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(54) **METHOD FOR THE PREPARATION OF 2,4-DIHYDROXYBUTYRATE**

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(57) **ABSTRACT**

A method for the preparation of 2,4-dihydroxybutyric acid from homoserine includes a first step of conversion of the primary amino group of homoserine to a carbonyl group to obtain 2-oxo-4-hydroxybutyrate, and a second step of reduction of the obtained 2-oxo-4-hydroxybutyrate (OHB) to 2,4-dihydroxybutyrate.

Specification includes a Sequence Listing.

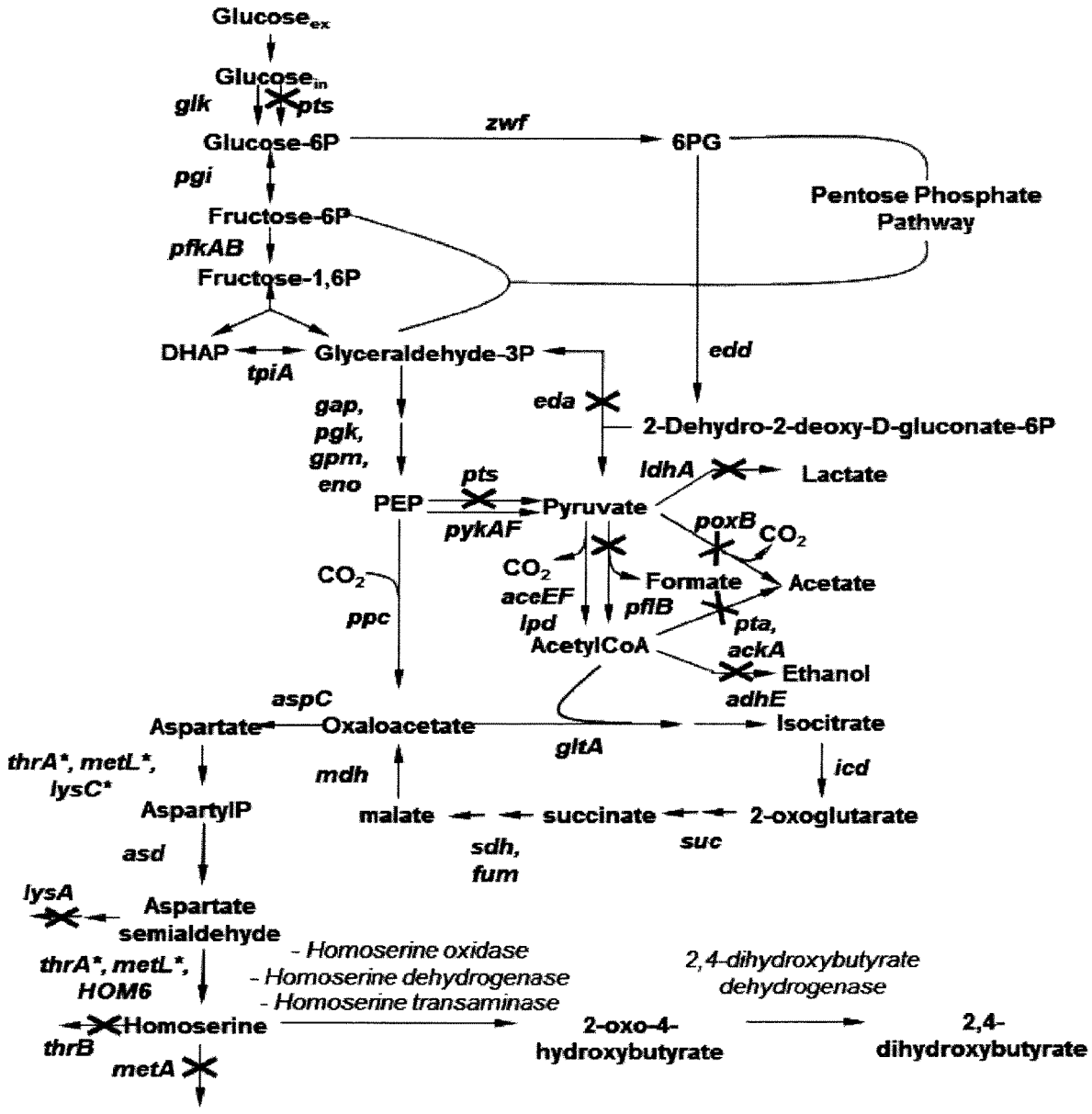
Glucose$_{ex}$
Glucose$_{in}$
glk
pts
Glucose-6P
zwf
6PG
pgi
Pentose Phosphate Pathway
Fructose-6P
pfkAB
Fructose-1,6P
DHAP
tpiA
Glyceraldehyde-3P
edd
gap, pgk, gpm, eno
eda
2-Dehydro-2-deoxy-D-gluconate-6P
ldhA
Lactate
pts
PEP
Pyruvate
pykAF
poxB
CO$_2$
CO$_2$
Formate
Acetate
aceEF lpd
pflB
pta, ackA
AcetylCoA
Ethanol
adhE
ppc
aspC
Aspartate
Oxaloacetate
Isocitrate
gltA
thrA*, metL*, lysC*
mdh
icd
AspartylP
malate
succinate
2-oxoglutarate
sdh, fum
suc
asd
lysA
Aspartate semialdehyde
thrA*, metL*, HOM6
- Homoserine oxidase
- Homoserine dehydrogenase
- Homoserine transaminase
2,4-dihydroxybutyrate dehydrogenase
Homoserine
2-oxo-4-hydroxybutyrate
2,4-dihydroxybutyrate
thrB
metA

FIGURE 1

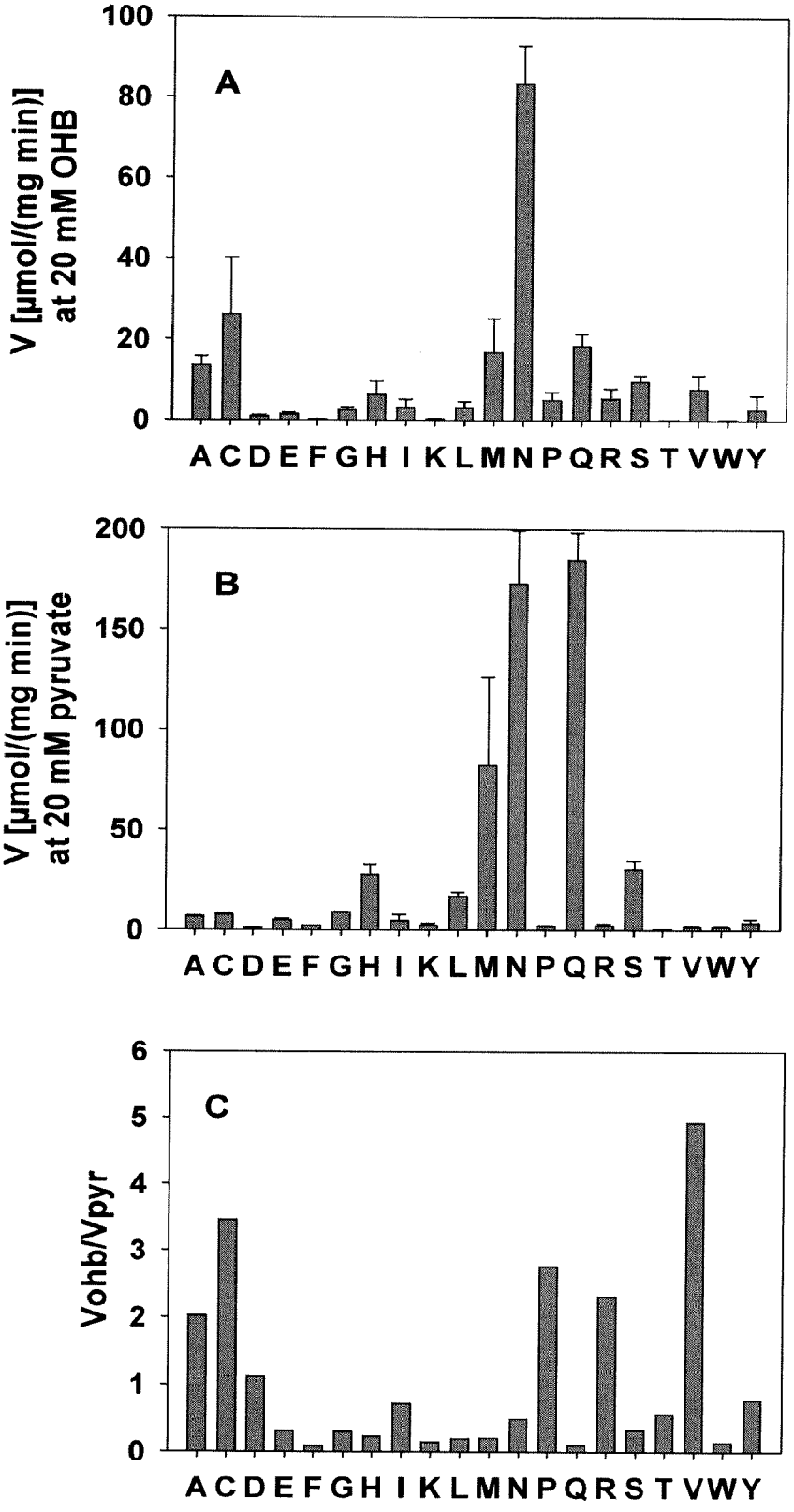
A
V [µmol/(mg min)] at 20 mM OHB
100
80
60
40
20
0
A C D E F G H I K L M N P Q R S T V W Y
B
V [µmol/(mg min)] at 20 mM pyruvate
200
150
100
50
0
A C D E F G H I K L M N P Q R S T V W Y
C
Vohb/Vpyr
6
5
4
3
2
1
0
A C D E F G H I K L M N P Q R S T V W Y

FIGURE 2

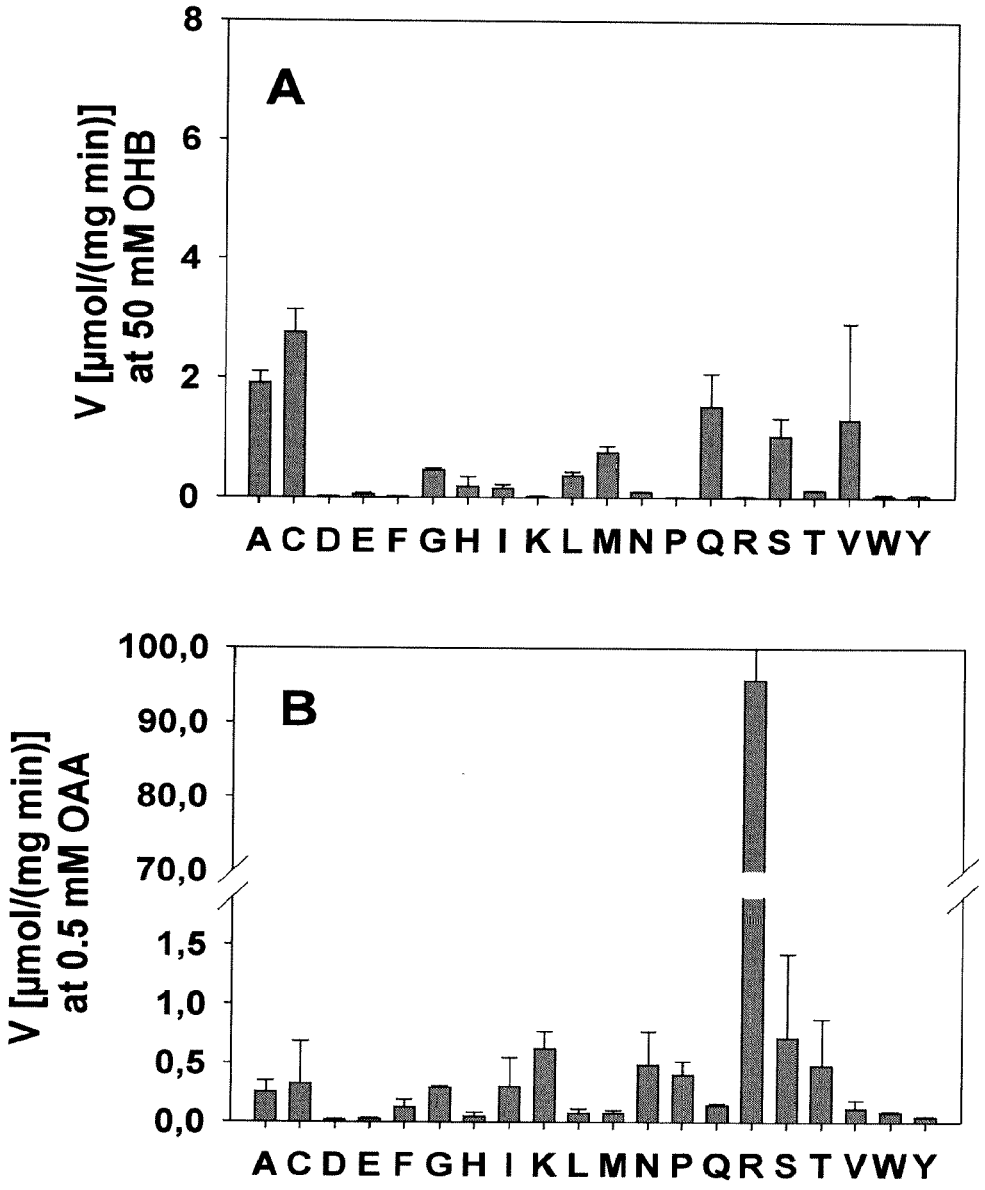
A
V [µmol/(mg min)] at 50 mM OHB
8
6
4
2
0
A C D E F G H I K L M N P Q R S T V W Y
B
V [µmol/(mg min)] at 0.5 mM OAA
100,0
90,0
80,0
70,0
1,5
1,0
0,5
0,0
A C D E F G H I K L M N P Q R S T V W Y

FIGURE 3

METHOD FOR THE PREPARATION OF 2,4-DIHYDROXYBUTYRATE

[0001] The present invention relates to a novel method for the preparation of 2,4-dihydroxybutyrate (2,4-DHB) from homoserine comprising a two-step pathway:

[0002] a first step of conversion of the primary amino group of homoserine to a carbonyl group to obtain 2-oxo-4-hydroxybutyrate, and

[0003] a second step of reduction of the obtained 2-oxo-4-hydroxybutyrate (OHB) to obtain 2,4-DHB.

[0004] The carboxylic acids cited within the present application are equally named under their salt (e.g. 2,4-dihydroxyburyrate) or acid forms (e.g. 2,4-dihydroxybutyric acid).

[0005] 2,4-dihydroxybutyric acid (equally 2,4-DHB or DHB) is a compound of considerable economic interest. DHB can be readily converted into α-hydroxy-γ-butyrolactone in aqueous media by adjusting the appropriate pH. α-hydroxy-γ-butyrolactone is a prominent precursor for the production of the methionine substitute 2-hydroxy-4-(methylthio)-butyrate (HMTB) (US 2009/318715) which has a large market in animal nutrition. At present, α-hydroxy-γ-butyrolactone is derived from γ-butryolactone by a multistage process that implies halogenation of the γ-butryolactone in position α, and subsequent substitution of the halogen atom by a hydroxyl group in alkaline medium (US 2009/318715).

[0006] From growing oil prices the need for the production of DHB from renewable resources arises. Microorganisms are capable of transforming biomass-derived raw material, e.g. sugars or organic acids, into a large variety of different chemical compounds (Werpy & Petersen, 2004). With the growing body of biochemical and genomic information it is possible to modify microorganisms such that they overproduce naturally occurring metabolic intermediates with high yield and productivity (Bailey, 1991). Optimization of production microorganisms often requires rational engineering of metabolic networks which ensures, among others, overexpression of enzymes required for the biosynthesis of the metabolite of interest, and alleviation of product feedback inhibition. Another possibility is the implementation of novel enzymatic systems that catalyze the production of a metabolite of interest.

[0007] Metabolic engineering approaches and enzymatic catalyses require detailed knowledge of the biochemistry and regulation of the metabolic pathway leading to the metabolite of interest. In the case of DHB production, this information is not available. Only few studies report the occurrence of DHB in patients with succinic semialdehyde dehydrogenase deficiency (Shinka et al., 2002) without, however, identifying enzymatic reactions implicated in DHB production. The zymotic or enzymatic production of DHB, therefore, requires (i) the identification of a thermodynamically feasible pathway which transforms an accessible precursor into DHB, (ii) the identification or construction of enzymes that are capable to catalyze individual reaction steps in the pathway and (iii) the functional expression of the pathway enzymes in an appropriate production organism. The present invention has as an objective to satisfy these needs.

[0008] Accordingly, one object of the present invention is a method of preparation of 2,4-DHB from homoserine comprising a two-step pathway (see FIG. **1**):

[0009] a first step of conversion of the primary amino group of homoserine to a carbonyl group to obtain OHB, and

[0010] a second step of reduction of the obtained OHB to 2,4-DHB.

[0011] The first and/or the second step(s) of the method of the invention can be catalyzed either by an enzyme encoded by an endogenous or a heterologous gene.

[0012] In the description, enzymatic activities are also designated by reference to the genes coding for the enzymes having such activity. The use of the denomination of the genes is not limited to a specific organism, but covers all the corresponding genes and proteins in other organisms (e.g. microorganisms, functional analogues, functional variants and functional fragments thereof as long as they retain the enzymatic activity).

[0013] Within a further aspect of the invention, the enzyme converting the primary amino group of homoserine to a carbonyl group to obtain OHB can be homoserine transaminase, homoserine dehydrogenase, or homoserine oxidase.

[0014] Within a further aspect of the invention, the enzyme having homoserine transaminase activity can be identified among enzymes having aspartate transaminase (EC2.6.1.1) activity, branched-chain-amino-acid transaminase (EC2.6.1.42) activity, or aromatic-amino-acid transaminase (EC2.6.1.57) activity.

[0015] Within a further aspect of the invention, the homoserine transaminase can be the branched-chain-amino-acid transaminase from *Escherichia coli*, Ec-IlvE, and *Lactococcus lactis*, Ll-BcaT, the aromatic-amino-acid transaminases from *E. coli*, Ec-TyrB, *L. lactis*, LI-AraT, and *Saccharomyces cerevisiae*, Sc-Aro8, or the aspartate transaminase from *E. coli*, Ec-AspC.

[0016] The second step of the method of the present invention is catalysed by an enzyme having OHB reductase activity. Within a further aspect of the invention, the enzyme having OHB reductase activity can be identified among enzymes having 2-hydroxyacid dehydrogenase activity, in particular among enzymes having lactate dehydrogenase (Ldh) (EC1.1.1.27, EC1.1.1.28), malate dehydrogenase (Mdh) (EC1.1.1.37, EC1.1.1.82, EC1.1.1.299) activity, or branched chain (D)-2-hydroxyacid dehydrogenase (EC1.1.1.272, EC1.1.1.345) activity. More specifically, the enzyme having homoserine transaminase activity is encoded by genes ilvE, tyrB, aspC, araT, bcaT, or ARO8.

[0017] In an even more specific aspect, the enzyme having homoserine transaminase activity is encoded by sequence set forth in SEQ ID No.59, SEQ ID No.61, SEQ ID No.63, SEQ ID No.65, SEQ ID No. 67 or SEQ ID No.69 or any sequence sharing a homology of at least 50% with said sequences or corresponds to SEQ ID No.60, SEQ ID No.62, SEQ ID No.64, SEQ ID No.66, SEQ ID No.68, SEQ ID No.70 or any sequence sharing a homology of at least 50% with said sequences.

[0018] Within a further aspect of the invention, the OHB reductase enzyme can be the (L)-lactate dehydrogenase from *Lactococcus lactis* (Ll-LdhA), from *Oryctalagus cuniculus* (Oc-LldhA), from *Geobacillus stearothermophilus* (Gs-Lldh), or from *Bacillus subtilis* (Bs-Ldh) , the (D)-lactate dehydrogenase from *Escherichia coli* (Ec-LdhA), the (L)-malate dehydrogenase from *Escherichia coli* (Ec-Mdh), or the branched chain (D)-2-hydroxyacid dehydrogenase from *Lactococcus lactis* (Ll-PanE).

[0019] In an even more specific aspect of the invention the OHB reductase enzyme is represented by the amino acid sequences SEQ ID No. 2, SEQ ID No. 4, SEQ ID No. 6, SEQ ID No. 8, SEQ ID No. 10, SEQ ID No. 12, SEQ ID No. 14, SEQ ID No. 288, SEQ ID No. 30, SEQ ID No. 32, SEQ ID No. 102, SEQ ID No. 104, SEQ ID No. 106, SEQ ID No. 108, SEQ ID No. 110, SEQ ID No. 112, SEQ ID No. 114, SEQ ID No. 116 or SEQ ID No. 118 or any sequence sharing a homology of at least 50% with said sequences, or is encoded by the nucleic acid sequences represented by SEQ ID No.1, SEQ ID No. 3, SEQ ID No. 5, SEQ ID No. 7, SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 287, SEQ ID No. 29, SEQ ID No. 31, SEQ ID No. 101, SEQ ID No. 103, SEQ ID No. 105, SEQ ID No. 107, SEQ ID No. 109, SEQ ID No.111, SEQ ID No. 113, SEQ ID No. 115 or SEQ ID No. 117 or any sequence sharing a homology of at least 50% with said sequences.

[0020] In a further aspect, the invention also deals with the use of an enzyme reducing OHB to 2,4-DHB as above described.

[0021] Proteins sharing substantial homology with the above enzymes are also another aspect of the invention such as functional variants or functional fragments.

[0022] The expression "substantial homology" covers homology with respect to structure and/or amino acid components and/or biological activity.

[0023] More generally, within the meaning of the invention the homology between two protein sequences can be determined by methods well known by the skilled man in the art. It is generally defined as a percentage of sequence identity between a reference sequence and the sequence of a protein of interest.

[0024] As used herein, "percent (%) sequence identity" with respect to the amino acid or nucleotide sequences identified herein is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues or nucleotides in an enzyme sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Methods for performing sequence alignment and determining sequence identity are known to the skilled artisan, may be performed without undue experimentation, and calculations of identity values may be obtained with definiteness. See, for example, Ausubel, et al., eds. (1995) Current Protocols in Molecular Biology, Chapter 19 (Greene Publishing and Wiley-Interscience, New York); and the ALIGN program (Dayhoff (1978) in Atlas of Protein Sequence and Structure 5:Suppl. 3 (National Biomedical Research Foundation, Washington, D.C.). A number of algorithms are available for aligning sequences and determining sequence identity and include, for example, the homology alignment algorithm of Needleman et al. (1970) J. Mol. Biol. 48:443; the local homology algorithm of Smith, et al. (1981) Adv. Appl. Math. 2:482; the search for similarity method of Pearson, et al. (1988) Proc. Natl. Acad. Sci. 85:2444; the Smith-Waterman algorithm (Meth. Mol. Biol. 70:173-187 (1997); and BLASTP, BLASTN, and BLASTX algorithms (see Altschul, et al. (1990) J. Mol. Biol. 215:403-410). Computerized programs using these algorithms are also available, and include, but are not limited to: ALIGN or Megalign (DNASTAR) software, or WU-BLAST-2 (Altschul, et al., Meth. Enzym., 266:460-480 (1996)); or GAP, BESTFIT, BLAST (Altschul, et al.), supra, FASTA, and TFASTA, available in the Genetics Computing Group (GCG) package, Version 8, Madison, Wis., USA; and CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, Calif. Those skilled in the art can determine appropriate parameters for measuring alignment, including algorithms needed to achieve maximal alignment over the length of the sequences being compared. Preferably, the sequence identity is determined using the default parameters determined by the program. Specifically, sequence identity can be determined by the Smith-Waterman homology search algorithm (Meth. Mol. Biol. 70:173-187 (1997)) as implemented in MSPRCH program (Oxford Molecular) using an affine gap search with the following search parameters: gap open penalty of 12, and gap extension penalty of 1. Preferably, paired amino acid comparisons can be carried out using the GAP program of the GCG sequence analysis software package of Genetics Computer Group, Inc., Madison, Wis., employing the blosum 62 amino acid substitution matrix, with a gap weight of 12 and a length weight of 2. With respect to optimal alignment of two amino acid sequences, the contiguous segment of the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference amino acid sequence will include at least 20 contiguous amino acid residues, and may be 30, 40, 50, or more amino acid residues. Corrections for increased sequence identity associated with inclusion of gaps in the derivative's amino acid sequence can be made by assigning gap penalties.

[0025] The enzymes according to the present invention having the same activity (either OHB reductase, or the enzyme converting the primary amino group of homoserine to a carbonyl group to obtain OHB) share at least about 50%, 70% or 85% amino acid sequence identity, preferably at least about 85% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, even more preferably at least about 95% amino acid sequence identity and yet more preferably 98% amino acid sequence identity. Preferably, any amino acid substitutions are "conservative amino acid substitutions" using L-amino acids, wherein one amino acid is replaced by another biologically similar amino acid. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/hydrophilicity, and/or steric bulk of the amino acid being substituted. Examples of conservative substitutions are those between the following groups: Gly/Ala, Val/Ile/Leu, Lys/Arg, Asn/Gln, Glu/Asp, Ser/Cys/Thr, and Phe/Trp/Tyr. A derivative may, for example, differ by as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0026] The term functional variant encompasses enzymes that may present substantial sequence modifications when compared to the sequences specifically described within the present application but that still retain the original enzymatic activity.

[0027] It also means that the sequence of the enzyme may comprise less amino acids than the original one but said truncated enzyme still retains the original enzymatic activity.

[0028] According to an aspect of the invention, the activity of the enzyme catalyzing the first and/or the second step of the method of the present invention is enhanced. This

enhancement can be measured by an enzymatic assay as described in Examples 1 or 4.

[0029] Improvement of said enzymes can be obtained by at least one mutation, said mutation(s) (i) improving the activity and/or substrate affinity of the mutated enzyme for homoserine or OHB respectively, and or (ii) decreasing the activity and/or substrate affinity of the mutated enzyme for their natural substrate.

[0030] Within the present invention, the expression "improve the activity and/or substrate affinity" means that the enzyme before mutation, was either

[0031] unable to use the substrate, and/or

[0032] synthesized the product of the reaction at a maximum specific rate at least three times lower, and/or

[0033] had an affinity for homoserine or OHB that was at least three times lower, and/or.

[0034] had a maximum specific activity on the natural substrate that was at least three times higher, and/or.

[0035] had an affinity for the natural substrate that was at least three times higher.

[0036] In a still further aspect the invention encompasses the nucleotide sequences encoding the enzymes catalyzing the first and the second step of the method of the invention.

[0037] In an even more specific aspect of the invention the OHB reductase enzyme is encoded by the nucleic acid sequences represented by SEQ ID No.1, SEQ ID No. 3, SEQ ID No. 5, SEQ ID No. 7, SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 287, SEQ ID No. 29, SEQ ID No. 31, SEQ ID No. 101, SEQ ID No. 103, SEQ ID No. 105, SEQ ID No. 107, SEQ ID No. 109, SEQ ID No.111, SEQ ID No. 113, SEQ ID No. 115 or SEQ ID No. 117 or any sequence sharing a homology of at least 50% with said sequences.

[0038] The OHB reductase according to the invention corresponds in a specific aspect to (L)-lactate dehydrogenase A comprising at least one mutation when a compared to the wild type enzyme in at least one of the positions V17, Q85, E89, I226, or A222. These positions are conserved in the lactate dehydrogenase family, and they are defined in this text by reference to the *Lactococcus lactis* (L)-lactate dehydrogenase A (SEQ ID No. 6). The skilled man in the art will then easily identify the corresponding amino acid residues in other lactate dehydrogenases by an alignment of the corresponding amino acid sequences. Therefore, the invention also provides for changes of these amino acids in other lactate dehydrogenase enzymes.

[0039] The OHB reductase according to the invention corresponds in a specific aspect to (L)-malate dehydrogenase comprising at least one mutation when compared to the wild type enzyme in at least one of the positions 112, R81, M85, D86, V93, G179, T211, or M227. These positions are conserved in the malate dehydrogenase family, and they are defined in this text by reference to the sequence of the *E. coli* (L)-malate dehydrogenase (SEQ ID No. 2). The man skilled in the art will easily identify the corresponding amino acid residues in other malate dehydrogenases by an alignment of the corresponding amino acid sequences. Therefore, the invention also provides for changes of these amino acids in other malate dehydrogenase enzymes.

[0040] In accordance with this invention, a "nucleic acid sequence" refers to a DNA or RNA molecule in single or double stranded form, preferably a DNA molecule. An "isolated DNA", as used herein, refers to a DNA which is not naturally-occurring or no longer in the natural environment wherein it was originally present, e.g., a DNA coding sequence associated with other regulatory elements in a chimeric gene, a DNA transferred into another host cell, or an artificial, synthetically-made DNA sequence having a different nucleotide sequence compared to any naturally-occurring DNA sequence.

[0041] The present invention also relates to a chimeric gene comprising, functionally linked to one another, at least one promoter which is functional in a host organism, a polynucleotide encoding anyone of the enzymes catalyzing first and second step of the method as defined according to the invention, and a terminator element that is functional in the same host organism. The various elements which a chimeric gene may contain are, firstly, elements regulating transcription, translation and maturation of proteins, such as a promoter, a sequence encoding a signal peptide or a transit peptide, or a terminator element constituting a polyadenylation signal and, secondly, a polynucleotide encoding a protein. The expression "functionally linked to one another" means that said elements of the chimeric gene are linked to one another in such a way that the function of one of these elements is affected by that of another. By way of example, a promoter is functionally linked to a coding sequence when it is capable of affecting the expression of said coding sequence. The construction of the chimeric gene according to the invention and the assembly of its various elements can be carried out using techniques well known to those skilled in the art. The choice of the regulatory elements constituting the chimeric gene depends essentially on the host organism in which they must function, and those skilled in the art are capable of selecting regulatory elements which are functional in a given host organism. The term "functional" is intended to mean capable of functioning in a given host organism.

[0042] The promoters which the chimeric gene according to the invention may contain are either constitutive or inducible. By way of example, the promoters used for expression in bacteria may be chosen from the promoters mentioned below. For expression in *Escherichia coli* mention may be made of the lac, trp, lpp, phoA, recA, araBAD, prou, cst-I, tetA, cadA, nar, tac, trc, lpp-lac, Psyn, cspA, PL, PL-9G-50, PR-PL, T7, [lambda]PL-PT7, T3-lac, T5-lac, T4 gene 32, nprM-lac, VHb and the protein A promoters or else the Ptrp promoter (WO 99/64607). For expression in Gram-positive bacteria such as *Corynebacteria* or *Streptomyces*, mention may be made of the PtipA or PS1 and PS2 (FR91/09870) promoters or those described in application EP0629699A2. For expression in yeasts and fungi, mention may be made of the *K. lactis* PLAC4 promoters or the *K. lactis* Ppgk promoter (patent application FR 91/05294), the *Trichoderma reesei* tef1 or cbh1 promoter (WO 94/04673), the *Penicillium funiculosum* his, csl or apf promoter (WO 00/68401) and the *Aspergillus* niger gla promoter.

[0043] According to the invention, the chimeric gene may also comprise other regulatory sequences, which are located between the promoter and the coding sequence, such as transcription activators (enhancers).

[0044] As such, the chimeric gene of the invention comprises in a specific embodiment at least, in the direction of transcription, functionally linked, a promoter regulatory sequence which is functional in a host organism, a nucleic acid sequence encoding a polynucleotide encoding anyone of the enzymes catalyzing first and second step of the

method as defined according to the invention and a terminator regulatory sequence which is functional in said host organism.

[0045] The present invention also relates to a cloning and/or expression vector comprising a chimeric gene according to the invention or a nucleic acid sequence of the invention. The vector according to the invention is of use for transforming a host organism and expressing in this organism anyone of the enzymes catalyzing the first and/or the second step(s) of the method of the present invention. This vector may be a plasmid, a cosmid, a bacteriophage or a virus. Preferentially, the transformation vector according to the invention is a plasmid. Generally, the main qualities of this vector should be able to maintain itself and to self-replicate in the cells of the host organism, in particular by virtue of the presence of an origin of replication, and to express anyone of the enzymes catalyzing the first and/or the second step(s) of the method of the present invention therein. For the purpose of stable transformation of a host organism, the vector may also integrate into the genome. The choice of such a vector, and also the techniques of insertion of the chimeric gene according to the invention into this vector are part of the general knowledge of those skilled in the art. Advantageously, the vector used in the present invention also contains, in addition to the chimeric gene according to the invention, a chimeric gene encoding a selectable marker. This selectable marker makes it possible to select the host organisms which are effectively transformed, i.e. those which incorporated the vector. According to a particular embodiment of the invention, the host organism to be transformed is a bacterium, a yeast, a fungus. Among the selectable markers which can be used, mention may be made of markers containing genes for resistance to antibiotics, such as, for example, the hygromycinphosphotransferase gene. Other markers may be genes to complement an auxotrophy, such as the pyrA, pyrB, pyrG, pyr4, arg4, argB and trpC genes, the molybdopterin synthase gene or that of acetamidase. Mention may also be made of genes encoding readily identifiable enzymes such as the GUS enzyme, or genes encoding pigments or enzymes regulating the production of pigments in the transformed cells. Such selectable marker genes are in particular described in patent applications WO 91/02071, WO 95/06128, WO 96/38567 and WO 97/04103.

[0046] The present invention also relates to modified microorganisms.

[0047] More specifically, the modified microorganism of the invention allows the preparation of 2,4-DHB from homoserine by a two-step pathway comprising:

[0048] a first step of conversion of the primary amino group of homoserine to a carbonyl group to obtain 2-oxo-4-hydroxybutyrate, and

[0049] a second step of reduction of the obtained 2-oxo-4-hydroxybutyrate to obtain 2,4-dihydroxybutyrate.

[0050] The enzymes involved in the two steps are those above described.

[0051] The term "microorganism" is intended to mean any lower unicellular organism into which the chimeric gene(s), nucleic acid(s) or vector(s) according to the invention may be introduced in order to produce 2,4-DHB. Preferably, the host organism is a microorganism, in particular a fungus, for example of the *Penicillium, Aspergillus* and more particularly *Aspergillusflavus, Chrysosporium* or *Trichoderma* genus, a yeast, in particular of the *Saccharomycetaceae, Pichiaceae* or *Schizosaccharomycetaceae*, most preferentially *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces marxianus*, or *Pichia jadinii, Pichia stipitis* or *Pichia pastoris*, a bacterium, preferentially selected among Enterobacteriaceae, Clostridiaceae, Bacillaceae, Streptomycetaceae, Streptococcaceae, Methylobacteriacae, and Corynebacteriaceae, most preferentially *Escherichia coli, Bacillus subtilis, Corynebacterium glutamicum, Clostridium acetobutylicum, Methylobacterium extorquens* or *Lactococcus lactis.*

[0052] The present invention also relates to modified microorganisms containing at least one chimeric gene according to the invention, either integrated into their genome or carried on an extra-chromosomal genetic element, for example a plasmid. In a more specific aspect of the invention, the transformed host organism comprises a nucleic acid of the invention encoding a polypeptide converting the primary amino acid group of homoserine to a carbonyl group to obtain OHB and/or a nucleic acid encoding a polypeptide reducing OHB in 2,4-DHB or a chimeric gene comprising a nucleic acid encoding a polypeptide converting the primary amino acid group of homoserine to a carbonyl group to obtain OHB, and/or a OHB reductase or an expression vector comprising a nucleic acid encoding a polypeptide converting the primary amino acid group of homoserine to a carbonyl group to obtain OHB, or a polypeptide having a OHB reductase activity.

[0053] Within a further aspect of the invention, the synthetic pathway for the conversion of homoserine into DHB is expressed in a microorganism with enhanced production of homoserine. Enhanced production of homoserine in microorganisms can be achieved by (i) overexpressing the enzymes aspartate kinase, aspartate semialdehyde dehydrogenase, and homoserine dehydrogenase, (ii) by rendering the aspartate kinase enzyme insensitive to product inhibition that can be brought about by lysine, methionine, or threonine, and (iii) by deletion of metabolic pathways that branch off the homoserine biosynthesis pathway. Overexpression of aspartate kinase, aspartate semialdehyde dehydrogenase, and homoserine dehydrogenase can be achieved by expressing the enzymes from a multicopy plasmid under the control of an appropriate constitutive or inducible promoter. Alternatively, overexpression of said enzymes can be achieved by deletion of transcriptional repressors that limit the transcription of genes coding for aspartate kinase, aspartate semialdehyde dehydrogenase, and homoserine dehydrogenase. Aspartate kinases can be rendered insensitive to inhibition by aspartate-derived amino acids by introducing appropriate mutations into their amino acid sequences. Entry points into metabolic pathways that branch off the homoserine biosynthesis pathway are catalyzed by enzymes having O-succinyl homoserine or O-acetyl homoserine synthase activity (entry into methionine biosynthesis), homoserine kinase activity (entry into threonine biosynthesis), or diaminopimelate decarboxylase activity (entry into lysine biosynthesis). Deletion of genes encoding proteins having said enzymatic activities avoids formation aspartate-derived amino acids and therefore aids homoserine formation.

[0054] Accordingly, deletion of the genes metA, thrB, and lysA in *E. coli* attenuates pathways that branch of the homoserine biosynthetic pathway. The increase of enzymatic activities of the homoserine pathway in *E. coli* can be achieved, for instance, by the overexpression of the bifunctional aspartate kinase-homoserine dehydrogenase mutant

thrA S345F (insensitive to threonine inhibition) and asd (both genes from *E. coli*); or by the overexpression of the monofunctional aspartate kinase mutant lysC E250K (insensitive to lysine), asd (both genes from *E. coli*), and the homoserine dehydrogenase gene HOM6 from *S cerevisiae*.

[0055] The microorganism of the invention may also have attenuated capacity to export homoserine which increases the intracellular availability of this amino acid. In order to achieve decreased homoserine export from the cells, permeases capable of exporting homoserine can be deleted. Such permeases may be identified by overexpressing genomic libraries in the microorganism and cultivating said microorganism at inhibitory concentrations of homoserine or structurally similar amino acids such as threonine, leucine, or aspartate (Zakataeva et al. 1999/FEBS Lett/452/228-232). Genes whose overexpression confers growth at increased concentrations of either of said amino acids are likely to participate in homoserine export.

[0056] In a further aspect, the microorganism of the invention being *Escherichia coli* carries deletions in the homoserine efflux transporters rhtA, rhtb, and/or rhtC.

[0057] Efficient production of DHB can be ensured by optimizing carbon flux repartitioning in the metabolic network of the host organism with respect to the optimization of cofactor supply for DHB synthesis, and attenuation of competing pathways that cause formation of metabolic by-products other than DHB. An important tool for strain improvement provides constraint-based flux balance analysis. This method allows calculating the theoretical yield of a given metabolic network depending on cultivation conditions, and facilitates identification of metabolic targets for overexpression or deletion. The experimental techniques used for overexpression and deletion of the metabolic target reaction are described (Example 8).

[0058] Accordingly, the microorganism of the invention may also exhibit enzymatic activities chosen among phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase, isocitrate lyase, pyruvate carboxylase, and hexose symporter permease which is increased, and/or at least one of the enzymatic activities chosen among lactate dehydrogenase, alcohol dehydrogenase, acetate kinase, phosphate acetyltransferase, pyruvate oxidase, isocitrate lyase, fumarase, 2-oxoglutarate dehydrogenase, pyruvate kinase, malic enzyme, phosphoglucose isomerase, phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase, pyruvate-formate lyase, succinic semialdehyde dehydrogenase, sugar-transporting phosphotransferase, ketohydroxyglutarate aldolase, homoserine-O-succinyl transferase, homoserine kinase, homoserine efflux transporter, diaminopimelate decarboxylase, and/or methylglyoxal synthase which is (are) decreased.

[0059] In a further aspect, the microorganism of the invention being *Escherichia coli* overexpresses at least one of the genes chosen among ppc, pck, aceA, galP, asd, thrA, metL, lysC all *E. coli*; pycA from *L. lactis*, and/or has at least one of the genes deleted chosen among ldhA, adhE, ackA, pta, poxB, focA, pflB, sad, gabABC, sfcA, maeB, ppc, pykA, pykF, mgsA, sucAB, ptsl, ptsG, pgi, fumABC, aldA, lldD, icIR, metA, thrB, lysA, eda, rhtA, rhtB, rhtC.

[0060] The present invention also encompasses a method of production of 2,4-DHB comprising the steps of

[0061] culturing the modified microorganism of the invention in an appropriate culture medium,

[0062] recovering 2,4-DHB from the culture medium. Said 2,4-DHB can be further purified.

[0063] Product separation and purification is very important factor enormously affecting overall process efficiency and product costs. Methods for product recovery commonly comprise the steps cell separation, as well as product purification, concentration and drying, respectively.

[0064] Cell Separation

[0065] Ultrafiltration and centrifugation can be used to separate cells from the fermentation medium. Cell separation from fermentation media is often complicated by high medium viscosity. Therefore, we can add additives such as mineral acids or alkali salts, or heating of the culture broth to optimize cell separation.

[0066] Product Recovery

[0067] A variety of ion-exchange chromatographic methods can be applied for the separation of DHB either before or after biomass removal. They include the use of primary cation exchange resins that facilitate separation of products according to their isoelectric point. Typically, the resin is charged with the solution, and retained product is eluted separately following increase of pH (e.g. by adding ammonium hydroxide) in the eluent. Another possibility is the use of ion-exchange chromatography using fixed or simulated moving bed resins. Different chromatographic steps may have to be combined in order to attain adequate product purity. Those purification methods are more economical compared with a costly crystallization step, also providing additional advantages and flexibility regarding the form of final product.

[0068] Product Concentration and Drying

[0069] The purification process can also comprise a drying step which may involve any suitable drying means such as a spray granulator, spray dryer, drum dryer, rotary dryer, and tunnel dryer. Concentrated DHB solutions can be obtained by heating fermentation broths under reduced pressure by steam at 130° C. using a multipurpose concentrator or thin film evaporator.

BRIEF DESCRIPTION OF THE FIGURES

[0070] FIG. **1**: Method of preparation of 2,4-DHB from homoserine comprising a two step pathway which employs a first step of conversion of the primary amino group of homoserine to a carbonyl group to obtain OHB, and a second step of reduction of the obtained OHB to 2,4-DHB.

[0071] FIG. **2**: Specific activities of purified *L. lactis* lactate dehydrogenase mutated in position Q85. (A) specific activities on OHB, (B) specific activities on pyruvate, (C) Substrate specificity expressed as ratio of Vmax values on OHB and pyruvate. Values higher than 1 in graph C indicate preference for OHB (no saturation of enzymatic activity was obtained on either substrate for mutated enzymes between 0 and 50 mM OHB or pyruvate). Activities were measured at a substrate concentration of 20 mM.

[0072] FIG. **3**: Specific activities of purified *E. coli* malate dehydrogenase mutated in position R81. (A) specific activities on OHB, (B) specific activities on oxaloacetate. Activities were measured at a substrate concentration of 20 mM OHB or 0.5 mM oxaloacetate.

[0073] The following non limiting examples illustrate the invention.

EXAMPLES

Example 1

Demonstration of OHB Reductase Activity

[0074] Construction of plasmids containing wild-type genes coding for lactate dehydrogenase or malate dehydrogenase:

[0075] The genes coding for (L)-malate dehydrogenase in *Escherichia coli*, Ec-mdh (SEQ ID No. 1), (D)-lactate dehydrogenase in *E. coli*, Ec-ldhA (SEQ ID No. 3), (L)-lactate dehydrogenase of *Lactococcus lactis*, Ll-ldhA (SEQ ID No. 5), (L)-lactate dehydrogenase of *Bacillus subtilis*, Bs-ldh (SEQ ID No. 7), (L)-lactate dehydrogenase of *Geobacillus stearothermophilus*, Gs-ldh (SEQ ID No. 9), the two isoforms of the (L)-lactate dehydrogenase of *Oryctalagus cuniculus*, Oc-ldhA (SEQ ID No. 11 and SEQ ID No. 13), were amplified by PCR using the high-fidelity polymerase Phusion™ (Fermentas) and the primers listed in Table 1. Genomic DNAs of *E. coli* MG1655, *L. Lactis* IL1403, and *B. subtilis* strain 168 were used as the template. The genes Oc-IdhA, and Gs-ldh were codon-optimized for expression in *E. coli* and synthesized by MWG Eurofins. The primers introduced restriction sites (Table 1) upstream of the start codon and downstream of the stop codon, respectively, facilitating the ligation of the digested PCR products into the corresponding sites of the pET28a+ (Novagen) expression vector using T4 DNA ligase (Fermentas). Ligation products were transformed into *E. coli* DH5α cells (NEB). The resulting pET28-Ec-mdh, pET28-Ec-ldhA, pET28-Ll-ldhA, pET28-Bs-ldh, pET28-Gs-ldh, and pET28-Oc-ldhA plasmids were isolated and shown by DNA sequencing to contain the correct full-length sequence of the *E. coli* mdh, *E. coli* ldhA, *L. lactis* ldhA, *B. subtilis* ldh, *G. stearothermophilus* ldh, and *O. cuniculus* ldhA genes, respectively. The corresponding protein sequences are represented by SEQ ID No. 2, SEQ ID No. 4, SEQ ID No. 6, SEQ ID No. 8, SEQ ID No. 10, SEQ ID No. 12 and SEQ ID No. 14, respectively.

TABLE 1

Primer sequences and restriction sites used for amplification and cloning of candidate enzymes

Gene	Forward and reverse primer sequence 5' - 3'	Restriction sites
Ec-mdh	TATAATCATATGAAAGTCGCAGTCCTC (SEQ ID No 15). TATAATGGATCCTTACTTATTAACGAACT C (SEQ ID No. 16)	NdeI BamHI
Ll-ldhA	TATAATCATATGGCTGATAAACAACGTAA AAAA (SEQ ID No. 17) TATAATGGATCCTTAGTTTTTAACTGCAG AAGCAAA (SEQ ID No. 18)	NdeI BamHI
Bs_ldh	TATAATGCTAGCATGATGAACAAACATGT AAATAAAGT (SEQ ID No. 19) TATAATGGATCCTTAGTTGACTTTTTGTT C (SEQ ID No. 20)	NdeI BamHI
Gs-ldh	Gene was delivered by MWG Eurofins™ in pET28a vector	NdeI BamHI
Oc-ldhA	TATAATGCTAGCATGGCGGCGTTGAAAGA C (SEQ ID No. 21) ATTATAGAATTCTTAAAATTGCAGTTCTT T (SEQ ID No. 22)	NheI EcoRI
Ll-panE	TATAATCATATGAGAATTACAATTGCCGG (SEQ ID No. 23) TATAATGGATCCTTATTTTGCTTTTAATA ACTCTTCTTTGC (SEQ ID No. 24)	NdeI BamHI
Ec-ldhA	TATAATCATATGAAACTCGCCGTTTATAG (SEQ ID No. 25) TATAATGGATCCTTAAACCAGTTCGTTCG G (SEQ ID No. 26)	NdeI BamHI

[0076] Expression of enzymes: *E. coli* BL21 (DE3) star cells were transformed with the appropriate plasmids using standard genetic protocols (Sambrook, Fritsch, & Maniatis, 1989). Enzymes with an N-terminal hexa-His tag were expressed in 50 mL LB cultures that were inoculated from an overnight culture at OD_{600} of 0.1 and grown to OD_{600} of 0.6 before protein expression was induced by addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to the culture medium. After 15 h of protein expression, cells were harvested by centrifugation at 4000 g at 4° C. for 10 min and discarding the supernatant. Cell pellets were stored at −20° C. until further analysis. Growth and protein expression were carried out at 25° C. Culture media contained 50 μg/mL kanamycin.

[0077] Purification of enzymes: Frozen cell pellets of expression cultures were resuspended in 0.5 mL of breakage buffer (50 mM Hepes, 300 mM NaCl, pH 7.5) and broken open by four successive rounds of sonication (sonication interval: 20 s, power output: 30%, sonicator: Bioblock Scientific, VibraCell™ 72437). Cell debris was removed by centrifuging the crude extracts for 15 min at 4° C. at 4000 g and retaining the clear supernatant. RNA and DNA were removed from the extracts by adding 15 mg/mL streptomycin sulfate (Sigma), centrifuging the samples at 13000 g for 10 min at 4° C. and retaining the supernatant. Clear protein extract was incubated for 1 h at 4° C. with 0.75 mL (bed volume) of Talon™ Cobalt affinity resin (Clontech). The suspension was centrifuged at 700 g in a table top centrifuge and supernatant was removed. The resin was washed with 10 bed volumes of wash buffer (50 mM Hepes, 300 mM NaCl, 15 mM Imidazole, pH 7.5) before proteins were eluted with 0.5 mL of elution buffer (50 mM Hepes, 300 mM NaCl, 250 mM Imidazole, pH 7.5). Purity of eluted enzymes was verified by SDS-PAGE analysis. Protein concentrations were estimated with the method of Bradford (Bradford (1976, Anal. Biochem. 72: 248-54). To stabilize the lactate dehydrogenase enzymes, the elution buffer was systematically exchanged by 100 mM phosphate buffer adjusted to pH 7. The protein sample was transferred to an Amicon™ Ultra centrifugal filter (cut-off 10 kDa), and centrifuged during 8 min at 4000 g at 4° C. to remove the buffer. The protein was diluted into phosphate buffer and the procedure was repeated 4 times.

[0078] Enzymatic assays: The reaction mixture contained 60 mM Hepes (pH 7), 50 mM potassium chloride, 5 mM

$MgCl_2$, 0.25 mM NADH, (optionally 5 mM fructose-1,6-bisphosphate) (all products from Sigma), and appropriate amounts of purified malate or lactate dehydrogenase or cell extract. Reactions were started by adding appropriate amounts of 2-oxo-4-hydroxybutyrate (OHB), pyruvate, or oxaloacetate (OAA). Enzymatic assays were carried out at 37° C. in 96-well flat bottomed microtiter plates in a final volume of 250 μL. The reactions were followed by the characteristic absorption of NADH at 340 nm (ε_{NADH}=6.22 mM^{-1} cm^{-1}) in a microplate reader (BioRad 680XR).

[0079] OHB was synthesized by incubating 125 mM homoserine with snake venom (L)-amino acid oxidase (1.25 U/mL, Sigma) and catalase (4400 U/mL, Sigma) in 100 mM Tris buffer at pH 7.8 for 90 min at 37° C. Subsequently, the reaction mixture was purified on an Amicon™ Ultra centrifugal filter with a cut-off of 10 kDa to eliminate the enzymes (method adapted from Wellner & Lichtenberg, 1971).

[0080] OHB was quantified by mixing 100 μL of the tested solution with 1 mL of a solution containing 1 M sodium arsenate and 1 M boric acid at pH 6.5. The mixture was incubated at room temperature for 30 min and the absorbance at 325 nm was used to quantify OHB. The relation between absorbance and concentration of the ketone was calibrated using pyruvate solutions of known concentrations (method adapted from (Wellner & Lichtenberg, 1971)). The typical OHB yield of the method was 90%.

[0081] Results: The kinetic parameters are listed in Table 2 for the tested enzymes on their natural substrates and OHB. Significant OHB reductase activity was found for all lactate dehydrogenases of different biological origin. Malate dehydrogenase, Mdh, of *E. coli* only had very minor activity on OHB. The branched chain 2-oxo-acid dehydrogenase, PanE, from *L. lactis* also had significant activity on OHB.

TABLE 2

Summary of kinetic parameters of selected candidate enzymes on their natural substrate and OHB

	Max. specific activity [μmol/(mg min)]		Substrate affinity, Km [mM]	
Enzyme	Natural substrate[a]	OHB[b]	Natural substrate[a]	OHB
Ec-Mdh	95.6	0.01	0.04	ns
Ll-Ldh	184	18	2.7	ns
Gs-Ldh	87.7	66.8	1.2	1.3
Bs-Ldh	170	15.7	nd	ns
Ll-PanE	nd	2.58	nd	ns
Oc-LdhA	68.3	6.5	1.5	13
Ec-LdhA	265	0.56	1.8	4.8

[a]Natural substrates for Mdh and Ldh are oxaloacetate and pyruvate, respectively

[b]When enzymes could not be saturated, maximum specific activity refers to the activity estimated at 20 mM substrate concentration

ns—not saturated

nd—not determined

Example 2

Construction of Lactate Dehydrogenase Enzymes with Improved OHB Reductase Activity

[0082] Site-directed mutagenesis of the *L. lactis* ldhA gene was carried out using the pET28-Ll-ldhA plasmid as the template. Point mutations to change the amino acid sequence were introduced by PCR (Phusion 1U, HF buffer 20% (v/v), dNTPs 0.2 mM, direct and reverse primers 0.04 μM each, template plasmid 30-50 ng, water) using the oligonucleotide pairs listed in Table 3. The genes mutated by PCR contained a new restriction site listed in Table 3 (introduced using silent mutations) in addition to the functional mutation to facilitate identification of mutated clones. The PCR products were digested by DpnI at 37° C. for 1 h to remove template DNA, and transformed into competent *E. coli* DH5α (NEB) cells. The mutated plasmids were identified by restriction site analysis and were verified to carry the desired mutations by DNA sequencing.

TABLE 3

Oligonucleotides used to mutate lactate dehydrogenase ldhA from *L. lactis* (nnk denotes a degenerated codon with k representing either thymine or cytosine)

Protein	Mutation	Primer sequences 5' - 3'	Restriction site
Ll-LdhA	Q85nnk	GTCTTGACTTCTGGTG CTCCANNKAAACCAGG TGAAACGCGTCTT (SEQ ID NO. 27) AAGACGCGTTTCACCT GGTTTMNNTGGAGCAC CAGAAGTCAAGAC (SEQ ID NO. 28)	MluI
Ll-LdhA	I226V	CGTGATGCTGCTTACT CGATCGTCGCTAAAAA AGGTG (SEQ ID No. 99) CACCTTTTTTAGCGAC GATCGAGTAAGCAGCA TCACG (SEQ ID No. 100)	PvuI

Mutant enzymes were expressed, purified and tested for OHB and pyruvate reductase activity as described in Example 1. The activity measurements for both substrates are summarized in FIG. **2**. The results demonstrate that the replacement of Gln85 by preferably alanine, cysteine, asparagine, or methionine yields an increase of the enzyme's specificity for OHB, and/or an increase in maximum specific OHB reductase activity.

The mutation Q85N in Ll-Ldh was combined with mutation I226V. It was demonstrated that this exchange had a major positive impact on substrate affinity for OHB.

TABLE 4

Summary of kinetic parameters of *L. lactis* lactate dehydrogenase A, Ll-LdhA, mutants on pyruvate and OHB

Mutant		Max. specific activity [μmol/(mg min)]		Km [mM]	
Enzyme	Seq ID	Pyruvate	OHB	Pyruvate	OHB
Q85N	SEQ ID No. 30	184	63.9	22.1	29.2
Q85NI226V	SEQ ID No. 32	11.5	4.9	1.4	3.3

Example 3

Construction of Malate Dehydrogenase Enzymes with Improved OHB Reductase Activity

[0083] Site-directed mutagenesis of the mdh gene from *E. coli* was carried out as described in Example 2 using the primers listed in Table 5. Plasmid pET28-Ec-mdh was used as the template.

TABLE 5

Oligonucleotides used to mutate malate dehydrogenase mdh from *E. coli*. (nnk denotes a degenerated codon with k representing either thymine or cytosine)

Protein	Mutation	Primer sequences 5' - 3'	Restr. site
Ec-Mdh	R81nnk	TTATCTCTGCAGGCGT AGCGNNKAAACCCGGG ATGGATCGTTC (SEQ ID No. 33) GAACGATCCATCCCGG GTTTMNNCGCTACGCC TGCAGAGATAA (SEQ ID No. 34)	SmaI
Ec-Mdh	R81AM85E	TTATCTCTGCAGGCGT AGCGGCTAAACCGGGT GAGGATCGTTCCGACC TG (SEQ ID NO. 35) CAGGTCGGAACGATCC TCACCCGGTTTAGCCG CTACGCCTGCAGAGAT AA (SEQ ID NO. 36)	no SmaI
Ec-Mdh	R81AM85Q	TTATCTCTGCAGGCGT AGCGGCTAAACCGGGT CAGGATCGTTCCGACC TG (SEQ ID NO. 37) CAGGTCGGAACGATCC TGACCCGGTTTAGCCG CTACGCCTGCAGAGAT AA (SEQ ID NO. 38)	no SmaI
Ec-Mdh	I12V	GTCGCAGTCCTCGGCG CCGCTGGCGGTGTCGG CCAGGCGCTTGCAC (SEQ ID NO. 39 GTGCAAGCGCCTGGCC GACACCGCCAGCGGCG CCGAGGACTGCGAC (SEQ ID NO. 40)	NarI
Ec-Mdh	G179D	CCG GTT ATT GGC GGC CAC TCT GAT GTT ACC ATT CTG CCG CTG CTG (SEQ ID NO. 41) CAGCAGCGGCAGAATG GTAACATCAGAGTGGC CGCCAATAACCGG (SEQ ID NO. 42)	EaeI
Ec-Mdh	R81AD86S	GGCGTAGCGGCTAAAC CGGGTATGTCTCGTTC CGACCTG (SEQ ID NO. 43)	no SmaI
		CAGGTCGGAACGAGAC ATACCCGGTTTAGCCG CTACGCC (SEQ ID NO. 44)	

Mutant enzymes were expressed, purified and tested for OHB and oxaloacetate reductase activity as described in Example 1. The activity measurements on OHB and oxaloacetate are summarized in FIG. **3**. The results demonstrate that replacement of Arg81 by alanine, cysteine, glycine, histidine, isoleucine, leucine, methionine, asparagine, glutamine, serine, threonine, or valine confer significant OHB reductase activity, and concomitant decrease of oxaloacetate reductase activity.

The mutation R81A in Ec-Mdh was combined with additional changes in the protein sequence. The results are listed in Table 6. It was demonstrated that the introduction of mutations M85Q, M85E, I12V, D86S or G179D result in an increased activity on OHB.

TABLE 6

Summary of kinetic parameters of *E. coli* malate dehydrogenase mutants on oxaloacetate (OAA) and OHB

Mutant		Max. specific activity [μmol/(mg min)]		Km [mM]	
Enzyme	Seq ID	OAA[a]	OHB[b]	OAA	OHB
Wild-type	SEQ ID No. 2	95	0.01	0.04	ns
R81A	SEQ ID No. 102	1.16	1.8	ns	ns
R81A M85Q	SEQ ID No. 104	0.5	4.99	ns	ns
R81A M85E	SEQ ID No. 106	1	3	ns	ns
R81A M85Q I12V	SEQ ID No. 108	1.84	18.9	ns	15
R81A M85E I12V	SEQ ID No. 110	2.2	12.54	ns	ns
R81A G179D	SEQ ID No. 112	0.37	4.16	ns	ns
R81A D86S	SEQ ID No. 1114	0.67	14.6	ns	ns
R81A I12V	SEQ ID No. 115	0.5	4.9	ns	ns
R81A G179D D86S	SEQ ID No. 118	0.54	19	ns	ns

[a]activity was measured at 0.5 mM oxaloacetate

[b]activity was measured at 20 mM OHB

ns—not saturated at concentrations of up to 50 mM of OHB and 0.5 mM of oxaloacetate

Example 4

Demonstration of Homoserine Transaminase Activity for Selected Transaminases

[0084] The genes coding for different transaminases in *E. coli, S. cerevisiae*, and *L. lactis* were amplified by PCR using the high-fidelity polymerase Phusion™ (Finnzymes) and the primers listed in Table 7. Genomic DNA of *E. coli* MG1655,

S. cerevisiae BY4741, and *L. lactis* IL1403 were used as the templates. The primers introduced restriction sites (Table 7) upstream of the start codon and downstream of the stop codon, respectively, facilitating the ligation of the digested PCR products into the corresponding sites of the pET28a+ (Novagen) expression vector using T4 DNA ligase (Biolabs). Ligation products were transformed into *E. coli* DH5a cells. The resulting plasmids were isolated and shown by DNA sequencing to contain the correct full-length sequence of the corresponding genes. The references to the corresponding protein sequences are listed in Table 7.

TABLE 7

Primer sequences and restriction sites used for amplification and cloning of candidate enzymes (Abbreviations used for source organism: Ec - *E. coli*, Sc - *S. cerevisiae*, Ll - *L. lactis*). All the genes were cloned into pET28a+ (Novagen), adding an N-terminal Hexa-HisTag.

Gene	Forward and reverse primer sequences 5' - 3'	Gene sequence	Protein sequence	Restriction sites
Ec-ilvE	tataatgctagcatgaccacgaagaaagctgattaca (SEQ ID No. 47) tataatggatccttattgattaacttgatctaacc (SEQ ID No. 48)	SEQ ID No. 59	SEQ ID No. 60	NheI BamHI
Ec-tyrB	Tataatgctagcgtgtttcaaaaagttgacg (SEQ ID No. 49) Tataatggatccttacatcaccgcagcaaac (SEQ ID No. 50)	SEQ ID No. 61	SEQ ID No. 62	NheI BamHI
Ec-aspC	Tataatgctagcatgtttgagaacattaccgc (SEQ ID No. 51) Tataatggatccttacagcactgccacaatcg (SEQ ID No. 52)	SEQ ID No. 63	SEQ ID No. 64	NheI BamHI
Ll-araT	Tataatgctagcatggatttattaaaaaaatttaaccctaa (SEQ ID No. 53) Tataatggatcctcagccacgtttttagtcacataa (SEQ ID No. 54)	SEQ ID No. 65	SEQ ID No. 66	NheI BamHI
Ll-bcaT	Tataatgctagcatggcaattaatttagactg (SEQ ID No. 55) Tataatggatccttaatcaactttaactatcc (SEQ ID No. 56)	SEQ ID No. 67	SEQ ID No. 68	NheI BamHI
Sc-ARO8	Tataatcatatgatcatgactttacctgaatcaaaaga (SEQ ID No. 57) Tataatggatccctatttggaaataccaaattcttcg (SEQ ID No. 58)	SEQ ID No. 69	SEQ ID No. 70	NheI BamHI

Enzymes were expressed and purified as described in Example 1, and tested for homoserine transaminase activity under the conditions described below.

[0085] Enzymatic assays: Transaminase activity of several candidate aminotransferases was quantified with 2-oxoglutarate as the amino group acceptor. Transaminase reactions were carried out using homoserine and the preferred amino acid of the enzymes. The reactions were followed by the amino acid-dependent oxidation of NADH in the coupled dehydrogenase reaction.

Transaminase Assays (Reaction Scheme)

[0086] Transaminase: Amino acid+2-oxoglutarate->2-oxo-acid+glutamate

Dehydrogenase: 2-oxo-acid+NADH->2-hydroxy-acid+NAD^+

[0087] The reaction mixture contained 60 mM Hepes (pH 7), 50 mM potassium chloride, 5 mM $MgCl_2$, 4 mM 2-oxoglutarate, 0.1 mM pyridoxal-5'-phosphate (PLP), 0.25 mM NADH, (optionally 5 mM fructose-1,6-bisphosphate) (all products from Sigma), 4 Units/mL of auxiliary 2-hydroxyacid dehydrogenase, and appropriate amounts of purified aminotransferase or cell extract. The auxiliary dehydrogenase enzyme was purified PanE from *L. lactis* in case of the amino acids phenylalanine and leucine (Chambellon, Rijnen, Lorquet, Gitton, van HylckamaVlieg, Wouters, &Yvon, 2009), malate dehydrogenase (Sigma) in case of aspartate, and rabbit muscle (L)-lactate dehydrogenase (Sigma) when homoserine was used as the starting substrate. Reactions were started by adding 50 mM of the amino acid.

[0088] Enzymatic assays were carried out at 37° C. in 96-well flat bottomed microtiter plates in a final volume of 250 μL. The reactions were followed by the characteristic absorption of NAD(P)H at 340 nm (ε_{NADPH}=6.22 mM^{-1} cm^{-1}) in a microplate reader (BioRad 680XR).

[0089] Results: The kinetic parameters of different aminotransferases are listed in Table 8. Significant homoserine transaminase activity was found for the listed transaminase enzymes.

TABLE 8

Transaminase activities of tested candidate enzymes on homoserine and their preferred amino acid substrate (Abbreviations used for source organism: Ec—*E. coli*, Sc—*S. cerevisiae*, Ll—*L. lactis*).

	Max. specific activity on different substrates [μmol/(min mg_{prot})]	
Enzyme	Homoserine*	Preferred amino acid
Ec-IlvE	0.077	$10.3^{(L)}$
Ec-TyrB	0.057	$9.03^{(P)}$
Ec-AspC	0.082	$74.031^{(A)}$
Ll-AraT	0.109	$11.72^{(P)}$
Ll-BcaT	0.028	$30.39^{(L)}$
Sc-ARO8	0.076	$20.5^{(P)}$

*activity measured at 50 mM homoserine,

Example 5

Construction of Plasmids for Overexpression of the Homoserine Pathway Enzymes

[0090] Construction of the Plasmids pTAC-op-HMS1 and pACT3-op-HMS1

[0091] The plasmid pET28-LYSCwt was constructed by amplifying the lysC gene by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers $^{5'}$CACGAGGTACATATGTCTGAAATTGTTGTCTCC$^{3'}$ (SEQ ID No. 71) and $^{5'}$CTTCCAGGGGATCCAGTATTTACTCAAAC$^{3'}$ (SEQ ID No. 72) that introduced a NdeI and BamHI restriction sites upstream of the start codon and downstream of the stop codon, respectively. Genomic DNA from *E. coli* MG1655 was used as the template. The PCR product was digested with NdeI and BamHI, ligated into the corresponding sites of the pET28a (Novagen) expression vector using T4 DNA ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pET28-LYSCwt plasmid was isolated and shown by DNA sequencing to contain the full-length lysC gene having the correct sequence (SEQ ID No. 73).

[0092] Site-directed mutagenesis of lysC to alleviate inhibition by lysine was carried out using the pET28-LYSCwt plasmid as the template. A point mutation to change the amino acid sequence in position 250 from glutamate to lysine (E250K, SEQ ID No. 36) was introduced by PCR (Phusion 1U, HF buffer 20% (v/v), dNTPs 0.2 mM, direct and reverse primers 0.04 μM each, template plasmid 50 ng, water) using the oligonucleotides $^{5'}$GCGTTTGCCGAAGCGGCAAAGATGGCCACTTTTG$^{3'}$ (SEQ ID No. 74) and $^{5'}$CAAAAGTGGCCATCTTTGCCGCTTCGGCAAACGC$^{3'}$ (SEQ ID No. 75). The PCR product (SEQ ID No. 35) was digested by DpnI at 37° C. for 1 h to remove template DNA, and transformed into competent *E. coli* DH5α (NEB) cells. The mutated plasmid pET28-LYSC* was identified by restriction site analysis and verified to carry the desired mutations by DNA sequencing.

[0093] The plasmid pET28-ASDwt was constructed by amplifying the asd gene of *E. coli* by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers $^{5'}$TATAATGCTAGCATGAAAAATGTTGGTTTTATCGG$^{3'}$ (SEQ ID No. 76) and $^{5'}$TATAATGGATCCTTACGCCAGTTGACGAAGC$^{3'}$ (SEQ ID No. 77) that introduced a NheI and BamHI restriction site upstream of the start codon and downstream of the stop codon, respectively. Genomic DNA from *E. coli* DH5α was used as the template. The PCR product was digested with NheI and BamHI, ligated into the corresponding sites of the pET28a (Novagen) expression vector using T4 DNA ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pET28-ASDwt plasmid was isolated and shown by DNA sequencing to contain the full-length asd gene having the correct sequence (SEQ ID No. 98).

[0094] The plasmid pET28-HOM6wt was constructed by amplifying the HOM6 gene of *S. cerevisiae* by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers $^{5'}$TATAATCATATGAGCACTAAAGTTGTTAATG$^{3'}$ (SEQ ID No. 78) and $^{5'}$TATAATGGATC-CCTAAAGTCTTTGAGCAATC$^{3'}$ (SEQ ID No. 79) that introduced a NdeI and BamHI restriction site upstream of the start codon and downstream of the stop codon, respectively. Genomic DNA from *S. cerevisiae* BY4741 was used as the template. The PCR product was digested with NdeI and BamHI, ligated into the corresponding sites of the pET28a (Novagen) expression vector using T4 ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pET28-HOM6wt plasmid was isolated and shown by DNA sequencing to contain the full-length HOM6 gene having the correct sequence (SEQ ID No. 97).

[0095] The plasmid pET28-LYSC* was used as the backbone for the construction of the pTAC-op-HMS plasmid that enabled the expression of lysine-insensitive aspartate kinase, aspartate semialdehyde dehydrogenase, and homoserine dehydrogenase from an inducible tac promoter.

[0096] The asd gene was obtained by PCR from pET28-asdwt. The whole coding region and part of the upstream region comprising the pET28 ribosome binding site (rbs) and the in-frame N-terminal His-Tag were amplified by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers $^{5'}$TATAAGGATCCGTTTAACTTTAAGAAGGAGATATACCATGGG$^{3'}$ (SEQ ID No. 80) and $^{5'}$TATAAGAATTCTTACGCCAGTTGACGAAG$^{3'}$ (SEQ ID No. 81) that introduced a BamHI and EcoRI restriction site upstream of the rbs and downstream of the stop codon, respectively. The PCR product was digested with BamHI and EcoRI, ligated into the corresponding sites of pET28-LYSC*, using T4 DNA ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pET28-LYSC*-ASD plasmid was isolated and shown by DNA sequencing to have the correct sequence.

[0097] The HOM6 gene was obtained by PCR from pET28-HOM6wt. The whole coding region and part of the upstream region comprising the pET28 ribosome binding site and the in-frame N-terminal His-Tag were amplified by PCR using high fidelity polymerase Phusion™ (Finnzymes), the direct primer $^{5'}$TATAAGCGGCCGCGTTTAACTTTAAGAAGGAGATAT$^{3'}$ (SEQ ID No. 82), and the reverse primer $^{5'}$TATAAACTCGAGCCTAAAGTCTTTGAGCAAT$^{3'}$ (SEQ ID No. 83) that introduced a NotI and a PspXI restriction site upstream of the rbs and downstream of the stop codon, respectively. The PCR product was digested with NotI and PspXI, ligated into the corresponding sites of pET28-LYSC*-ASD, using T4 DNA ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pET28-op-HMS1 plasmid was isolated and shown by DNA sequencing to have the correct sequence.

[0098] The 5' upstream promoter region simultaneously regulating the expression of the three genes (i.e. the T7 promoter in pET28a+) can be replaced with any other

promoter, inducible or constitutive, by digesting the plasmids with SphI and XbaI and cloning another promoter region with suitable restriction sites.

[0099] In the present non-exclusive example, the T7 promoter of the pET28a+backbone was replaced by the artificial IPTG-inducible tac promoter (de Boer et al., 1983). The tac promoter was obtained from plasmid pEXT20 (Dykxhoorn et al., 1996) by digesting this plasmid with SphI and XbaI. The DNA fragment containing the promoter was purified and cloned into SphI and XbaI digested pET28-op-HMS1 obtaining pTAC-op-HMS1. The resulting pTAC-op-HMS plasmid was isolated and shown by DNA sequencing to have the correct sequence.

[0100] The operon containing the coding sequences of lysC*, asd, and HOM6 was PCR amplified from the plasmid pTAC-op-HMS1 using the primers 5'-TATAAAGATCTTA-GAAATAATTTTGTTTA-3' (SEQ ID No. 84) and 5'-TATAATCTAGACTAAAGTCTTTGAGCAAT-3' (SEQ ID No. 85) which introduced a BglII and a XbaI restriction site at the 5' and the 3' end, respectively, of the PCR fragment. The fragment was purified, digested with BglII and XbaI and cloned into the corresponding sites of pACT3 (Dykxhoorn et al., 1996) to obtain the vector pACT3-op-HMS1. The resulting pACT3-op-HMS1 plasmid was isolated and shown by DNA sequencing to have the correct sequence.

Construction of the Plasmids pEXT20-op-HMS2 and pACT3-op-HMS2

[0101] The plasmid pET28-thrAwt was constructed by amplifying the *E. coli* thrA gene encoding bifunctional enzyme aspartate kinase/homoserine dehydrogenase I by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers 5'-TATAATCATATGC-GAGTGTTGAAGTTCG-3' (SEQ ID No. 86) and 5'-TATAATGGATCCTCAGACTCCTAACTTCCA-3' (SEQ ID No. 87) that introduced a NdeI and BamHI restriction sites upstream of the start codon and downstream of the stop codon, respectively. Genomic DNA from *E. coli* MG1655 was used as the template. The PCR product was digested with NdeI and BamHI, ligated into the corresponding sites of the pET28a+ (Novagen) expression vector using T4 DNA ligase (Biolabs), and transformed into NEB 5-alpha competent *E. coli* cells (NEB). The resulting pET28-thrAwt plasmid was isolated and shown by DNA sequencing to contain the full-length thrA gene having the correct sequence (SEQ ID No.88). The corresponding protein is represented by SEQ ID No.89.

[0102] An aspartate kinase/homoserine dehydrogenase with strongly decreased sensitivity for inhibition by threonine was constructed by site directed mutagenesis, replacing serine in position 345 with phenylalanine (S345F). Site-directed mutagenesis was carried out using the direct and reverse primers 5'-TGT CTCGAGCCCGTATTTTCGTGGTGCTG-3' (SEQ ID No. 90) and 5'-CAGCACCACGAAAATACGGG CTCGAGACA-3' (SEQ ID No.91) and the pET28-thrAwt plasmid as the template. A single point mutation to change the amino acid sequence was introduced by PCR (Phusion 1U, HF buffer 20% (v/v), dNTPs 0.2 mM, direct and reverse primers 0.04 μM each, template plasmid 30-50 ng, water). Plasmids created by PCR contained a new restriction site for XhoI (underlined) introduced by silent mutation in addition to the functional mutation to facilitate identification of mutated clones. The PCR products were digested by DpnI at 37° C. for 1 h to remove template DNA, and transformed into DH5α competent *E. coli* cells (NEB). The mutated plasmid pET_Ec_thrA_S345F was identified by restriction site analysis and verified to carry the desired mutation by DNA sequencing.

[0103] The thrAS345F coding region of the bifunctional *E. coli* aspartate kinase/homoserine dehydrogenase was obtained by PCR using the plasmid pET_Ec_thrA_S345F as the template (SEQ ID No. 92). The whole coding region was amplified by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers 5'-TATAAT GAGCTCGTTTAACTTTAAGAAGGAGATATACCATG CGAGTGTTGA AGTTCGGCG-3' (SEQ ID No. 93) and 5'-TATAATCCCGGGTCAGACTCCTAACTTCCA-3' (SEQ ID No. 94) that introduced a SacI and XmaI restriction site (underlined) upstream of the start codon and downstream of the stop codon, respectively. The direct primer includes the ribosome binding site (bold face) sequence of pET28. The PCR product was digested with SacI and XmaI, ligated into the corresponding sites of either pEXT20 or pACT3 (Dykxhoorn, St Pierre, & Linn, 1996), using T4 DNA ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pEXT20-op-HMS2_step1 and pACT3-op-HMS2_step1 plasmids were isolated and shown by DNA sequencing to have the correct sequence.

[0104] *Escherichia coli* aspartate semialdehyde dehydrogenase asd was amplified by PCR using high fidelity polymerase Phusion™ (Finnzymes) and the direct and reverse primers 5'-TATAAT CCCGGGGTTTAACTTTAAGAAGGAGATATACCATG AAAAATGTTG GTTTTATCGGC-3' (SEQ ID No. 95) and 5'-TATAATGGATCCTTACGCCAGTTGACGAAG-3' (SEQ ID No. 96) that introduced a XmaI and BamHI restriction site upstream of the start codon and downstream of the stop codon, respectively(SEQ ID No. 98). The direct primer includes the ribosome binding site sequence of pET28. Genomic DNA of *E coli* MG1655 was used as the template. The PCR product was digested with XmaI and BamHI, ligated into the corresponding sites of pEXT20-op-HMS2_step1 and pACT3-op-HMS2_step1, directly downstream the *E. coli* thrA gene, using T4 DNA ligase (Biolabs), and transformed into *E. coli* DH5α cells. The resulting pEXT20-op-HMS2 and pACT3-op-HMS2 plasmids were isolated and shown by DNA sequencing to have the correct sequence.

Example 6

Construction of Plasmids for Overexpression of Phosphoenolpyruvate (PEP) Carboxykinase, PEP Carboxylase, Pyruvate Kinase, Pyruvate Carboxylase, Isocitrate Lyase Enzymes and the Galactose Symporter Permease

[0105] The plasmid pACT3-pck harbouring the PEP carboxykinase encoding pck gene of *E. coli* was constructed by amplifying the pck coding sequence using genomic DNA from *E. coli* MG1655 as the template and the forward and reverse primers, respectively, $^{5'}$TATAATCCCGGGATGCGCGTTAACAATGGTTT-GACC$^{3'}$ (SEQ ID No. 119) and $^{5'}$TATAATTCTAGATTA-CAGTTTCGGACCAGCCG$^{3'}$ (SEQ ID No. 120). The DNA fragment was digested with XmaI and XbaI, ligated into the corresponding sites of the pACT3 expression vector (Dykxhoorn et al., 1996) using T4 DNA ligase (Biolabs), and

transformed into *E. coli* DH5α cells. The transformants were selected on solid LB medium containing chloramphenicol (25 μg/mL). The resulting plasmid was isolated and correct insertion of the pck gene was verified by sequencing. Plasmids pACT3-aceA, pACT3-ppc, pACT3-galP, pACT3-pck and pACT3-pycA harbouring, respectively, aceA, ppc, galP, or pck (all *E. coli*) or pycA from *Lactococcus lactis* were constructed analogously using the primers listed in Table 9.

TABLE 9

Primers used for construction of plasmids for gene overexpression. Restriction sites used for cloning into pACT3 are underlined

Gene	Primer	Linker	Sequence
Ec_pck	Ec_pck_clon_for	XmaI	tataatcccgggatgcgcgttaacaatggtttgacc (SEQ ID No. 121)
	Ec_pck_clon_rev	XbaI	tataattctagattacagtttcggaccagccg (SEQ ID No. 122)
Ec_ppc	Ec_ppc_clon_for	XmaI	tataatcccgggatgaacgaacaatattcc (SEQ ID No. 123)
	Ec_ppc_clon_rev	XbaI	tataattctagattagccggtattacgcat (SEQ ID No. 124)
Ec_aceA	Ec_aceA_clon_for	XmaI	tataatcccgggatgaaaacccgtacacaacaaatt (SEQ ID No. 125)
	Ec_aceA_clon_rev	XbaI	tataattctagattagaactgcgattcttcag (SEQ ID No. 126)
Ll_pycA	Ll_pycA_clon_for	XmaI	tataatcccgggatgaaaaaactactcgtcgccaat (SEQ ID No. 127)
	Ll_pycA_clon_rev	XbaI	tataattctagattaattaatttcgattaaca (SEQ ID No. 128)
Ec_galP	Ec_galP_clon_for	XmaI	tataatcccgggatgcctgacgctaaaaaacaggggcggt (SEQ ID No. 129)
	Ec_galP_clon_rev	XbaI	tataattctagattaatcgtgagcgcctatttc (SEQ ID No. 130)

Example 7

Construction of the Plasmid for Overexpression of the Homoserine Transaminase and the OHB Reductase

[0106] The coding sequence of the branched chain amino transferase, IlvE, from *E. coli* was PCR amplified using the forward and reverse primers 5'-ACAATTTCACACAGGAAACAGAATTCGAGCTCGGTACCGTTTAACTTTAAG AAGGAGATATACCATGACCACGAAGAAAGCTGATTAC-3' (SEQ ID No. 131) and 5'-GGATAACTTTTTTACGTTGTTTATCAGCCATGGTATATCTCCTTCTTAAAGT TAAACGGATCCTTATTGATTAACTTG-3' (SEQ ID No. 132), respectively, and plasmid pET28-Ec-ilvE (Example 4) as the template. The coding sequence of lactate dehydrogenase, LdhA, from *L. lactis* was PCR amplified using the forward and reverse primers 5'-TAATATGGATCCGTTTAACTTTAAGAAGGAGATATACCATGGCTGATAAAC AACGTAAAAAAGTTATCC-3' (SEQ ID No. 133) and 5'-CAATGCGGAATATTGTTCGTTCATGGTATATCTCCTTCTTA AAGTTAAACTC TAGATTAGTTTTTAACTGCAGAAGCAAATTC-3' (SEQ ID No. 134), respectively, and plasmid pET28-Ll-ldhA (Example 1) as the template. The amplified PCR fragments were fused in an overlap extension PCR by adding 150 ng of each fragment to 50 μL of the reaction mix and running a PCR using primers 5'-ACAATTTCACACAGGAAACAGAATTCGAGCTCGGTACCGTTTAACTTTAAG AAGGAGATATACCATGACCACGAAGAAAGCTGATTAC-3' (SEQ ID No. 135) and 5'-CAATGCGGAATATTGTTCGTTCATGGTATATCTCCTTCTTAAAGTTAAACTC TAGATTAGTTTTTAACTGCAGAAGCAAATTC-3' (SEQ ID No. 136). The resulting PCR fragment was purified, digested with KpnI and XbaI, and ligated into the corresponding sites of pEXT20 (Dykxhoorn, St Pierre, & Linn, 1996) using T4 DNA ligase (Fermentas). The ligation product was transformed into *E. coli* DH5α. The resulting plasmid pEXT20-DHB was isolated and shown by DNA sequencing to contain the correct full-length coding sequences of Ec-ilvE and Ll-ldhA. The plasmid was then transformed into *E. coli* MG1655-derived mutant strains and tested regarding DHB production.

Example 8

Construction of Optimized Strains for DHB Production

[0107] Several genes were disrupted in *E. coli* strain MG1655 in order to optimise carbon flux repartitioning and cofactor supply for DHB production. Gene deletions were carried out using phage transduction method, or the lambda red recombinase method according to Datsenko et al. (Datsenko & Wanner, 2000).

Protocol for Introduction of Gene Deletions Using the Phage Transduction Method:

[0108] Strains carrying the desired single deletions were obtained from the Keio collection (Baba et al., 2006). Phage lysates of single deletion mutants were prepared by inoculating 10 mL of LB medium containing 50 μg/mL kanamycin, 2 g/L glucose, and 5 mM $CaCl_2$ with 100 μL of overnight precultures. Following an incubation of 1 h at 37° C., 200 μL of phage lysate prepared from the wild-type MG1655 strain were added, and cultures were incubated for another 2-3 h until cell lysis had completed. After addition of 200 μL chloroform, cell preparations were first vigorously vortexted and then centrifuged for 10 min at 4500×g. The clear lysate was recovered and stored at 4° C.

[0109] The receptor strain was prepared for phage transduction by an overnight cultivation at 37° C. in LB medium. A volume of 1.5 mL of the preculture was centrifuged at 1500×g for 10 min. The supernatant was discarded and the cell pellet was resuspended in 600 μl of a solution containing 10 mM $MgSO_4$ and 5 mM $CaCl_2$. The transduction was carried out by mixing 100 μL of the solution containing the

receptor strain with 100 μL of lysate and incubating this mixture at 30° C. for 30 min. Thereafter, 100 μL of a 1M sodium citrate solution were added followed by vigorous vortexing. After addition of 1 mL LB medium, the cell suspension was incubated at 37° C. for 1 h before spreading the cells on LB agar dishes containing 50 μg/mL kanamycin. Clones able to grow in presence of the antibiotic were confirmed by colony PCR to contain the desired deletion using the primers listed in Table 11. After the introduction of each gene deletion, the antibiotic marker was removed as described above following the method of (Cherepanov & Wackernagel, 1995). The deletions ΔldhA, ΔadhE, ΔmetA, ΔthrB, ΔrhtB, and ΔlldD were successively introduced by the described method.

Protocol for Introduction of Gene Deletions Using the Lambda-Red Recombinase Method:

[0110] The deletion cassettes were prepared by PCR using high fidelity polymerase Phusion™ (Finnzymes), and the FRT-flanked kanamycin resistance gene (kan) of plasmid pKD4 as the template (Datsenko & Wanner, 2000). Sense primers contained sequences corresponding to the 5' end of each targeted gene (underlined) followed by 20 bp corresponding to the FRT-kan-FRT cassette of pKD4. Anti-sense primers contained sequences corresponding to the 3' end region of each targeted gene (underlined) followed by 20 bp corresponding to the cassette. The primers are described in Table 10. PCR products were digested with DpnI and purified prior to transformation.

[0111] *E. coli* MG1655 strain was rendered electro-competent by growing the cells to an OD_{600} of 0.6 in LB liquid medium at 37° C., concentrating the cells 100-fold, and washing them twice with ice-cold 10% glycerol. The cells were transformed with plasmid pKD46 (Datsenko & Wanner, 2000) by electroporation (2.5 kV, 200 Ω, 25 μF, in 2 mm gap cuvettes). Transformants were selected at 30° C. on ampicillin (100 μg/mL) LB solid medium.

[0112] Disruption cassettes were transformed into electro-competent *E. coli* strains harbouring the lambda Red recombinase-expressing plasmid pKD46. The cells were grown at 30° C. in liquid SOB medium containing ampicillin (100 μg/mL). The lambda red recombinase system was induced by adding 10 mM arabinose when OD_{600} of the cultures reached 0.1. Cells were further grown to an OD_{600} of 0.6 before they were harvested by centrifugation, washed twice with ice-cold 10% glycerol, and transformed with the disruption cassette by electroporation. After an overnight phenotypic expression at 30° C. in LB liquid medium, cells were plated on solid LB medium containing 25 μg/mL kanamycin. Transformants were selected after cultivation at 30° C.

[0113] The gene replacement was verified by colony PCR using Crimson Taq polymerase (NEB). A first reaction was carried out with the flanking locus-specific primers (see Table 11) to verify simultaneous loss of the parental fragment and gain of the new mutant specific fragment. Two additional reactions were done by using one locus-specific primer together with one of the corresponding primers k1 rev, or k2 for (see Table 11) that align within the FRT-kanamycin resistance cassette (sense locus primer/k1 rev and k2for/reverse locus primer).

[0114] The resistance gene (FRT-kan-FRT) was subsequently excised from the chromosome using the FLP recombinase-harbouring plasmid pCP20 (Cherepanov & Wackernagel, 1995) leaving a scar region containing one FRT site. pCP20 is an ampicillin and CmR plasmid that shows temperature-sensitive replication and thermal induction of FLP recombinase synthesis. Kanamycin resistant mutants were transformed with pCP20, and ampicillin-resistant transformants were selected at 30° C. Transformants were then grown on solid LB medium at 37° C. and tested for loss of all antibiotic resistances. Excision of the FRT-kanamycin cassette was analysed by colony PCR using crimson taq polymerase and the flanking locus-specific primers (Table 11). Multiple deletions were obtained by repeating the above described steps.

TABLE 10

Primers used for gene disruptions.
Sequences homologous to target genes are underlined

Gene	Primer	Sequence
ldhA	Δ_ldhA_for	gaaggttgcgcctacactaagcatagttgttgatgagtgtaggctggagctgcttc (SEQ ID No. 137)
	Δ_ldhA_rev	ttaaaccagttcgttcgggcaggtttcgccttttttcatgggaattagccatggtcc SEQ ID No. 138)
adhE	Δ_adhE_for	atggctgttactaatgtcgctgaacttaacgcactcgtagaagcgtgtgtaggctggagctgcttc (SEQ ID No. 139)
	Δ_adhE_rev	ttaagcggatttttttcgctttttttctcagctttagccggagcagccatatgaatatcctccttag (SEQ ID No. 140)
ackA	Δ_ackA_for	atgtcgagtaagttagtactggttctgaactgcggtagttcttcagtgtaggctggagctgcttc (SEQ ID No. 141
	Δ_ackA_rev	tcaggcagtcaggcggctcgcgtcttgcgcgataaccagttcttccatatgaatatcctccttag (SEQ ID No. 142)
focA-pflB	Δ_focA-pflB_for	ttactccgtatttgcataaaaaccatgcgagttacgggcctataagtgtaggctggagctgcttc (SEQ ID No. 143)
	Δ_focA-pflB_rev	atagattgagtgaaggtacgagtaataacgtcctgctgctgttctcatatgaatatcctccttag (SEQ ID No. 144)
pta	Δ_pta_for	gtgtcccgtattattatgctgatccctaccggaaccagcgtcggtgtgtaggctggagctgcttc (SEQ ID No. 145)
	Δ_pta_rev	ttactgctgctgtgcagactgaatcgcagtcagcgcgatggtgtacatatgaatatcctccttag (SEQ ID No. 146)

TABLE 10-continued

Primers used for gene disruptions.
Sequences homologous to target genes are underlined

Gene	Primer	Sequence
poxB	Δ_poxB_for	atgaaacaaacggttgcagcttatatcgccaaaacactcgaatcggtgtaggctggagctgcttc (SEQ ID No. 147)
	Δ_poxB_rev	ttaccttagccagtttgttttcgccagttcgatcacttcatcacccatatgaatatcctccttag (SEQ ID No. 148)
sad	Δ_sad_for	atgaccattactccggcaactcatgcaatttcgataaatcctgccgtgtaggctggagctgcttc (SEQ ID No. 149)
	Δ_sad_rev	tcagatccggtctttccacaccgtctggatattacagaattcgtgcatatgaatatcctccttag (SEQ ID No. 150)
gabD	Δ_gabD_for	atgaaacttaacgacagtaacttattccgccagcaggcgttgattgtgtaggctggagctgcttc (SEQ ID No. 151)
	Δ_gabD_rev	ttaaagaccgatgcacatatatttgatttctaagtaatcttcgatcatatgaatatcctccttag (SEQ ID No. 152)
gadA	Δ_gadA_for	atggaccagaagctgttaacggatttccgctcagaactactcgatgtgtaggctggagctgcttc (SEQ ID No. 153)
	Δ_gadA_rev	tcaggtgtgtttaaagctgttctgctgggcaataccctgcagtttcatatgaatatcctccttag (SEQ ID No. 154)
gadB	Δ_gadB_for	atggataagaagcaagtaacggatttaaggtcggaactactcgatgtgtaggctggagctgcttc (SEQ ID No. 155)
	Δ_gadB_rev	tcaggtatgtttaaagctgttctgttgggcaataccctgcagtttcatatgaatatcctccttag (SEQ ID No. 156)
gadC	Δ_gadC_for	atggctacatcagtacagacaggtaaagctaagcagctcacattagtgtaggctggagctgcttc (SEQ ID No. 157)
	Δ_gadC_rev	ttagtgtttcttgtcattcatcacaatatagtgtggtgaacgtgccatatgaatatcctccttag (SEQ ID No. 158)
sfcA	Δ_sfcA_for	atggaaccaaaaacaaaaaaacagcgttcgctttatatcccttacgtgtaggctggagctgcttc (SEQ ID No. 159)
	Δ_sfcA_rev	ttagatggaggtacggcggtagtcgcggtattcggcttgccagaacatatgaatatcctccttag (SEQ ID No. 160)
maeB	Δ_maeB_for	atggatgaccagttaaaacaaagtgcacttgatttccatgaatttgtgtaggctggagctgcttc (SEQ ID No. 161)
	Δ_maeB_rev	ttacagcggttgggtttgcgcttctaccacggccagcgccaccatcatatgaatatcctccttag (SEQ ID No. 162)
ppc	Δ_ppc_for	atgaacgaacaatattccgcattgcgtagtaatgtcagtatgctcgtgtaggctggagctgcttc (SEQ ID No. 163)
	Δ_ppc_rev	ttagccggtattacgcatacctgccgcaatcccggcaatagtgaccatatgaatatcctccttag (SEQ ID No. 164)
pykA	Δ_pykA_for	atgtccagaaggcttcgcagaacaaaaatcgttaccacgttaggcgtgtaggctggagctgcttc (SEQ ID No. 165)
	Δ_pykA_rev	ttactctaccgttaaaatacgcgtggtattagtagaacccacggtcatatgaatatcctccttag (SEQ ID No. 166)
pykF	Δ_pykF_for	atgaaaaagaccaaaattgtttgcaccatcggaccgaaaaccgaagtgtaggctggagctgcttc (SEQ ID No. 167)
	Δ_pykF_rev	ttacaggacgtgaacagatgcggtgttagtagtgccgctcggtaccatatgaatatcctccttag (SEQ ID No. 168)
mgsA	Δ_mgsA_for	atggaactgacgactcgcactttacctgcgcggaaacatattgcggtgtaggctggagctgcttc (SEQ ID No. 169)
	Δ_mgsA_rev	ttacttcagacggtccgcgagataacgctgataatcggggatcagcatatgaatatcctccttag (SEQ ID No. 170)
iclR	Δ_iclR_for	atggtcgcacccattcccgcgaaacgcggcagaaaacccgccgttgtgtaggctggagctgcttc (SEQ ID No. 171)
	Δ_iclR_rev	tcagcgcattccaccgtacgccagcgtcacttccttcgccgctttcatatgaatatcctccttag (SEQ ID No. 172)
icd	Δ_icd_for	atggaaagtaaagtagttgttccggcacaaggcaagaagatcaccgtgtaggctggagctgcttc (SEQ ID No. 173)
	Δ_icd_rev	ttacatgttttcgatgatcgcgtcaccaaactctgaacatttcagcatatgaatatcctccttag (SEQ ID No. 174)

TABLE 10-continued

Primers used for gene disruptions.
Sequences homologous to target genes are underlined

Gene	Primer	Sequence
sucA	Δ_sucA_for	atgcagaacagcgctttgaaagcctggttggactcttcttacctcgtgtaggctggagctgcttc (SEQ ID No. 175)
	Δ_sucA_rev	ttattcgacgttcagcgcgtcattaaccagatcttgttgctgtttcatatgaatatcctccttag (SEQ ID No. 176)
sucB	Δ_sucB_for	atgagtagcgtagatattctggtccctgacctgcctgaatccgtagtgtaggctggagctgcttc (SEQ ID No. 177)
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frdB	Δ_frdB_for	atggctgagatgaaaaacctgaaaattgaggtggtgcgctataacgtgtaggctggagctgcttc (SEQ ID No. 181)
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	Δ_thrB_rev	ttagttttccagtactcgtgcgcccgccgtatccagccggcaaatcatatgaatatcctccttag (SEQ ID No. 200)
lysA	Δ_lysA_for	atgccacattcactgttcagcaccgataccgatctcaccgccgaagtgtaggctggagctgcttc (SEQ ID No. 201)
	Δ_lysA_rev	ttaaagcaattccagcgccagtaattcttcgatggtctggcgacgcatatgaatatcctccttag (SEQ ID No. 202)

TABLE 10-continued

Primers used for gene disruptions.
Sequences homologous to target genes are underlined

Gene	Primer	Sequence
eda	Δ_eda_for	atgaaaaactggaaaacaagtgcagaatcaatcctgaccaccggcgtgtaggctggagctgcttc (SEQ ID No. 203)
	Δ_eda_rev	ctcgatcgggcattttgacttttacagcttagcgccttctacagccatatgaatatcctccttag (SEQ ID No. 204)
recA	Δ_recA_for	atggctatcgacgaaaacaaacagaaagcgttggcggcagcactggtgtaggctggagctgcttc (SEQ ID No. 205)
	Δ_recA_rev	ttaaaaatcttcgttagtttctgctacgccttcgctatcatctaccatatgaatatcctccttag (SEQ ID No. 206)
asd	Δ_asd_for	atgaaaaatgttggttttatcggctggcgcggtatggtcggctccgtgtaggctggagctgcttc (SEQ ID No. 207)
	Δ_asd_rev	ttacgccagttgacgaagcatccgacgcagcggctccgcggccccatatgaatatcctccttag (SEQ ID No. 208)

TABLE 11

Primer pairs used for
verification of gene disruptions

Deleted gene	Sequence (5' - 3') Forwardprimer	Reverse primer
K2 for/ k1 rev	cggtgccctgaatgaactgc (SEQ ID No. 209)	cagtcatagccgaatagcct (SEQ ID No. 210)
ldhA	atacgtgtcccgagcggtag (SEQ ID No. 211)	tacacatcccgccatcagca (SEQ ID No. 212)
adhE	Gaagtaaacgggaaaatcaa (SEQ ID No. 213)	Agaagtggcataagaaaacg (SEQ ID No. 214)
ackA	ccattggctgaaaattacgc (SEQ ID No. 215)	gttccattgcacggatcacg (SEQ ID No. 216)
focA_pflB	atgccgtagaagccgccagt (SEQ ID No. 217)	tgttggtgcgcagctcgaag (SEQ ID No. 218)
pta	gcaaatctggtttcatcaac (SEQ ID No. 219)	tcccttgcacaaaacaaagt (SEQ ID No. 220)
poxB	ggatttggttctcgcataat (SEQ ID No. 221)	agcattaacggtagggtcgt (SEQ ID No. 222)
sad	gctgattctcgcgaataaac (SEQ ID No. 223)	aaaaacgttcttgcgcgtct (SEQ ID No. 224)
gabD	tctgtttgtcaccaccccgc (SEQ ID No. 225)	Aagccagcacctggaagcag (SEQ ID No. 226)
gadA	aagagctgccgcaggaggat (SEQ ID No. 227)	gccgccctcttaagtcaaat (SEQ ID No. 228)
gadB	ggattttagcaatattcgct (SEQ ID No. 229)	cctaatagcaggaagaagac (SEQ ID No. 230)
gadC	gctgaactgttgctggaaga (SEQ ID No. 231)	ggcgtgcttttacaactaca (SEQ ID No. 232)
sfcA	tagtaaataacccaaccggc (SEQ ID No. 233)	tcagtgagcgcagtgtttta (SEQ ID No. 234)
maeB	attaatggtgagagtttgga (SEQ ID No. 235)	tgcttttttttattattcgc (SEQ ID No. 236)
ppc	gctttataaaagacgacgaa (SEQ ID No. 237)	gtaacgacaattccttaagg (SEQ ID No. 238)

TABLE 11-continued

Deleted gene	Primer pairs used for verification of gene disruptions Sequence (5' - 3') Forwardprimer	Reverse primer
pykA	tttatatgcccatggtttct (SEQ ID No. 239)	atctgttagaggcggatgat (SEQ ID No. 240)
pykF	ctggaacgttaaatctttga (SEQ ID No. 241)	ccagtttagtagctttcatt (SEQ ID No. 242)
iclR	gatttgttcaacattaactcatcgg (SEQ ID No. 243)	tgcgattaacagacaccctt (SEQ ID No. 244)
mgsA	tctcaggtgctcacagaaca (SEQ ID No. 245)	tatggaagaggcgctactgc (SEQ ID No. 246)
icd	cgacctgctgcataaacacc (SEQ ID No. 247)	tgaacgctaaggtgattgca (SEQ ID No. 248)
sucA	acgtagacaagagctcgcaa (SEQ ID No. 249)	catcacgtacgactgcgtcg (SEQ ID No. 250)
sucB	tgcaactttgtgctgagcaa (SEQ ID No. 251)	tatcgcttccgggcattgtc (SEQ ID No. 252)
frdA	Aaatcgatctcgtcaaatttcagac (SEQ ID No. 253)	aggaaccacaaatcgccata (SEQ ID No. 254)
frdB	gacgtgaagattactacgct (SEQ ID No. 255)	agttcaatgctgaaccacac (SEQ ID No. 256)
frdC	tagccgcgaccacggtaagaaggag (SEQ ID No. 257)	cagcgcatcacccggaaaca (SEQ ID No. 258)
frdD	atcgtgatcattaacctgat (SEQ ID No. 259)	ttaccctgataaattaccgc (SEQ ID No. 260)
ptsG	ccatccgttgaatgagtttt (SEQ ID No. 261)	tggtgttaactggcaaaatc (SEQ ID No. 262)
ptsI	gtgacttccaacggcaaaag (SEQ ID No. 263)	ccgttggtttgatagcaata (SEQ ID No. 264)
lacI	Gaatctggtgtatatggcga (SEQ ID No. 265)	Tcttcgctattacgccagct (SEQ ID No. 266)
lldD	Cgtcagcggatgtatctggt (SEQ ID No. 267)	Gcggaatttctggttcgtaa (SEQ ID No. 268)
pgi	Ttgtcaacgatggggtcatg (SEQ ID No. 269)	Aaaaatgccgacataacgtc (SEQ ID No. 270)
lysA	Tctcaaagcgcgcaagttcg (SEQ ID No. 271)	Ggtattgatgtaccgggtgagatt (SEQ ID No. 272)
metA	Tcgacagaacgacaccaaat (SEQ ID No. 273)	Cactgtgaacgaaggatcgt (SEQ ID No. 274)
thrB	Tgttggcaatattgatgaag (SEQ ID No. 275)	Gacatcgctttcaacattgg (SEQ ID No. 276)
eda	Gacagacaggcgaactgacg (SEQ ID No. 277)	Gcgcagatttgcagattcgt (SEQ ID No. 278)
recA	Tggcggcagtgaagagaagc (SEQ ID No. 279)	Gcaataacgcgctcgtaatc (SEQ ID No. 280)
asd	Acaaagcaggataagtcgca (SEQ ID No. 281)	Gacttcaggtaaggctgtga (SEQ ID No. 282)
rhtA	CAGAGAACTGCGTAAGTATTACGCA (SEQ ID No. 283)	TAGTGGTAACAAGCGTGAAAAACAA (SEQ ID No. 284)

TABLE 11-continued

Deleted gene	Primer pairs used for verification of gene disruptions: Sequence (5' - 3') Forwardprimer	Reverse primer
rhtB	ATGAAGACTCCGTAAACGTTTCCCC (SEQ ID No. 285)	CAAAAATAGACACACCGGGAGTTCA (SEQ ID No. 286)

[0115] The plasmid co-expressing aspartate kinase, aspartate semialdehyde dehydrogenase, and homoserine dehydrogenase (pACT3-op-HMS1) was transformed together with the plasmid expressing the homoserine transaminase and the OHB reductase (pEXT2O-DHB) into the optimized host strains. Transformants were selected on solid LB medium containing chloramphenicol (25 μg/mL) and ampicillin (100 μg/mL). Non-exclusive examples of constructed strains are listed in Table 12.

TABLE 12

Examples of strains constructed for DHB production

Strain	Relevant Genotype
MG1655	Wild-type
ECE73	ΔldhA ΔadhE ΔmetA ΔthrB
ECE74	ΔldhA ΔadhE ΔmetA ΔthrB pACT3-op-HMS1
ECE75	ΔldhA ΔadhE ΔmetA ΔthrB pEXT20-DHB
ECE76	ΔldhA ΔadhE ΔmetA ΔthrB pACT3-op-HMS1 pEXT20-DHB
ECE77	ΔldhA ΔadhE ΔmetA ΔthrB ΔlldD pACT3-op-HMS1 pEXT20-DHB
ECE78	ΔldhA ΔadhE ΔmetA ΔthrB ΔrhtB pACT3-op-HMS1 pEXT20-DHB

[0116] It is understood that removal of the lacI gene from the backbone of the above described plasmids along with the genomic deletion of lacI in the host strain may render protein expression from above described plasmids constitutive.

Example 9

Demonstration of the Zymotic Production of DHB Via the Homoserine-OHB Pathway

[0117] Strains and cultivation conditions: Experiments were carried out with strains listed in Table 12. All cultivations were carried out at 37° C. on an Infors rotary shaker running at 170 rpm. Overnight cultures (3 mL medium in test tube) were inoculated from glycerol stocks and used to adjust an initial OD_{600} of 0.05 in 100 mL growth cultures cultivated in 500 mL shake flasks. IPTG was added at a concentration of 1 mmol/L when OD_{600} in the growth cultures reached 0.8. One liter culture medium contained, 20 g glucose, 18 g Na_2HPO_4*12 H_2O, 3 g KH_2PO_4, 0.5 g NaCl, 2 g NH_4Cl, 0.5 g $MgSO_4$*7 H_2O, 0.015 $CaCl_2$*2 H_2O, 1 mL of 0.06 mol/L $FeCl_3$ stock solution prepared in 100 times diluted concentrated HCl, 2 mL of 10 mM thiamine HCl stock solution, 20 g MOPS and 1 mL of trace element solution (containing per liter: 0.04 g Na_2EDTA*2H_2O, 0.18 g $CoCl_2$*6 H_2O, ZnSO4*7 H_2O, 0.04 g Na_2MoO4*2 H_2O, 0.01 g H_3BO_3, 0.12 g $MnSO_4$*H_2O, 0.12 g $CuCl_2$*H2O.). Medium pH was adjusted to 7 and medium was filter-sterilized. The antibiotics kanamycin sulphate, ampicillin, and chloramphenicol were added at concentrations of 50 mg/L, 100 mg/L, and 25 mg/L, respectively, when necessary.

[0118] Estimation of DHB concentration by LC-MS analyses: Liquid anion exchange chromatography was performed on an ICS-3000 system from Dionex (Sunnyvale, USA) equipped with an automatic eluent (KOH) generator system (RFIC, Dionex), and an autosampler (AS50, Dionex) holding the samples at 4° C. Analytes were separated on an IonPac AS11 HC (250×2 mm, Dionex) column protected by an AG11 HC (50×2 mm, Dionex) pre-column. Column temperature was held at 25° C., flow rate was fixed at 0.25 mL/min, and analytes were eluted applying the KOH gradient described earlier (Groussac E, Ortiz M & Francois J (2000): Improved protocols for quantitative determination of metabolites from biological samples using high performance ionic-exchange chromatography with conductimetric and pulsed amperometric detection. *Enzyme. Microb. Technol.* 26, 715-723). Injected sample volume was 15 μL. For background reduction, an ASRS ultra II (2 mm, external water mode, 75 mA) anion suppressor was used. Analytes were quantified using a mass-sensitive detector (MSQ Plus, Thermo) running in ESI mode (split was ⅓, nitrogen pressure was 90 psi, capillary voltage was 3.5 kV, probe temperature was 450° C.).

[0119] Results:

[0120] After 24 h cultivation, the DHB concentration in the supernatant of different strains was quantified by LC-MS analyses. The strains ECE73, ECE74, ECE75, and ECE76 had produced 0 mg/L, 3.7 mg/L, 0.67 mg/L, and 11.9 mg/L of DHB, respectively.

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SEQUENCE LISTING

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tctggtgctc cacaaaaacc aggtgaaact cgtcttgacc ttgttgaaaa aaatcttcgt    300

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gctgctaacc cagttgatat cttgacatac gctacttgga aattctcagg tttccctaaa    420

aaccgcgttg taggttcagg tacttcactt gatactgcac gtttccgtca agcattggca    480

gaaaaagttg atgttgacgc tcgttcaatc cacgcataca tcatgggtga acacggtgac    540

tcagaatttg ccgtttggtc acacgctaac gttgctggtg ttaaattgga acaatggttc    600

caagaaaatg actaccttaa cgaagctgaa atcgttgaat tgtttgaatc tgtacgtgat    660

gctgcttact caatcatcgc taaaaaaggt gcaacattct atggtgtcgc tgtagctctt    720

gctcgtatta ctaaagcaat tcttgatgat gaacatgcag tacttccagt atcagtattc    780

caagatggac aatatggcgt aagcgactgc taccttggtc aaccagctgt agttggtgct    840

gaaggtgttg ttaacccaat ccacattcca ttgaatgatg ctgaaatgca aaaaatggaa    900

gcttctggtg ctcaattgaa agcaatcatt gacgaagctt ttgctaaaga agaatttgct    960

tctgcagtta aaaactaa                                                  978
```

<210> SEQ ID NO 6
<211> LENGTH: 325
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 6

```
Met Ala Asp Lys Gln Arg Lys Lys Val Ile Leu Val Gly Asp Gly Ala
1               5                   10                  15

Val Gly Ser Ser Tyr Ala Phe Ala Leu Val Asn Gln Gly Ile Ala Gln
            20                  25                  30

Glu Leu Gly Ile Val Asp Leu Phe Lys Glu Lys Thr Gln Gly Asp Ala
        35                  40                  45

Glu Asp Leu Ser His Ala Leu Ala Phe Thr Ser Pro Lys Lys Ile Tyr
    50                  55                  60

Ser Ala Asp Tyr Ser Asp Ala Ser Asp Ala Asp Leu Val Val Leu Thr
65                  70                  75                  80

Ser Gly Ala Pro Gln Lys Pro Gly Glu Thr Arg Leu Asp Leu Val Glu
                85                  90                  95
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Lys Asn Leu Arg Ile Thr Lys Asp Val Val Thr Lys Ile Val Ala Ser
            100                 105                 110

Gly Phe Lys Gly Ile Phe Leu Val Ala Ala Asn Pro Val Asp Ile Leu
        115                 120                 125

Thr Tyr Ala Thr Trp Lys Phe Ser Gly Phe Pro Lys Asn Arg Val Val
    130                 135                 140

Gly Ser Gly Thr Ser Leu Asp Thr Ala Arg Phe Arg Gln Ala Leu Ala
145                 150                 155                 160

Glu Lys Val Asp Val Asp Ala Arg Ser Ile His Ala Tyr Ile Met Gly
                165                 170                 175

Glu His Gly Asp Ser Glu Phe Ala Val Trp Ser His Ala Asn Val Ala
            180                 185                 190

Gly Val Lys Leu Glu Gln Trp Phe Gln Glu Asn Asp Tyr Leu Asn Glu
        195                 200                 205

Ala Glu Ile Val Glu Leu Phe Glu Ser Val Arg Asp Ala Ala Tyr Ser
    210                 215                 220

Ile Ile Ala Lys Lys Gly Ala Thr Phe Tyr Gly Val Ala Val Ala Leu
225                 230                 235                 240

Ala Arg Ile Thr Lys Ala Ile Leu Asp Asp Glu His Ala Val Leu Pro
                245                 250                 255

Val Ser Val Phe Gln Asp Gly Gln Tyr Gly Val Ser Asp Cys Tyr Leu
            260                 265                 270

Gly Gln Pro Ala Val Val Gly Ala Glu Gly Val Val Asn Pro Ile His
        275                 280                 285

Ile Pro Leu Asn Asp Ala Glu Met Gln Lys Met Glu Ala Ser Gly Ala
    290                 295                 300

Gln Leu Lys Ala Ile Ile Asp Glu Ala Phe Ala Lys Glu Glu Phe Ala
305                 310                 315                 320

Ser Ala Val Lys Asn
                325
```

<210> SEQ ID NO 7
<211> LENGTH: 966
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 7

```
atgatgaaca aacatgtaaa taaagtagct ttaatcggag cgggttttgt tggaagcagt     60

tatgcatttg cgttaattaa ccaaggaatc acagatgagc ttgtggtcat tgatgtaaat    120

aaagaaaaag caatgggcga tgtgatggat ttaaaccacg gaaaggcgtt tgcgccacaa    180

ccggtcaaaa catcttacgg aacatatgaa gactgcaagg atgctgatat tgtctgcatt    240

tgcgccggag caaaccaaaa acctggtgag acacgccttg aattagtaga aaagaacttg    300

aagattttca aaggcatcgt tagtgaagtc atggcgagcg gatttgacgg cattttctta    360

gtcgcgacaa atccggttga tatcctgact tacgcaacat ggaaattcag cggcctgcca    420

aaagagcggg tgattggaag cggcacaaca cttgattctg cgagattccg tttcatgctg    480

agcgaatact ttggcgcagc gcctcaaaac gtacacgcgc atattatcgg agagcacggc    540

gacacagagc ttcctgtttg gagccacgcg aatgtcggcg gtgtgccggt cagtgaactc    600

gttgagaaaa acgatgcgta caaacaagag gagctggacc aaattgtaga tgatgtgaaa    660
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aacgcagctt accatatcat tgagaaaaaa ggcgcgactt attatggggt tgcgatgagt 720

cttgctcgca ttacaaaagc cattcttcat aatgaaaaca gcatattaac tgtcagcaca 780

tatttggacg ggcaatacgg tgcagatgac gtgtacatcg gtgtgccggc tgtcgtgaat 840

cgcggaggga tcgcaggtat cactgagctg aacttaaatg agaaagaaaa agaacagttc 900

cttcacagcg ccggcgtcct taaaaacatt ttaaaacctc attttgcaga acaaaaagtc 960

aactaa 966

<210> SEQ ID NO 8
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 8

Met Met Asn Lys His Val Asn Lys Val Ala Leu Ile Gly Ala Gly Phe
1 5 10 15

Val Gly Ser Ser Tyr Ala Phe Ala Leu Ile Asn Gln Gly Ile Thr Asp
20 25 30

Glu Leu Val Val Ile Asp Val Asn Lys Glu Lys Ala Met Gly Asp Val
35 40 45

Met Asp Leu Asn His Gly Lys Ala Phe Ala Pro Gln Pro Val Lys Thr
50 55 60

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

Cys Ala Gly Ala Asn Gln Lys Pro Gly Glu Thr Arg Leu Glu Leu Val
85 90 95

Glu Lys Asn Leu Lys Ile Phe Lys Gly Ile Val Ser Glu Val Met Ala
100 105 110

Ser Gly Phe Asp Gly Ile Phe Leu Val Ala Thr Asn Pro Val Asp Ile
115 120 125

Leu Thr Tyr Ala Thr Trp Lys Phe Ser Gly Leu Pro Lys Glu Arg Val
130 135 140

Ile Gly Ser Gly Thr Thr Leu Asp Ser Ala Arg Phe Arg Phe Met Leu
145 150 155 160

Ser Glu Tyr Phe Gly Ala Ala Pro Gln Asn Val His Ala His Ile Ile
165 170 175

Gly Glu His Gly Asp Thr Glu Leu Pro Val Trp Ser His Ala Asn Val
180 185 190

Gly Gly Val Pro Val Ser Glu Leu Val Glu Lys Asn Asp Ala Tyr Lys
195 200 205

Gln Glu Glu Leu Asp Gln Ile Val Asp Asp Val Lys Asn Ala Ala Tyr
210 215 220

His Ile Ile Glu Lys Lys Gly Ala Thr Tyr Tyr Gly Val Ala Met Ser
225 230 235 240

Leu Ala Arg Ile Thr Lys Ala Ile Leu His Asn Glu Asn Ser Ile Leu
245 250 255

Thr Val Ser Thr Tyr Leu Asp Gly Gln Tyr Gly Ala Asp Asp Val Tyr
260 265 270

Ile Gly Val Pro Ala Val Val Asn Arg Gly Gly Ile Ala Gly Ile Thr
275 280 285

Glu Leu Asn Leu Asn Glu Lys Glu Lys Glu Gln Phe Leu His Ser Ala
290 295 300

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Gly Val Leu Lys Asn Ile Leu Lys Pro His Phe Ala Glu Gln Lys Val
305 310 315 320

Asn

<210> SEQ ID NO 9
<211> LENGTH: 954
<212> TYPE: DNA
<213> ORGANISM: Geobacillus sterarothermophillus

<400> SEQUENCE: 9

atgaagaaca atggtggagc gcgtgttgtg gtaattggcg cgggttttgt gggtgccagc 60

tatgttttcg cgttaatgaa ccaaggtatt gcagacgaga ttgtcctgat tgacgcgaat 120

gaatccaaag cgattgggga cgccatggat ttcaaccacg gtaaagtgtt tgctccgaaa 180

ccggtcgata tctggcatgg cgattacgac gattgtcgcg atgccgatct ggtggtcatc 240

tgcgctggtg caaaccagaa acccggtgaa actcgtctgg atcttgttga caagaacatt 300

gccatctttc ggtctattgt cgaaagcgtg atggcaagtg ggtttcaggg actgtttctg 360

gttgccacca atccggtaga catcctgacg tatgctacct ggaaattcag cggcttaccg 420

catgaacgtg ttatcggcag tggtaccatt cttgatacgg cacgttttcg cttcctgttg 480

ggagagtact tctccgttgc ccctcagaat gtgcatgcct acatcattgg ggaacatggc 540

gataccgaat tgccagtgtg gtcgcaagcg tatattggtg taatgccgat tcgcaaactg 600

gtggaatcga aaggcgaaga agcccagaaa gacttggaac gcatctttgt caacgtacgc 660

gatgcagcgt atcagatcat cgagaaaaaa ggtgcgacct attacggcat cgcaatgggc 720

ttagctcgtg taactcgggc tattctgcac aacgagaacg cgattctcac agtgtcagcg 780

tatctcgatg ggctgtatgg cgaacgcgat gtgtacattg gcgttccagc cgtcatcaat 840

cgcaatggca ttcgtgaggt gattgaaatc gaactgaacg atgacgagaa gaatcgcttc 900

catcactctg cggctacact gaaaagcgtt ctcgcacgtg cgtttacgcg ctaa 954

<210> SEQ ID NO 10
<211> LENGTH: 317
<212> TYPE: PRT
<213> ORGANISM: Geobacillus sterarothermophillus

<400> SEQUENCE: 10

Met Lys Asn Asn Gly Gly Ala Arg Val Val Val Ile Gly Ala Gly Phe
1 5 10 15

Val Gly Ala Ser Tyr Val Phe Ala Leu Met Asn Gln Gly Ile Ala Asp
20 25 30

Glu Ile Val Leu Ile Asp Ala Asn Glu Ser Lys Ala Ile Gly Asp Ala
35 40 45

Met Asp Phe Asn His Gly Lys Val Phe Ala Pro Lys Pro Val Asp Ile
50 55 60

Trp His Gly Asp Tyr Asp Asp Cys Arg Asp Ala Asp Leu Val Val Ile
65 70 75 80

Cys Ala Gly Ala Asn Gln Lys Pro Gly Glu Thr Arg Leu Asp Leu Val
85 90 95

Asp Lys Asn Ile Ala Ile Phe Arg Ser Ile Val Glu Ser Val Met Ala
100 105 110

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

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        115                 120                 125

Leu Thr Tyr Ala Thr Trp Lys Phe Ser Gly Leu Pro His Glu Arg Val
    130                 135                 140

Ile Gly Ser Gly Thr Ile Leu Asp Thr Ala Arg Phe Arg Phe Leu Leu
145                 150                 155                 160

Gly Glu Tyr Phe Ser Val Ala Pro Gln Asn Val His Ala Tyr Ile Ile
                165                 170                 175

Gly Glu His Gly Asp Thr Glu Leu Pro Val Trp Ser Gln Ala Tyr Ile
            180                 185                 190

Gly Val Met Pro Ile Arg Lys Leu Val Glu Ser Lys Gly Glu Glu Ala
        195                 200                 205

Gln Lys Asp Leu Glu Arg Ile Phe Val Asn Val Arg Asp Ala Ala Tyr
    210                 215                 220

Gln Ile Ile Glu Lys Lys Gly Ala Thr Tyr Tyr Gly Ile Ala Met Gly
225                 230                 235                 240

Leu Ala Arg Val Thr Arg Ala Ile Leu His Asn Glu Asn Ala Ile Leu
                245                 250                 255

Thr Val Ser Ala Tyr Leu Asp Gly Leu Tyr Gly Glu Arg Asp Val Tyr
            260                 265                 270

Ile Gly Val Pro Ala Val Ile Asn Arg Asn Gly Ile Arg Glu Val Ile
        275                 280                 285

Glu Ile Glu Leu Asn Asp Asp Glu Lys Asn Arg Phe His His Ser Ala
    290                 295                 300

Ala Thr Leu Lys Ser Val Leu Ala Arg Ala Phe Thr Arg
305                 310                 315


<210> SEQ ID NO 11
<211> LENGTH: 999
<212> TYPE: DNA
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 11

atggcggcgt tgaaagacca actgattcat aacctttaa aagaggaaca tgtgccgcag     60

aacaaaatta ccgttgtagg cgtaggtgca gttggtatgg cctgtgccat tagcatcctg    120

atgaaagact tggcggatga acttgctctg gtcgatgtaa tggaggataa actgaaaggc    180

gaaatgatgg acttgcagca tgggtcgctg tttcttcgca cacccaagat cgtaagcggc    240

aaagattact ccgtgactgc aaattccaaa ttggtcatca ttaccgccgg agcacgccag    300

caagaaggtg aaagccgcct gaacctggtg caacggaacg tcaacatttt caaatttatc    360

attccgaacg tggtgaaata ctctccacac tgcaaactcc tcgttgttag taaccctgtt    420

gacatcctga cgtatgttgc ctggaaaatt agcggcttcc cgaagaatcg cgtaattggc    480

tcaggatgca atctggattc ggcgcgtttt cgctatctga tgggcgaacg tttaggtgtt    540

catgcactgt catgccacgg gtggattctg ggtgaacatg gcgatagttc tgtgcctgtg    600

tggtctggca tgaatgtggc tggtgtgtca ctgaaaacgt tacacccaga acttggcact    660

gacgcggata aagagcagtg gaaacaggta cacaaacagg tggtcgatag cgcgtatgag    720

gtcatcaaac tgaaaggtta caccacatgg gccattgggc tgagtgtcgc cgatctggct    780

gagagcatta tgaagaatct ccgtcgtgtt catccgatct ccacgatgct caaaggtctg    840

tatgggatca aagaggacgt tttcttaagt gtgccgtgtg tcctgggtca gaatggcatt    900
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tcggatgtgg tcaaggtgac cttaacctcg gaagaagaag cgcacctgaa gaagagcgcg 960

gataccttgt ggggaatcca gaaagaactg caattttaa 999

<210> SEQ ID NO 12
<211> LENGTH: 332
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 12

Met Ala Ala Leu Lys Asp Gln Leu Ile His Asn Leu Leu Lys Glu Glu
1 5 10 15

His Val Pro Gln Asn Lys Ile Thr Val Val Gly Val Gly Ala Val Gly
20 25 30

Met Ala Cys Ala Ile Ser Ile Leu Met Lys Asp Leu Ala Asp Glu Leu
35 40 45

Ala Leu Val Asp Val Met Glu Asp Lys Leu Lys Gly Glu Met Met Asp
50 55 60

Leu Gln His Gly Ser Leu Phe Leu Arg Thr Pro Lys Ile Val Ser Gly
65 70 75 80

Lys Asp Tyr Ser Val Thr Ala Asn Ser Lys Leu Val Ile Ile Thr Ala
85 90 95

Gly Ala Arg Gln Gln Glu Gly Glu Ser Arg Leu Asn Leu Val Gln Arg
100 105 110

Asn Val Asn Ile Phe Lys Phe Ile Ile Pro Asn Val Val Lys Tyr Ser
115 120 125

Pro His Cys Lys Leu Leu Val Val Ser Asn Pro Val Asp Ile Leu Thr
130 135 140

Tyr Val Ala Trp Lys Ile Ser Gly Phe Pro Lys Asn Arg Val Ile Gly
145 150 155 160

Ser Gly Cys Asn Leu Asp Ser Ala Arg Phe Arg Tyr Leu Met Gly Glu
165 170 175

Arg Leu Gly Val His Ala Leu Ser Cys His Gly Trp Ile Leu Gly Glu
180 185 190

His Gly Asp Ser Ser Val Pro Val Trp Ser Gly Met Asn Val Ala Gly
195 200 205

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

Glu Gln Trp Lys Gln Val His Lys Gln Val Val Asp Ser Ala Tyr Glu
225 230 235 240

Val Ile Lys Leu Lys Gly Tyr Thr Thr Trp Ala Ile Gly Leu Ser Val
245 250 255

Ala Asp Leu Ala Glu Ser Ile Met Lys Asn Leu Arg Arg Val His Pro
260 265 270

Ile Ser Thr Met Leu Lys Gly Leu Tyr Gly Ile Lys Glu Asp Val Phe
275 280 285

Leu Ser Val Pro Cys Val Leu Gly Gln Asn Gly Ile Ser Asp Val Val
290 295 300

Lys Val Thr Leu Thr Ser Glu Glu Glu Ala His Leu Lys Lys Ser Ala
305 310 315 320

Asp Thr Leu Trp Gly Ile Gln Lys Glu Leu Gln Phe
325 330

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<210> SEQ ID NO 13
<211> LENGTH: 1014
<212> TYPE: DNA
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 13

atggcaacgc tgaaagagaa attgatcgca cctgtcgccg ataacgaagc ggctgttccg 60

aacaacaaaa ttaccgtagt aggcgtcggt caagtaggca tggcgtgtgc gatttccatt 120

ctcggcaaaa gtttagcgga cgaactggca cttgtcgatg tcttggaaga taaactgaaa 180

ggtgaaatga tggatttaca gcatggttcg ctgtttctcc agacacccaa aattgtggcg 240

gataaagatt acagtgtgac tgcgaacagc aagatcgtag ttgtcaccgc cggagtccgt 300

caacaggaag gtgaatcacg cctgaacttg gtgcaacgca atgtgaatgt gttcaaattc 360

atcatcccgc agattgttaa gtatagcccg aactgcatca tcattgtcgt cagcaaccct 420

gagtgtctgg ttgacatcct gacgtacgtt acctggaaac tctccggact gccgaaacac 480

cgcgtaattg gctcgggttg caatctggac agcgctcgtt ttcggtatct tatggccgag 540

aaattaggta ttcacccatc tagttgtcat ggatggattc tgggtgaaca tggcgatagc 600

tctgtggcag tatggtctgg cgttaacgtt gcgggtgtgt cgttgcaaga actgaatccg 660

gagatgggga ccgataatga tagcgaaaat tggaaagagg tgcacaaaat ggtggtggaa 720

agcgcctatg aagtgattaa gctgaaaggg tacaccaact gggcaattgg cttatcagtt 780

gcggatctta tcgagtccat gctgaagaat ctgtcacgca ttcatccggt ttccacaatg 840

gtgaaaggca tgtatgggat cgaaaacgaa gtgtttctgt ctttaccatg catcctgaat 900

gctcgtggcc tcacttcggt gattaatcag aagctgaaag atgacgaagt tgcccagctg 960

aagaaaagtg ccgatacgct gtgggacatt cagaaagacc tgaaagacct ttaa 1014

<210> SEQ ID NO 14
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 14

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

Ala Ala Val Pro Asn Asn Lys Ile Thr Val Val Gly Val Gly Gln Val
20 25 30

Gly Met Ala Cys Ala Ile Ser Ile Leu Gly Lys Ser Leu Ala Asp Glu
35 40 45

Leu Ala Leu Val Asp Val Leu Glu Asp Lys Leu Lys Gly Glu Met Met
50 55 60

Asp Leu Gln His Gly Ser Leu Phe Leu Gln Thr Pro Lys Ile Val Ala
65 70 75 80

Asp Lys Asp Tyr Ser Val Thr Ala Asn Ser Lys Ile Val Val Val Thr
85 90 95

Ala Gly Val Arg Gln Gln Glu Gly Glu Ser Arg Leu Asn Leu Val Gln
100 105 110

Arg Asn Val Asn Val Phe Lys Phe Ile Ile Pro Gln Ile Val Lys Tyr
115 120 125

Ser Pro Asn Cys Ile Ile Ile Val Val Ser Asn Pro Glu Cys Leu Val
130 135 140

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Asp Ile Leu Thr Tyr Val Thr Trp Lys Leu Ser Gly Leu Pro Lys His
145 150 155 160

Arg Val Ile Gly Ser Gly Cys Asn Leu Asp Ser Ala Arg Phe Arg Tyr
165 170 175

Leu Met Ala Glu Lys Leu Gly Ile His Pro Ser Ser Cys His Gly Trp
180 185 190

Ile Leu Gly Glu His Gly Asp Ser Ser Val Ala Val Trp Ser Gly Val
195 200 205

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

Asp Asn Asp Ser Glu Asn Trp Lys Glu Val His Lys Met Val Val Glu
225 230 235 240

Ser Ala Tyr Glu Val Ile Lys Leu Lys Gly Tyr Thr Asn Trp Ala Ile
245 250 255

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

Arg Ile His Pro Val Ser Thr Met Val Lys Gly Met Tyr Gly Ile Glu
275 280 285

Asn Glu Val Phe Leu Ser Leu Pro Cys Ile Leu Asn Ala Arg Gly Leu
290 295 300

Thr Ser Val Ile Asn Gln Lys Leu Lys Asp Asp Glu Val Ala Gln Leu
305 310 315 320

Lys Lys Ser Ala Asp Thr Leu Trp Asp Ile Gln Lys Asp Leu Lys Asp
325 330 335

Leu

<210> SEQ ID NO 15
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 15

tataatcata tgaaagtcgc agtcctc 27

<210> SEQ ID NO 16
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 16

tataatggat ccttacttat taacgaactc 30

<210> SEQ ID NO 17
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 17

tataatcata tggctgataa acaacgtaaa aaa 33

<210> SEQ ID NO 18

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<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 18

tataatggat ccttagtttt taactgcaga agcaaa 36

<210> SEQ ID NO 19
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 19

tataatgcta gcatgatgaa caaacatgta aataaagt 38

<210> SEQ ID NO 20
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 20

tataatggat ccttagttga ctttttgttc 30

<210> SEQ ID NO 21
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 21

tataatgcta gcatggcggc gttgaaagac 30

<210> SEQ ID NO 22
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 22

attatagaat tcttaaaatt gcagttcttt 30

<210> SEQ ID NO 23
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 23

tataatcata tgagaattac aattgccgg 29

<210> SEQ ID NO 24
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 24

tataatggat ccttattttg cttttaataa ctcttctttg c 41

<210> SEQ ID NO 25
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 25

tataatcata tgaaactcgc cgtttatag 29

<210> SEQ ID NO 26
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 26

tataatggat ccttaaacca gttcgttcgg 30

<210> SEQ ID NO 27
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 27

gtcttgactt ctggtgctcc annkaaacca ggtgaaacgc gtctt 45

<210> SEQ ID NO 28
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(24)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 28

aagacgcgtt tcacctggtt tmnntggagc accagaagtc aagac 45

<210> SEQ ID NO 29
<211> LENGTH: 978
<212> TYPE: DNA
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 29

atggctgata aacaacgtaa aaaagttatc cttgtaggtg acggtgctgt aggttcatca 60

tacgcttttg ctcttgtaaa ccaagggatt gcacaagaat taggaattgt tgacctttt 120

aaagaaaaaa ctcaaggaga tgcagaagac ctttctcatg ccttggcatt tacttcacct 180

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aaaaagattt actctgcaga ctactctgat gcaagcgacg ctgacctcgt agtcttgacg    240

tctggtgctc caaataaacc aggtgaaact cgtcttgacc ttgttgaaaa aaatcttcgt    300

atcactaaag atgttgtcac taaaattgtt gcttcaggtt tcaaaggaat cttccttgtt    360

gctgctaacc cagttgatat cttgacatac gctacttgga aattctcagg tttccctaaa    420

aaccgcgttg taggttcagg tacttcactt gatactgcac gtttccgtca agcattggca    480

gaaaaagttg atgttgacgc tcgttcaatc cacgcataca tcatgggtga acacggtgac    540

tcagaatttg ccgtttggtc acacgctaac gttgctggtg ttaaattgga acaatggttc    600

caagaaaatg actaccttaa cgaagctgaa atcgttgaat tgtttgaatc tgtacgtgat    660

gctgcttact caatcatcgc taaaaaaggt gcaacattct atggtgtcgc tgtagctctt    720

gctcgtatta ctaaagcaat tcttgatgat gaacatgcag tacttccagt atcagtattc    780

caagatggac aatatggcgt aagcgactgc taccttggtc aaccagctgt agttggtgct    840

gaaggtgttg ttaacccaat ccacattcca ttgaatgatg ctgaaatgca aaaaatggaa    900

gcttctggtg ctcaattgaa agcaatcatt gacgaagctt ttgctaaaga agaatttgct    960

tctgcagtta aaaactaa                                                  978


<210> SEQ ID NO 30
<211> LENGTH: 325
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 30

Met Ala Asp Lys Gln Arg Lys Lys Val Ile Leu Val Gly Asp Gly Ala
1               5                   10                  15

Val Gly Ser Ser Tyr Ala Phe Ala Leu Val Asn Gln Gly Ile Ala Gln
            20                  25                  30

Glu Leu Gly Ile Val Asp Leu Phe Lys Glu Lys Thr Gln Gly Asp Ala
        35                  40                  45

Glu Asp Leu Ser His Ala Leu Ala Phe Thr Ser Pro Lys Lys Ile Tyr
    50                  55                  60

Ser Ala Asp Tyr Ser Asp Ala Ser Asp Ala Asp Leu Val Val Leu Thr
65                  70                  75                  80

Ser Gly Ala Pro Asn Lys Pro Gly Glu Thr Arg Leu Asp Leu Val Glu
                85                  90                  95

Lys Asn Leu Arg Ile Thr Lys Asp Val Val Thr Lys Ile Val Ala Ser
            100                 105                 110

Gly Phe Lys Gly Ile Phe Leu Val Ala Ala Asn Pro Val Asp Ile Leu
        115                 120                 125

Thr Tyr Ala Thr Trp Lys Phe Ser Gly Phe Pro Lys Asn Arg Val Val
    130                 135                 140

Gly Ser Gly Thr Ser Leu Asp Thr Ala Arg Phe Arg Gln Ala Leu Ala
145                 150                 155                 160

Glu Lys Val Asp Val Asp Ala Arg Ser Ile His Ala Tyr Ile Met Gly
                165                 170                 175

Glu His Gly Asp Ser Glu Phe Ala Val Trp Ser His Ala Asn Val Ala
            180                 185                 190

Gly Val Lys Leu Glu Gln Trp Phe Gln Glu Asn Asp Tyr Leu Asn Glu
        195                 200                 205

Ala Glu Ile Val Glu Leu Phe Glu Ser Val Arg Asp Ala Ala Tyr Ser
```

-continued

```
    210                 215                 220

Ile Ile Ala Lys Lys Gly Ala Thr Phe Tyr Gly Val Ala Val Ala Leu
225                 230                 235                 240

Ala Arg Ile Thr Lys Ala Ile Leu Asp Asp Glu His Ala Val Leu Pro
                245                 250                 255

Val Ser Val Phe Gln Asp Gly Gln Tyr Gly Val Ser Asp Cys Tyr Leu
            260                 265                 270

Gly Gln Pro Ala Val Val Gly Ala Glu Gly Val Val Asn Pro Ile His
        275                 280                 285

Ile Pro Leu Asn Asp Ala Glu Met Gln Lys Met Glu Ala Ser Gly Ala
    290                 295                 300

Gln Leu Lys Ala Ile Ile Asp Glu Ala Phe Ala Lys Glu Glu Phe Ala
305                 310                 315                 320

Ser Ala Val Lys Asn
                325
```

<210> SEQ ID NO 31
<211> LENGTH: 978
<212> TYPE: DNA
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 31

```
atggctgata aacaacgtaa aaaagttatc cttgtaggtg acggtgctgt aggttcatca     60

tacgcttttg ctcttgtaaa ccaagggatt gcacaagaat taggaattgt tgaccttttt    120

aaagaaaaaa ctcaaggaga tgcagaagac ctttctcatg ccttggcatt tacttcacct    180

aaaaagattt actctgcaga ctactctgat gcaagcgacg ctgacctcgt agtcttgacg    240

tctggtgctc caaataaacc aggtgaaact cgtcttgacc ttgttgaaaa aaatcttcgt    300

atcactaaag atgttgtcac taaaattgtt gcttcaggtt tcaaaggaat cttccttgtt    360

gctgctaacc cagttgatat cttgacatac gctacttgga aattctcagg tttccctaaa    420

aaccgcgttg taggttcagg tacttcactt gatactgcac gtttccgtca agcattggca    480

gaaaaagttg atgttgacgc tcgttcaatc cacgcataca tcatgggtga acacggtgac    540

tcagaatttg ccgtttggtc acacgctaac gttgctggtg ttaaattgga acaatggttc    600

caagaaaatg actaccttaa cgaagctgaa atcgttgaat tgtttgaatc tgtacgtgat    660

gctgcttact cgatcgtcgc taaaaaaggt gcaacattct atggtgtcgc tgtagctctt    720

gctcgtatta ctaaagcaat tcttgatgat gaacatgcag tacttccagt atcagtattc    780

caagatggac aatatggcgt aagcgactgc taccttggtc aaccagctgt agttggtgct    840

gaaggtgttg ttaacccaat ccacattcca ttgaatgatg ctgaaatgca aaaaatggaa    900

gcttctggtg ctcaattgaa agcaatcatt gacgaagctt ttgctaaaga agaatttgct    960

tctgcagtta aaaactaa                                                  978
```

<210> SEQ ID NO 32
<211> LENGTH: 325
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 32

```
Met Ala Asp Lys Gln Arg Lys Lys Val Ile Leu Val Gly Asp Gly Ala
1               5                   10                  15
```

-continued

```
Val Gly Ser Ser Tyr Ala Phe Ala Leu Val Asn Gln Gly Ile Ala Gln
            20                  25                  30

Glu Leu Gly Ile Val Asp Leu Phe Lys Glu Lys Thr Gln Gly Asp Ala
        35                  40                  45

Glu Asp Leu Ser His Ala Leu Ala Phe Thr Ser Pro Lys Lys Ile Tyr
    50                  55                  60

Ser Ala Asp Tyr Ser Asp Ala Ser Asp Ala Asp Leu Val Val Leu Thr
65                  70                  75                  80

Ser Gly Ala Pro Asn Lys Pro Gly Glu Thr Arg Leu Asp Leu Val Glu
                85                  90                  95

Lys Asn Leu Arg Ile Thr Lys Asp Val Val Thr Lys Ile Val Ala Ser
            100                 105                 110

Gly Phe Lys Gly Ile Phe Leu Val Ala Ala Asn Pro Val Asp Ile Leu
        115                 120                 125

Thr Tyr Ala Thr Trp Lys Phe Ser Gly Phe Pro Lys Asn Arg Val Val
    130                 135                 140

Gly Ser Gly Thr Ser Leu Asp Thr Ala Arg Phe Arg Gln Ala Leu Ala
145                 150                 155                 160

Glu Lys Val Asp Val Asp Ala Arg Ser Ile His Ala Tyr Ile Met Gly
                165                 170                 175

Glu His Gly Asp Ser Glu Phe Ala Val Trp Ser His Ala Asn Val Ala
            180                 185                 190

Gly Val Lys Leu Glu Gln Trp Phe Gln Glu Asn Asp Tyr Leu Asn Glu
        195                 200                 205

Ala Glu Ile Val Glu Leu Phe Glu Ser Val Arg Asp Ala Ala Tyr Ser
    210                 215                 220

Ile Val Ala Lys Lys Gly Ala Thr Phe Tyr Gly Val Ala Val Ala Leu
225                 230                 235                 240

Ala Arg Ile Thr Lys Ala Ile Leu Asp Asp Glu His Ala Val Leu Pro
                245                 250                 255

Val Ser Val Phe Gln Asp Gly Gln Tyr Gly Val Ser Asp Cys Tyr Leu
            260                 265                 270

Gly Gln Pro Ala Val Val Gly Ala Glu Gly Val Val Asn Pro Ile His
        275                 280                 285

Ile Pro Leu Asn Asp Ala Glu Met Gln Lys Met Glu Ala Ser Gly Ala
    290                 295                 300

Gln Leu Lys Ala Ile Ile Asp Glu Ala Phe Ala Lys Glu Glu Phe Ala
305                 310                 315                 320

Ser Ala Val Lys Asn
                325


<210> SEQ ID NO 33
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(22)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 33

ttatctctgc aggcgtagcg nnkaaacccg ggatggatcg ttc                      43
```

-continued

<210> SEQ ID NO 34
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 34

gaacgatcca tcccgggttt mnncgctacg cctgcagaga taa 43

<210> SEQ ID NO 35
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 35

ttatctctgc aggcgtagcg gctaaaccgg gtgaggatcg ttccgacctg 50

<210> SEQ ID NO 36
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 36

caggtcggaa cgatcctcac ccggtttagc cgctacgcct gcagagataa 50

<210> SEQ ID NO 37
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 37

ttatctctgc aggcgtagcg gctaaaccgg gtcaggatcg ttccgacctg 50

<210> SEQ ID NO 38
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 38

caggtcggaa cgatcctgac ccggtttagc cgctacgcct gcagagataa 50

<210> SEQ ID NO 39
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 39

gtcgcagtcc tcggcgccgc tggcggtgtc ggccaggcgc ttgcac 46

-continued

<210> SEQ ID NO 40
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 40

gtgcaagcgc ctggccgaca ccgccagcgg cgccgaggac tgcgac 46

<210> SEQ ID NO 41
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 41

ccggttattg gcggccactc tgatgttacc attctgccgc tgctg 45

<210> SEQ ID NO 42
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 42

cagcagcggc agaatggtaa catcagagtg gccgccaata accgg 45

<210> SEQ ID NO 43
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 43

ggcgtagcgg ctaaaccggg tatgtctcgt tccgacctg 39

<210> SEQ ID NO 44
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 44

caggtcggaa cgagacatac ccggtttagc cgctacgcc 39

<210> SEQ ID NO 45
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 45

acggatccag aacgccggct atgaagtggt tgaagcg 37

<210> SEQ ID NO 46
<211> LENGTH: 37
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 46

cgcttcaacc acttcatagc cggcgttctg gatccgt 37

<210> SEQ ID NO 47
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 47

tataatgcta gcatgaccac gaagaaagct gattaca 37

<210> SEQ ID NO 48
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 48

tataatggat ccttattgat taacttgatc taacc 35

<210> SEQ ID NO 49
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 49

tataatgcta gcgtgtttca aaaagttgac g 31

<210> SEQ ID NO 50
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 50

tataatggat ccttacatca ccgcagcaaa c 31

<210> SEQ ID NO 51
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 51

tataatgcta gcatgtttga gaacattacc gc 32

<210> SEQ ID NO 52
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

-continued

<400> SEQUENCE: 52

tataatggat ccttacagca ctgccacaat cg 32

<210> SEQ ID NO 53
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 53

tataatgcta gcatggattt attaaaaaaa tttaacccta a 41

<210> SEQ ID NO 54
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 54

tataatggat cctcagccac gttttttagt cacataa 37

<210> SEQ ID NO 55
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 55

tataatgcta gcatggcaat taatttagac tg 32

<210> SEQ ID NO 56
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 56

tataatggat ccttaatcaa ctttaactat cc 32

<210> SEQ ID NO 57
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 57

tataatcata tgatcatgac tttacctgaa tcaaaaga 38

<210> SEQ ID NO 58
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 58

tataatggat ccctatttgg aaataccaaa ttcttcg 37

-continued

<210> SEQ ID NO 59
<211> LENGTH: 930
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 59

atgaccacga agaaagctga ttacatttgg ttcaatgggg agatggttcg ctgggaagac 60

gcgaaggtgc atgtgatgtc gcacgcgctg cactatggca cttcggtttt tgaaggcatc 120

cgttgctacg actcgcacaa aggaccggtt gtattccgcc atcgtgagca tatgcagcgt 180

ctgcatgact ccgccaaaat ctatcgcttc ccggtttcgc agagcattga tgagctgatg 240

gaagcttgtc gtgacgtgat ccgcaaaaac aatctcacca gcgcctatat ccgtccgctg 300

atcttcgtcg gtgatgttgg catgggagta aacccgccag cgggatactc aaccgacgtg 360

attatcgctg ctttcccgtg gggagcgtat ctgggcgcag aagcgctgga gcaggggatc 420

gatgcgatgg tttcctcctg gaaccgcgca gcaccaaaca ccatcccgac ggcggcaaaa 480

gccggtggta actacctctc ttccctgctg gtgggtagcg aagcgcgccg ccacggttat 540

caggaaggta tcgcgctgga tgtgaacggt tatatctctg aaggcgcagg cgaaaacctg 600

tttgaagtga aagatggtgt gctgttcacc ccaccgttca cctcctccgc gctgccgggt 660

attacccgtg atgccatcat caaactggcg aaagagctgg gaattgaagt acgtgagcag 720

gtgctgtcgc gcgaatccct gtacctggcg gatgaagtgt ttatgtccgg tacggcggca 780

gaaatcacgc cagtgcgcag cgtagacggt attcaggttg gcgaaggccg ttgtggcccg 840

gttaccaaac gcattcagca agccttcttc ggcctcttca ctggcgaaac cgaagataaa 900

tggggctggt tagatcaagt taatcaataa 930

<210> SEQ ID NO 60
<211> LENGTH: 309
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 60

Met Thr Thr Lys Lys Ala Asp Tyr Ile Trp Phe Asn Gly Glu Met Val
1 5 10 15

Arg Trp Glu Asp Ala Lys Val His Val Met Ser His Ala Leu His Tyr
20 25 30

Gly Thr Ser Val Phe Glu Gly Ile Arg Cys Tyr Asp Ser His Lys Gly
35 40 45

Pro Val Val Phe Arg His Arg Glu His Met Gln Arg Leu His Asp Ser
50 55 60

Ala Lys Ile Tyr Arg Phe Pro Val Ser Gln Ser Ile Asp Glu Leu Met
65 70 75 80

Glu Ala Cys Arg Asp Val Ile Arg Lys Asn Asn Leu Thr Ser Ala Tyr
85 90 95

Ile Arg Pro Leu Ile Phe Val Gly Asp Val Gly Met Gly Val Asn Pro
100 105 110

Pro Ala Gly Tyr Ser Thr Asp Val Ile Ile Ala Ala Phe Pro Trp Gly
115 120 125

Ala Tyr Leu Gly Ala Glu Ala Leu Glu Gln Gly Ile Asp Ala Met Val
130 135 140

Ser Ser Trp Asn Arg Ala Ala Pro Asn Thr Ile Pro Thr Ala Ala Lys

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```
145                 150                 155                 160

Ala Gly Gly Asn Tyr Leu Ser Ser Leu Leu Val Gly Ser Glu Ala Arg
                165                 170                 175

Arg His Gly Tyr Gln Glu Gly Ile Ala Leu Asp Val Asn Gly Tyr Ile
            180                 185                 190

Ser Glu Gly Ala Gly Glu Asn Leu Phe Glu Val Lys Asp Gly Val Leu
        195                 200                 205

Phe Thr Pro Pro Phe Thr Ser Ser Ala Leu Pro Gly Ile Thr Arg Asp
    210                 215                 220

Ala Ile Ile Lys Leu Ala Lys Glu Leu Gly Ile Glu Val Arg Glu Gln
225                 230                 235                 240

Val Leu Ser Arg Glu Ser Leu Tyr Leu Ala Asp Glu Val Phe Met Ser
                245                 250                 255

Gly Thr Ala Ala Glu Ile Thr Pro Val Arg Ser Val Asp Gly Ile Gln
            260                 265                 270

Val Gly Glu Gly Arg Cys Gly Pro Val Thr Lys Arg Ile Gln Gln Ala
        275                 280                 285

Phe Phe Gly Leu Phe Thr Gly Glu Thr Glu Asp Lys Trp Gly Trp Leu
    290                 295                 300

Asp Gln Val Asn Gln
305
```

<210> SEQ ID NO 61
<211> LENGTH: 1194
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 61

```
gtgtttcaaa aagttgacgc ctacgctggc gacccgattc ttacgcttat ggagcgtttt     60

aaagaagacc ctcgcagcga caaagtgaat ttaagtatcg gtctgtacta caacgaagac    120

ggaattattc cacaactgca agccgtggcg gaggcggaag cgcgcctgaa tgcgcagcct    180

catggcgctt cgctttattt accgatggaa gggcttaact gctatcgcca tgccattgcg    240

ccgctgctgt ttggtgcgga ccatccggta ctgaaacaac agcgcgtagc aaccattcaa    300

acccttggcg gctccggggc attgaaagtg ggcgcggatt tcctgaaacg ctacttcccg    360

gaatcaggcg tctgggtcag cgatcctacc tgggaaaacc acgtagcaat attcgccggg    420

gctggattcg aagtgagtac ttacccctgg tatgacgaag cgactaacgg cgtgcgcttt    480

aatgacctgt tggcgacgct gaaaacatta cctgcccgca gtattgtgtt gctgcatcca    540

tgttgccaca acccaacggg tgccgatctc actaatgatc agtgggatgc ggtgattgaa    600

attctcaaag cccgcgagct tattccattc ctcgatattg cctatcaagg atttggtgcc    660

ggtatggaag aggatgccta cgctattcgc gccattgcca gcgctggatt acccgctctg    720

gtgagcaatt cgttctcgaa aattttctcc ctttacggcg agcgcgtcgg cggactttct    780

gttatgtgtg aagatgccga agccgctggc cgcgtactgg ggcaattgaa agcaacagtt    840

cgccgcaact actccagccc gccgaatttt ggtgcgcagg tggtggctgc agtgctgaat    900

gacgaggcat tgaaagccag ctggctggcg gaagtagaag agatgcgtac tcgcattctg    960

gcaatgcgtc aggaattggt gaaggtatta agcacagaga tgccagaacg caatttcgat   1020

tatctgctta atcagcgcgg catgttcagt tataccggtt taagtgccgc tcaggttgac   1080
```

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cgactacgtg aagaatttgg tgtctatctc atcgccagcg gtcgcatgtg tgtcgccggg 1140

ttaaatacgg caaatgtaca acgtgtggca aaggcgtttg ctgcggtgat gtaa 1194

<210> SEQ ID NO 62
<211> LENGTH: 397
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 62

Val Phe Gln Lys Val Asp Ala Tyr Ala Gly Asp Pro Ile Leu Thr Leu
1 5 10 15

Met Glu Arg Phe Lys Glu Asp Pro Arg Ser Asp Lys Val Asn Leu Ser
20 25 30

Ile Gly Leu Tyr Tyr Asn Glu Asp Gly Ile Ile Pro Gln Leu Gln Ala
35 40 45

Val Ala Glu Ala Glu Ala Arg Leu Asn Ala Gln Pro His Gly Ala Ser
50 55 60

Leu Tyr Leu Pro Met Glu Gly Leu Asn Cys Tyr Arg His Ala Ile Ala
65 70 75 80

Pro Leu Leu Phe Gly Ala Asp His Pro Val Leu Lys Gln Gln Arg Val
85 90 95

Ala Thr Ile Gln Thr Leu Gly Gly Ser Gly Ala Leu Lys Val Gly Ala
100 105 110

Asp Phe Leu Lys Arg Tyr Phe Pro Glu Ser Gly Val Trp Val Ser Asp
115 120 125

Pro Thr Trp Glu Asn His Val Ala Ile Phe Ala Gly Ala Gly Phe Glu
130 135 140

Val Ser Thr Tyr Pro Trp Tyr Asp Glu Ala Thr Asn Gly Val Arg Phe
145 150 155 160

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

Leu Leu His Pro Cys Cys His Asn Pro Thr Gly Ala Asp Leu Thr Asn
180 185 190

Asp Gln Trp Asp Ala Val Ile Glu Ile Leu Lys Ala Arg Glu Leu Ile
195 200 205

Pro Phe Leu Asp Ile Ala Tyr Gln Gly Phe Gly Ala Gly Met Glu Glu
210 215 220

Asp Ala Tyr Ala Ile Arg Ala Ile Ala Ser Ala Gly Leu Pro Ala Leu
225 230 235 240

Val Ser Asn Ser Phe Ser Lys Ile Phe Ser Leu Tyr Gly Glu Arg Val
245 250 255

Gly Gly Leu Ser Val Met Cys Glu Asp Ala Glu Ala Ala Gly Arg Val
260 265 270

Leu Gly Gln Leu Lys Ala Thr Val Arg Arg Asn Tyr Ser Ser Pro Pro
275 280 285

Asn Phe Gly Ala Gln Val Val Ala Ala Val Leu Asn Asp Glu Ala Leu
290 295 300

Lys Ala Ser Trp Leu Ala Glu Val Glu Glu Met Arg Thr Arg Ile Leu
305 310 315 320

Ala Met Arg Gln Glu Leu Val Lys Val Leu Ser Thr Glu Met Pro Glu
325 330 335

Arg Asn Phe Asp Tyr Leu Leu Asn Gln Arg Gly Met Phe Ser Tyr Thr

-continued

```
            340                 345                 350

Gly Leu Ser Ala Ala Gln Val Asp Arg Leu Arg Glu Glu Phe Gly Val
        355                 360                 365

Tyr Leu Ile Ala Ser Gly Arg Met Cys Val Ala Gly Leu Asn Thr Ala
    370                 375                 380

Asn Val Gln Arg Val Ala Lys Ala Phe Ala Ala Val Met
385                 390                 395


<210> SEQ ID NO 63
<211> LENGTH: 1191
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 63

atgtttgaga acattaccgc cgctcctgcc gacccgattc tgggcctggc cgatctgttt     60

cgtgccgatg aacgtcccgg caaaattaac ctcgggattg gtgtctataa agatgagacg    120

ggcaaaaccc cggtactgac cagcgtgaaa aaggctgaac agtatctgct cgaaaatgaa    180

accaccaaaa attacctcgg cattgacggc atccctgaat ttggtcgctg cactcaggaa    240

ctgctgtttg gtaaaggtag cgccctgatc aatgacaaac gtgctcgcac ggcacagact    300

ccgggggggca ctggcgcact acgcgtggct gccgatttcc tggcaaaaaa taccagcgtt    360

aagcgtgtgt gggtgagcaa cccaagctgg ccgaaccata agagcgtctt taactctgca    420

ggtctggaag ttcgtgaata cgcttattat gatgcggaaa atcacactct tgacttcgat    480

gcactgatta acagcctgaa tgaagctcag gctggcgacg tagtgctgtt ccatggctgc    540

tgccataacc caaccggtat cgaccctacg ctggaacaat ggcaaacact ggcacaactc    600

tccgttgaga aaggctggtt accgctgttt gacttcgctt accagggttt tgcccgtggt    660

ctggaagaag atgctgaagg actgcgcgct ttcgcggcta tgcataaaga gctgattgtt    720

gccagttcct actctaaaaa ctttggcctg tacaacgagc gtgttggcgc ttgtactctg    780

gttgctgccg acagtgaaac cgttgatcgc gcattcagcc aaatgaaagc ggcgattcgc    840

gctaactact ctaacccacc agcacacggc gcttctgttg ttgccaccat cctgagcaac    900

gatgcgttac gtgcgatttg ggaacaagag ctgactgata tgcgccagcg tattcagcgt    960

atgcgtcagt tgttcgtcaa tacgctgcag gaaaaaggcg caaaccgcga cttcagcttt   1020

atcatcaaac agaacggcat gttctccttc agtggcctga caaaagaaca agtgctgcgt   1080

ctgcgcgaag agtttggcgt atatgcggtt gcttctggtc gcgtaaatgt ggccgggatg   1140

acaccagata acatggctcc gctgtgcgaa gcgattgtgg cagtgctgta a            1191


<210> SEQ ID NO 64
<211> LENGTH: 396
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 64

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

Ala Asp Leu Phe Arg Ala Asp Glu Arg Pro Gly Lys Ile Asn Leu Gly
            20                  25                  30

Ile Gly Val Tyr Lys Asp Glu Thr Gly Lys Thr Pro Val Leu Thr Ser
        35                  40                  45
```

-continued

```
Val Lys Lys Ala Glu Gln Tyr Leu Leu Glu Asn Glu Thr Thr Lys Asn
    50                  55                  60

Tyr Leu Gly Ile Asp Gly Ile Pro Glu Phe Gly Arg Cys Thr Gln Glu
65                  70                  75                  80

Leu Leu Phe Gly Lys Gly Ser Ala Leu Ile Asn Asp Lys Arg Ala Arg
                85                  90                  95

Thr Ala Gln Thr Pro Gly Gly Thr Gly Ala Leu Arg Val Ala Ala Asp
            100                 105                 110

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

Ser Trp Pro Asn His Lys Ser Val Phe Asn Ser Ala Gly Leu Glu Val
    130                 135                 140

Arg Glu Tyr Ala Tyr Tyr Asp Ala Glu Asn His Thr Leu Asp Phe Asp
145                 150                 155                 160

Ala Leu Ile Asn Ser Leu Asn Glu Ala Gln Ala Gly Asp Val Val Leu
                165                 170                 175

Phe His Gly Cys Cys His Asn Pro Thr Gly Ile Asp Pro Thr Leu Glu
            180                 185                 190

Gln Trp Gln Thr Leu Ala Gln Leu Ser Val Glu Lys Gly Trp Leu Pro
        195                 200                 205

Leu Phe Asp Phe Ala Tyr Gln Gly Phe Ala Arg Gly Leu Glu Glu Asp
    210                 215                 220

Ala Glu Gly Leu Arg Ala Phe Ala Ala Met His Lys Glu Leu Ile Val
225                 230                 235                 240

Ala Ser Ser Tyr Ser Lys Asn Phe Gly Leu Tyr Asn Glu Arg Val Gly
                245                 250                 255

Ala Cys Thr Leu Val Ala Ala Asp Ser Glu Thr Val Asp Arg Ala Phe
            260                 265                 270

Ser Gln Met Lys Ala Ala Ile Arg Ala Asn Tyr Ser Asn Pro Pro Ala
        275                 280                 285

His Gly Ala Ser Val Val Ala Thr Ile Leu Ser Asn Asp Ala Leu Arg
    290                 295                 300

Ala Ile Trp Glu Gln Glu Leu Thr Asp Met Arg Gln Arg Ile Gln Arg
305                 310                 315                 320

Met Arg Gln Leu Phe Val Asn Thr Leu Gln Glu Lys Gly Ala Asn Arg
                325                 330                 335

Asp Phe Ser Phe Ile Ile Lys Gln Asn Gly Met Phe Ser Phe Ser Gly
            340                 345                 350

Leu Thr Lys Glu Gln Val Leu Arg Leu Arg Glu Glu Phe Gly Val Tyr
        355                 360                 365

Ala Val Ala Ser Gly Arg Val Asn Val Ala Gly Met Thr Pro Asp Asn
    370                 375                 380

Met Ala Pro Leu Cys Glu Ala Ile Val Ala Val Leu
385                 390                 395


<210> SEQ ID NO 65
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 65

atggatttat taaaaaaatt taaccctaat ttagataaaa ttgaaatttc attgattcgt     60
```

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```
cagtttgacc aacaggtttc atctattcct gatgttatta agttgacttt gggagaacct    120

gatttttata cgcctgagca tgttaaacaa gcagggattg tggcgattga aaataatcaa    180

agtcattata ctggaatggc tggtttacta gaactacgtc aggcagctag tgaatttatg    240

aataaaaaat atggtttatc ttatgcagca gaagatgaaa ttttagttac tgttggagta    300

acggaagcca tttctagtgt tttgttatca attttggttg ctggtgatga agttttgatt    360

cccgcgcctg catatcctgg ttatgagcca ttaattacgc ttgctggcgg ttctttggtt    420

gaaattgata caagagctaa tgattttgtt cttacgcctg agatgcttga acaagcgatt    480

gtcgagcgtg agggaaaagt taaggccgtt attttgaatt atccagcaaa tcctacaggg    540

gtaacttata atcgggggca aattaaggct ttagctgaag ttttgaaaaa gcatgaagta    600

tttgtgattg ctgatgaagt ttattctgaa ctaaattata ctgaccaacc gcatgtgtca    660

attgctgaat atgcacctga gcaaacaatc gttcttaatg gtttatcaaa atcgcatgcg    720

atgactggtt ggcggattgg attaatcttt gcagcgcgtg aattagtggc acagattatt    780

aagactcacc aatatttggt gacttcggct tcaactcagt cacagtttgc agcgattgaa    840

gctttgaaaa atggtgctta tgatgctctt ccgatgaaaa aagaatatct taaacgtcgt    900

gattatatta ttgaaaagat gtcagacctt ggtttcaaaa ttattgaacc agatggagct    960

ttctacattt ttgcaaaaat tccagctgat ttagaacaag attcattcaa atttgctgtg   1020

gattttgcaa aagaaaatgc agttgccatt attcctggta tcgcttttgg tcagtacggt   1080

gaaggatttg tccgcttatc ttatgcggct tcaatggata tgattgagca agcaatggca   1140

agattgacgg attatgtgac taaaaaacgt ggctga                             1176
```

<210> SEQ ID NO 66
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 66

```
Met Asp Leu Leu Lys Lys Phe Asn Pro Asn Leu Asp Lys Ile Glu Ile
1               5                   10                  15

Ser Leu Ile Arg Gln Phe Asp Gln Gln Val Ser Ser Ile Pro Asp Val
            20                  25                  30

Ile Lys Leu Thr Leu Gly Glu Pro Asp Phe Tyr Thr Pro Glu His Val
        35                  40                  45

Lys Gln Ala Gly Ile Val Ala Ile Glu Asn Asn Gln Ser His Tyr Thr
    50                  55                  60

Gly Met Ala Gly Leu Leu Glu Leu Arg Gln Ala Ala Ser Glu Phe Met
65                  70                  75                  80

Asn Lys Lys Tyr Gly Leu Ser Tyr Ala Ala Glu Asp Glu Ile Leu Val
                85                  90                  95

Thr Val Gly Val Thr Glu Ala Ile Ser Ser Val Leu Leu Ser Ile Leu
            100                 105                 110

Val Ala Gly Asp Glu Val Leu Ile Pro Ala Pro Ala Tyr Pro Gly Tyr
        115                 120                 125

Glu Pro Leu Ile Thr Leu Ala Gly Gly Ser Leu Val Glu Ile Asp Thr
    130                 135                 140

Arg Ala Asn Asp Phe Val Leu Thr Pro Glu Met Leu Glu Gln Ala Ile
145                 150                 155                 160
```

-continued

```
Val Glu Arg Glu Gly Lys Val Lys Ala Val Ile Leu Asn Tyr Pro Ala
                165                 170                 175

Asn Pro Thr Gly Val Thr Tyr Asn Arg Gly Gln Ile Lys Ala Leu Ala
            180                 185                 190

Glu Val Leu Lys Lys His Glu Val Phe Val Ile Ala Asp Glu Val Tyr
        195                 200                 205

Ser Glu Leu Asn Tyr Thr Asp Gln Pro His Val Ser Ile Ala Glu Tyr
    210                 215                 220

Ala Pro Glu Gln Thr Ile Val Leu Asn Gly Leu Ser Lys Ser His Ala
225                 230                 235                 240

Met Thr Gly Trp Arg Ile Gly Leu Ile Phe Ala Ala Arg Glu Leu Val
                245                 250                 255

Ala Gln Ile Ile Lys Thr His Gln Tyr Leu Val Thr Ser Ala Ser Thr
            260                 265                 270

Gln Ser Gln Phe Ala Ala Ile Glu Ala Leu Lys Asn Gly Ala Tyr Asp
        275                 280                 285

Ala Leu Pro Met Lys Lys Glu Tyr Leu Lys Arg Arg Asp Tyr Ile Ile
    290                 295                 300

Glu Lys Met Ser Asp Leu Gly Phe Lys Ile Ile Glu Pro Asp Gly Ala
305                 310                 315                 320

Phe Tyr Ile Phe Ala Lys Ile Pro Ala Asp Leu Glu Gln Asp Ser Phe
                325                 330                 335

Lys Phe Ala Val Asp Phe Ala Lys Glu Asn Ala Val Ala Ile Ile Pro
            340                 345                 350

Gly Ile Ala Phe Gly Gln Tyr Gly Glu Gly Phe Val Arg Leu Ser Tyr
        355                 360                 365

Ala Ala Ser Met Asp Met Ile Glu Gln Ala Met Ala Arg Leu Thr Asp
    370                 375                 380

Tyr Val Thr Lys Lys Arg Gly
385                 390


<210> SEQ ID NO 67
<211> LENGTH: 1023
<212> TYPE: DNA
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 67

atggcaatta atttagactg ggaaaattta ggattcagct atcggaactt acctttttcgt     60

tatatcgctc gttttaaaga tggaaaatgg agtgctggag aactaacagg agataatcaa    120

cttcatatta gtgaatcatc acctgctttg cattatggtc aacaaggttt tgaaggatta    180

aaagcctatc gaacaaagga tggttcaatc caacttttcc gtcctgacca aaatgctgct    240

cgtttgcaaa atacggcgcg tcgactttgc atggcagaag ttccaactga aatgtttatt    300

gatgcagtta aacaagtggt gaaagcaaac gaagattttg tgcctcctta cggaacgggt    360

gcaacgctct atctccgtcc acttttgatt ggggttggtg acgttattgg ggtgaaacct    420

gctgatgaat atattttcac cgtttttgct atgccggttg gttcttattt taaaggcgga    480

ttggctcctt caaaatttgt aatttcaaga gattatgata gggcagctcc acttggtaca    540

ggtggtgcca aagttggagg aaattatgca gcttctttac aagcagaagt tggtgccaaa    600

gcttcaggct atgcagatgc aatttatctt gacccaagca cacatactaa aattgaagaa    660

gtcggggcag caaatttctt tggaattaca gccgataatg aatttatcac accattgagt    720
```

-continued

```
ccatcaatct taccttcaat tactaaatat tctcttcttt atttagctga acatcgtttg    780

ggactcaaag cgattgaggg tgaagtttat gccaaagatt taggtaaatt tgttgaagca    840

ggagcttgtg gcacagcggc aattatctct ccaattggtc gtattgacga tggagaagat    900

tcttacattt tccattcaga aacagaagta ggaccaacgg ttaaacgttt atatgatgag    960

ttggttggca ttcagtttgg tgatgttgaa gcaccagaag gctggatagt taaagttgat   1020

taa                                                                 1023
```

<210> SEQ ID NO 68
<211> LENGTH: 340
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 68

```
Met Ala Ile Asn Leu Asp Trp Glu Asn Leu Gly Phe Ser Tyr Arg Asn
1               5                  10                  15

Leu Pro Phe Arg Tyr Ile Ala Arg Phe Lys Asp Gly Lys Trp Ser Ala
            20                  25                  30

Gly Glu Leu Thr Gly Asp Asn Gln Leu His Ile Ser Glu Ser Ser Pro
        35                  40                  45

Ala Leu His Tyr Gly Gln Gln Gly Phe Glu Gly Leu Lys Ala Tyr Arg
    50                  55                  60

Thr Lys Asp Gly Ser Ile Gln Leu Phe Arg Pro Asp Gln Asn Ala Ala
65                  70                  75                  80

Arg Leu Gln Asn Thr Ala Arg Arg Leu Cys Met Ala Glu Val Pro Thr
                85                  90                  95

Glu Met Phe Ile Asp Ala Val Lys Gln Val Val Lys Ala Asn Glu Asp
            100                 105                 110

Phe Val Pro Pro Tyr Gly Thr Gly Ala Thr Leu Tyr Leu Arg Pro Leu
        115                 120                 125

Leu Ile Gly Val Gly Asp Val Ile Gly Val Lys Pro Ala Asp Glu Tyr
    130                 135                 140

Ile Phe Thr Val Phe Ala Met Pro Val Gly Ser Tyr Phe Lys Gly Gly
145                 150                 155                 160

Leu Ala Pro Ser Lys Phe Val Ile Ser Arg Asp Tyr Asp Arg Ala Ala
                165                 170                 175

Pro Leu Gly Thr Gly Gly Ala Lys Val Gly Gly Asn Tyr Ala Ala Ser
            180                 185                 190

Leu Gln Ala Glu Val Gly Ala Lys Ala Ser Gly Tyr Ala Asp Ala Ile
        195                 200                 205

Tyr Leu Asp Pro Ser Thr His Thr Lys Ile Glu Glu Val Gly Ala Ala
    210                 215                 220

Asn Phe Phe Gly Ile Thr Ala Asp Asn Glu Phe Ile Thr Pro Leu Ser
225                 230                 235                 240

Pro Ser Ile Leu Pro Ser Ile Thr Lys Tyr Ser Leu Leu Tyr Leu Ala
                245                 250                 255

Glu His Arg Leu Gly Leu Lys Ala Ile Glu Gly Glu Val Tyr Ala Lys
            260                 265                 270

Asp Leu Gly Lys Phe Val Glu Ala Gly Ala Cys Gly Thr Ala Ala Ile
        275                 280                 285

Ile Ser Pro Ile Gly Arg Ile Asp Asp Gly Glu Asp Ser Tyr Ile Phe
```

-continued

```
    290                 295                 300

His Ser Glu Thr Glu Val Gly Pro Thr Val Lys Arg Leu Tyr Asp Glu
305                 310                 315                 320

Leu Val Gly Ile Gln Phe Gly Asp Val Glu Ala Pro Glu Gly Trp Ile
                325                 330                 335

Val Lys Val Asp
            340
```

<210> SEQ ID NO 69
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 69

```
atgactttac ctgaatcaaa agacttttct tacttgtttt cggatgaaac caatgctcgt     60

aaaccatccc cattgaaaac ctgcatccat cttttccaag atcctaacat tatcttttg    120

ggtggtggcc tgccattaaa agattatttc ccatgggata atctatctgt agattcaccc    180

aagcctcctt ttccccaggg tattggagct ccaattgacg agcagaattg cataaaatac    240

accgtcaaca aagattacgc tgataaaagt gccaatcctt ccaacgatat tcctttgtca    300

agagctttgc aatacgggtt cagtgctggt caacctgaac tattaaactt cattagagat    360

cataccaaga ttatccacga tttgaagtat aaggactggg acgttttagc cactgcaggt    420

aacacaaatg cctgggaatc tactttaaga gtcttttgta accgaggtga tgtcatctta    480

gttgaggcac attctttttc ctcttcattg gcttctgcag aggctcaagg tgtcattacc    540

ttccccgtgc caattgacgc tgatggtatc attcctgaaa aattagctaa agtcatggaa    600

aactggacac ctggtgctcc taaaccaaag ttgttataca ctattccaac gggccaaaat    660

ccaactggta cttccattgc agaccataga aaggaggcaa tttacaagat cgctcaaaag    720

tacgacttcc taattgtgga agatgaacct tattatttct tacaaatgaa tccctacatc    780

aaagacttga aggaaagaga gaaggcacaa agttctccaa agcaggacca tgacgaattt    840

ttgaagtcct tggcaaacac tttcctttcc ttggatacag aaggccgtgt tattagaatg    900

gattcctttt caaaagtttt ggccccaggg acaagattgg gttggattac tggttcatcc    960

aaaatcttga agccttactt gagtttgcat gaaatgacga ttcaagcccc agcaggtttt   1020

acacaagttt tggtcaacgc tacgctatcc aggtggggtc aaaagggtta cttggactgg   1080

ttgcttggcc tgcgtcatga atacactttg aaacgtgact gtgccatcga tgccctttac   1140

aagtatctac cacaatctga tgctttcgtg atcaatcctc caattgcagg tatgtttttc   1200

accgtgaaca ttgacgcatc tgtccaccct gagtttaaaa caaaatacaa ctcagaccct   1260

taccagctag aacagagtct ttaccacaaa gtggttgaac gtggtgtttt agtggttccc   1320

ggttcttggt tcaagagtga gggtgagacg gaacctcctc aacccgctga atctaaagaa   1380

gtcagtaatc caaacataat tttcttcaga ggtacctatg cagctgtctc tcctgagaaa   1440

ctgactgaag gtctgaagag attaggtgat actttatacg aagaatttgg tatttccaaa   1500

tag                                                                 1503
```

<210> SEQ ID NO 70
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

-continued

<400> SEQUENCE: 70

```
Met Thr Leu Pro Glu Ser Lys Asp Phe Ser Tyr Leu Phe Ser Asp Glu
1               5                  10                  15

Thr Asn Ala Arg Lys Pro Ser Pro Leu Lys Thr Cys Ile His Leu Phe
            20                  25                  30

Gln Asp Pro Asn Ile Ile Phe Leu Gly Gly Gly Leu Pro Leu Lys Asp
        35                  40                  45

Tyr Phe Pro Trp Asp Asn Leu Ser Val Asp Ser Pro Lys Pro Pro Phe
    50                  55                  60

Pro Gln Gly Ile Gly Ala Pro Ile Asp Glu Gln Asn Cys Ile Lys Tyr
65                  70                  75                  80

Thr Val Asn Lys Asp Tyr Ala Asp Lys Ser Ala Asn Pro Ser Asn Asp
                85                  90                  95

Ile Pro Leu Ser Arg Ala Leu Gln Tyr Gly Phe Ser Ala Gly Gln Pro
            100                 105                 110

Glu Leu Leu Asn Phe Ile Arg Asp His Thr Lys Ile Ile His Asp Leu
        115                 120                 125

Lys Tyr Lys Asp Trp Asp Val Leu Ala Thr Ala Gly Asn Thr Asn Ala
    130                 135                 140

Trp Glu Ser Thr Leu Arg Val Phe Cys Asn Arg Gly Asp Val Ile Leu
145                 150                 155                 160

Val Glu Ala His Ser Phe Ser Ser Ser Leu Ala Ser Ala Glu Ala Gln
                165                 170                 175

Gly Val Ile Thr Phe Pro Val Pro Ile Asp Ala Asp Gly Ile Ile Pro
            180                 185                 190

Glu Lys Leu Ala Lys Val Met Glu Asn Trp Thr Pro Gly Ala Pro Lys
        195                 200                 205

Pro Lys Leu Leu Tyr Thr Ile Pro Thr Gly Gln Asn Pro Thr Gly Thr
    210                 215                 220

Ser Ile Ala Asp His Arg Lys Glu Ala Ile Tyr Lys Ile Ala Gln Lys
225                 230                 235                 240

Tyr Asp Phe Leu Ile Val Glu Asp Glu Pro Tyr Tyr Phe Leu Gln Met
                245                 250                 255

Asn Pro Tyr Ile Lys Asp Leu Lys Glu Arg Glu Lys Ala Gln Ser Ser
            260                 265                 270

Pro Lys Gln Asp His Asp Glu Phe Leu Lys Ser Leu Ala Asn Thr Phe
        275                 280                 285

Leu Ser Leu Asp Thr Glu Gly Arg Val Ile Arg Met Asp Ser Phe Ser
    290                 295                 300

Lys Val Leu Ala Pro Gly Thr Arg Leu Gly Trp Ile Thr Gly Ser Ser
305                 310                 315                 320

Lys Ile Leu Lys Pro Tyr Leu Ser Leu His Glu Met Thr Ile Gln Ala
                325                 330                 335

Pro Ala Gly Phe Thr Gln Val Leu Val Asn Ala Thr Leu Ser Arg Trp
            340                 345                 350

Gly Gln Lys Gly Tyr Leu Asp Trp Leu Leu Gly Leu Arg His Glu Tyr
        355                 360                 365

Thr Leu Lys Arg Asp Cys Ala Ile Asp Ala Leu Tyr Lys Tyr Leu Pro
    370                 375                 380

Gln Ser Asp Ala Phe Val Ile Asn Pro Pro Ile Ala Gly Met Phe Phe
```

-continued

385 390 395 400

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

Asn Ser Asp Pro Tyr Gln Leu Glu Gln Ser Leu Tyr His Lys Val Val
420 425 430

Glu Arg Gly Val Leu Val Val Pro Gly Ser Trp Phe Lys Ser Glu Gly
435 440 445

Glu Thr Glu Pro Pro Gln Pro Ala Glu Ser Lys Glu Val Ser Asn Pro
450 455 460

Asn Ile Ile Phe Phe Arg Gly Thr Tyr Ala Ala Val Ser Pro Glu Lys
465 470 475 480

Leu Thr Glu Gly Leu Lys Arg Leu Gly Asp Thr Leu Tyr Glu Glu Phe
485 490 495

Gly Ile Ser Lys
500

<210> SEQ ID NO 71
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 71

cacgaggtac atatgtctga aattgttgtc tcc 33

<210> SEQ ID NO 72
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 72

cttccagggg atccagtatt tactcaaac 29

<210> SEQ ID NO 73
<211> LENGTH: 1350
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 73

atgtctgaaa ttgttgtctc caaatttggc ggtaccagcg tagctgattt tgacgccatg 60

aaccgcagcg ctgatattgt gctttctgat gccaacgtgc gtttagttgt cctctcggct 120

tctgctggta tcactaatct gctggtcgct ttagctgaag gactggaacc tggcgagcga 180

ttcgaaaaac tcgacgctat ccgcaacatc cagtttgcca ttctggaacg tctgcgttac 240

ccgaacgtta tccgtgaaga gattgaacgt ctgctggaga acattactgt tctggcagaa 300

gcggcggcgc tggcaacgtc tccggcgctg acagatgagc tggtcagcca cggcgagctg 360

atgtcgaccc tgctgtttgt tgagatcctg cgcgaacgcg atgttcaggc acagtggttt 420

gatgtacgta aagtgatgcg taccaacgac cgatttggtc gtgcagagcc agatatagcc 480

gcgctggcgg aactggccgc gctgcagctg ctcccacgtc tcaatgaagg cttagtgatc 540

acccagggat ttatcggtag cgaaaataaa ggtcgtacaa cgacgcttgg ccgtggaggc 600

-continued

agcgattata cggcagcctt gctggcggag gctttacacg catctcgtgt tgatatctgg 660

accgacgtcc cgggcatcta caccaccgat ccacgcgtag tttccgcagc aaaacgcatt 720

gatgaaatcg cgtttgccga agcggcagag atggcaactt ttggtgcaaa agtactgcat 780

ccggcaacgt tgctacccgc agtacgcagc gatatcccgg tctttgtcgg ctccagcaaa 840

gacccacgcg caggtggtac gctggtgtgc aataaaactg aaaatccgcc gctgttccgc 900

gctctggcgc ttcgtcgcaa tcagactctg ctcactttgc acagcctgaa tatgctgcat 960

tctcgcggtt tcctcgcgga agttttcggc atcctcgcgc ggcataatat ttcggtagac 1020

ttaatcacca cgtcagaagt gagcgtggca ttaacccttg ataccaccgg ttcaacctcc 1080

actggcgata cgttgctgac gcaatctctg ctgatggagc tttccgcact gtgtcgggtg 1140

gaggtggaag aaggtctggc gctggtcgcg ttgattggca atgacctgtc aaaagcctgc 1200

ggcgttggca aagaggtatt cggcgtactg gaaccgttca acattcgcat gatttgttat 1260

ggcgcatcca gccataacct gtgcttcctg gtgcccggcg aagatgccga gcaggtggtg 1320

caaaaactgc atagtaattt gtttgagtaa 1350

<210> SEQ ID NO 74
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 74

gcgtttgccg aagcggcaaa gatggccact tttg 34

<210> SEQ ID NO 75
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 75

caaaagtggc catctttgcc gcttcggcaa acgc 34

<210> SEQ ID NO 76
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 76

tataatgcta gcatgaaaaa tgttggtttt atcgg 35

<210> SEQ ID NO 77
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 77

tataatggat ccttacgcca gttgacgaag c 31

-continued

<210> SEQ ID NO 78
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 78

tataatcata tgagcactaa agttgttaat g 31

<210> SEQ ID NO 79
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 79

tataatggat ccctaaagtc tttgagcaat c 31

<210> SEQ ID NO 80
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 80

tataaggatc cgtttaactt taagaaggag atataccatg gg 42

<210> SEQ ID NO 81
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 81

tataagaatt cttacgccag ttgacgaag 29

<210> SEQ ID NO 82
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 82

tataagcggc cgcgtttaac tttaagaagg agatat 36

<210> SEQ ID NO 83
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 83

tataaactcg agcctaaagt ctttgagcaa t 31

<210> SEQ ID NO 84
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 84

tataaagatc ttagaaataa ttttgttta 29

<210> SEQ ID NO 85
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 85

tataatctag actaaagtct ttgagcaat 29

<210> SEQ ID NO 86
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 86

tataatcata tgcgagtgtt gaagttcg 28

<210> SEQ ID NO 87
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 87

tataatggat cctcagactc ctaacttcca 30

<210> SEQ ID NO 88
<211> LENGTH: 2463
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 88

atgcgagtgt tgaagttcgg cggtacatca gtggcaaatg cagaacgttt tctgcgtgtt 60

gccgatattc tggaaagcaa tgccaggcag gggcaggtgg ccaccgtcct ctctgccccc 120

gccaaaatca ccaaccacct ggtggcgatg attgaaaaaa ccattagcgg ccaggatgct 180

ttacccaata tcagcgatgc cgaacgtatt tttgccgaac ttttgacggg actcgccgcc 240

gcccagccgg ggttcccgct ggcgcaattg aaaactttcg tcgatcagga atttgcccaa 300

ataaaacatg tcctgcatgg cattagtttg ttggggcagt gcccggatag catcaacgct 360

gcgctgattt gccgtggcga gaaaatgtcg atcgccatta tggccggcgt attagaagcg 420

cgcggtcaca acgttactgt tatcgatccg gtcgaaaaac tgctggcagt ggggcattac 480

ctcgaatcta ccgtcgatat tgctgagtcc acccgccgta ttgcggcaag ccgcattccg 540

gctgatcaca tggtgctgat ggcaggtttc accgccggta atgaaaaagg cgaactggtg 600

gtgcttggac gcaacggttc cgactactct gctgcggtgc tggctgcctg tttacgcgcc 660

gattgttgcg agatttggac ggacgttgac ggggtctata cctgcgaccc gcgtcaggtg 720

cccgatgcga ggttgttgaa gtcgatgtcc taccaggaag cgatggagct ttcctacttc 780

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ggcgctaaag ttcttcaccc ccgcaccatt acccccatcg cccagttcca gatcccttgc 840

ctgattaaaa ataccggaaa tcctcaagca ccaggtacgc tcattggtgc cagccgtgat 900

gaagacgaat taccggtcaa gggcatttcc aatctgaata acatggcaat gttcagcgtt 960

tctggtccgg ggatgaaagg gatggtcggc atggcggcgc gcgtctttgc agcgatgtca 1020

cgcgcccgta tttccgtggt gctgattacg caatcatctt ccgaatacag catcagtttc 1080

tgcgttccac aaagcgactg tgtgcgagct gaacgggcaa tgcaggaaga gttctacctg 1140

gaactgaaag aaggcttact ggagccgctg gcagtgacgg aacggctggc cattatctcg 1200

gtggtaggtg atggtatgcg caccttgcgt gggatctcgg cgaaattctt tgccgcactg 1260

gcccgcgcca atatcaacat tgtcgccatt gctcagggat cttctgaacg ctcaatctct 1320

gtcgtggtaa ataacgatga tgcgaccact ggcgtgcgcg ttactcatca gatgctgttc 1380

aataccgatc aggttatcga agtgtttgtg attggcgtcg gtggcgttgg cggtgcgctg 1440

ctggagcaac tgaagcgtca gcaaagctgg ctgaagaata aacatatcga cttacgtgtc 1500

tgcggtgttg ccaactcgaa ggctctgctc accaatgtac atggccttaa tctggaaaac 1560

tggcaggaag aactggcgca agccaaagag ccgtttaatc tcgggcgctt aattcgcctc 1620

gtgaaagaat atcatctgct gaacccggtc attgttgact gcacttccag ccaggcagtg 1680

gcggatcaat atgccgactt cctgcgcgaa ggtttccacg ttgtcacgcc gaacaaaaag 1740

gccaacacct cgtcgatgga ttactaccat cagttgcgtt atgcggcgga aaaatcgcgg 1800

cgtaaattcc tctatgacac caacgttggg gctggattac cggttattga gaacctgcaa 1860

aatctgctca atgcaggtga tgaattgatg aagttctccg gcattctttc tggttcgctt 1920

tcttatatct tcggcaagtt agacgaaggc atgagtttct ccgaggcgac cacgctggcg 1980

cgggaaatgg gttataccga accggacccg cgagatgatc tttctggtat ggatgtggcg 2040

cgtaaactat tgattctcgc tcgtgaaacg ggacgtgaac tggagctggc ggatattgaa 2100

attgaacctg tgctgcccgc agagtttaac gccgagggtg atgttgccgc ttttatggcg 2160

aatctgtcac aactcgacga tctctttgcc gcgcgcgtgg cgaaggcccg tgatgaagga 2220

aaagttttgc gctatgttgg caatattgat gaagatggcg tctgccgcgt gaagattgcc 2280

gaagtggatg gtaatgatcc gctgttcaaa gtgaaaaatg gcgaaaacgc cctggccttc 2340

tatagccact attatcagcc gctgccgttg gtactgcgcg gatatggtgc gggcaatgac 2400

gttacagctg ccggtgtctt tgctgatctg ctacgtaccc tctcatggaa gttaggagtc 2460

tga 2463

<210> SEQ ID NO 89
<211> LENGTH: 820
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 89

Met Arg Val Leu Lys Phe Gly Gly Thr Ser Val Ala Asn Ala Glu Arg
1 5 10 15

Phe Leu Arg Val Ala Asp Ile Leu Glu Ser Asn Ala Arg Gln Gly Gln
20 25 30

Val Ala Thr Val Leu Ser Ala Pro Ala Lys Ile Thr Asn His Leu Val
35 40 45

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Ala Met Ile Glu Lys Thr Ile Ser Gly Gln Asp Ala Leu Pro Asn Ile
    50                  55                  60

Ser Asp Ala Glu Arg Ile Phe Ala Glu Leu Leu Thr Gly Leu Ala Ala
65                  70                  75                  80

Ala Gln Pro Gly Phe Pro Leu Ala Gln Leu Lys Thr Phe Val Asp Gln
                85                  90                  95

Glu Phe Ala Gln Ile Lys His Val Leu His Gly Ile Ser Leu Leu Gly
            100                 105                 110

Gln Cys Pro Asp Ser Ile Asn Ala Ala Leu Ile Cys Arg Gly Glu Lys
        115                 120                 125

Met Ser Ile Ala Ile Met Ala Gly Val Leu Glu Ala Arg Gly His Asn
    130                 135                 140

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

Leu Glu Ser Thr Val Asp Ile Ala Glu Ser Thr Arg Arg Ile Ala Ala
                165                 170                 175

Ser Arg Ile Pro Ala Asp His Met Val Leu Met Ala Gly Phe Thr Ala
            180                 185                 190

Gly Asn Glu Lys Gly Glu Leu Val Val Leu Gly Arg Asn Gly Ser Asp
        195                 200                 205

Tyr Ser Ala Ala Val Leu Ala Ala Cys Leu Arg Ala Asp Cys Cys Glu
    210                 215                 220

Ile Trp Thr Asp Val Asp Gly Val Tyr Thr Cys Asp Pro Arg Gln Val
225                 230                 235                 240

Pro Asp Ala Arg Leu Leu Lys Ser Met Ser Tyr Gln Glu Ala Met Glu
                245                 250                 255

Leu Ser Tyr Phe Gly Ala Lys Val Leu His Pro Arg Thr Ile Thr Pro
            260                 265                 270

Ile Ala Gln Phe Gln Ile Pro Cys Leu Ile Lys Asn Thr Gly Asn Pro
        275                 280                 285

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

Pro Val Lys Gly Ile Ser Asn Leu Asn Asn Met Ala Met Phe Ser Val
305                 310                 315                 320

Ser Gly Pro Gly Met Lys Gly Met Val Gly Met Ala Ala Arg Val Phe
                325                 330                 335

Ala Ala Met Ser Arg Ala Arg Ile Ser Val Val Leu Ile Thr Gln Ser
            340                 345                 350

Ser Ser Glu Tyr Ser Ile Ser Phe Cys Val Pro Gln Ser Asp Cys Val
        355                 360                 365

Arg Ala Glu Arg Ala Met Gln Glu Glu Phe Tyr Leu Glu Leu Lys Glu
    370                 375                 380

Gly Leu Leu Glu Pro Leu Ala Val Thr Glu Arg Leu Ala Ile Ile Ser
385                 390                 395                 400

Val Val Gly Asp Gly Met Arg Thr Leu Arg Gly Ile Ser Ala Lys Phe
                405                 410                 415

Phe Ala Ala Leu Ala Arg Ala Asn Ile Asn Ile Val Ala Ile Ala Gln
            420                 425                 430

Gly Ser Ser Glu Arg Ser Ile Ser Val Val Val Asn Asn Asp Asp Ala
        435                 440                 445

Thr Thr Gly Val Arg Val Thr His Gln Met Leu Phe Asn Thr Asp Gln
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    450                 455                 460

Val Ile Glu Val Phe Val Ile Gly Val Gly Gly Val Gly Gly Ala Leu
465                 470                 475                 480

Leu Glu Gln Leu Lys Arg Gln Gln Ser Trp Leu Lys Asn Lys His Ile
                485                 490                 495

Asp Leu Arg Val Cys Gly Val Ala Asn Ser Lys Ala Leu Leu Thr Asn
            500                 505                 510

Val His Gly Leu Asn Leu Glu Asn Trp Gln Glu Glu Leu Ala Gln Ala
        515                 520                 525

Lys Glu Pro Phe Asn Leu Gly Arg Leu Ile Arg Leu Val Lys Glu Tyr
    530                 535                 540

His Leu Leu Asn Pro Val Ile Val Asp Cys Thr Ser Ser Gln Ala Val
545                 550                 555                 560

Ala Asp Gln Tyr Ala Asp Phe Leu Arg Glu Gly Phe His Val Val Thr
                565                 570                 575

Pro Asn Lys Lys Ala Asn Thr Ser Ser Met Asp Tyr Tyr His Gln Leu
            580                 585                 590

Arg Tyr Ala Ala Glu Lys Ser Arg Arg Lys Phe Leu Tyr Asp Thr Asn
        595                 600                 605

Val Gly Ala Gly Leu Pro Val Ile Glu Asn Leu Gln Asn Leu Leu Asn
    610                 615                 620

Ala Gly Asp Glu Leu Met Lys Phe Ser Gly Ile Leu Ser Gly Ser Leu
625                 630                 635                 640

Ser Tyr Ile Phe Gly Lys Leu Asp Glu Gly Met Ser Phe Ser Glu Ala
                645                 650                 655

Thr Thr Leu Ala Arg Glu Met Gly Tyr Thr Glu Pro Asp Pro Arg Asp
            660                 665                 670

Asp Leu Ser Gly Met Asp Val Ala Arg Lys Leu Leu Ile Leu Ala Arg
        675                 680                 685

Glu Thr Gly Arg Glu Leu Glu Leu Ala Asp Ile Glu Ile Glu Pro Val
    690                 695                 700

Leu Pro Ala Glu Phe Asn Ala Glu Gly Asp Val Ala Ala Phe Met Ala
705                 710                 715                 720

Asn Leu Ser Gln Leu Asp Asp Leu Phe Ala Ala Arg Val Ala Lys Ala
                725                 730                 735

Arg Asp Glu Gly Lys Val Leu Arg Tyr Val Gly Asn Ile Asp Glu Asp
            740                 745                 750

Gly Val Cys Arg Val Lys Ile Ala Glu Val Asp Gly Asn Asp Pro Leu
        755                 760                 765

Phe Lys Val Lys Asn Gly Glu Asn Ala Leu Ala Phe Tyr Ser His Tyr
    770                 775                 780

Tyr Gln Pro Leu Pro Leu Val Leu Arg Gly Tyr Gly Ala Gly Asn Asp
785                 790                 795                 800

Val Thr Ala Ala Gly Val Phe Ala Asp Leu Leu Arg Thr Leu Ser Trp
                805                 810                 815

Lys Leu Gly Val
            820


<210> SEQ ID NO 90
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 90

tgtctcgagc ccgtattttc gtggtgctg 29

<210> SEQ ID NO 91
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 91

cagcaccacg aaaatacggg ctcgagaca 29

<210> SEQ ID NO 92
<211> LENGTH: 2463
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid

<400> SEQUENCE: 92

atgcgagtgt tgaagttcgg cggtacatca gtggcaaatg cagaacgttt tctgcgtgtt 60

gccgatattc tggaaagcaa tgccaggcag gggcaggtgg ccaccgtcct ctctgccccc 120

gccaaaatca ccaaccacct ggtggcgatg attgaaaaaa ccattagcgg ccaggatgct 180

ttacccaata tcagcgatgc cgaacgtatt tttgccgaac ttttgacggg actcgccgcc 240

gcccagccgg ggttcccgct ggcgcaattg aaaactttcg tcgatcagga atttgcccaa 300

ataaaacatg tcctgcatgg cattagtttg ttggggcagt gcccggatag catcaacgct 360

gcgctgattt gccgtggcga gaaaatgtcg atcgccatta tggccggcgt attagaagcg 420

cgcggtcaca acgttactgt tatcgatccg gtcgaaaaac tgctggcagt ggggcattac 480

ctcgaatcta ccgtcgatat tgctgagtcc acccgccgta ttgcggcaag ccgcattccg 540

gctgatcaca tggtgctgat ggcaggtttc accgccggta atgaaaaagg cgaactggtg 600

gtgcttggac gcaacggttc cgactactct gctgcggtgc tggctgcctg tttacgcgcc 660

gattgttgcg agatttggac ggacgttgac ggggtctata cctgcgaccc gcgtcaggtg 720

cccgatgcga ggttgttgaa gtcgatgtcc taccaggaag cgatggagct ttcctacttc 780

ggcgctaaag ttcttcaccc ccgcaccatt acccccatcg cccagttcca gatcccttgc 840

ctgattaaaa ataccggaaa tcctcaagca ccaggtacgc tcattggtgc cagccgtgat 900

gaagacgaat taccggtcaa gggcatttcc aatctgaata acatggcaat gttcagcgtt 960

tctggtccgg ggatgaaagg gatggtcggc atggcggcgc gcgtctttgc agcgatgtct 1020

cgagcccgta ttttcgtggt gctgattacg caatcatctt ccgaatacag catcagtttc 1080

tgcgttccac aaagcgactg tgtgcgagct gaacgggcaa tgcaggaaga gttctacctg 1140

gaactgaaag aaggcttact ggagccgctg gcagtgacgg aacggctggc cattatctcg 1200

gtggtaggtg atggtatgcg caccttgcgt gggatctcgg cgaaattctt tgccgcactg 1260

gcccgcgcca atatcaacat tgtcgccatt gctcagggat cttctgaacg ctcaatctct 1320

gtcgtggtaa ataacgatga tgcgaccact ggcgtgcgcg ttactcatca gatgctgttc 1380

aataccgatc aggttatcga agtgtttgtg attggcgtcg gtggcgttgg cggtgcgctg 1440

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ctggagcaac tgaagcgtca gcaaagctgg ctgaagaata aacatatcga cttacgtgtc 1500

tgcggtgttg ccaactcgaa ggctctgctc accaatgtac atggccttaa tctggaaaac 1560

tggcaggaag aactggcgca agccaaagag ccgtttaatc tcgggcgctt aattcgcctc 1620

gtgaaagaat atcatctgct gaacccggtc attgttgact gcacttccag ccaggcagtg 1680

gcggatcaat atgccgactt cctgcgcgaa ggtttccacg ttgtcacgcc gaacaaaaag 1740

gccaacacct cgtcgatgga ttactaccat cagttgcgtt atgcggcgga aaaatcgcgg 1800

cgtaaattcc tctatgacac caacgttggg gctggattac cggttattga gaacctgcaa 1860

aatctgctca atgcaggtga tgaattgatg aagttctccg gcattctttc tggttcgctt 1920

tcttatatct tcggcaagtt agacgaaggc atgagtttct ccgaggcgac cacgctggcg 1980

cgggaaatgg gttataccga accggacccg cgagatgatc tttctggtat ggatgtggcg 2040

cgtaaactat tgattctcgc tcgtgaaacg ggacgtgaac tggagctggc ggatattgaa 2100

attgaacctg tgctgcccgc agagtttaac gccgagggtg atgttgccgc ttttatggcg 2160

aatctgtcac aactcgacga tctctttgcc gcgcgcgtgg cgaaggcccg tgatgaagga 2220

aaagttttgc gctatgttgg caatattgat gaagatggcg tctgccgcgt gaagattgcc 2280

gaagtggatg gtaatgatcc gctgttcaaa gtgaaaaatg gcgaaaacgc cctggccttc 2340

tatagccact attatcagcc gctgccgttg gtactgcgcg gatatggtgc gggcaatgac 2400

gttacagctg ccggtgtctt tgctgatctg ctacgtaccc tctcatggaa gttaggagtc 2460

tga 2463

<210> SEQ ID NO 93
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 93

tataatgagc tcgtttaact ttaagaagga gatataccat gcgagtgttg aagttcggcg 60

<210> SEQ ID NO 94
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 94

tataatcccg ggtcagactc ctaacttcca 30

<210> SEQ ID NO 95
<211> LENGTH: 62
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 95

tataatcccg gggtttaact ttaagaagga gatataccat gaaaaatgtt ggttttatcg 60

gc 62

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<210> SEQ ID NO 96
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 96

tataatggat ccttacgcca gttgacgaag 30

<210> SEQ ID NO 97
<211> LENGTH: 1080
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid

<400> SEQUENCE: 97

atgagcacta aagttgttaa tgttgccgtt atcggtgccg gtgttgttgg ttcagctttc 60

ttggatcaat tgttagccat gaagtctacc attacttaca atctagttct tttggctgaa 120

gctgagcgtt ctttaatctc caaggacttt tctccattaa atgttggttc tgattggaag 180

gctgctttag cagcctccac tactaaaacg ttgcctttgg atgatttaat tgctcatttg 240

aagacttcac ctaagccagt cattttggtt gataacactt ccagcgctta cattgctggt 300

ttttacacta agtttgtcga aaatggtatt tccattgcta ctccaaacaa gaaggccttt 360

tcctctgatt tggctacctg gaaggctctt ttctcaaata agccaactaa cggttttgtc 420

tatcatgaag ctaccgtcgg tgctggtttg cctatcatca gtttcttaag agaaattatt 480

caaaccggtg acgaagttga aaaaattgaa ggtatcttct ctggtactct atcttatatt 540

ttcaacgagt tctccactag tcaagctaac gacgtcaaat tctctgatgt tgtcaaagtt 600

gctaaaaaat tgggttatac tgaaccagat ccaagagatg atttgaatgg gttggatgtt 660

gctagaaagg ttaccattgt tggtaggata tctggtgtgg aagttgaatc tccaacttcc 720

ttccctgtcc agtctttgat tccaaaacca ttggaatctg tcaagtctgc tgatgaattc 780

ttggaaaaat tatctgatta cgataaagat ttgactcaat tgaagaagga agctgccact 840

gaaaataagg tattgagatt cattggtaaa gtcgatgttg ccaccaaatc tgtgtctgta 900

ggaattgaaa agtacgatta ctcacaccca ttcgcatcat tgaagggatc agataacgtt 960

atttccatca agactaagcg ttacaccaat cctgttgtca ttcaaggtgc cggtgccggt 1020

gctgccgtta ctgccgctgg tgttttgggt gatgttatca agattgctca aagactttag 1080

<210> SEQ ID NO 98
<211> LENGTH: 1104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 98

atgaaaaatg ttggttttat cggctggcgc ggtatggtcg gctccgttct catgcaacgc 60

atggttgaag agcgcgactt cgacgccatt cgccctgtct tcttttctac ttctcagctt 120

ggccaggctg cgccgtcttt tggcggaacc actggcacac ttcaggatgc ctttgatctg 180

gaggcgctaa aggccctcga tatcattgtg acctgtcagg gcggcgatta taccaacgaa 240

atctatccaa agcttcgtga aagcggatgg caaggttact ggattgacgc agcatcgtct 300

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ctgcgcatga aagatgacgc catcatcatt cttgaccccg tcaatcagga cgtcattacc 360

gacggattaa ataatggcat caggactttt gttggcggta actgtaccgt aagcctgatg 420

ttgatgtcgt tgggtggttt attcgccaat gatcttgttg attgggtgtc cgttgcaacc 480

taccaggccg cttccggcgg tggtgcgcga catatgcgtg agttattaac ccagatgggc 540

catctgtatg gccatgtggc agatgaactc gcgaccccgt cctctgctat tctcgatatc 600

gaacgcaaag tcacaacctt aacccgtagc ggtgagctgc cggtggataa ctttggcgtg 660

ccgctggcgg gtagcctgat tccgtggatc gacaaacagc tcgataacgg tcagagccgc 720

gaagagtgga aagggcaggc ggaaaccaac aagatcctca acacatcttc cgtaattccg 780

gtagatggtt tatgtgtgcg tgtcggggca ttgcgctgcc acagccaggc attcactatt 840

aaattgaaaa aagatgtgtc tattccgacc gtggaagaac tgctggctgc gcacaatccg 900

tgggcgaaag tcgttccgaa cgatcgggaa atcactatgc gtgagctaac cccagctgcc 960

gttaccggca cgctgaccac gccggtaggc cgcctgcgta agctgaatat gggaccagag 1020

ttcctgtcag cctttaccgt gggcgaccag ctgctgtggg gggccgcgga gccgctgcgt 1080

cggatgcttc gtcaactggc gtaa 1104

<210> SEQ ID NO 99
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 99

cgtgatgctg cttactcgat cgtcgctaaa aaaggtg 37

<210> SEQ ID NO 100
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 100

caccttttt agcgacgatc gagtaagcag catcacg 37

<210> SEQ ID NO 101
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 101

atgaaagtcg cagtcctcgg cgctgctggc ggtattggcc aggcgcttgc actactgtta 60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc 120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg tttttctggt 180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg 240

gctaaacccg ggatggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac 300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg 360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa 420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa 480

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ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt 540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct 600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc 660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt 720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac 780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct 840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag 900

aaagatatcg ccctgggcga agagttcgtt aataagtaa 939

<210> SEQ ID NO 102
<211> LENGTH: 317
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 102

Met Asp His Arg Ala Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly
1 5 10 15

Ile Gly Gln Ala Leu Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly
20 25 30

Ser Glu Leu Ser Leu Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala
35 40 45

Val Asp Leu Ser His Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser
50 55 60

Gly Glu Asp Ala Thr Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile
65 70 75 80

Ser Ala Gly Val Ala Ala Lys Pro Gly Met Asp Arg Ser Asp Leu Phe
85 90 95

Asn Val Asn Ala Gly Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys
100 105 110

Thr Cys Pro Lys Ala Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr
115 120 125

Thr Val Ala Ile Ala Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp
130 135 140

Lys Asn Lys Leu Phe Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn
145 150 155 160

Thr Phe Val Ala Glu Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val
165 170 175

Pro Val Ile Gly Gly His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser
180 185 190

Gln Val Pro Gly Val Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr
195 200 205

Lys Arg Ile Gln Asn Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly
210 215 220

Gly Gly Ser Ala Thr Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly
225 230 235 240

Leu Ser Leu Val Arg Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys
245 250 255

Ala Tyr Val Glu Gly Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro
260 265 270

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Leu Leu Leu Gly Lys Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr
        275                 280                 285

Leu Ser Ala Phe Glu Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu
    290                 295                 300

Lys Lys Asp Ile Ala Leu Gly Glu Glu Phe Val Asn Lys
305                 310                 315


<210> SEQ ID NO 103
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 103

atgaaagtcg cagtcctcgg cgctgctggc ggtattggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg tttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240

gctaaaccgg gtcaggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac    300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg    360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa    420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa    480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt    540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct    600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc    660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt    720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac    780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct    840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag    900

aaagatatcg ccctgggcga agagttcgtt aataagtaa                           939


<210> SEQ ID NO 104
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 104

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Ile Gly Gln Ala Leu
1               5                   10                  15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
            20                  25                  30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
        35                  40                  45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
    50                  55                  60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65                  70                  75                  80

Ala Lys Pro Gly Gln Asp Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
                85                  90                  95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
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            100                 105                 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
        115                 120                 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
    130                 135                 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145                 150                 155                 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
                165                 170                 175

His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
            180                 185                 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
        195                 200                 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
    210                 215                 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225                 230                 235                 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
                245                 250                 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
            260                 265                 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
        275                 280                 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310


<210> SEQ ID NO 105
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 105

atgaaagtcg cagtcctcgg cgctgctggc ggtattggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg tttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240

gctaaaccgg gtgaggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac    300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg    360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa    420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa    480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt    540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct    600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc    660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt    720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac    780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct    840
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atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag 900

aaagatatcg ccctgggcga agagttcgtt aataagtaa 939

<210> SEQ ID NO 106
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 106

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Ile Gly Gln Ala Leu
1 5 10 15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
20 25 30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
35 40 45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
50 55 60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65 70 75 80

Ala Lys Pro Gly Glu Asp Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
85 90 95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
100 105 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
115 120 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
130 135 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145 150 155 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
165 170 175

His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
180 185 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
195 200 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
210 215 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225 230 235 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
245 250 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
260 265 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
275 280 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
290 295 300

Leu Gly Glu Glu Phe Val Asn Lys
305 310

<210> SEQ ID NO 107
<211> LENGTH: 939

-continued

<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 107

atgaaagtcg cagtcctcgg cgccgctggc ggtgtcggcc aggcgcttgc actactgtta 60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc 120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg tttttctggt 180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg 240

gctaaaccgg gtcaggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac 300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg 360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa 420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa 480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt 540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct 600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc 660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt 720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac 780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct 840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag 900

aaagatatcg ccctgggcga agagttcgtt aataagtaa 939

<210> SEQ ID NO 108
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 108

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Val Gly Gln Ala Leu
1 5 10 15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
20 25 30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
35 40 45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
50 55 60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65 70 75 80

Ala Lys Pro Gly Gln Asp Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
85 90 95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
100 105 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
115 120 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
130 135 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145 150 155 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly

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               165                 170                 175

His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
            180                 185                 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
        195                 200                 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
    210                 215                 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225                 230                 235                 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
                245                 250                 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
            260                 265                 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
        275                 280                 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310
```

<210> SEQ ID NO 109
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 109

```
atgaaagtcg cagtcctcgg cgccgctggc ggtgtcggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg tttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240

gctaaaccgg gtgaggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac    300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg    360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa    420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa    480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt    540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct    600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc    660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt    720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac    780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct    840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag    900

aaagatatcg ccctgggcga agagttcgtt aataagtaa                           939
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<210> SEQ ID NO 110
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 110

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```
Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Val Gly Gln Ala Leu
1               5                  10                  15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
            20                  25                  30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
        35                  40                  45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
    50                  55                  60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65                  70                  75                  80

Ala Lys Pro Gly Glu Asp Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
                85                  90                  95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
            100                 105                 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
        115                 120                 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
    130                 135                 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145                 150                 155                 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
                165                 170                 175

His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
            180                 185                 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
        195                 200                 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
    210                 215                 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225                 230                 235                 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
                245                 250                 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
            260                 265                 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
        275                 280                 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310


<210> SEQ ID NO 111
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 111

atgaaagtcg cagtcctcgg cgctgctggc ggtattggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg ttttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240
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gctaaacccg ggatggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac 300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg 360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa 420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa 480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggcca ctctgatgtt 540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct 600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc 660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt 720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac 780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct 840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag 900

aaagatatcg ccctgggcga agagttcgtt aataagtaa 939

<210> SEQ ID NO 112
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 112

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Ile Gly Gln Ala Leu
1 5 10 15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
20 25 30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
35 40 45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
50 55 60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65 70 75 80

Ala Lys Pro Gly Met Asp Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
85 90 95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
100 105 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
115 120 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
130 135 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145 150 155 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
165 170 175

His Ser Asp Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
180 185 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
195 200 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
210 215 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg

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225                 230                 235                 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
                245                 250                 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
            260                 265                 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
        275                 280                 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310


<210> SEQ ID NO 113
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 113

atgaaagtcg cagtcctcgg cgctgctggc ggtattggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg tttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240

gctaaacccg ggatgtctcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac    300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg    360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa    420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa    480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt    540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct    600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc    660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt    720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac    780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct    840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag    900

aaagatatcg ccctgggcga agagttcgtt aataagtaa                           939


<210> SEQ ID NO 114
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 114

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Ile Gly Gln Ala Leu
1               5                   10                  15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
            20                  25                  30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
        35                  40                  45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
    50                  55                  60
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Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65                  70                  75                  80

Ala Lys Pro Gly Met Ser Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
                85                  90                  95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
            100                 105                 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
        115                 120                 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
    130                 135                 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145                 150                 155                 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
                165                 170                 175

His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
            180                 185                 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
        195                 200                 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
    210                 215                 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225                 230                 235                 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
                245                 250                 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
            260                 265                 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
        275                 280                 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310


<210> SEQ ID NO 115
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 115

atgaaagtcg cagtcctcgg cgctgctggc ggtgtcggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg ttttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240

gctaaacccg ggatggatcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac    300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg    360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa    420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa    480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggtca ctctggtgtt    540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct    600
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gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc 660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt 720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac 780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct 840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag 900

aaagatatcg ccctgggcga agagttcgtt aataagtaa 939

<210> SEQ ID NO 116
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 116

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Val Gly Gln Ala Leu
1 5 10 15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
20 25 30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
35 40 45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
50 55 60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65 70 75 80

Ala Lys Pro Gly Met Asp Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
85 90 95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
100 105 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
115 120 125

Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
130 135 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145 150 155 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
165 170 175

His Ser Gly Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
180 185 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
195 200 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
210 215 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225 230 235 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
245 250 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
260 265 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
275 280 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala

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    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310


<210> SEQ ID NO 117
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 117

atgaaagtcg cagtcctcgg cgctgctggc ggtattggcc aggcgcttgc actactgtta     60

aaaacccaac tgccttcagg ttcagaactc tctctgtatg atatcgctcc agtgactccc    120

ggtgtggctg tcgatctgag ccatatccct actgctgtga aaatcaaagg ttttttctggt    180

gaagatgcga ctccggcgct ggaaggcgca gatgtcgttc ttatctctgc aggcgtagcg    240

gctaaacccg ggatgtctcg ttccgacctg tttaacgtta acgccggcat cgtgaaaaac    300

ctggtacagc aagttgcgaa aacctgcccg aaagcgtgca ttggtattat cactaacccg    360

gttaacacca cagttgcaat tgctgctgaa gtgctgaaaa aagccggtgt ttatgacaaa    420

aacaaactgt tcggcgttac cacgctggat atcattcgtt ccaacacctt tgttgcggaa    480

ctgaaaggca aacagccagg cgaagttgaa gtgccggtta ttggcggcca ctctgatgtt    540

accattctgc cgctgctgtc acaggttcct ggcgttagtt ttaccgagca ggaagtggct    600

gatctgacca aacgcatcca gaacgcgggt actgaagtgg ttgaagcgaa ggccggtggc    660

gggtctgcaa ccctgtctat gggccaggca gctgcacgtt ttggtctgtc tctggttcgt    720

gcactgcagg gcgaacaagg cgttgtcgaa tgtgcctacg ttgaaggcga cggtcagtac    780

gcccgtttct tctctcaacc gctgctgctg ggtaaaaacg gcgtggaaga gcgtaaatct    840

atcggtaccc tgagcgcatt tgaacagaac gcgctggaag gtatgctgga tacgctgaag    900

aaagatatcg ccctgggcga agagttcgtt aataagtaa                           939


<210> SEQ ID NO 118
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 118

Met Lys Val Ala Val Leu Gly Ala Ala Gly Gly Ile Gly Gln Ala Leu
1               5                   10                  15

Ala Leu Leu Leu Lys Thr Gln Leu Pro Ser Gly Ser Glu Leu Ser Leu
            20                  25                  30

Tyr Asp Ile Ala Pro Val Thr Pro Gly Val Ala Val Asp Leu Ser His
        35                  40                  45

Ile Pro Thr Ala Val Lys Ile Lys Gly Phe Ser Gly Glu Asp Ala Thr
    50                  55                  60

Pro Ala Leu Glu Gly Ala Asp Val Val Leu Ile Ser Ala Gly Val Ala
65                  70                  75                  80

Ala Lys Pro Gly Met Ser Arg Ser Asp Leu Phe Asn Val Asn Ala Gly
                85                  90                  95

Ile Val Lys Asn Leu Val Gln Gln Val Ala Lys Thr Cys Pro Lys Ala
            100                 105                 110

Cys Ile Gly Ile Ile Thr Asn Pro Val Asn Thr Thr Val Ala Ile Ala
        115                 120                 125
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Ala Glu Val Leu Lys Lys Ala Gly Val Tyr Asp Lys Asn Lys Leu Phe
    130                 135                 140

Gly Val Thr Thr Leu Asp Ile Ile Arg Ser Asn Thr Phe Val Ala Glu
145                 150                 155                 160

Leu Lys Gly Lys Gln Pro Gly Glu Val Glu Val Pro Val Ile Gly Gly
                165                 170                 175

His Ser Asp Val Thr Ile Leu Pro Leu Leu Ser Gln Val Pro Gly Val
            180                 185                 190

Ser Phe Thr Glu Gln Glu Val Ala Asp Leu Thr Lys Arg Ile Gln Asn
        195                 200                 205

Ala Gly Thr Glu Val Val Glu Ala Lys Ala Gly Gly Gly Ser Ala Thr
    210                 215                 220

Leu Ser Met Gly Gln Ala Ala Ala Arg Phe Gly Leu Ser Leu Val Arg
225                 230                 235                 240

Ala Leu Gln Gly Glu Gln Gly Val Val Glu Cys Ala Tyr Val Glu Gly
                245                 250                 255

Asp Gly Gln Tyr Ala Arg Phe Phe Ser Gln Pro Leu Leu Leu Gly Lys
            260                 265                 270

Asn Gly Val Glu Glu Arg Lys Ser Ile Gly Thr Leu Ser Ala Phe Glu
        275                 280                 285

Gln Asn Ala Leu Glu Gly Met Leu Asp Thr Leu Lys Lys Asp Ile Ala
    290                 295                 300

Leu Gly Glu Glu Phe Val Asn Lys
305                 310


<210> SEQ ID NO 119
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 119

tataatcccg ggatgcgcgt taacaatggt ttgacc                              36


<210> SEQ ID NO 120
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 120

tataattcta gattacagtt tcggaccagc cg                                  32


<210> SEQ ID NO 121
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 121

tataatcccg ggatgcgcgt taacaatggt ttgacc                              36


<210> SEQ ID NO 122
<211> LENGTH: 32
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 122

tataattcta gattacagtt tcggaccagc cg 32

<210> SEQ ID NO 123
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 123

tataatcccg ggatgaacga acaatattcc 30

<210> SEQ ID NO 124
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 124

tataattcta gattagccgg tattacgcat 30

<210> SEQ ID NO 125
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 125

tataatcccg ggatgaaaac ccgtacacaa caaatt 36

<210> SEQ ID NO 126
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 126

tataattcta gattagaact gcgattcttc ag 32

<210> SEQ ID NO 127
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 127

tataatcccg ggatgaaaaa actactcgtc gccaat 36

<210> SEQ ID NO 128
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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<400> SEQUENCE: 128

tataattcta gattaattaa tttcgattaa ca 32

<210> SEQ ID NO 129
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 129

tataatcccg ggatgcctga cgctaaaaaa caggggcggt 40

<210> SEQ ID NO 130
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 130

tataattcta gattaatcgt gagcgcctat ttc 33

<210> SEQ ID NO 131
<211> LENGTH: 88
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 131

acaatttcac acaggaaaca gaattcgagc tcggtaccgt ttaactttaa gaaggagata 60

taccatgacc acgaagaaag ctgattac 88

<210> SEQ ID NO 132
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 132

ggataacttt tttacgttgt ttatcagcca tggtatatct ccttcttaaa gttaaacgga 60

tccttattga ttaacttg 78

<210> SEQ ID NO 133
<211> LENGTH: 69
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 133

taatatggat ccgtttaact ttaagaagga gatataccat ggctgataaa caacgtaaaa 60

aagttatcc 69

<210> SEQ ID NO 134
<211> LENGTH: 83
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 134

caatgcggaa tattgttcgt tcatggtata tctccttctt aaagttaaac tctagattag 60

tttttaactg cagaagcaaa ttc 83

<210> SEQ ID NO 135
<211> LENGTH: 88
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 135

acaatttcac acaggaaaca gaattcgagc tcggtaccgt ttaactttaa gaaggagata 60

taccatgacc acgaagaaag ctgattac 88

<210> SEQ ID NO 136
<211> LENGTH: 83
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 136

caatgcggaa tattgttcgt tcatggtata tctccttctt aaagttaaac tctagattag 60

tttttaactg cagaagcaaa ttc 83

<210> SEQ ID NO 137
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 137

gaaggttgcg cctacactaa gcatagttgt tgatgagtgt aggctggagc tgcttc 56

<210> SEQ ID NO 138
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 138

ttaaaccagt tcgttcgggc aggtttcgcc tttttcatgg gaattagcca tggtcc 56

<210> SEQ ID NO 139
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 139

atggctgtta ctaatgtcgc tgaacttaac gcactcgtag agcgtgtgta ggctggagct 60

gcttc 65

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<210> SEQ ID NO 140
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 140

ttaagcggat tttttcgctt ttttctcagc tttagccgga gcagccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 141
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 141

atgtcgagta agttagtact ggttctgaac tgcggtagtt cttcagtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 142
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 142

tcaggcagtc aggcggctcg cgtcttgcgc gataaccagt tcttccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 143
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 143

ttactccgta tttgcataaa aaccatgcga gttacgggcc tataagtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 144
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 144

atagattgag tgaaggtacg agtaataacg tcctgctgct gttctcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 145
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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<400> SEQUENCE: 145

gtgtcccgta ttattatgct gatccctacc ggaaccagcg tcggtgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 146
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 146

ttactgctgc tgtgcagact gaatcgcagt cagcgcgatg gtgtacatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 147
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 147

atgaaacaaa cggttgcagc ttatatcgcc aaaacactcg aatcggtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 148
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 148

ttaccttagc cagtttgttt tcgccagttc gatcacttca tcacccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 149
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 149

atgaccatta ctccggcaac tcatgcaatt tcgataaatc ctgccgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 150
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 150

tcagatccgg tctttccaca ccgtctggat attacagaat tcgtgcatat gaatatcctc 60

cttag 65

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<210> SEQ ID NO 151
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 151

atgaaactta acgacagtaa cttattccgc cagcaggcgt tgattgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 152
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 152

ttaaagaccg atgcacatat atttgatttc taagtaatct tcgatcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 153
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 153

atggaccaga agctgttaac ggatttccgc tcagaactac tcgatgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 154
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 154

tcaggtgtgt ttaaagctgt tctgctgggc aataccctgc agtttcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 155
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 155

atggataaga agcaagtaac ggatttaagg tcggaactac tcgatgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 156
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 156

tcaggtatgt ttaaagctgt tctgttgggc aataccctgc agtttcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 157
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 157

atggctacat cagtacagac aggtaaagct aagcagctca cattagtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 158
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 158

ttagtgtttc ttgtcattca tcacaatata gtgtggtgaa cgtgccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 159
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 159

atggaaccaa aaacaaaaaa acagcgttcg ctttatatcc cttacgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 160
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 160

ttagatggag gtacggcggt agtcgcggta ttcggcttgc cagaacatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 161
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 161

atggatgacc agttaaaaca aagtgcactt gatttccatg aatttgtgta ggctggagct 60

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gcttc 65

<210> SEQ ID NO 162
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 162

ttacagcggt tgggtttgcg cttctaccac ggccagcgcc accatcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 163
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 163

atgaacgaac aatattccgc attgcgtagt aatgtcagta tgctcgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 164
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 164

ttagccggta ttacgcatac ctgccgcaat cccggcaata gtgaccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 165
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 165

atgtccagaa ggcttcgcag aacaaaaatc gttaccacgt taggcgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 166
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 166

ttactctacc gttaaaatac gcgtggtatt agtagaaccc acggtcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 167
<211> LENGTH: 65

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 167

atgaaaaaga ccaaaattgt ttgcaccatc ggaccgaaaa ccgaagtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 168
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 168

ttacaggacg tgaacagatg cggtgttagt agtgccgctc ggtaccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 169
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 169

atggaactga cgactcgcac tttacctgcg cggaaacata ttgcggtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 170
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 170

ttacttcaga cggtccgcga gataacgctg ataatcgggg atcagcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 171
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 171

atggtcgcac ccattcccgc gaaacgcggc agaaaacccg ccgttgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 172
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 172

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tcagcgcatt ccaccgtacg ccagcgtcac ttccttcgcc gctttcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 173
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 173

atggaaagta aagtagttgt tccggcacaa ggcaagaaga tcaccgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 174
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 174

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cttag 65

<210> SEQ ID NO 175
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 175

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gcttc 65

<210> SEQ ID NO 176
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 176

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cttag 65

<210> SEQ ID NO 177
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 177

atgagtagcg tagatattct ggtccctgac ctgcctgaat ccgtagtgta ggctggagct 60

gcttc 65

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<210> SEQ ID NO 178
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 178

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cttag 65

<210> SEQ ID NO 179
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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gcttc 65

<210> SEQ ID NO 180
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 180

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cttag 65

<210> SEQ ID NO 181
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 181

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gcttc 65

<210> SEQ ID NO 182
<211> LENGTH: 65
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 182

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cttag 65

<210> SEQ ID NO 183
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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<400> SEQUENCE: 183

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gcttc 65

<210> SEQ ID NO 184
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 184

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cttag 65

<210> SEQ ID NO 185
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 185

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gcttc 65

<210> SEQ ID NO 186
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 186

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cttag 65

<210> SEQ ID NO 187
<211> LENGTH: 65
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 187

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gcttc 65

<210> SEQ ID NO 188
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 188

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cttag 65

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<210> SEQ ID NO 189
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 189

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gcttc 65

<210> SEQ ID NO 190
<211> LENGTH: 65
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 190

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cttag 65

<210> SEQ ID NO 191
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 191

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gcttc 65

<210> SEQ ID NO 192
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 192

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cttag 65

<210> SEQ ID NO 193
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 193

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gcttc 65

<210> SEQ ID NO 194
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 194

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cttag 65

<210> SEQ ID NO 195
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 195

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gcttc 65

<210> SEQ ID NO 196
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 196

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cttag 65

<210> SEQ ID NO 197
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 197

atgccgattc gtgtgccgga cgagctaccc gccgtcaatt tcttggtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 198
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 198

ttaatccagc gttggattca tgtgccgtag atcgtatggc gtgatcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 199
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 199

atggttaaag tttatgcccc ggcttccagt gccaatatga gcgtcgtgta ggctggagct 60

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gcttc 65

<210> SEQ ID NO 200
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 200

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cttag 65

<210> SEQ ID NO 201
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 201

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gcttc 65

<210> SEQ ID NO 202
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 202

ttaaagcaat tccagcgcca gtaattcttc gatggtctgg cgacgcatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 203
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 203

atgaaaaact ggaaaacaag tgcagaatca atcctgacca ccggcgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 204
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 204

ctcgatcggg cattttgact tttacagctt agcgccttct acagccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 205
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 205

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gcttc 65

<210> SEQ ID NO 206
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 206

ttaaaaatct tcgttagttt ctgctacgcc ttcgctatca tctaccatat gaatatcctc 60

cttag 65

<210> SEQ ID NO 207
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 207

atgaaaaatg ttggttttat cggctggcgc ggtatggtcg gctccgtgta ggctggagct 60

gcttc 65

<210> SEQ ID NO 208
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 208

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cttag 65

<210> SEQ ID NO 209
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 209

cggtgccctg aatgaactgc 20

<210> SEQ ID NO 210
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 210

cagtcatagc cgaatagcct 20

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<210> SEQ ID NO 211
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 211

atacgtgtcc cgagcggtag 20

<210> SEQ ID NO 212
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 212

tacacatccc gccatcagca 20

<210> SEQ ID NO 213
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 213

gaagtaaacg ggaaaatcaa 20

<210> SEQ ID NO 214
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 214

agaagtggca taagaaaacg 20

<210> SEQ ID NO 215
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 215

ccattggctg aaaattacgc 20

<210> SEQ ID NO 216
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 216

gttccattgc acggatcacg 20

<210> SEQ ID NO 217
<211> LENGTH: 20

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 217

atgccgtaga agccgccagt 20

<210> SEQ ID NO 218
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 218

tgttggtgcg cagctcgaag 20

<210> SEQ ID NO 219
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 219

gcaaatctgg tttcatcaac 20

<210> SEQ ID NO 220
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 220

tcccttgcac aaaacaaagt 20

<210> SEQ ID NO 221
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 221

ggatttggtt ctcgcataat 20

<210> SEQ ID NO 222
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 222

agcattaacg gtagggtcgt 20

<210> SEQ ID NO 223
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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<400> SEQUENCE: 223

gctgattctc gcgaataaac 20

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 224

aaaaacgttc ttgcgcgtct 20

<210> SEQ ID NO 225
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 225

tctgtttgtc accaccccgc 20

<210> SEQ ID NO 226
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 226

aagccagcac ctggaagcag 20

<210> SEQ ID NO 227
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 227

aagagctgcc gcaggaggat 20

<210> SEQ ID NO 228
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 228

gccgccctct taagtcaaat 20

<210> SEQ ID NO 229
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 229

ggattttagc aatattcgct 20

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<210> SEQ ID NO 230
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 230

cctaatagca ggaagaagac 20

<210> SEQ ID NO 231
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 231

gctgaactgt tgctggaaga 20

<210> SEQ ID NO 232
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 232

ggcgtgcttt tacaactaca 20

<210> SEQ ID NO 233
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 233

tagtaaataa cccaaccggc 20

<210> SEQ ID NO 234
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 234

tcagtgagcg cagtgtttta 20

<210> SEQ ID NO 235
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 235

attaatggtg agagtttgga 20

<210> SEQ ID NO 236
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 236

tgctttttt tattattcgc 20

<210> SEQ ID NO 237
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 237

gctttataaa agacgacgaa 20

<210> SEQ ID NO 238
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 238

gtaacgacaa ttccttaagg 20

<210> SEQ ID NO 239
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 239

tttatatgcc catggtttct 20

<210> SEQ ID NO 240
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 240

atctgttaga ggcggatgat 20

<210> SEQ ID NO 241
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 241

ctggaacgtt aaatctttga 20

<210> SEQ ID NO 242
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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<400> SEQUENCE: 242

ccagtttagt agctttcatt 20

<210> SEQ ID NO 243
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 243

gatttgttca acattaactc atcgg 25

<210> SEQ ID NO 244
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 244

tgcgattaac agacaccctt 20

<210> SEQ ID NO 245
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 245

tctcaggtgc tcacagaaca 20

<210> SEQ ID NO 246
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 246

tatggaagag gcgctactgc 20

<210> SEQ ID NO 247
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 247

cgacctgctg cataaacacc 20

<210> SEQ ID NO 248
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 248

tgaacgctaa ggtgattgca 20

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<210> SEQ ID NO 249
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 249

acgtagacaa gagctcgcaa 20

<210> SEQ ID NO 250
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 250

catcacgtac gactgcgtcg 20

<210> SEQ ID NO 251
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 251

tgcaactttg tgctgagca 19

<210> SEQ ID NO 252
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 252

tatcgcttcc gggcattgtc 20

<210> SEQ ID NO 253
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 253

aaatcgatct cgtcaaattt cagac 25

<210> SEQ ID NO 254
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 254

aggaaccaca aatcgccata 20

<210> SEQ ID NO 255
<211> LENGTH: 20

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 255

gacgtgaaga ttactacgct 20

<210> SEQ ID NO 256
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 256

agttcaatgc tgaaccacac 20

<210> SEQ ID NO 257
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 257

tagccgcgac cacggtaaga aggag 25

<210> SEQ ID NO 258
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 258

cagcgcatca cccggaaaca 20

<210> SEQ ID NO 259
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 259

atcgtgatca ttaacctgat 20

<210> SEQ ID NO 260
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 260

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<210> SEQ ID NO 261
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer for amplification

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<400> SEQUENCE: 261

ccatccgttg aatgagtttt 20

<210> SEQ ID NO 262
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 262

tggtgttaac tggcaaaatc 20

<210> SEQ ID NO 263
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 263

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<210> SEQ ID NO 264
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 264

ccgttggttt gatagcaata 20

<210> SEQ ID NO 265
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 265

gaatctggtg tatatggcga 20

<210> SEQ ID NO 266
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 266

tcttcgctat tacgccagct 20

<210> SEQ ID NO 267
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 267

cgtcagcgga tgtatctggt 20

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 268

gcggaatttc tggttcgtaa 20

<210> SEQ ID NO 269
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 269

ttgtcaacga tggggtcatg 20

<210> SEQ ID NO 270
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 270

aaaaatgccg acataacgtc 20

<210> SEQ ID NO 271
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<400> SEQUENCE: 271

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<212> TYPE: DNA
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<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 272

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<210> SEQ ID NO 273
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 273

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<210> SEQ ID NO 274
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

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<210> SEQ ID NO 275
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<210> SEQ ID NO 277
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<223> OTHER INFORMATION: Primer for amplification

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<220> FEATURE:
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<210> SEQ ID NO 279
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 279

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<210> SEQ ID NO 280
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<212> TYPE: DNA
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<210> SEQ ID NO 281
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<220> FEATURE:
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<400> SEQUENCE: 281

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<210> SEQ ID NO 282
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 282

cacttcaggt aaggctgtga 20

<210> SEQ ID NO 283
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 283

cagagaactg cgtaagtatt acgca 25

<210> SEQ ID NO 284
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 284

tagtggtaac aagcgtgaaa aacaa 25

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<211> LENGTH: 25
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer for amplification

<400> SEQUENCE: 285

atgaagactc cgtaaacgtt tcccc 25

<210> SEQ ID NO 286
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 286

caaaaataga cacaccggga gttca 25

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<210> SEQ ID NO 287
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 287

atgagaatta caattgccgg tgcaggagcg atggggagtc gttttggttt aatgcttcat 60

aaaggtggca atgaagtaac ccttatagat ggatggcctg aacacgttaa agcgattaaa 120

gagcatggtt tgcgagctaa ttacaatgga gaagaactca ccgctcatct atcggttgag 180

ttacaatctg agatttcttc taaagaaaaa acagatttaa ttattttgtt tacaaaagcc 240

atgcaattag ataagatgct acaagatatt aaaccattaa ttgacgagca taccaaggta 300

ctttgcttac taaatggaat tggtcacgaa gatactatag aaaaatatgt ttcgaaaaat 360

aatatcttta ttggtaatac tatgtggact gctggattag aaggtccagg taaagctaaa 420

ttatttggtg atggttcggt tgagctacaa aatcttattt caggtgagga agaaacagct 480

aaaaagttag cagaaatatt atcagaatcg ggactgaatg ctaaatattc taacaatatt 540

cattattcta tttatagaaa agcttgtgtt aatggaacaa tgaatgggct ttgtactatt 600

ttagacacta atatggccgg attaggtgaa acaaaaccag cacatgatat ggttgttact 660

attgttaatg aatttgcagc agtagcaaaa tttgagaatg taaaccttga tattgctgaa 720

gtagttcagc acgttgaaac atgttttgat ccatctacaa ttggattaca ttacccttct 780

atgtatcagg atttgattaa aaataatcga ttgacagaga ttgactatat taatggggct 840

gtttcacgta aaggtaaaaa atataatgta gccacacctt attgtgattt cttaacacaa 900

ttagttcaca gcaaagaaga gttattaaaa gcaaaataa 939

<210> SEQ ID NO 288
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 288

Met Arg Ile Thr Ile Ala Gly Ala Gly Ala Met Gly Ser Arg Phe Gly
1 5 10 15

Leu Met Leu His Lys Gly Gly Asn Glu Val Thr Leu Ile Asp Gly Trp
20 25 30

Pro Glu His Val Lys Ala Ile Lys Glu His Gly Leu Arg Ala Asn Tyr
35 40 45

Asn Gly Glu Glu Leu Thr Ala His Leu Ser Val Glu Leu Gln Ser Glu
50 55 60

Ile Ser Ser Lys Glu Lys Thr Asp Leu Ile Ile Leu Phe Thr Lys Ala
65 70 75 80

Met Gln Leu Asp Lys Met Leu Gln Asp Ile Lys Pro Leu Ile Asp Glu
85 90 95

His Thr Lys Val Leu Cys Leu Leu Asn Gly Ile Gly His Glu Asp Thr
100 105 110

Ile Glu Lys Tyr Val Ser Lys Asn Asn Ile Phe Ile Gly Asn Thr Met
115 120 125

Trp Thr Ala Gly Leu Glu Gly Pro Gly Lys Ala Lys Leu Phe Gly Asp
130 135 140

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Gly Ser Val Glu Leu Gln Asn Leu Ile Ser Gly Glu Glu Glu Thr Ala
145                 150                 155                 160

Lys Lys Leu Ala Glu Ile Leu Ser Glu Ser Gly Leu Asn Ala Lys Tyr
                165                 170                 175

Ser Asn Asn Ile His Tyr Ser Ile Tyr Arg Lys Ala Cys Val Asn Gly
            180                 185                 190

Thr Met Asn Gly Leu Cys Thr Ile Leu Asp Thr Asn Met Ala Gly Leu
        195                 200                 205

Gly Glu Thr Lys Pro Ala His Asp Met Val Val Thr Ile Val Asn Glu
    210                 215                 220

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

Val Val Gln His Val Glu Thr Cys Phe Asp Pro Ser Thr Ile Gly Leu
                245                 250                 255

His Tyr Pro Ser Met Tyr Gln Asp Leu Ile Lys Asn Asn Arg Leu Thr
            260                 265                 270

Glu Ile Asp Tyr Ile Asn Gly Ala Val Ser Arg Lys Gly Lys Lys Tyr
        275                 280                 285

Asn Val Ala Thr Pro Tyr Cys Asp Phe Leu Thr Gln Leu Val His Ser
    290                 295                 300

Lys Glu Glu Leu Leu Lys Ala Lys
305                 310
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1-22. (canceled)

23. A method for the preparation of 2,4-dihydroxybutyrate (2,4-DHB) from homoserine comprising:

deaminating homoserine to form 2-oxo-4-hydroxybutyrate (OHB), where the deamination of homoserine is catalyzed by an enzyme having homoserine transaminase activity, wherein the enzyme having homoserine transaminase activity is produced via a transformed host microorganism that comprises a first chimeric gene including a first nucleic acid sequence encoding the enzyme having homoserine transaminase activity for converting the primary amino acid group of homoserine to a carbonyl group to obtain OHB; and

reducing the OHB to form 2,4-DHB, where the reduction of OHB is catalyzed by an enzyme having OHB reductase activity, wherein the enzyme having OHB reductase activity is produced via the transformed host microorganism, which further comprises a second chimeric gene including a second nucleic acid sequence encoding the enzyme having OHB reductase activity for reducing OHB to 2,4-DHB.

24. The method of claim **23**, wherein the enzyme having homoserine transaminase activity is selected from the group consisting of enzymes classified in E.C. 2.6.1.1, E.C. 2.6.1.2, E.C. 2.6.1.42, E.C. 2.6.1.57 or E.C. 2.6.1.88.

25. The method of claim **24**, wherein the enzyme having homoserine transaminase activity is selected from:

a transaminase having a sequence SEQ ID NO: 64 or encoded by the gene aspC,

a transaminase having a sequence SEQ ID NO: 60 or encoded by the gene ilvE,

a transaminase having a sequence SEQ ID NO: 68 or encoded by the gene bcaT,

a transaminase having a sequence SEQ ID NO: 62 or encoded by the gene tyrB,

a transaminase having a sequence SEQ ID NO: 66 or encoded by the gene araT,

a transaminase having a sequence SEQ ID NO: 70 or encoded by the gene ARO8,

a transaminase encoded by the gene alaC,

a transaminase encoded by the gene mtnE,

a transaminase encoded by the gene ybdL;

or is selected from any sequence sharing a sequence identity of at least 90% with at least one of the sequences of said enzymes.

26. The method of claim **23**, wherein the enzyme having OHB reductase activity is selected from the group consisting of lactate dehydrogenases classified in E.C.1.1.1.27 or E.C.1.1.1.28, malate dehydrogenases classified in E.C.1.1.1.37, E.C.1.1.1.82 or E.C.1.1.1.299, or branched-chain 2-hydroxyacid dehydrogenases classified in E.C.1.1.1.272 or E.C.1.1.1.345.

27. The method of claim **26**, wherein the enzyme having OHB reductase activity is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 288, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116 or SEQ ID NO: 118, or is selected from any sequence sharing a sequence identity of at least 90% with at least one of said sequences.

28. The method of claim **27**, wherein the enzyme having OHB reductase activity is selected from the group consisting of (D)-lactate dehydrogenase from *Escherichia coli* (SEQ ID NO: 4), (L)-lactate dehydrogenase from *Lactococcus lactis* (SEQ ID NO: 6), the two isoforms of (L)-lactate

dehydrogenase from *Oryctalagus cuniculus* (SEQ ID NO: 12 and SEQ ID NO: 14), (L)-lactate dehydrogenase from *Geobacillus stearothermophilus* (SEQ ID NO: 10), (L)-lactate dehydrogenase from *Bacillus subtilis* (SEQ ID NO: 8), (L)-malate dehydrogenase from *Escherichia coli* (SEQ ID NO: 2), branched chain (D)-2-hydroxyacid dehydrogenase from *Lactococcus lactis*, and dehydrogenases having an amino acid sequence sharing a sequence identity of at least 90% with at least one of said sequences.

29. The method of claim **28**, wherein the enzyme having OHB reductase activity is

a lactate dehydrogenase comprising at least one mutation in position V17, Q85, E89, 1226, or A222, said positions being defined by reference to the L-Lactis LdhA (SEQ. ID NO: 6); or

a malate dehydrogenase comprising at least one mutation in position A112, R81, M85, D86, V93, G179, T211, or M227 said positions being defined by reference to the *E. coli* Mdh (SEQ ID NO: 2).

30. The method of claim **23**, wherein the enzyme having homoserine transaminase activity is selected from the group consisting of enzymes classified in E.C. 2.6.1.1, E.C. 2.6.1.2, E.C. 2.6.1.42, E.C. 2.6.1.57 or E.C. 2.6.1.88, and wherein the enzyme having OHB reductase activity is a lactate dehydrogenase classified in E.C.1.1.1.27 or E.C.1.1.1.28, a malate dehydrogenase classified in E.C.1.1.1.37, E.C.1.1.1.82 or E.C.1.1.1.299, or a branched-chain 2-hydroxyacid dehydrogenase classified in E.C.1.1.1.272 or E.C.1.1.1.345.

31. A modified microorganism for the preparation of 2,4-dihydroxybutyrate (2,4-DHB) from homoserine via a two-step pathway comprising:

deaminating homoserine to form 2-oxo-4-hydroxybutyrate (OHB), where the deamination of homoserine is catalyzed by an enzyme having homoserine transaminase activity, and

reducing the OHB to form 2,4-DHB, where the reduction of OHB is catalyzed by an enzyme having OHB reductase activity;

wherein

the modified microorganism is a host microorganism that has been transformed to enhance production of 2,4-DHB compared to a non-transformed host microorganism, the transformed host microorganism comprising:

a first chimeric gene including a first nucleic acid sequence encoding the enzyme having homoserine transaminase activity for converting the primary amino acid group of homoserine to a carbonyl group to obtain OHB, and

a second chimeric gene including a second nucleic acid sequence encoding the enzyme having OHB reductase activity for reducing OHB in 2,4-DHB.

32. The modified microorganism of claim **31**, wherein the transformed host microorganism has been further transformed to enhance production of homoserine compared to the non-transformed host microorganism.

33. The modified microorganism of claim **32**, wherein the enhanced production of homoserine comprises overexpressing one or more additional enzymes selected from the group consisting of aspartate kinase, aspartate semialdehyde dehydrogenase and homoserine dehydrogenase, wherein the overexpression of said one or more enzymes is realized by expressing the enzymes from a multicopy plasmid.

34. The modified microorganism of claim **31**, wherein the modified microorganism is a bacterium, a yeast, or a fungus.

35. The modified microorganism of claim **31**, wherein

the expression of at least of one the enzymatic activities chosen among phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase, isocitrate lyase, pyruvate carboxylase, and hexose symporter permease is increased, and/or

at least one of the enzymatic activities chosen among lactate dehydrogenase, alcohol dehydrogenase, acetate kinase, phosphate acetyltransferase, pyruvate oxidase, isocitrate lyase, fumarase, 2-oxoglutarate dehydrogenase, pyruvate kinase, malic enzyme, phosphoglucose isomerase, phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxykinase, pyruvate-formate lyase, succinic semialdehyde dehydrogenase, sugar-transporting phosphotransferase, ketohydroxyglutarate aldolase, homoserine-O-succinyl transferase, homoserine kinase, homoserine efflux transporter, diaminopimelate decarboxylase, and/or methylglyoxal synthase is decreased.

36. The modified microorganism of claim **34**, the modified microorganism being *Escherichia coli*, which

overexpresses at least one of the genes chosen among ppc (phosphoenol pyruvate carboxylase), pck, aceA, galP, asd, thrA, metL, lysC all *E coli*; pycA from *L lactis*, and/or

has at least one of the genes deleted chosen among IdhA, adhE, ackA, pta, poxB, focA, pflB, sad, gabABC, sfcA, maeB, ppc, pykA, pykF, mgsA, sucAB, ptsl, ptsG, pgi, fumABCaldA, HdD, icIR, metA, thrB, lysA, eda, rthA, rthB, and rthC.

37. The modified microorganism of claim **31**, wherein the enzyme having homoserine transaminase activity is selected from the group consisting of enzymes classified in E.C. 2.6.1.1, E.C. 2.6.1.2, E.C. 2.6.1.42, E.C. 2.6.1.57 or E.C. 2.6.1.88, and/or wherein the enzyme having OHB reductase activity is a lactate dehydrogenase classified in E.C.1.1.1.27 or E.C.1.1.1.28, a malate dehydrogenase classified in E.C. 1.1.1.37, E.C.1.1.1.82 or E.C.1.1.1.299, or a branched-chain 2-hydroxyacid dehydrogenase classified in E.C.1.1.1.272 or E.C.1.1.1.345.

38. A method of production of 2,4-DHB comprising the steps of

culturing the modified microorganism of claim **31** in an appropriate culture medium,

recovering 2,4-DHB from the culture medium.

39. The method of claim **38** wherein the 2,4-DHB is further purified.

* * * * *