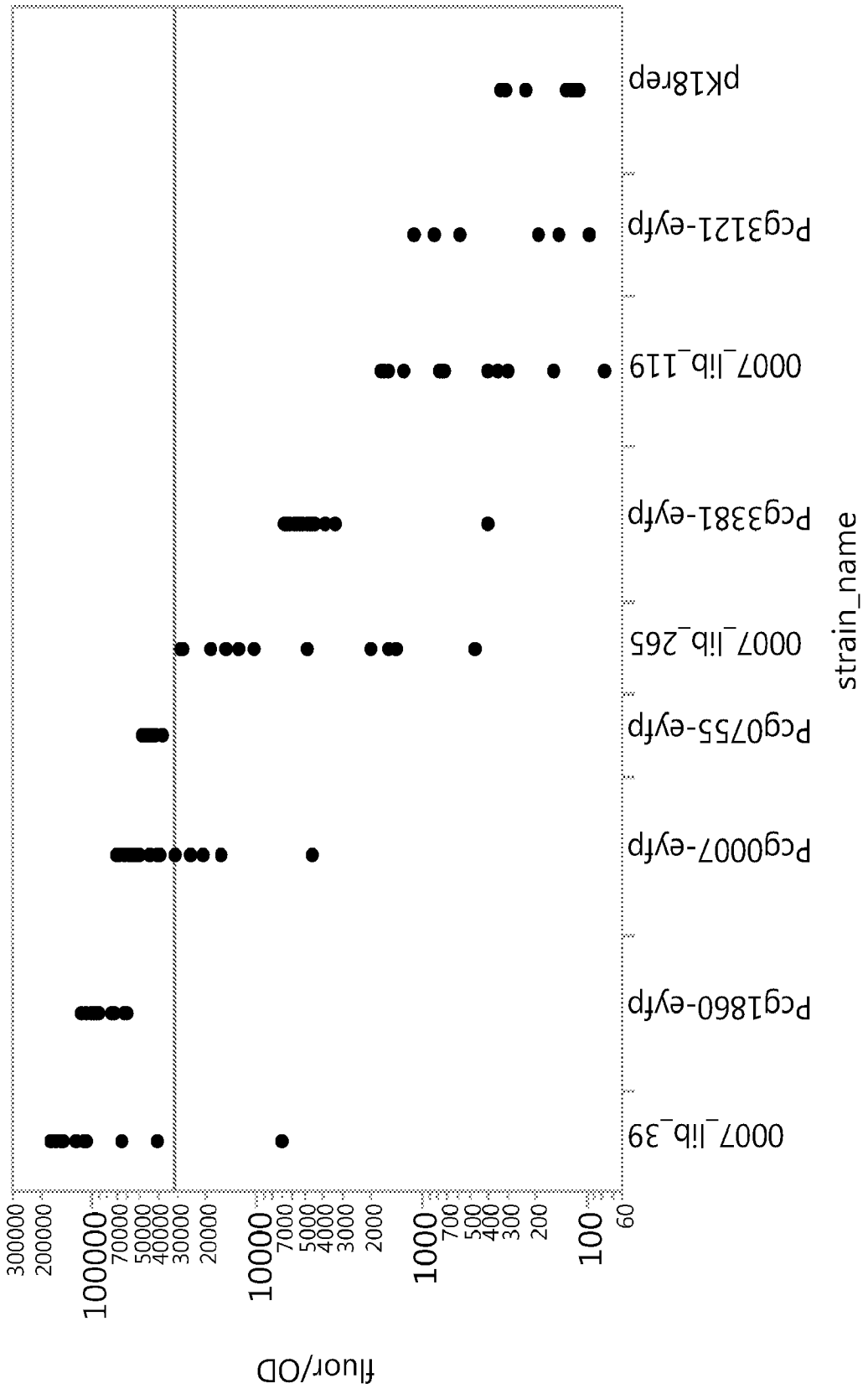
FIG. 1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500
400
300
200
100
60
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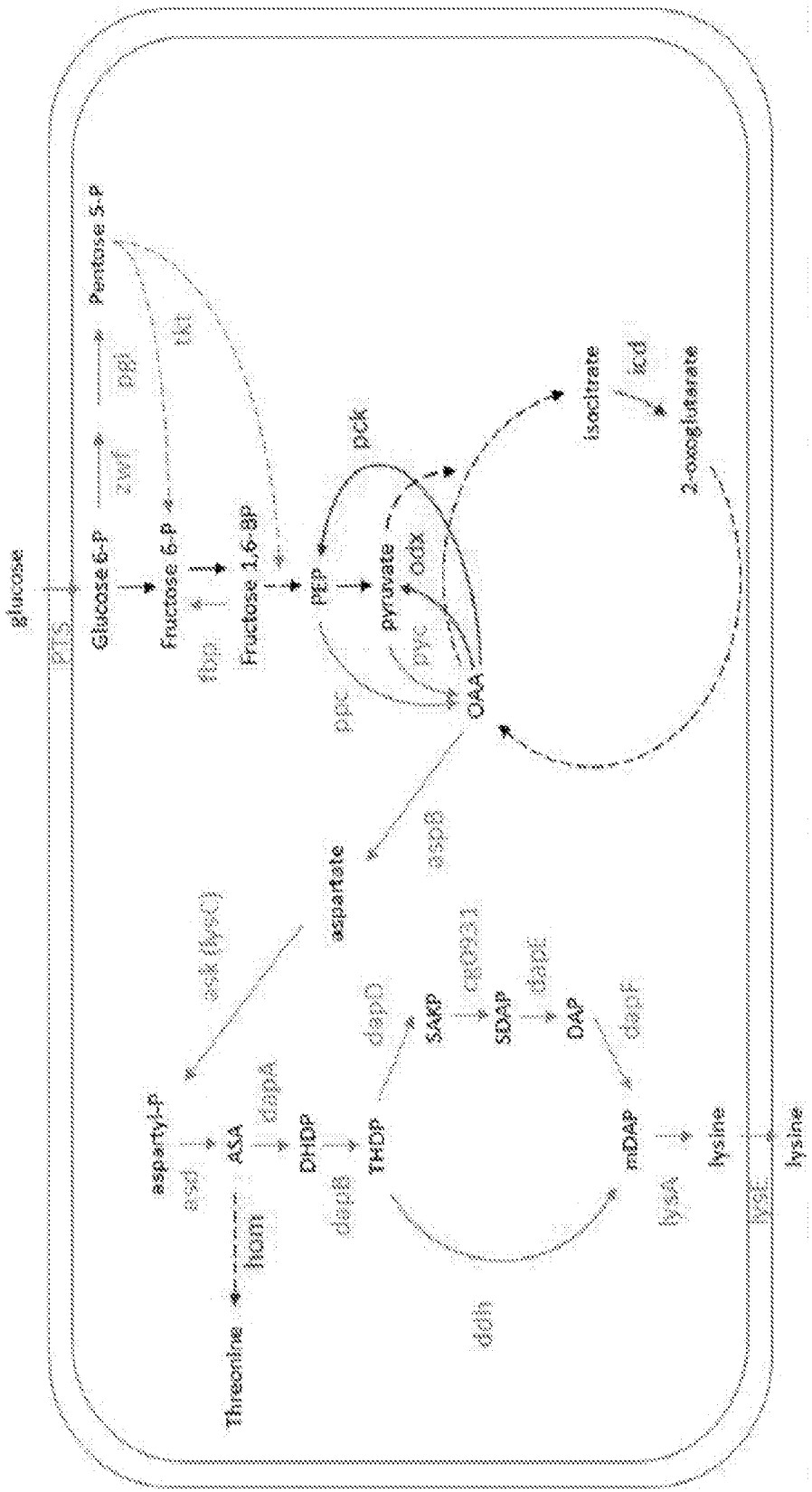
FIG. 2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pyruvate
ppc
pyc
odx
OAA
Isocitrate
icd
2-oxoglutarate
aspartate
aspB
ask (lysC)
aspartyl-P
asd
ASA
dapA
DHDP
dapB
THDP
dapD
SAKP
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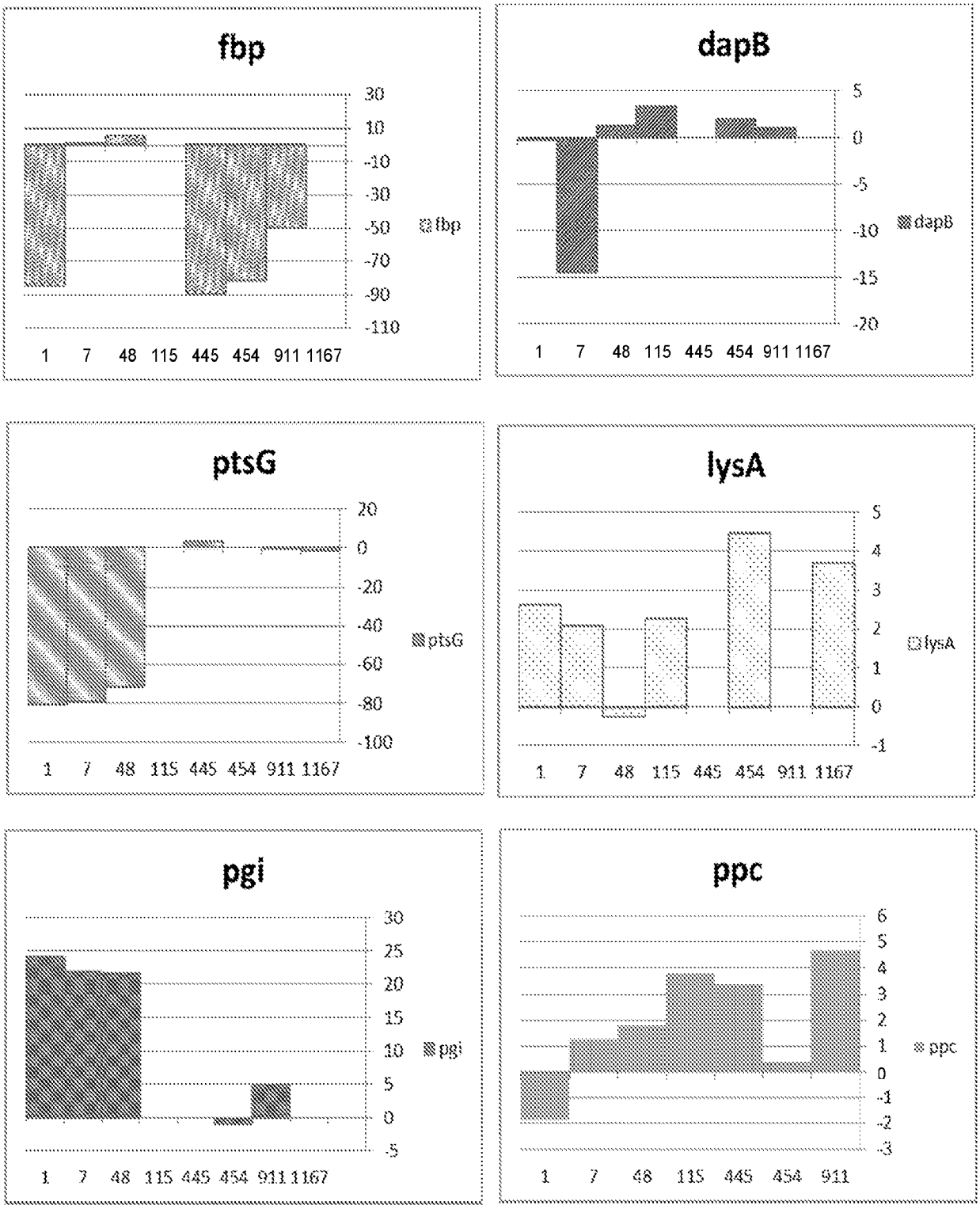
lysE
lysine
hom
Threonine
dapB

FIG. 3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-15
-20
dapB
1 7 48 115 445 454 911 1167
ptsG
20
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-60
-80
-100
ptsG
1 7 48 115 445 454 911 1167
lysA
5
4
3
2
1
0
-1
lysA
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pgi
30
25
20
15
10
5
0
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pgi
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ppc
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ppc
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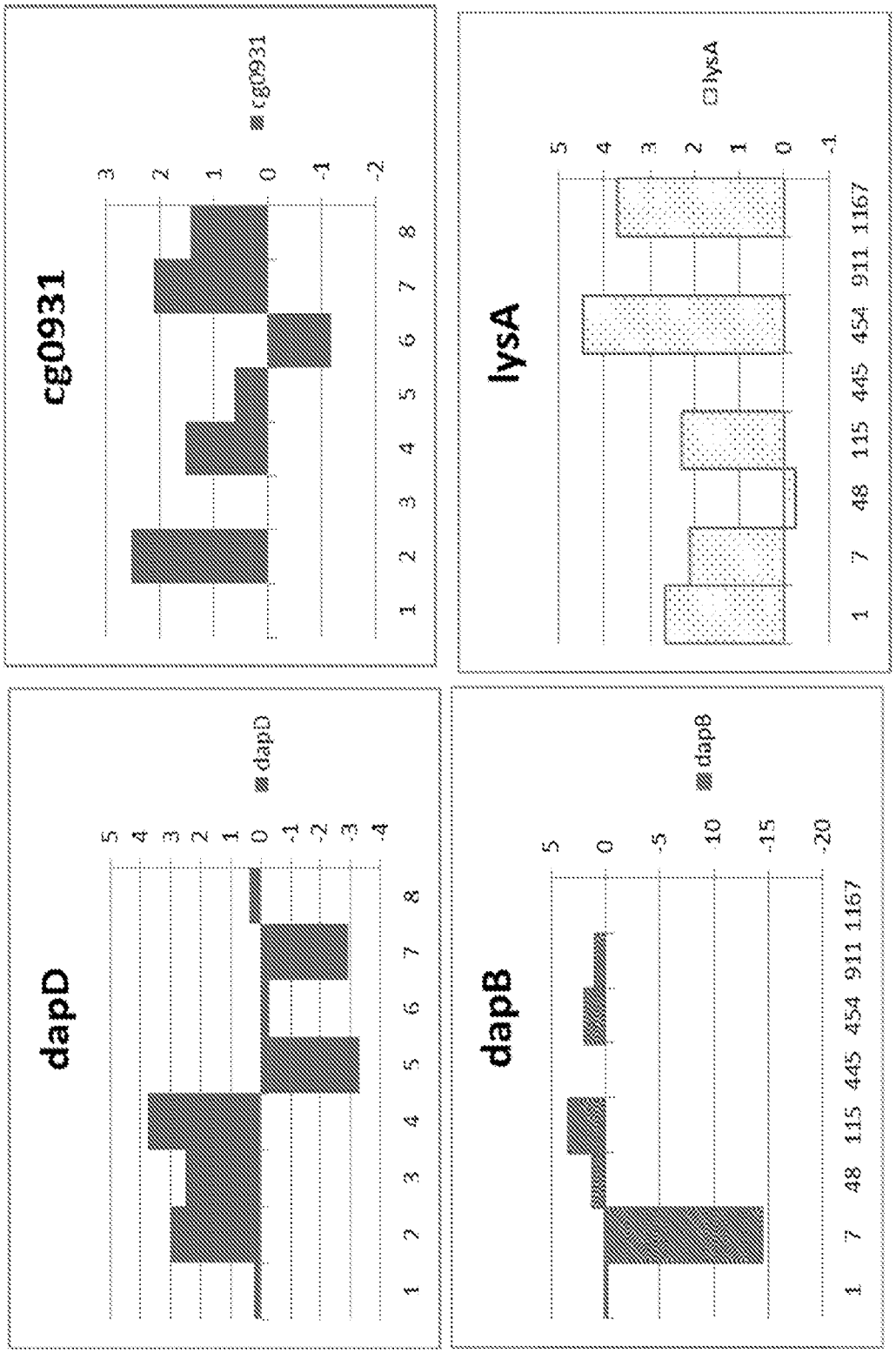
FIG. 4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0
-1
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dapD
dapD
5
4
3
2
1
0
-1
-2
-3
-4
1 2 3 4 5 6 7 8
dapB
dapB
5
0
-5
-10
-15
-20
1 7 48 115 445 454 911 1167

FIG. 5