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(57) Abstract

The present invention relates to methods for ummunoassay of analytes employing mutant glucose-6-phosphate dehydrogenase (G6PDH) enzymes as labels. In particular, the invention relates to the use of conjugates of an analyte or analyte analog and a mutant NAD^+ dependent G6PDH differing from any precursor G6PDH by the deletion, substitution, or insertion, or any combination thereof of at least one amino acid per subunit. The invention also involves the construction of several mutations in precursor glucose-6-phosphate dehydrogenase (G6PDH) enzymes. Typically, the mutations involve deletion or substitution of one or more lysine residues, or introduction of one or more cysteine residues by insertion of cysteine to precursor G6PDH or substitution of precursor G6PDH amino acids residues with cysteine. The present invention also relates to conjugates of the subject enzymes and specific binding pair members, kits useful in performing the methods of the invention, cell lines producing the subject enzymes, DNA sequences encoding the subject enzymes, and vectors containing DNA encoding the subject enzymes and designed to allow a host cell to produce the subject enzymes.

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HOMOGENEOUS IMMUNOASSAYS USING MUTANT GLUCOSE-6-PHOSPHATE DEHYDROGENASES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to homogeneous immunoassays for analytes and compositions of matter that are useful in conducting such immunoassays. Homogeneous immunoassays have the advantage of not requiring separation steps. Such assays, however, are limited by the difficulty of selecting antibodies which will modulate the activity of a label that is normally bound to the antibodies or an analog of the analyte.

The present invention relates to methods for immunoassay of analytes employing mutant glucose-6-phosphate dehydrogenase (G6PDH) enzymes as labels. In particular, the invention relates to the use of conjugates of an analyte and a mutant NAD^+ dependent G6PDH of bacterial origin differing from any precursor G6PDH by the deletion, substitution, or insertion, or any combination thereof of at least one amino acid per subunit. The invention also involves the construction of several mutations in a precursor glucose-6-phosphate dehydrogenase (G6PDH) enzymes. Typically, the mutations involve deletion or substitution of one or more lysine residues, or introduction of one or more cysteine residues by insertion of cysteine to a precursor G6PDH or substitution of precursor G6PDH amino acid residues with cysteine. The present invention also relates to conjugates of the subject enzymes and specific binding pair members, cell lines producing the subject enzymes, DNA sequences encoding the subject enzymes, and plasmids containing DNA encoding the subject enzymes and designed to allow a host cell to produce the subject enzymes.

2. Brief Description of the Related Art

Adams, M. J., H. R. Levy, and K. Moffat; 1983; Crystallization and preliminary X-ray data for glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*; J. Bio. Chem. 258:5867-5868; discloses the crystallization and preliminary X-ray data for glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*.

Barnell, W. O., K. C. Yi, and T. Conway; 1990; Sequence and genetic organization of a *Zymomonas mobilis* gene cluster that encodes several enzymes of glucose metabolism; J. Bacteriology 172:7227-7240; discloses cloning, sequence and organization of *Zymomonas mobilis* genes encoding glycolytic pathway enzymes, including glucose-6-phosphate dehydrogenase. The information is said to be useful as a tool for studying the contribution of gene expression to flux control at each step of the

pathway.

Bhadbhade, M. M., M. J. Adams, T. G. Flynn, and H. R. Levy; 1987; Sequence identity between a lysine-containing peptide from *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase and an active site peptide from human erythrocyte glucose-6-phosphate dehydrogenase; FEBS Lett. 211:243-246; discloses the sequence identity between a lysine-containing peptide from *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase and an active site peptide from human erythrocyte glucose-6-phosphate dehydrogenase.

Gasser, F., and M. Hontebeyrie; 1977; Immunological relationships of glucose-6-phosphate dehydrogenase of *Leuconostoc mesenteroides* NCDO 768 (=ATCC 12291); Int. J. Systematic Bact. 27:6-8; discloses the immunological cross-reactivity patterns of antibodies capable of recognizing *Leuconostoc mesenteroides* with various *Leuconostoc* strains

Haghighi, B., T. G. Flynn, and H. R. Levy; 1982; Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides;* Isolation and sequence of a peptide containing an essential lysine; Biochemistry 21:6415-6420; discloses the interaction of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* with pyridoxal 5'-phosphate and sodium borohydride.

Heilmann, H. J., H. J. Mägert, and H. G. Gassen; 1988; Identification and isolation of glucose-6-phosphate dehydrogenase genes of *Bacillus megaterium* M1286 and their expression in *Escherichia coli;* Eur. J. Biochem. 174:485-490; discloses the identification and isolation of glucose dehydrogenase genes of *Bacillus megaterium* M1286 and their expression in *Escherichia coli*.

Hey, Y., and P. D. G. Dean; 1983; Tandem dye-ligand chromatography and biospecific elution applied to the purification of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides;* Biochem. J. 209:363-371; discloses the purification of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*.

Hontebeyrie, M.; and F. Gasser; 1975; Comparative immunological relationships of two distinct sets of isofunctional dehydrogenases in the genus *Leuconostoc;* Int. J. Systematic Bact. 25:1-6; discloses the immunological cross-reactivity patterns of antibodies capable of recognizing *Leuconostoc lactis* with various *Leuconostoc* strains and heterofermentative lactobacilli.

Ishaque, A., M. Milhausen, and H. R. Levy; 1974; On the absence of cysteine in glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides;* Biochem. Biophys. Res. Commn. *59*:894-901; discloses the complete lack of cysteine in *Leuconostoc mesenteroides*.

Jarsch, M., and G. Lang; Cloning and overexpression of glucose-6-phosphate dehydrogenase from *Leuconostoc dextranicus;* Canadian Patent Application Number 2,045,838 A1 (published January 31, 1992); discloses recombinant glucose-6-phosphate dehydrogenase enzymes derived from

Leuconostoc dextranicus having improved temperature stability.

Jeffery, J., L. Hobbs, and H. Jörnvall; 1985; Glucose-6-phosphate dehydrogenase from *Saccharomyces cerevisiae*: characterization of a reactive lysine residue labeled with acetylsalicylic acid; Biochem. **24**:666-671: discloses the characterization of a reactive lysine residue that reacts with acetylsalicylic acid.

Jeffery, J., I. Wood, A. Macleod, R. Jeffery, and H. Jörnvall; 1989; Glucose-6-phosphate dehydrogenase; Biochem. Biophys. Res. Commn. *160*:1290-1295; discloses the characterization of a reactive lysine residue in the *Pichia jadinii* glucose-6-phosphate dehydrogenase enzyme. The information is said to reveal a limited structural variation in a functionally significant segment of the enzyme.

Lee, W. T., T. G. Flynn, C. Lyons, and H. R. Levy; 1991; Cloning of the gene and amino acid sequence for glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*; J. Bio. Chem. **266**:13028-13034; discloses the cloning of the *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase gene and the amino acid sequence of the enzyme, derived from partial sequencing of the DNA. The information is said to be useful for site-directed mutagenesis studies of those structural features of *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase that allow for NAD^+ binding and utilization.

Lee, W. T., and H. R. Levy; 1992; Lysine-21 of *Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase participates in substrate binding through charge-charge interaction; Protein Science 1:329-353; discloses the purification and kinetic characterization of Lys-21-Arg and Lys-21-Gln mutants of glucose-6-phosphate dehydrogenase in order to determine the function of Lys-21.

Levy, H. R.; 1979; glucose-6-phosphate dehydrogenases; Advances in Enzymology **48**:97-192; discloses the isolation, structure, and catalytic activity of glucose-6-phosphate dehydrogenases.

Levy, H. R., and W. T. Lee; Cloned *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase genes and methods of making same; International Patent Application Number PCT/US91/07715 (International publication WO 92/07078, April 30, 1992) discloses the isolation, PCR amplification and cloning of a gene for glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* into plasmid pUC19 for expression in *E. coli*.

Murphy, N. B., D. J. McConnell, and T. F. R. Schwarz; 1987; Expression of the gene for NAD^+-dependent glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* cloned in *Escherichia coli* K-12; J. Bacteriology **169**:334-339; discloses the expression of the gene for NAD^+-dependent glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* cloned in *Escherichia coli* K-12.

Olive, C., and H. R. Levy; 1967; The preparation and some properties of crystalline glucose 6-phosphate dehydrogenase from *Leuconostoc*

mesenteroides; Biochemistry 6:730-736; discloses the purification of glucose-6-phosphate dehydrogenase from extracts of *Leuconostoc mesenteroides*.

Rowley, D. L., and R. E. Wolf; 1991; Molecular characterization of the *Escherichia coli* K-12 *zwf* gene encoding glucose-6-phosphate dehydrogenase; J. Bacteriology 173:968-977; discloses cloning and sequencing of *Escherichia coli* K-12 glucose-6-phosphate dehydrogenase. The information is said to be useful in the search for an internal complementary sequence that functions as a cis-acting antisense RNA by forming a long-range secondary structure that sequesters the ribosome binding site.

Rubenstein, K. E., and R. K. Leute; Antibody Steric Hindrance Immunoassay with Two Antibodies; U.S. Patent 3,935,074, January 27, 1976 (filed December 17, 1973); discloses an antibody steric hindrance immunoassay using two antibodies.

Rubenstein, K. E., and E. F. Ullman; Enzyme amplification Assay; U.S. Patent 3,817,837, June 18, 1974 (filed November 6, 1972); discloses homogeneous immunoassays.

Skold, C., I. Gibbons, D. Gould, and E. F. Ullman; 1987; Monoclonal antibodies to glucose-6-phosphate dehydrogenase (G6PDH) form cyclic 1:1 complexes with G6PDH and act as regulatory subunits; J. Immunology 138:3408-3414; and **Skold, C., D. R. Gould, and E. F. Ullman**; Methods for modulating ligand-receptor interactions and their application; U.S. Patent 4,727,022, February 23, 1988 (filed March 14, 1984); discloses the preparation of inhibitory antibodies to G6PDH from *L. mesenteroides*.

Yoshida, R. A., and E. T. Maggio; Antienzyme Homogeneous Competitive Binding Assay; U.S. Patent 4,233,401, November 11, 1980 (filed July 14, 1977); discloses a protein binding assay for a member of an immunological pair whereby an enzyme-ligand conjugate is employed in combination with an enzyme inhibitor that can be an antibody.

SUMMARY OF THE INVENTION

One aspect of the present invention relates to methods for homogeneous immunoassay of an analyte in a sample suspected of containing the analyte. The methods comprise the steps of: (1) combining in a liquid medium: (a) the sample to be assayed, (b) a conjugate of (i) an analyte analog and (ii) a mutant NAD^+ dependent G6PDH differing from any precursor G6PDH by the deletion, substitution, or insertion or any combination thereof of at least one amino acid per subunit, (c) a receptor for the analyte, and (d) substrates for the G6PDH; (2) determining the enzymatic activity of the G6PDH in the medium; and (3) comparing the enzymatic activity to the enzymatic activity observed with a sample containing the analyte.

Another aspect of the present invention relates to methods for determining the amount of a specific binding pair (sbp) member in a sample suspected of containing the sbp member. The methods comprise the steps of: (a) combining in an assay medium: (1) the sample, (2) a conjugate of an enzyme and an analog of the sbp member, wherein the enzyme is a mutant bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least two amino acid mutations per subunit as compared to precursor G6PDH, and (3) an sbp partner of the sbp member capable of binding the conjugate, and (b) determining the activity of the enzyme.

Another aspect of the present invention relates to methods for determining the presence or amount of an analyte in a sample suspected of containing the analyte. The methods comprise the steps of: a) combining in an assay medium: 1) the sample, 2) a conjugate of an analyte analog and an enzyme, wherein the enzyme is a mutant glucose-6-phosphate dehydrogenase (G6PDH) derived from an organism selected from the group consisting of *Leuconostoc mesenteroides*, *Leuconostoc citreum*, *Leuconostoc lactis*, and *Leuconostoc dextranicus* wherein the G6PDH has at least one amino acid mutation per subunit as compared to precursor G6PDH wherein at least one of the mutations comprises the introduction of a cysteine residue proximate to an epitope recognized by an inhibitory anti-G6PDH antibody capable of simultaneously binding to two of the subunits within the same G6PDH molecule, 3) an antibody capable of binding the analyte and the analyte analog conjugate, and 4) substrates for the enzyme; and b) measuring the activity of the enzyme.

Another aspect of the present invention relates to improved methods for determining the presence of a ligand in a sample suspected of containing the ligand. The assay to be improved includes the steps of: a) bringing together in an aqueous medium: 1) the sample, 2) enzyme-bound-ligand, and 3) receptor capable of binding to the ligand and the enzyme-bound-ligand, wherein the receptor is at a concentration sufficient to substantially change the enzymatic activity of the enzyme-bound-ligand in the absence of the ligand; b) determining the enzymatic activity of the

enzyme-bound-ligand in the medium; and the improvement comprises employing as the enzyme a mutant glucose-6-phosphate dehydrogenase (G6PDH) having at least two amino acid mutations as compared to a precursor G6PDH.

Another aspect of the present invention relates to compositions comprising a specific binding pair member conjugated to a mutant NAD^+ dependent bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one amino acid mutation per subunit as compared to precursor G6PDH.

Another aspect of the present invention relates to a mutant glucose-6-phosphate dehydrogenase (G6PDH) enzyme that is the expression product of a mutated DNA sequence encoding a glucose-6-phosphate dehydrogenase, the mutant DNA sequence being derived from a precursor glucose-6-phosphate dehydrogenase by the deletion, insertion or substitution of one or more amino acids in the precursor glucose-6-phosphate dehydrogenase. Preferably, the G6PDH is an NAD^+ dependent bacterial G6PDH, more preferably the mutant DNA sequence is derived from a precursor glucose-6-phosphate dehydrogenase by the deletion, insertion or substitution of two or more amino acids in the precursor glucose-6-phosphate dehydrogenase.

Another aspect of the present invention relates to mutant glucose-6-phosphate dehydrogenase (G6PDH) enzymes having at least one mutation per subunit as compared to precursor G6PDH wherein the mutation is proximate to an epitopic site recognized by an anti-G6PDH antibody capable of inhibiting the activity of the precursor G6PDH.

Another aspect of the present invention relates to mutant DNA sequences encoding such glucose-6-phosphate dehydrogenase (G6PDH) enzymes. These mutant DNA sequences are derived from a precursor DNA sequence which encodes a naturally-occurring or recombinant precursor enzyme. The mutant DNA sequences are derived by modifying the precursor DNA sequence to encode the substitution, deletion or insertion of at least one amino acid residue encoded by the precursor DNA sequence. These recombinant DNA sequences encode glucose-6-phosphate dehydrogenase mutant enzymes having a novel amino acid sequence.

Further the invention relates to expression vectors containing such mutant glucose-6-phosphate dehydrogenase DNA sequences as well as host cells transformed with such vectors which are capable or producing such mutant enzymes.

Another aspect of the present invention relates to improved assay reagents for use in the determination of an analyte in a sample suspected of containing the analyte. The assay reagents include an analyte-label conjugate. The improvement comprises employing as the label mutant NAD^+ dependent glucose-6-phosphate dehydrogenase (G6PDH) enzymes having at least one mutation per subunit as compared to precursor G6PDH wherein the mutations are proximate to an epitopic site recognized by an anti-G6PDH antibody capable of inhibiting the activity of the precursor G6PDH.

Another aspect of the present invention relates to kits for

conducting an assay for the determination of a specific binding pair (sbp) member. The kits comprise in packaged combination an sbp partner of the sbp member and a composition which comprises the sbp member or an analog of the sbp member conjugated to a mutant NAD^+ dependent bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one amino acid mutation per subunit as compared to precursor G6PDH.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is the DNA sequence of the gene encoding G6PDH from *L. mesenteroides* strain ATCC 12291 (SEQ ID NO:1).

FIG. 2 is the amino acid sequence of the G6PDH from *L. mesenteroides* strain ATCC 12291 (SEQ ID NO:2).

FIG. 3 is the DNA sequence of the gene encoding G6PDH from *L. citreum* strain NCIMB 3351 (SEQ ID NO:3).

FIG. 4 is the amino acid sequence of the G6PDH from *L. citreum* strain NCIMB 3351 (SEQ ID NO:4).

FIG. 5 is the DNA sequence of the gene encoding G6PDH from *L. lactis* strain NCDO 546 (SEQ ID NO:5).

FIG. 6 is the amino acid sequence of the G6PDH from *L. lactis* strain NCDO 546 (SEQ ID NO:6).

FIG. 7 is the DNA sequence of the gene encoding G6PDH from *L. dextranicus* strain ATCC 19255 (SEQ ID NO:7).

FIG. 8 is the amino acid sequence of the G6PDH from *L. dextranicus* strain ATCC 19255 (SEQ ID NO:8).

FIG. 9 is plasmid pJS12.

FIG. 10 is vector pDR540.

FIG. 11 is plasmid pJS14.

FIG. 12 is plasmid pKK233-2

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention relates to methods for immunoassay of analytes employing mutant glucose-6-phosphate dehydrogenase (G6PDH) enzymes as labels. In particular, the invention relates to the use of conjugates of an analog of an analyte to be determined and a mutant NAD^+ dependent G6PDH differing from any precursor G6PDH by the deletion, substitution, or insertion, or any combination thereof of at least one amino acid per subunit. The invention also involves the construction of multiple mutations in precursor glucose-6-phosphate dehydrogenase (G6PDH) genes. Typically, the mutations involve deletion or substitution of one or more lysine residues, or introduction of one or more cysteine residues by insertion of cysteine to precursor G6PDH or substitution of precursor G6PDH amino acid residues with cysteine. The present invention also relates to conjugates of the subject enzymes and specific binding pair members, cell lines producing the subject enzymes, DNA and amino acid sequences encoding the subject enzymes, and plasmids (vectors) containing DNA encoding the subject enzymes and designed to allow a host cell to produce the subject enzymes.

Before proceeding further with the description of the specific embodiments of the invention, a number of terms will be defined.

DEFINITIONS

Analyte *** The compound or composition to be measured, the material of interest. The analyte is a member of a specific binding pair (sbp) and may be a ligand, which is mono- or polyvalent, usually antigenic or haptenic, and is a single compound or plurality of compounds which share at least one common epitopic or determinant site.

The polyvalent ligand analytes will normally be poly(amino acids), i.e., polypeptides and proteins, polysaccharides, nucleic acids, and combinations thereof. The precise nature of some of the analytes together with numerous examples thereof are disclosed in U.S. Patent 4,299,916 to Litman, et al., particularly at columns 16 to 23, the disclosure of which is incorporated herein by reference.

For the most part, the polyepitopic ligand analytes employed in the subject invention will have a molecular weight of at least about 5,000, more usually at least about 10,000. In the poly(amino acid) category, the poly(amino acids) of interest will generally be from about 5,000 to 5,000,000 molecular weight, more usually from about 20,000 to 1,000,000 molecular weight; among the hormones of interest, the molecular weights will usually range from about 5,000 to 60,000 molecular weight.

A wide variety of proteins may be considered as to the family of proteins having similar structural features, proteins having particular biological functions, proteins related to specific microorganisms, particularly disease causing microorganisms, etc.

The monoepitopic ligand analytes will generally be from about 100 to 5,000 molecular weight, more usually from 125 to 2,000 molecular weight. The analytes of interest include drugs, metabolites, pesticides, pollutants, and the like. Included among drugs of interest are the alkaloids. Among the alkaloids are morphine alkaloids, which includes morphine, codeine, heroin, dextroamphetamine, their derivatives and metabolites; cocaine alkaloids, which include cocaine and benzoyl ecgonine, their derivatives and metabolites, ergot alkaloids, which include the diethylamide of lysergic acid; steroid alkaloids; iminazoyl alkaloids; quinazoline alkaloids, isoquinoline alkaloids; quinoline alkaloids, which include quinine and quinidine; diterpene alkaloids, their derivatives and metabolites.

The next group of drugs includes steroids, which includes the estrogens, estogens, androgens, andrenocortical steroids, bile acids, cardiotonic glycosides and aglycones, which includes digoxin and digoxigenin, saponins and sapogenins, their derivatives and metabolites. Also included are the steroid mimetic substances, such as diethylstilbestrol.

The next group of drugs is lactams having from 5 to 6 annular members, which include the barbiturates, e.g. phenobarbital and secobarbital, diphenylhydantoin, primidone, ethosuximide, and their metabolites.

The next group of drugs is aminoalkylbenzenes, with alkyl of from 2 to 3 carbon atoms, which includes the amphetamines, catecholamines, which includes ephedrine, L-dopa, epinephrine, narceine, papaverine, and their metabolites.

The next group of drugs is benzheterocyclics which include oxazepam, chlorpromazine, tegretol, imipramine, their derivatives and metabolites, the heterocyclic rings being azepines, diazepines and phenothiazines.

The next group of drugs is purines, which includes theophylline, caffeine, their metabolites and derivatives.

The next group of drugs includes those derived from marijuana, which includes cannabinol and tetrahydrocannabinol.

The next group of drugs includes the vitamins such as A, B, e.g. B_{12}, C, D, E and K, folic acid, thiamine.

The next group of drugs is prostaglandins, which differ by the degree and sites of hydroxylation and unsaturation.

The next group of drugs is antibiotics, which include penicillin, chloromycetin, actinomycetin, tetracycline, terramycin, the metabolites and derivatives.

The next group of drugs is the nucleosides and nucleotides, which include ATP, FMN, adenosine, guanosine, thymidine, and cytidine with their appropriate sugar and phosphate substituents.

The next group of drugs is miscellaneous individual drugs which include methadone, meprobamate, serotonin, meperidine, amitriptyline, nortriptyline, cotinine, lidocaine, procainamide, n-acetylprocainamide, propranolol, griseofulvin, valproic acid, butyrophenones, antihistamines, anticholinergic drugs, such as atropine, their metabolites and derivatives.

Metabolites related to diseased states include spermine, galactose, phenylpyruvic acid, and porphyrin Type 1.

The next group of drugs is aminoglycosides, such as gentamicin, kanamycin, tobramycin, and amikacin.

Among pesticides of interest are polyhalogenated biphenyls, phosphate esters, thiophosphates, carbamates, polyhalogenated sulfenamides, their metabolites and derivatives.

Also included are hormones such as progesterone, testosterone, thyroid hormones, and so forth.

For receptor analytes, the molecular weights will generally range from 10,000 to $2X10^8$, more usually from 10,000 to 10^6. For immunoglobulins, IgA, IgG, IgE and IgM, the molecular weights will generally vary from about 160,000 to about 10^6. Enzymes will normally range from about 10,000 to 1,000,000 in molecular weight. Natural receptors vary widely, generally being at least about 25,000 molecular weight and may be 10^6 or higher molecular weight, including such materials as avidin, DNA, RNA, thyroxine binding globulin, thyroxine binding prealbumin, transcortin, etc.

A particularly preferred group of analytes are drugs including, by

way of example and not limitation, drugs selected from the group consisting of digoxin, vancomycin, thyroxine, cyclosporin, folate, rapamycin, vitamin B12, mycophenolate, FK506 and tetrahydrocannabinol.

Sample Suspected of Containing Analyte *** Any sample which is reasonably suspected of containing analyte can be analyzed by the method of the present invention. Such samples can include human, animal or man-made samples. The sample can be prepared in any convenient medium which does not interfere with the assay. Typically, the sample is an aqueous solution or a natural fluid, preferably, urine, whole blood, serum, plasma, cerebral-spinal fluid, or saliva more preferably, serum.

Measuring the Amount of Analyte *** Quantitative, semiquantitative, and qualitative methods as well as all other methods for determining analyte are considered to be methods of measuring the amount of analyte. For example, a method which merely detects the presence or absence of analyte in a sample suspected of containing an analyte is considered to be included within the scope of the present invention.

Synonyms for the phrase "measuring the amount of analyte" which are contemplated within the scope of the present invention include, but are not limited to, detecting, measuring, or determining analyte; detecting, measuring, or determining the presence of analyte; and detecting, or determining the amount of analyte.

Member of a Specific Binding Pair *** A member of a specific binding pair (sbp member) is one of two different molecules, having an area on the surface or in a cavity which specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as ligand and receptor (antiligand), sbp member and sbp partner, or the like. These will usually be members of an immunological pair such as antigen-antibody, although other specific binding pairs, such as biotin-avidin, hormones-hormone receptors, nucleic acid duplexes, IgG-protein A, DNA-DNA, DNA-RNA, and the like, are not immunological pairs but are specific binding pairs.

Ligand *** Any organic compound for which a receptor naturally exists or can be prepared. For example, in one context of the present invention, the analyte is a ligand and the present invention provides methods for determining the concentration of the analyte which is a ligand.

Receptor *** A receptor is any compound or composition capable of recognizing a particular spatial and polar organization of a molecule. These organized areas of a molecule are referred to as epitopic or determinant sites. Illustrative naturally occurring receptors include: antibodies, enzymes, Fab fragments, poly(nucleic acids), complement component Clq, thyroxine binding globulin, lectins, protein A, and the like. Receptors are also referred to as antiligands.

Ligand, analyte or sbp member analog *** A modified ligand or ligand surrogate, modified analyte or analyte surrogate, or modified sbp member or

sbp member surrogate which can compete with the analogous ligand, analyte or sbp member for a receptor, antiligand, sbp partner, or the like, the modification providing means to join a ligand analog, analyte analog, or sbp member analog to another molecule. The ligand analog, analyte analog, or sbp member analog will usually differ from the ligand, analyte, or sbp member by more than replacement of a hydrogen with a bond which links the ligand analog, analyte analog, or sbp member analog to a hub or label, but need not. The term ligand surrogate, analyte surrogate, or sbp member surrogate refers to a compound having the capability of specifically binding a receptor complementary to the ligand, analyte or sbp member. Thus, the ligand surrogate, analyte surrogate, or sbp member surrogate can bind to the receptor in a manner similar to the ligand, analyte, or sbp member. The surrogate could be, for example, an antibody directed against the idiotype of an antibody to the ligand or analyte.

Linking Group *** A linking group is a portion of a structure which connects 2 or more substructures. A linking group has at least 1 uninterrupted chain of atoms extending between the substructures. The atoms of a linking group are themselves connected by chemical bonds. The number of atoms in a linking group is determined by counting the atoms other than hydrogen.

Conjugate *** A conjugate is a molecule comprised of two or more substructures bound together, optionally through a linking group, to form a single structure. The binding can be made either by a direct connection (e.g. a chemical bond) between the subunits or by use of a linking group. Within the context of the present invention, a conjugate is a G6PDH enzyme attached to a hapten, sbp member or analyte analog.

Conjugation *** Conjugation is any process wherein two subunits are linked together to form a conjugate. The conjugation process can be comprised of any number of steps.

Hapten *** Haptens are capable of binding specifically to corresponding antibodies, but usually do not themselves act as immunogens for preparation of the antibodies. Antibodies which recognize a hapten can be prepared against compounds comprised of the hapten linked to an immunogenic carrier. Haptens are a subset of ligands.

Signal Producing System *** The signal producing system is utilized in assays for analytes and may have one or more components, at least one component being a mutant G6PDH of the invention. The signal producing system generates a signal that relates to the presence or amount of analyte in a sample. The signal producing system includes all of the reagents required to produce a measurable signal.

For purposes of the present invention, the signal-producing system includes at least one enzyme and at least one substrate, and may include two or more enzymes and a plurality of substrates, and may include a combination of enzymes, where the substrate of one enzyme is the product of the other enzyme; provided throughout that at least one enzyme of the

signal producing system is a mutant G6PDH of the invention.

Typically, the mutant G6PDH is conjugated to an sbp member analogous to the analyte. When the label is not conjugated to an sbp member analogous to the analyte, the label is normally bound to an sbp member complementary to the analyte.

Other components of the signal producing system can include substrates, enhancers, activators, chemiluminescent compounds, cofactors, inhibitors, scavengers, metal ions, specific binding substances required for binding of signal generating substances, coenzymes, substances that react with enzymic products, other enzymes and catalysts, and the like.

The signal producing system provides a signal detectable by external means, normally by measurement of electromagnetic radiation, desirably by visual examination. For the most part, the signal producing system includes a chromophoric substrate and mutant G6PDH enzyme of the invention, where chromophoric substrates are enzymatically converted to dyes which absorb light in the ultraviolet or visible region, phosphors or fluorescers.

Ancillary Materials *** Various ancillary materials will frequently be employed in an assay in accordance with the present invention. For example, buffers will normally be present in the assay medium, as well as stabilizers for the assay medium and the assay components. Frequently, in addition to these additives, additional proteins may be included, such as albumins, or surfactants, particularly non-ionic surfactants, binding enhancers, e.g., polyalkylene glycols, or the like.

Sensitivity *** Is used in the sense of detection limit, i.e., the smallest amount of an analyte giving a signal that is distinguishable from the signal obtained in the absence of analyte.

Substantial Change in Enzyme Activity *** A change in activity of an enzyme sufficient to allow detection of an analyte when the enzyme is used as a label in an assay for the analyte. Typically, the enzyme's activity is reduced 10-100% preferably 20-99%, more preferably 30-95%.

Inhibitory Antibody *** An antibody capable of inhibiting the activity of an enzyme or an enzyme-ligand conjugate upon binding an epitope present on the enzyme. Such antibodies are distinguished from anti-ligand antibodies capable of inhibiting the enzyme activity of enzyme-ligand conjugates upon binding to the ligand.

Expression Vector *** A DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of said DNA in a suitable host. Such control sequences may include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome-binding sites, and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert.

Recombinant or Mutant G6PDH *** G6PDH in which the DNA sequence

encoding the naturally occurring G6PDH is modified to produce a mutant DNA sequence which encodes the substitution, insertion or deletion of one or more amino acids in the G6PDH sequence.

G6PDH *** A naturally occurring G6PDH or a recombinant G6PDH. Preferably an bacterial or fungal G6PDH, more preferably a bacterial G6PDH. Particularly preferred G6PDHs are bacterial NAD^+ dependent G6PDHs.

Precursor G6PDH *** A naturally occurring G6PDH enzyme as well as a recombinant G6PDH enzyme having a sequence substantially identical to a naturally occurring G6PDH. The amino acid sequence of a G6PDH mutant is derived from the precursor G6PDH amino acid sequence by the substitution or deletion of one or more amino acids of the precursor amino acid sequence or the insertion of one or more amino acids into the precursor amino acid sequence. Such modification is of the precursor DNA sequence which encodes the amino acid sequence of the precursor G6PDH rather than manipulation of the precursor G6PDH enzyme per se.

Host Strains or Cells *** Generally procaryotic or eucaryotic hosts and include any transformable microorganism in which the expression of G6PDH can be achieved.

SPECIFIC EMBODIMENTS

Homogeneous enzyme immunoassays depend on the availability of enzyme-sbp member conjugates whose enzyme activity can be strongly modulated on binding of the sbp partner. The present invention provides methods, enzymes, enzyme-sbp member conjugates, reagents and kits of reagents for conducting assays that are useful in immunoassays, especially homogeneous immunoassays.

One aspect of the present invention relates to methods for homogeneous immunoassay of an analyte in a sample suspected of containing the analyte. The methods comprise the steps of: (1) combining in a liquid medium: (a) the sample to be assayed, (b) a conjugate of the analyte or an analyte analog with a mutant NAD^+ dependent G6PDH differing from any precursor G6PDH by the deletion, substitution, or insertion or any combination thereof of at least one amino acid per subunit, (c) a receptor for the analyte, and (d) substrates for the G6PDH; (2) determining the enzymatic activity of the G6PDH in the medium; and (3) comparing the activity to the enzymatic activity observed with a sample containing the analyte.

Accordingly, the present invention includes a mutant G6PDH differing from any precursor G6PDH by the deletion, substitution, or insertion or any combination thereof of at least one amino acid per subunit as well as a conjugate of such a mutant G6PDH and an analog of the analyte or a receptor for the analyte. The mutations are selected to improve the properties of G6PDH conjugate. Typically, the improvement involves the performance of the G6PDH as a detectable label in binding assays, particularly, immunoassays. Frequently, the immunoassay to be improved is a homogeneous immunoassay wherein the G6PDH is conjugated to a specific binding pair (sbp) member and the improvement comprises an increase in stability or modulation of enzyme activity upon binding of the sbp partner or a higher activity of the enzyme conjugate relative to precursor enzyme.

The DNA encoding any NAD^+ utilizing G6PDH is suitable for performing the mutations of the present invention. The encoded enzyme may be capable of using both $NADP^+$ and NAD^+ such as *L. mesenteroides*, *A. suboxydans*, *P. aeruginosa*, *Pseudomonas* W6, *H. eutropha* H-16, *Hydrogenomonas facilis*, *Arthrobacter* 7C, *A. beijerickii*, *T. ferrooxidans*, *B. licheniformis*, *P. denitrificans*, *C. crescentus*, *L. lactis*, and *R. spheroides*. Alternatively, the encoded enzyme may be capable of using NAD^+ as a preferred cofactor such as *P. fluorescens* and one of the G6PDHs from *P. multivorans*, or may be NAD^+ specific such as one of the G6PDHs from *A. xylinum*.

For example, *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenases (G6PDH) are dimeric enzymes that have the ability to catalyze the oxidation of D-glucose-6-phosphate to D-glucono-δ-lactone-6-phosphate by utilizing either NAD^+ or $NADP^+$. This property of using NAD^+

differentiates these enzymes from human G6PDH, which utilizes only $NADP^+$ effectively, and allows *L. mesenteroides*-specific G6PDH activity to be measured in the presence of human G6PDH, as for example in human samples. Glucose-6-phosphate dehydrogenases from *L. mesenteroides* are used in current EMIT homogeneous immunoassays (EMIT is a Trademark of Syva Company, Palo Alto, California, U.S.A.).

Two preferred genera of bacteria from which to select DNA encoding G6PDH are *Leuconostoc* and *Zymomonas*. Within these genera *L. mesenteroides*, *L. citreum*, *L. lactis*, *L. dextranicum*, and *Z. mobilis* are preferred, *L. mesenteroides*, *L. citreum*, *L. lactis* being particularly preferred. Because G6PDH from *Leuconostoc* does not contain cysteine residues, it is preferred for mutation strategies wherein one or more cysteine residues are introduced.

Table 1 describes exemplary strains of a variety of *Leuconostoc* species. Such strains are purely exemplary and do not limit the selection of DNA suitable for use within the context of the present invention to that of any particular genus, species or strain. Among the most preferred strains from which to select DNA encoding G6PDH are *Leuconostoc mesenteroides* strain ATCC 12291, *Leuconostoc citreum* strain NCIMB 3351, *Leuconostoc lactis* strain NCDO 546, and *Leuconostoc dextranicum* strain ATCC 19255.

Table 1

Organisms from the American Type Culture Collection (ATCC)[1]

Leuconostoc amelibiosum: ATCC 10882, and ATCC 13146.
Leuconostoc carnosum: ATCC 49367.
Leuconostoc citreum: ATCC 49370.
Leuconostoc gelidum: ATCC 49366.
Leuconostoc lactis: ATCC 15520, and ATCC 19256.
Leuconostoc mesenteroides* subsp. *cremoris: ATCC 19254.
Leuconostoc mesenteroides* subsp. *dextranicum: ATCC 8086, ATCC 17071, ATCC 17072, and ATCC 19255.
Leuconostoc mesenteroides* subsp. *mesenteroides: ATCC 8293, ATCC 9135, ATCC 10830, ATCC 10830a, ATCC 10877, ATCC 10878, ATCC 10879, ATCC 10880, ATCC 10883, ATCC 11449, ATCC 12291, ATCC 14430, ATCC 14935, ATCC 23386, ATCC 27258.
Leuconostoc oenos: ATCC 23277, ATCC 23278, ATCC 23279, ATCC 27307, ATCC 27308, ATCC 27309, ATCC 27310, ATCC 27311, ATCC 39401, and ATCC 39402.
Leuconostoc paramesenteroides: ATCC 33313.
Leuconostoc pseudomesenteroides: ATCC 49371.
Leuconostoc sp.: ATCC 21435, ATCC 21436, and ATCC 21437.

Organism from the National Collections of Industrial and Marine Bacteria (NCIMB)[2]

Leuconostoc carnosum: NCIMB 12898.
Leuconostoc citreum: NCIMB 3351.
Leuconostoc gelidum: NCIMB 12897.
Leuconostoc mesenteroides subsp. *cremoris* AL: NCIMB 12008.
Leuconostoc mesenteroides subsp. *dextranicum* AL: NCIMB 2706, NCIMB 3355, NCIMB 3356, NCIMB 3740, NCIMB 8189, NCIMB 8723, NCIMB 9312, NCIMB 9313, and NCIMB 12007.
Leuconostoc mesenteroides subsp. *mesenteroides* AL: NCIMB 3352, NCIMB 3354, NCIMB 3739, NCIMB 6109, NCIMB 6992, NCIMB 8013, NCIMB 8015, NCIMB 8023, NCIMB 8029, NCIMB 8172, NCIMB 8590, NCIMB 8710, NCIMB 8724, NCIMB 9260, NCIMB 9314, NCIMB 9315, NCIMB 9316, NCIMB 9319, and NCIMB 10488.
Leuconostoc oenos AL: NCIMB 11648.
Leuconostoc pseudomesenteroides: NCIMB 8699.

[1] Data format and abbreviations as in American Type Culture Collection, Catalogue of Bacteria and Phages, Eighteenth edition, 1992, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852-1776.

[2] Data format and abbreviations as in The National Collections of Industrial and Marine Bacteria Limited, Catalogue of Strains, 1990, NCIMB Ltd., 23 St., Machar Drive, Aberdeen, Scotland, U.K. AB2 1RY.

The DNA sequence of the G6PDH gene from *L. mesenteroides* strain ATCC 12291, determined as described below, is provided as Figure 1 (SEQ ID NO:1) and the amino acid sequence for the corresponding G6PDH is provided as Figure 2 (SEQ ID NO:2). The DNA sequence of the G6PDH gene from *L. citreum* strain NCIMB 3351, determined as described below, is provided as Figure 3 (SEQ ID NO:3) and the amino acid sequence for the corresponding G6PDH is provided as Figure 4 (SEQ ID NO:4). The DNA sequence of the G6PDH gene from *L. lactis* strain NCDO 546, determined as described below, is provided as Figure 5 (SEQ ID NO:5) and the amino acid sequence for the corresponding G6PDH is provided as Figure 6 (SEQ ID NO:6). The DNA sequence of the G6PDH gene from *L. dextranicum* strain ATCC 19255, as described in Canadian Patent Application Number 2,045,838 A1, is provided as Figure 7 (SEQ ID NO:7) and the amino acid sequence for the corresponding G6PDH is provided as Figure 8 (SEQ ID NO:8). Exemplary mutations are described below. These are exemplary approaches and in no way limit the scope of the present invention. For example, mutations, other than those set forth below, could provide increased stability, modulability or specific activity of the

mutant G6PDH as compared to the precursor G6PDH by inserting, deleting, or substituting amino acids at sites affecting such properties.

First Mutation Scheme

The immunoassay to be improved by the present invention is one wherein the label enzyme (G6PDH), is conjugated to a specific binding pair (sbp) member. As described below, the enzymatic activity of such a conjugate is modulated, usually inhibited, by binding of an sbp partner to the conjugate. G6PDH-sbp member conjugates are generally formed by covalently binding or conjugating an sbp member to a G6PDH through a linker to a reactive amino acid such as tyrosine, lysine, cysteine, histidine and the like.

However, not all reactive amino acids will react with an sbp member to form a conjugate that is readily modulatable in a homogeneous immunoassay. For example, linking of a hapten to *Leuconostoc mesenteroides* wild type G6PDH through a carboxyl or tyrosine gives poorly modulatable conjugates. If a hapten is linked to G6PDH through the free primary amino group of lysine residues and/or the N-terminus of the enzyme, the resulting conjugate mixture usually has reduced activity and may have limited ability to be modulated by anti-hapten antibody because binding of the sbp member to different groups on the enzyme can result in deactivation, destabilization, reduced modulability, or no affect depending on the particular group or set of groups that becomes bound.

Accordingly, one mutation scheme for improving the performance of G6PDH as a label in homogeneous immunoassays provides enzymes that form conjugates having higher enzymatic activity when the sbp member is not bound to its sbp partner and having a greater reduction in enzymatic activity when the sbp member is bound to its sbp partner relative to the corresponding properties of a precursor enzyme conjugated to a like number of sbp members.

Exemplary of such a scheme is the elimination of lysine residues that, when conjugated to sbp members, contribute little to the modulability by an sbp partner. These lysines can be eliminated by deleting them from the protein sequence or, preferably, by substituting them with other amino acids that have no detrimental effect on the protein stability. The replacement amino acids are any amino acids that do not substantially, adversely affect the activity or stability of the enzyme and preferably an amino acid that preserves the charge of the enzyme such as arginine or histidine.

The mutations of this scheme involve deletion or substitution of one, preferably two, more preferably at least four lysine amino acid residues per subunit. The mutations preferably further involve substitution of the lysines by a different amino acid residue.

Lysines to be deleted or substituted can be selected in any manner. For example, they can be replaced one at a time, examining the enzyme

activity of the conjugate with and without sbp partner, to determine suitable mutants. Preferably, if it is known which lysines upon conjugation produce inactive conjugates or do not contribute to modulation, then these lysines are candidates for deletion or substitution. Lysines of this type can be identified, for example, by systematically deleting lysines that react during conjugation of a precursor enzyme where these lysines can be identified for example by sequencing tryptic digestion fragments of the conjugate to determine which amino acids are linked to sbp members.

One class of mutant G6PDH enzymes having such mutations are those mutant G6PDH enzymes having lysines deleted or substitution in one or more amino acid residue positions selected from the group consisting of positions 5, 19, 31, 32, 37, 55, 63, 96, 105, 128, 131, 148, 204, 208, 252, 259, 265, 273, 282, 298, 311, 338, 343, 352, 376, 382, 386, 408, 409, 441, 454, 461, 472, and 484 in G6PDH from *L. mesenteroides* strain ATCC 12291, preferably from positions 5, 19, 37, 128, 131, 204, 252, 265, 282, 338, 343, 454, 461, and 472. Preferably, at least two lysines are deleted or substituted, more preferably at least 4, most preferably 6 or more lysines are deleted or substituted wherein at least 1 and preferably at least 4 of the lysines substituted are selected from the above group of positions.

Another class of mutant G6PDH enzymes having such mutations are those mutant G6PDH enzymes having lysines deleted or substituted from one or more positions selected from the group consisting of positions 5, 19, 31, 32, 55, 69, 96, 105, 128, 131, 148, 162, 204, 208, 252, 259, 265, 273, 282, 298, 311, 338, 343, 352, 376, 382, 386, 408, 441, 454, 461, 472, and 484 in G6PDH from *L. citreum* strain NCIMB 3351, preferably from positions 5, 19, 69, 128, 131, 204, 252, 265, 282, 338, 343, 454, 461, and 472. Preferably, at least two lysines are deleted or substituted, more preferably at least 4, most preferably 6 or more lysines are deleted or substituted wherein at least 1 and preferably at least 4 of the lysines substituted are selected from the above group of positions.

Another class of G6PDH enzymes having such mutations are those mutant G6PDH enzymes having lysines deleted or substituted from one or more positions selected from the group consisting of positions 5, 19, 31, 32, 55, 69, 96, 105, 128, 131, 148, 159, 204, 208, 252, 259, 265, 273, 282, 298, 311, 338, 343, 352, 382, 386, 408, 441, 454, 461, 472, and 484 in G6PDH from *L. lactis* strain NCDO 546, preferably from positions 5, 19, 128, 131, 204, 252, 265, 282, 338, 343, 454, 461, and 472. Preferably, at least two lysines are deleted or substituted, more preferably at least 4, most preferably 6 or more lysines are deleted or substituted wherein at least 1 and preferably at least 4 of the lysines substituted are selected from the above group of positions.

Another class of G6PDH enzymes having such mutations are those mutant G6PDH enzymes having lysines deleted or substituted from one or more positions selected from the group consisting of positions 5, 19, 31, 32,

55, 63, 96, 128, 131, 148, 204, 208, 252, 259, 265, 273, 282, 298, 311, 338, 343, 352, 376, 382, 386, 408, 409, 441, 454, 461, 472, and 484 in G6PDH from *L. dextranicum* strain ATCC 19255, preferably from positions 5, 19, 128, 131, 204, 252, 265, 282, 338, 343, 454, 461, and 472. Preferably, at least two lysines are deleted or substituted, more preferably at least 4, most preferably 6 or more lysines are deleted or substituted wherein at least 1 and preferably at least 4 of the lysines substituted are selected from the above group of positions.

Generally, any precursor G6PDH having a high degree of homology with the exemplary strains listed above is a candidate for the above exemplary mutations. Such homology is not a requirement of the first mutation strategy. However, homology is one method of identifying candidate strains. The degree of homology may be 50-80%, preferably 80-85%, more preferably >85%.

Second Mutation Scheme

In homogeneous binding assays using sbp member-enzyme conjugates, it is sometimes difficult to attach a given sbp member to a label enzyme so that the resulting conjugate has substantial enzyme activity when not bound to an sbp partner but has substantially reduced enzyme activity (is inhibited) when bound to an sbp partner. One reason for this difficulty may be that there are no sites on the enzyme to which an sbp member can be attached that will provide the desired inhibition. This can occur when all sites that have functional groups to which an sbp member can be bound to the enzyme are incapable of providing inhibition or when binding of an sbp member to a functional group capable of providing inhibition leads to substantial loss of enzyme activity. Exemplary functional groups to which an sbp member may be bound include by way of example and not limitation, hydroxyl, carboxyl, amino, sulfhydryl, and the like. Exemplary of such functional groups to which an sbp member may be bound are the hydroxy group of tyrosine, serine, and threonine; the carboxy group of aspartic acid and glutamic acid; the amine group of lysine; and the sulfhydryl group of cysteine.

Accordingly another exemplary mutation scheme of the present invention results in improving the inhibitability of enzyme-sbp member conjugates by providing one or more sbp member attachment sites at locations on the enzyme not otherwise having such attachment sites. Providing attachment sites can involve adding one or more new residues (having such a functional group) to a protein sequence or substituting one or more existing residues (not having such a functional group) with new residues (having such a functional group).

For example, because G6PDH from many strains of *L. mesenteroides* lack cysteine residues, cysteine residues can be provided (by insertion or substitution) that allow sbp members to be bound (through free sulfhydryl of the new cysteine residues) to sites on the enzyme not otherwise

available. The number of cysteine residues introduced depends on the nature of the assay to be performed. Generally, a small number of cysteines at sites selected to allow for the formation of conjugates that are inhibitable upon binding of the sbp member is preferred. Accordingly, the mutations of this scheme preferably involve introduction of cysteine residues, usually up to 8 cysteines, per subunit, preferably up to 4 cysteines and most preferably, a single cysteine residue per subunit.

One class of preferred mutant G6PDH enzymes are those mutant G6PDH enzymes having at least one cysteine residue introduced between amino acids 35 and 70, preferably, 45 and 60, more preferably, 45 and 57 in G6PDH from *Leuconostoc mesenteroides* strain ATCC 12291, *Leuconostoc citreum* strain NCIMB 3351, *Leuconostoc lactis* strain NCDO 546, or *Leuconostoc dextranicum* strain ATCC 19255 wherein the cysteine preferably replaces one of these amino acids, but need not.

Table 2 lists exemplary cysteine introductions consistent with this mutation scheme. Such mutations are purely exemplary and do not limit suitable mutations within the context of the present invention.

Table 2

Cysteine Introduction Mutations

Leuconostoc mesenteroides ATCC 12291

Ala-45-Cys
Arg-46-Cys
Gln-47-Cys
Ala-48-Cys
Leu-49-Cys
Asn-50-Cys
Asp-51-Cys
Asp-52-Cys
Glu-53-Cys
Phe-54-Cys
Lys-55-Cys
Gln-56-Cys
Leu-57-Cys
Val-58-Cys
Arg-59-Cys
Asp-60-Cys
Lys-128-Cys
Lys-182-Cys

Leuconostoc citreum NCIMB 3351

Lys-21-Cys
Ala-45-Cys
Arg-46-Cys
Gln-47-Cys

Asp-48-Cys
Leu-49-Cys
Thr-50-Cys
Asp-51-Cys
Ala-52-Cys
Glu-53-Cys
Phe-54-Cys
Lys-55-Cys
Gln-56-Cys
Leu-57-Cys
Val-58-Cys
Arg-59-Cys
Glu-60-Cys
Asp-48-Ala, and Ala-52-Cys

Leuconostoc lactis NCDO 546

Ala-45-Cys
Arg-46-Cys
Gln-47-Cys
Asp-48-Cys
Leu-49-Cys
Thr-50-Cys
Glu-51-Cys
Asp-52-Cys
Glu-53-Cys
Phe-54-Cys
Lys-55-Cys
Gln-56-Cys
Leu-57-Cys
Val-58-Cys
Arg-59-Cys
Asp-60-Cys

Leuconostoc dextranicus ATCC 19255

Ala-45-Cys
Arg-46-Cys
Gln-47-Cys
Asp-48-Cys
Leu-49-Cys
Thr-50-Cys
Glu-51-Cys
Asp-52-Cys
Glu-53-Cys
Phe-54-Cys
Lys-55-Cys

Gln-56-Cys
Leu-57-Cys
Val-58-Cys
Arg-59-Cys
Asp-60-Cys

Third Mutation Scheme

Another exemplary mutation scheme involves inhibitory epitopic sites (IES) present on a given enzyme. Suitable inhibitory epitopic sites as well as methods for using such sites in assays is contained in **Skold, C., D. R. Gould, and E. F. Ullman** (Methods for modulating ligand-receptor interactions and their application; U.S. Patent 4,727,022, February 23, 1988; filed March 14, 1984) which is incorporated in its entirety herein by reference, particularly, column 1, lines 40-59, and column 2, lines 26-38. An IES is a site on an enzyme that binds an inhibitory antibody (IA) such that, when the IA is bound, the activity of the enzyme is substantially reduced but, when the IA is not bound, the enzyme is active. There may be one or a plurality of IES's present on a given enzyme. Normally G6PDH will have at least two IES's. In some cases the IA may have to bind two IES's simultaneously to substantially reduce the enzyme activity. Often the enzyme will be a multiunit enzyme having a number of identical subunits, each bearing one or more IES's.

In this third mutation scheme, an enzyme having an IES is modified to provide sbp member attachment sites at one or more locations proximate to the IES so that when an sbp partner of the sbp member is bound to the enzyme-sbp member conjugate, the inhibitory antibodies will be inhibited from binding to the IES. In this scheme, an immunoassay is available which utilizes the enzyme conjugate; an sbp partner, usually an antibody capable of binding both the analyte and the conjugate; an inhibitory anti-G6PDH antibody; the G6PDH substrates glucose-6-phosphate (G6P) and NAD^+ or analogs thereof; and ancillary reagents. The activity of the enzyme conjugate is substantially inhibited in the presence of analyte (an sbp member), and active in the absence of analyte. When analyte is present, it competes with enzyme-sbp member conjugate for binding to the sbp partner (an antibody capable of binding analyte), and less sbp partner is then available to bind the conjugate. Inhibitory antibody also competes with sbp partner for binding to the enzyme sbp conjugate. Because binding of the sbp partner to the conjugate does not substantially inhibit the enzyme activity, the enzyme is active when the sbp partner binds. However, when the sbp partner is bound to analyte it cannot compete with the inhibitory antibody for binding to the conjugate, and the enzyme activity is reduced.

An IES can be naturally present on a precursor enzyme or can be created by modification of a precursor enzyme. A site for specific attachment of a ligand proximate to an IES will frequently not be available

on a precursor enzyme but may be introduced by a mutation. Usually there will be at least one, frequently two or more IES's per subunit. Because G6PDH's are typically comprised of at least 2 identical subunits, a given G6PDH may have one or more pairs of identical inhibitory epitopic sites. Certain antibodies are capable of binding simultaneously to each member of such a pair. In such a case the inhibitory antibody may be able to bridge the subunits when bound to the inhibitory epitopic sites.

Inhibitory monoclonal antibodies can be obtained by the process discussed by Milstein and Kohler and reported in *Nature* 1975, *256*, 495-7. The details of this process are well known and will not be repeated here. However, basically it involves injecting a host, usually a mouse or other suitable animal, with an immunogen. Generally, the immunogen is the enzyme against which inhibitory antibodies are to be prepared. However, fragments of the enzyme or synthetic poly(peptides) mimicking the IES of the enzyme may also be used as the immunogen. Such enzyme fragment or synthetic poly(peptide) immunogens may incorporate one of the immunogenic carriers common in the art. Cells are then taken from the spleen of the animal. Alternatively, the host may be unsensitized spleen cells, which are sensitized to the immunogen *in vitro*. The resulting cells are fused with myeloma cells. The result is a hybrid cell, referred to as a "hybridoma" that can be cultured *in vitro*. The population of hybridomas is screened and manipulated so as to isolate individual clones, each of which secretes a single antibody to the antigen.

Skold, C., I. Gibbons, D. Gould, and E. F. Ullman (1987; Monoclonal antibodies to glucose-6-phosphate dehydrogenase (G6PDH) form cyclic 1:1 complexes with G6PDH and act as regulatory subunits; J. Immunology 138:3408-3414; which is incorporated herein by reference) describes the preparation of inhibitory antibodies to G6PDH from *L. mesenteroides*. In particular, the "Materials and Methods" section beginning at page 3408, column 2, paragraph 3, and ending after page 3409, column 1, paragraph 8, and the "Results" section beginning at page 3409, column 1, paragraph 8, and ending after page 3412, column 1, last paragraph, are incorporated herein by reference. Further, the above incorporated U.S. Patent 4,727,002 discloses the preparation of such antibodies, in particular Example 5, column 11, line 1, to column 12, line 17, and Example 1, column 7, line 36, to column 8, line 14, are incorporated herein by reference.

For a given enzyme/inhibitory antibody pair, the IES can be located in a variety of ways, For example, epitope mapping such as described by **Geysen, H. M., S. J. Rodda, T. J. Manson, G. Tribbick, and P. G. Schoofs** (1987; Strategies for epitope analysis using peptide synthesis; *J. Immunol. Methods* 102:259-274) can be performed. Alternatively, the binding of IAs to various differing strains of G6PDH, such as described below, with known amino acid sequences can be determined. By comparing amino acid sequences among the binders and non-binders of the particular IA a number of possible binding sites can usually be determined. The exact site can subsequently be

determined either by mutagenizing a binding enzyme to incorporate a non-binding amino acid sequence in a region suspected of being an IES and subsequently checking whether IA binding has been prevented or by splicing regions of a binding enzyme into a non-binding enzyme and determining whether an IES has been created.

Once the IES has been located, an sbp member attachment site can be added by introducing an amino acid residue proximate to the IES having a side chain bearing a functional group capable of reacting to form an enzyme-sbp member conjugate. Preferably the side chain will have a sulfhydryl group or a disulfide but side chains having other groups such as amino, imidazole and indole and hydroxyl can be used. Frequently the amino acid that is introduced will replace an amino acid that is normally found in the precursor enzyme.

The location of the new sbp member attachment site is selected so that, when sbp partner is bound to an enzyme-sbp member conjugate, an inhibitory antibody is substantially incapable of binding the conjugate. The new sbp member attachment site is also located so that the inhibitory antibody is capable of binding and inhibiting the conjugate when sbp partner is absent.

The new sbp member attachment site is preferably proximate to the IES. It may be characterized as proximate in 3-dimensional space or proximate along the amino acid sequence of the enzyme. If the attachment site is characterized as proximate in a spacial sense, it is usually within about 35 Å of an amino acid residue of the IES, preferably within about 30 to 3 Å, most preferably, 15 to 3 Å. If the attachment site is characterized as proximate in a sequence sense, it is preferably within about 10 amino acid residues of an amino acid residue of the IES, more preferably, within about 6 to 1, most preferably, 4 to 1.

A mutation of this scheme is any mutation proximate to an epitopic site which is recognized by an anti-G6PDH antibody capable of inhibiting the activity of the precursor G6PDH. The antibody may but need not be capable of simultaneously binding to two of the subunits within the same G6PDH molecule. One preferred mutation is the introduction (or insertion) of at least one cysteine residue per subunit.

Representative precursor G6PDH enzymes suitable for introducing such mutations include *L. mesenteroides* strain ATCC 12291, *L. lactis* strain NCDO 546 and *L. dextranicus* strain ATCC 19255. Four or more cysteine residues can be introduced by insertion or substitution between amino acids 45 and 60, and preferably, no more than 2, more preferably, a single cysteine can be introduced. Preferably, a single cysteine is introduced by substitution of the naturally present amino acid at position 51-56, most preferably position 52.

The mutant G6PDH conjugates of the present invention can be utilized in an assay of an analyte in a sample suspected of containing analyte. In one embodiment the assay comprises the steps of contacting the sample with

antibodies capable of binding the analyte and an enzyme conjugate of the analyte and measuring the activity of the enzyme which relates to the amount of analyte in the sample. The improvement provided in this aspect of the invention is the utilization of the mutant G6PDH conjugates of the invention. The enzyme activity is determined by adding glucose-6-phosphate and NAD^+ or $NADP^+$, usually NAD^+ to the assay medium and detecting either the disappearance of one of these substrates or the appearance of NADH, NADPH or D-glucono-δ-lactone-6-phosphate.

In another configuration of an assay for the determination of analyte in an optionally pretreated sample suspected of containing analyte, the sample is contacted with antibodies for analyte inhibitory antibodies for G6PDH and a mutant G6PDH-analyte conjugate of the invention recognized by the antibodies for the analyte and the inhibitory antibodies for G6PDH. The method further includes measuring the enzyme activity in relation to the amount of analyte present in the sample. Analyte or analyte analog is conjugated, optionally through a linking group that will usually be less than about 50, preferably less than 20, more preferably less than about 6 atoms other than hydrogen having a chain of usually not more than about 35, preferably less than 15, more preferably less than 4 atoms in length, to a mutant G6PDH of the invention may be employed as the G6PDH conjugate.

The compositions of the invention have special application to homogeneous immunoassays for analytes exemplified by enzyme multiplied immunoassay techniques (e.g. see U.S. Patent 3,817,837), and enzyme protection immunoassays (e.g. see U.S. Patent 4,233,401) other homogeneous enzyme immunoassays are discussed in "Enzyme-Immunoassay" by Edward T. Maggio, CRC Press Incorporated, Boca Raton, Florida, 1980. Assays using inhibitory antibodies as described above are described in the above incorporated U.S. Patent 4,727,022, in particular, column 5, line 16, to column 6, line 51, are incorporated herein by reference. The disclosures of the above patents not otherwise incorporated are incorporated herein by reference in their entirety as to the description of the particular assay methods mentioned.

The assay is preferably homogeneous and will normally be carried out in an aqueous buffered medium at a moderate pH, generally that which provides optimum assay sensitivity.

The aqueous medium may be solely water or may include from 0 to 40 volume percent of a cosolvent. The pH for the medium will usually be in the range of about 4 to 11, more usually in the range of about 5 to 10, and preferably in the range of about 6.5 to 9.5. The pH will usually be a compromise between optimum binding of the binding members of any specific binding pairs and the pH optimum for other reagents of the assay such as members of the signal producing system.

Various buffers may be used to achieve the desired pH and maintain the pH during the determination. Illustrative buffers include borate, phosphate, carbonate, tris, barbital and the like. The particular buffer

employed is not critical to this invention, but in an individual assay one or another buffer may be preferred.

Moderate temperatures are normally employed for carrying out the assay and usually constant temperatures during the period of the measurement, particularly for rate determinations. Incubation temperatures will normally range from about 5° to 45°C, more usually from about 15° to 40°C. Temperatures during measurements will generally range from about 10° to 50°, more usually from about 15° to 40°C.

The concentration of analyte that may be assayed will generally vary from about 10^{-5} to 10^{-13} M, more usually from about 10^{-6} to 10^{-8} M. Considerations such as whether the assay is qualitative, semiquantitative or quantitative (relative to the amount of analyte present in the sample), the particular detection technique and the concentration of the analyte will normally determine the concentrations of the various reagents.

While the concentrations of the various reagents in the assay medium will generally be determined by the concentration range of interest of analyte, the final concentration of each of the reagents will normally be determined empirically to optimize the sensitivity of the assay over the range. That is, a variation in concentration of analyte which is of significance should provide an accurately measurable signal difference.

While the order of addition may be varied widely, there will be certain preferences depending on the nature of the assay. The simplest order of addition is to add all the materials simultaneously and determine the effect that the assay medium has on the signal. Alternatively, the reagents are combined sequentially. Optionally, an incubation step is involved subsequent to each addition, generally ranging from about 10 seconds to 6 hours, more usually from about 30 seconds to 1 hour.

In a homogeneous assay after all of the reagents have been combined either simultaneously or sequentially, the signal is determined. The signal is related to the amount of analyte in the sample tested. For example, when carrying out the enzyme multiplied immunoassay technique, as described in U.S. Patent 3,817,837 (1974) the disclosure of which is incorporated by reference, for the detection of analyte by means of the present invention, a sample suspected of containing analyte is combined in an aqueous medium either simultaneously or sequentially with an antibody and a mutant G6PDH-analyte analog conjugate of the present invention.

Generally, substrates for the mutant G6PDH such as glucose-6-phosphate and NAD^+, $NADP^+$ or a derivative thereof are added which result in the formation of a chromogenic or fluorogenic product upon enzyme catalyzed reaction. The analyte in the sample and the mutant G6PDH-analyte analog conjugate compete for sites on the antibody. The enzyme activity in the medium is then determined, usually by spectrophotometric means, and is compared to the enzyme activity determined when a calibrator or reference sample is tested in which a known amount of analyte is present. Typically, the calibrator or reference sample is tested in a manner substantially the

same as the sample suspected of containing analyte. Generally, a comparison can be made of the result from an unknown sample with the results of assay runs on several standard samples. The standard samples will typically contain differing, but known, concentrations of the analyte to be determined. Preferably, the concentration ranges present in the standard samples will span the range of suspected analyte concentrations in the unknown samples.

A similar procedure is employed for carrying out enzyme protection assays as described in U.S. Patent 4,233,401 except that the mutant G6PDH analyte analog conjugate is capable of inhibition by an anti-G6PDH antibody which is included in the assay mixture.

Another aspect of the present invention relates to compositions comprising a specific binding pair member conjugated to a mutant NAD^+ dependent bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one amino acid mutation per subunit as compared to precursor G6PDH. Analyte or analyte analog conjugated, optionally through a linking group of less than about 50, preferably less than 20, more preferably less than about 6 atoms other than hydrogen having a chain of usually not more than about 35, preferably less than 15, more preferably less than 4 atoms in length, to a mutant G6PDH of the invention may be employed as the conjugate.

One embodiment of such a conjugate is illustrated by the formula:

$$ENZ-(-L-sbp)_n$$

wherein ENZ is a mutant bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one cysteine residue per subunit, L is a bond to the sulfur atom of the cysteine or a linking group having a chain of 1 to 20 atoms bonded to the sulfur atom of the cysteine, sbp is a specific binding pair member, and n is a number from 1 to 8. Preferably, L is a bond or linking group having a chain of less than 15 atoms, more preferably, L is a linking group having a chain of less than 4 atoms.

Conjugates of the invention are prepared by linking a hapten to a mutant G6PDH. When a functional group suitable for linking the hapten is not available a linking group will be introduced that contains a suitable functional group. The linking group may comprise a chain of atoms of 0 to 50 usually less than 15, more usually less than 6 in length. The length of the linking group will normally be determined empirically so as to provide G6PDH conjugates that have high modulation of activity by antibodies, low loss of activity upon conjugation, and high stability. The functional groups suitable for attaching the hapten to the enzyme will usually be an activated ester or alkylating agent when the amino acid(s) that are to be conjugated on the G6PDH have amino or hydroxyl groups and will usually be alkylating agents or desulfurizes when the amino acid(s) that are to be conjugated on the G6PDH are cysteine. A large number of suitable functional groups are available for attaching to aminos and alcohols such as activated esters including imidic esters, sulfonic esters and phosphate

esters, activated nitriles, aldehydes, ketones, alkylating agents and the like.

Conjugation of haptens to proteins using these and other attaching groups are well known in the art and are described in reviews such as for example, Maggio, E.T. "Enzyme-Immunoassay" (CRC Press, Boca Raton, FL, 1980), Chapter 4, contains an assortment of conjugation techniques; pages 81-88 of which are incorporated herein by reference.

Following reaction of mutant G6PDH with a hapten to form a conjugate the product is then optionally purified as may be required. The purification and characterization of poly(amino acid)-hapten conjugates has been described in detail Maggio, *et. al*; "enzyme-immunoassay" (CRC Press, Boca Raton, FL, 1980), Chapter 4, pages 86-88 of which are incorporated herein by reference. For example, if the conjugate is a mutant G6PDH-hapten conjugate, the purification can be by dialysis against aqueous/organic and aqueous solutions such as water/DMF or water, or by gel filtration chromatography on supports such as Sephadex, and the like.

Alternatively, the conjugation can involve binding of a hapten to a free thio group present on an amino acid side chain of the enzyme (e.g. cysteine). Such conjugation involves alkylation of the thio sulfur atom by treatment with an electrophilic compound such as an α,β-unsaturated amide, ketone, ester, or the like, or an alkylating agent such as a reactive halide or sulfonate or the like or reaction with an active disulfide such as a 2-nitro-4-carboxyphenyl disulfide. Specific examples by way of illustration and not limitation include α-bromoamides, maleimides, vinyl sulfones, α-iodoketones, triflates and the like.

Conjugation reactions with G6PDH can be affected by a number of factors. These include, but are not confined to, pH, temperature, buffer, ionic strength, substances which may protect the enzyme active site, amount and type of cosolvent, reaction time, and activation chemistry. For each enzyme-hapten combination, appropriate manipulation of these variables can lead to conjugates which are improved in one or more of the following properties: 1) reduced deactivation for a given amount of inhibition; 2) larger standard curve; 3) improved assay precision; 4) enhanced thermal stability. Keeping in mind that these variables are not independent, the following observations can be made. A range of pH values from 5.0 to 9.5 can usually be used for conjugation reactions. These reactions are generally carried out at 0-40°C, preferably 4-20°C. A number of buffers and salts, both alone and in combination, can be used for such reactions. These include Tris, bicarbonate, phosphate, pyrophosphate, EDTA, KCl, NaCl, and many others. The active site may be protected by substrates (i.e. G6P), cofactors (NAD^+, NADH, $NADP^+$, NADPH) and cofactor analogs (thio-NAD^+, thio-NADH, thio-$NADP^+$, or thio-NADPH), and compounds which react reversibly with lysine (i.e. pyridoxal) to reduce deactivation of the enzyme during conjugation. Cosolvents which may enhance hapten solubility include, but are not limited to, dimethylformamide, carbitol, dimethyl

sulfoxide, 1-Methyl-2-pyrrolidinone, and 1,3-Dimethyl-3,4,5,6-tetrahydro 2(1H)-pyrimidinone. These may be useful as 1-30% of the reaction volume. Reactions can vary from 15 minutes to many days, depending on the activation chemistry. Carboxylic compounds may be activated to form esters with N-Hydroxysuccinimide or its sulfo-analog, or to mixed anhydrides through reaction with carbitol chloroformate or t-butylchloroformate, or may be coupled directly using carbodiimides such as EDAC. For reaction with cysteine thiols on the enzyme, the hapten should contain a good leaving group such as I, Br or tosyl; alternatively, the hapten can contain a thiol, preferably activated with 2,2'dithiodipyridine or DTNB. Another method of conjugation, described in **Rowley, G. L., D. Leung, and P. Singh** (U.S. Patent 4,220,722, September 2, 1980, filed February 10, 1978) involves modification of the enzyme with bromoacetyl containing reactants; the bromo groups are subsequently reacted with thiol-containing haptens. The reaction of enzyme with bromoacetyl modifier, and the bromoacetyl enzyme with the thiolated hapten, are subject to the same reaction condition variables described above.

Another aspect of the present invention relates to improved assay reagents for use in the determination of an analyte in a sample suspected of containing the analyte. The assay reagents include an analyte-label conjugate; and the improvement comprises employing as the label mutant glucose-6-phosphate dehydrogenase (G6PDH) enzymes having at least one mutation per subunit as compared to precursor G6PDH wherein the mutations are proximate to an epitopic site recognized by an anti-G6PDH antibody capable of inhibiting the activity of the precursor G6PDH. Preferably, the label conjugate reagent is comprised of the conjugate and an aqueous medium, the pH medium being optimized to balance, among any other considerations, the activity and stability of the label conjugate. In one of its preferred embodiments, the present invention relates to aqueous mutant G6PDH-sbp member conjugate reagents wherein the pH is 6-10, preferably 7-9, more preferably 7.5 - 8.5.

Another aspect of the present invention relates to kits for conducting an assay for the determination of a specific binding pair (sbp) member. The kits comprise in packaged combination an sbp partner of the sbp member and a composition which comprises the sbp member or an analog of the sbp member conjugated to a mutant NAD^+ dependent bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one amino acid mutation per subunit as compared to precursor G6PDH. Additionally the kits may contain an inhibitory anti-G6PDH antibody.

Surface active additives, including bulking agents such as BLG or PEG; defoamers and surfactants such as tween-20, plurafac A38, triton X-100, pluronic 25R2, RSA, BSA, Mod-u-cyte, sol-u-pro, or the like; and other materials commonly used in the art can be added to improve solubility and reduce nonspecific binding of reagents and analyte to surfaces.

Anti-microbial agents such as azide thimerosal , gentamicin and the

like can be added to assay reagents in order to extend the storage life of the reagents. In one of its most preferred embodiments, the present invention relates to assay reagents containing anti-microbial agents.

To enhance the versatility of the subject invention, the reagents can be provided in packaged combination, in the same or separate containers, so that the ratio of the reagents provides for substantial optimization of the method and assay. The reagents may each be in separate containers or various reagents can be combined in one or more containers depending on the cross-reactivity and stability of the reagents. The kit may comprise as one or more reagents antibodies capable of binding analyte or inhibitory antibodies capable of inhibiting a G6PDH-analyte analog conjugate of the invention. The kit may also include the conjugates of the invention. The kit can further include other packaged reagents for conducting an assay including members of the signal producing system, ancillary reagents, and so forth.

Various ancillary materials will frequently be employed in an assay in accordance with the present invention. For example, buffers will normally be present in the assay medium, as well as stabilizers for the assay medium and the assay components. Frequently, in addition to these additives, additional proteins may be included, such as albumins, or surfactants, particularly non-ionic surfactants, binding enhancers, e.g., polyalkylene glycols, or the like.

Another aspect of the present invention relates to a mutant glucose-6-phosphate dehydrogenase (G6PDH) enzyme that is the expression product of a mutated DNA sequence encoding a glucose-6-phosphate dehydrogenase, the mutant DNA sequence being derived from a precursor glucose-6-phosphate dehydrogenase by the deletion, insertion or substitution of one or more amino acids in the precursor glucose-6-phosphate dehydrogenase. Preferably, the G6PDH is an NAD^+ dependent bacterial G6PDH, more preferably the mutant DNA sequence is derived from a precursor glucose-6-phosphate dehydrogenase by the deletion, insertion or substitution of two or more amino acids in the precursor glucose-6-phosphate dehydrogenase.

Another aspect of the present invention relates to mutant glucose-6-phosphate dehydrogenase (G6PDH) enzymes having at least one mutation per subunit as compared to precursor G6PDH wherein the mutation is proximate to an epitopic site recognized by an anti-G6PDH antibody capable of inhibiting the activity of the precursor G6PDH.

To prepare the glucose-6-phosphate dehydrogenases (G6PDHs) and G6PDH conjugates described in this invention, it is first necessary to obtain genomic DNA that codes for the production of G6PDH from suitable **Leuconostoc** organisms. These DNAs can be cloned in **E. coli** cloning vectors. Colonies containing recombinant cloning vectors harboring the gene for G6PDH may be screened for by colony or plaque hybridization using labeled probes specific for the gene. Alternatively, the gene encoding G6PDH can be isolated by means of the polymerase chain reaction (PCR) using

appropriate oligodeoxynucleotide primers. Suitable oligodeoxynucleotide probes and primers can be readily synthesized based on the nucleotide sequences of the G6PDH genes described below.

Once the gene encoding a particular G6PDH has been isolated and expressed, mutations causing changes in the amino acid sequence of the protein can be introduced by any of a number of methods of site-specific mutagenesis. Specific mutations which result in G6PDHs with improved properties for use as immunoassay conjugates are described below. Methods for the purification of non-recombinant **Leuconostoc** G6PDHs are well known in the literature. These same methods can be successfully applied to mutant recombinant G6PDHs as described below. Finally, the methods for the production of conjugates useful in immunoassays employing these purified mutant recombinant G6PDHs and the use of these conjugates in improved homogeneous immunoassays are described in the examples below.

The basic molecular biological techniques employed in accomplishing features of this invention, such as DNA and plasmid isolation, restriction enzyme digestion, DNA ligation, purification and characterization of DNAs by polyacrylamide and agarose gel electrophoresis, labeling and hybridization of DNAs, Southern blotting, maintenance and growth of bacterial strains, and other general techniques are all well know in the literature. Specifically, the general techniques of molecular biology are described in "Molecular Cloning A Laboratory Manual" by Sambrook, J., Fritsch, E.F., and Maniatis, T. published by Cold Spring Harbor Laboratory Press, 2nd edition, 1989, or "A Practical Guide to Molecular Cloning" by Bernard Perbal published by John Wiley & Sons, New York, 1984. Specific methods for obtaining the gene for G6PDH and expressing and purifying the protein are described below.

Another aspect of the present invention relates to mutant DNA sequences encoding such glucose-6-phosphate dehydrogenases (G6PDH) enzymes. These mutant DNA sequences are derived from a precursor DNA sequence which encodes a naturally-occurring or recombinant precursor enzyme. The mutant DNA sequences are derived by modifying the precursor DNA sequence to encode the substitution, deletion or insertion of at least one amino acid residue encoded by the precursor DNA sequence. These recombinant DNA sequences encode glucose-6-phosphate dehydrogenase mutant enzymes having a novel amino acid sequence.

Further the invention relates to expression vectors containing such mutant glucose-6-phosphate dehydrogenase DNA sequences as well as host cells transformed with such vectors which are capable or producing such mutant enzymes.

Another aspect of the present invention relates to engineered DNA sequences characterized by a nucleotide sequence encoding a glucose-6-phosphate dehydrogenase (G6PDH) enzyme produced by an organism for expression in a unicellular host, wherein the G6PDH has at least two amino acid mutations as compared to precursor G6PDH of the organism.

Further the invention relates to expression vectors containing such mutant glucose-6-phosphate dehydrogenase DNA sequences as well as host cells transformed with such vectors which are capable or producing such mutant enzymes.

Another aspect of the present invention relates to plasmids characterized by containing an engineered native DNA sequence encoding a glucose-6-phosphate dehydrogenase (G6PDH) enzyme for expression in a unicellular host, wherein the G6PDH has at least two amino acid mutations as compared to precursor G6PDH of the organism.

Another aspect of the present invention relates to transformed unicellular hosts characterized by the presence of a plasmid with an engineered DNA sequence encoding a glucose-6-phosphate dehydrogenase (G6PDH) enzyme wherein the G6PDH has at least two amino acid mutations as compared to precursor G6PDH of the organism.

The requirements for an expression vector will include a selectable, with an intracellular expression system from a strong, inducible promoter. To optimize expression, a vector having its own ribosome binding site (rbs) is preferred, rather than engineering the G6PDH gene. An example of such a plasmid, shown in figure 9, is pJS12 based on the vector pDR540, shown in figure 10, an ampicillin-selectable plasmid having a tac promoter and a Bam HI cloning site. The first two bases of the Bam HI site (GAATTC) complete the sequence of the second rbs (AGGA) following the promoter. To achieve a desirable spacing (7-9 bases) between the rbs and the G6PDH start codon, the 5' end of a PCR fragment containing the gene, for example, can be designed so that the ATG is spaced 4 bases away from the end of the Bam HI site.

Another example of a useful plasmid, shown in figure 11, is pJS14 based on pKK233-2, shown in figure 12. This expression vector has several improvements as compared to pJS12 based on pDR540. It carries an Nco I site (CCATGG), the ATG of which corresponds to the properly positioned start codon of the expressed protein. This start codon site eliminates the need to properly space the rbs and ATG sites. The vector also contains a transcriptional terminator sequence following the multiple cloning site, which reduces or eliminates run-on transcription from a plasmid.

Once the desired G6PDH DNA fragment has been successfully cloned into the expression vector, the resulting cells are tested for expression of desired G6PDH and absence of expression of native G6PDH. This is most readily done by enzymatic assay for activity of the recombinant G6PDH in the presence of NAD^+. Alternatively, western blots are performed on cell extracts using polyclonal antibodies that recognize the desired G6PDH but do not recognize other related but undesirable enzymes such as any native enzyme produced by the host cell without the plasmid. For example, a subunit of G6PDH from *L. mesenteroides* appears as a protein of approximately 54 kDa (on a denaturing polyacrylamide gel) and cross-reacts with the antibodies. This protein is present in cells containing the

expression plasmid, but is absent in extracts of cells without the plasmid. Levels of the specific G6PDH protein generally increases when the cultures are incubated in the presence of isopropylthio-β-galactoside (IPTG), demonstrating that protein production is inducible.

The following examples further describe the specific embodiments of the invention. These are typical illustrative examples and are intended to describe and not to limit the scope of the invention.

EXAMPLES

Preparation of Genomic DNA from Leuconostoc organisms

Genomic DNA was prepared from **Leuconostoc** organisms by the method of Murphy, N.B., McConnell, D.J., and Schwarz, T.F.R. *J. Bact. 169*(1), 334-339 (1987). Specific instructions for bacterial growth and DNA isolation are given on pages 334 and 335.

Cloning and expression of Leuconostoc G6PDHs

The cloning and expression of **Leuconostoc mesenteroides** G6PDH has been described by Lee, W.T., Flynn, T.G., Lyons, C., and Levy, H.R. *J. Biol. Chem. 266*(20), 13028-13034 (1991). This technology has further been set forth in International Patent Publication Number WO92/07078 (International Application Number PCT/US91/07715).

Alternatively, the probe TG46 (5' AGG-TAG-TGG-TCA-ATA-CGG-AAT-AGT-TGG-TTA-TCA-TCA-AAT-GCG-TTT-T 3') can be used in Southern blotting experiments to identify restriction fragments containing the G6PDH gene. Specifically, in the cloning of G6PDH from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (NCIMB) 3351 (**Leuconostoc citreum**), Southern blot hybridization of NCIMB 3351 genomic DNA with the 46 base pair (bp) TG46 probe showed strongly hybridizing 2.7 kilo base pair (kb) **Kpn** I and 2.5 kb **Hind** III fragments. **Kpn** I and **Hind** III digested NCIMB 3351 genomic DNA was size fractionated by agarose gel electrophoresis and libraries in the plasmid pUC18 were prepared in **E. coli** JM101. Colony hybridization with TG46 resulted in the isolation of clones containing overlapping **Kpn** I and **Hind** III fragments. In addition to the complete coding region for G6PDH, these clones contained approximately 1.7 kb of 5' flanking (**Kpn** I clones) and approximately 1.1 kb 3' flanking sequences (**Hind** III clones). Approximately 0.8 kb downstream of the gene was a unique **Mlu** I site.

The 2.5 kb **Hind** III fragment, containing all but the first 20 codons of the gene, was cloned between the unique **Hind** III and **Nco** I sites of pKK233-2 (Pharmacia Molecular Biologicals, Piscataway, NJ) along with a synthetic DNA duplex which spanned pKK233-2's unique **Nco** I and **Hind** III sites and encoded the first 20 amino acids of NCIMB 3351 G6PDH as derived from the DNA sequence of the cloned **Kpn** I fragment. The synthetic linker sequences were: 5'-C-ATG-GTT-GCA-GAA-ATC-AAA-ACA-TTA-GTT-ACT-TTT-TTT-GGT-GGA-ACT-GGT-GAT-TTA-GCA-AAG-CGT-A-3' and, 5' AG-CTT-ACG-CTT-TGC-TAA-ATC-ACC-AGT-TCC-ACC-AAA-AAA-AGT-AAC-TAA-TGT-TTT-GAT-TTC-TGC-AAC 3'. The

resulting plasmid was transformed into JM101 or HB101 and expressed the wild type G6PDH from **Leuconostoc citreum** (NCIMB 3351). It was called 3351/pKK233-2.

By another method, G6PDH from American Type Culture Collection (ATCC) 12291 (**Leuconostoc mesenteroides**) was cloned by polymerase chain reaction amplification of the gene from genomic DNA and direct ligation of the restricted amplification product into an expression vector. The polymerase chain reaction (PCR) is described by Saiki, R.K., Scharf, S., Faloone, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. in **Science 230**, 1350-1354 (1985) and in United States Patent 4,683,202. The use of this technology in DNA cloning is described in United States Patent 4,800,159. Further the use of thermostable DNA polymerase in this process is described in United States Patent 4,889,818. Methods for accomplishing PCR are further described in "The Polymerase Chain Reaction" by Erlich, H.A., Gibbs, R., and Kazazian, Jr., H.H. eds. published by the Cold Spring Harbor Laboratory Press (1989), especially pages 1-31. In the specific G6PDH cloning described here, two oligos were designed for the PCR reaction to create the entire G6PDH coding region from amplification of ATCC 12291 genomic DNA. The 5' PCR oligo, 5'-T-TTT-TGG-GAT-CCA-TCC-ATG-GTT-TCA-GAA-ATC-AAG-ACG-TTA-GTA-ACT-TTC-TTT-GG-3' provides an internal **Bam** HI site, an internal **Nco** I site (which includes the ATG of the initiation codon) and sequence hybridizing to the first 38 bases of the coding region. The 3' PCR oligo, 5' T-TTT-TTC-TAG-ATT-AAG-TTA-ACC-TTT-AAA-CAC-CCA-AGC-ATC-ACC-ATT-GGC-AGC-CAA-TAA-TTT-ATC-GGA-TG 3' provided the coding sequence for the last 17 amino acids of the protein, a stop codon, and an internal **Xba** I site. ATCC 12291 genomic DNA was used as a template to amplify the desired fragment in 30 cycles, using 94°C denaturation, 42°C hybridization, and 72°C synthesis. **Taq** polymerase was purchased from Perkin-Elmer Cetus (Norwalk, CT) and used in conjunction with the Perkin-Elmer Cetus, DNA Thermal Cycler 480. The expression vector, pKK233-2 (Pharmacia Molecular Biologicals, Piscataway, NJ.) was prepared by cleaving it at the **Hind** III site, filling in the overhanging ends with Klenow fragment and ligating in a phosphorylated **Xba** I linker (d(pCTCTAGAG) and d(pGCTCTAGAG) purchased from New England Biolabs, Beverly, MA). The order of restriction enzyme sites, from the **trc** promoter, was then **Nco** I, **Pst** I and **Xba** I. A 1.5 kb fragment that included the entire coding sequence of G6PDH, beginning with the ATG of the start codon, was isolated by complete digest with **Xba** I and partial digestion (in some strains, for example ATCC 12291, the G6PDH gene may contain an internal **Nco** I site) with **Nco** I and cloned into the modified, **Nco** I, **Xba** I-cut pKK233-2.

DNA Sequencing and amino acid sequences of G6PDH Genes

Once the gene for G6PDH has been obtained, its complete nucleotide sequence may be determined by the method of Sanger, **et al.** (**Proc. Natl. Acad. Sci., USA 74** (12), 5463-5467 (1977)). Such sequencing may be

accomplished by the transfer of the DNA clones described above into suitable commercially available M13 vectors such as M13mp18 or M13mp19 (Messing, J., "Methods in Enzymology" **101** (part C); Recombinant DNA pp. 20-78. R. Wu, L. Grossman, K. Moldave (eds.) Academic Press, New York and Yanisch-Perron, C., Vieira, J. and Messing, J. *Gene 33*, 103-119 (1985). Alternatively, techniques have been published and are well known in the literature for determining the nucleotide sequence of a DNA directly from a double stranded vectors (see for example, Ku-chuan Hsiao, **Nucleic Acids Res.** 19 (10), 2787 (1991)).

DNA sequencing was accomplished using a Sequenase Kit (United States Biochemical Corp., Cleveland, Ohio) according to the instructions provided by the manufacturer and the above references. In addition to sequencing primers located within the cloning vector, other oligodeoxynucleotides useful as DNA sequencing primers for G6PDH from ATCC 12291 are described in **Table 3**. The DNA sequence of the G6PDH gene derived from **Leuconostoc mesenteroides** (ATCC 12291) is shown in Figure 1 . The DNA sequence of the G6PDH gene derived from **Leuconostoc citreum** (NCIMB 3351) and its flanking DNA is shown in Figure 3. Additional DNA sequencing and hybridization primers may be readily deduced from the sequences.

Once the DNA sequence of the G6PDH gene was determined, the amino acid sequence of the gene could be deduced from the genetic code. The amino acid sequence of the G6PDH enzyme from **Leuconostoc mesenteroides** (ATCC 12291) is shown in Figure 2. The amino acid sequence of the G6PDH enzyme from **Leuconostoc citreum** (NCIMB 3351) is shown in Figure 4. Amino terminal and tryptic fragment amino acid sequencing of authentic G6PDH enzyme isolated from these strains showed that the correct and complete genes had been isolated by the genomic and PCR cloning. In some cases, recombinant proteins expressed in **E. coli** have an additional methionine residue at the amino terminus of the protein. The presence or absence of an amino terminal methionine had no significant detrimental effect on the specific activity or conjugation properties of the proteins described in this invention.

Table 3. DNA Sequencing Primers for G6PDH from ATCC 12291.

Name	Sequence[a]	5' start position[b]	Orientation[c]
271	GAA-CGC-CTC-AGC-TTG-TGC	273	-
272	GAC-ACA-GCC-GGT-GCA-TTG	730	+
273	GCT-AGT-AGG-CCT-TCT-GAC	458	-
274	GTG-GTG-CTT-TAT-CTG-CGG	1417	-
281	CGT-CAG-ATA-CAG-TCC-AAC-C	1264	-
282	CCG-CAG-ATA-AAG-CAC-CAC	1400	+
283	CAC-GTT-CTT-CGA-CAC-CCA	718	-
285	GCG-CTT-ACC-TGA-ACG-GAC	1065	-
287	GCA-CCA-TAT-TGT-GCA-CGA-AC	920	-
288	CCT-TCA-TCG-CCG-GCG-AAT-T	989	+
294	CCA-AAG-GGT-GCT-ATC-GAA	1171	+
295	GAC-ACA-GCT-GCC-GAA-CTC	511	+
296	GAC-GTA-ACA-GAT-GCT-GCT	301	+
297	GGT-GGC-ACT-GGT-GAC-TTG	82	+
300	GAA-AAC-TGA-TGG-GTA-AAG-C	129	-
301	TCA-GTT-GCG-CCA-CGT-TTC	397	+
302	CGC-AGC-CTT-TAA-TGC-TTT-G	846	+
303	GCT-GAC-TGG-AAT-GGC-GTT	1339	+
364	ACG-CCA-GAA-CCA-TAC-GAA	1279	+

a. DNA sequences are written 5' to 3'
b. Numbering refers to figure 1.
c. Oligodeoxynucleotides reading 5' to 3' directly into amino acid sequence such as shown figure 2 are designated +; oligodeoxynucleotides complimentary to the nucleotide sequence in figure 1 are designated - in orientation.

Mutagenesis of Recombinant Leuconostoc G6PDH

Several methods are available for the introduction of specific nucleotide changes in cloned genes. These methods, including that of Kunkel, T.A. *Proc. Natl. Acad. Sci., U.S.A. 82*, 488-492 (1985), are reviewed and described in detail in most laboratory manuals of molecular biology. Specific methodologies can be found in "Current Protocols in Molecular Biology", Ausubel, F.M., Brent, R., Kingston, R.E., Morre, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. eds. published by Greene Publishing Associates and Wiley-Interscience, John Wiley & Sons, New York, especially pages 8.0.1 to 8.4.7 and in particular the section 8.1 entitled Oligonucleotide-Directed Mutagenesis without Phenotypic Selection. An additional source of methodology for the introduction and characterization of specific mutation can be found in "Molecular Cloning A Laboratory Manual", by Sambrook, J., Fritsch, E.F., and Maniates, T. published by Cold Spring Harbor Laboratory Press, 2nd edition, Chapter 15.51 to 15.80, especially 15.74 through 15.79.

The M13 site-specific mutagenesis techniques described above were employed in the isolation of the mutants listed in **Table 4**. The nomenclature used there to designate the mutants gives the one letter amino acid code for the native or "precursor" amino acid, the position of that amino acid along the polypeptide chain, and then the one letter amino acid code for the amino acid that replaces the precursor residue in the mutant form of the enzyme. Thus, for example "K343R" means that the K (lysine)

normally present at amino acid position 343 has been replaced by R (arginine).

Table 4 Mutagenic oligodeoxynucleotides for M13 site-specific mutagenesis of G6PDH genes.

Mutant	Strain	Sequence[a]	Oligo No.
K343R	ATCC 12291	CTT-AGC-TGC-TCG-TCA-GAC-ACG GGT-TGA-TAT-CGT-CTT-TAA- GGC-TGG-TAC-CTT-TAA-CTT-TG	487 526
K338R	ATCC 12291	GTC-CCA-TTC-TAC-GTA-CGT- TCA-GGT-CGT-CGC-TTA-GCT-G	488
K282R	ATCC 12291	GAA-GTT-AAC-CGT-TAC-TTT- GTT-AGA-GCT-CAA-TAT-GGT-G	508
K461R	ATCC 12291	AAG-CAC-CAC-TCG-AGA-CTT- ACC-GTT-CGG-GCT-CAA	509
K472R	ATCC 12291	GCA-TCC-GAT-CGT-TTA-TTG- GCT-G	511
K5R	NCIMB 3351	GTT-CGA-GAA-ATC-CGT-ACA- CTA-GTT-ACT-TT	534
K454R	ATCC 12291	CGT-TTA-TAC-TGC-AGA-TCG- TGC-ACC-ACT-TG	537
K182C	ATCC 12291	CTA-CGT-GGG-TTG-CGA- AAT-GGT-TC	569
K182C	NCIMB 3351	GAT-CAT-TAC-CTG-GGT- TGC-GAA-ATG-GTC-C	570
L180C	ATCC 12291	CTA-TTC-CGT-ATT-GAC-CAC TAC-TGT-GGT-AAG-GAA-ATG- GTT-CAA-AAC-ATT-GCT-GCC-C	514
K252R	NCIMB 3351	CTT-GCT-ATG-GAA-CGT- CCA-GAT-TCA	630
K252C	NCIMB 3351	CTT-GCT-ATG-GAA-TGC-CCA- GAT-TCA	632
A52K	NCIMB 3351	ATT-TGA-CTG-ATA-AGG-AAT- TCA-AGC-A	660
K128R	NCIMB 3351	CTG-TGG-CAC-CGC-GGT-TCT- TTG-GGA-CAA-TTG-CAC-GTT- ATC-TCA-AG	662
K128R/ K131R	NCIMB 3351	GAC-AAT-TGC-ACG-TTA-TCT- CCG-TTC-AGA-GGG-T	663
K77R	NCIMB 3351	TGA-AAA-GTG-TTC-GAT-GAA- TGC-CTC-GGC	540
K37C	ATCC 12291	TAT-AAA-AAA-GGA-TAT-CTG- CAA-TGC-CAT-TTT-GCC-ATT	678
A2C	NCIMB 3351	GAC-CAT-GGT-TTG-CGA-AAT- CAA-AAC-ATT-AG	738

a. Sequences are written 5' to 3'.

Besides site specific mutagenesis in M13 vectors, specific mutations can also be introduced in plasmid DNA by PCR. Higuchi, R., Krummel, B., and Saiki, R.K. have described these techniques in **Nucleic Acids Res.** 16(15), 7351-7367 (1985). This technique has the advantage that no transfer of genes between M13 vectors and plasmid expression vectors (such as pKK233-2 used here) is necessary. This mutagenesis technique involves the amplification in a first round of two independent fragments of the gene. These consist of flanking wild type primers derived from either the vector outside of the gene insert or from some distant position within the gene itself and complimentary internal primers located precisely at the site of the mutation. These internal primers contain wild type sequence except for the mutation and in some cases also a modified restriction site in the vicinity of the mutation. This latter feature allows detection of recombinant genes carrying the desired mutation after the ligation and transformation processes. Two separate amplification reactions consisting of either one of the flanking primers together with the mutant primer facing it on the other strand were carried out and the presence and quantity of the correct fragment was tested by electrophoresis.

These fragments were electrophoretically separated on SeaChem GTG grade agarose (FMC BioProducts, Rockland, ME) and gel purified using Elu-Quik glass beads (Schleicher & Schuell, Keene, NH) according to the manufacturers' instructions. The purified DNAs were used as templates for a second round of PCR together with the original flanking primers in the same tube. Amplification of this mix led to production of a template from which a mutant fragment of DNA could be excised by restriction. Restriction sites were generally chosen so as to release a fragment of between 100-300 bp. Following agarose-gel purification, as above, this fragment could then be inserted into a precut vector from which had been removed the same wild type piece of DNA. Successful clones of mutant genes could be tested in several ways, including restriction analysis looking for the non-coding mutated site present in the original pair of primers as well as by direct sequencing of the region.

PCR reactions were performed in a buffer containing 10mM Tris-HCl, pH 8.3, 2mM $MgCl_2$, 50mM KCl, 50μM of each of the four normal deoxyribonucleoside triphosphates and 2.5 units of Amplitaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). Primers were generally approximately 1μM in concentration. Cycling was as follows: 1 min at 94°C followed by 30 cycles of 30 sec at 94°C, 30 sec at 50°C and 1 min at 72°C followed by 1 cycle of 30 sec at 94°C, 30 sec at 50°C and 4 min at 72°C after which reactions were stored at 4°C.

The PCR technique described above was employed to isolate the mutants listed in **Table 5**.

Table 5. Mutagenic oligodeoxynucleotides for PCR Site-specific mutagenesis of G6PDH genes.

Mutant	Strain	Sequence[a]	Oligo No.
K128R	ATCC 12291	AGC-TAG-TAG-GCC-TTC-TGA-CTT-AAG-ATA-ACG-GGC-AAT-TGT-ACC	451
K131R	ATCC 12291	AGC-TAG-TAG-GCC-TTC-TGA-ACG-AAG-ATA-TTT-GGC	452
K252R	ATCC 12291	GAA-TGA-TTC-TGG-ACG-TTC-CAT-CGC-TAA	469
K252R		TTA-GCG-ATG-GAA-CGT-CCA-GAA-TCA-TTC	470
K148R	ATCC 12291	GAG-GTA-CCG-AAA-GGA-CGT-TCA-ATC	471
K148R		GAT-TGA-ACG-TCC-TTT-CGG-TAC-CTC	472
K259R	ATCC 12291	TCA-TTC-ACT-GAC-CGT-GAT-ATC-CGT-GCC	477
K259R		GGC-ACG-GAT-ATC-ACG-GTC-AGT-GAA-TGA	478
K265R	ATCC 12291	ATT-CGT-GCC-GCG-CGC-AAC-GCA-GCC-TTT	479
K265R		AAA-GGC-TGC-GTT-GCG-CGC-GGC-ACG-AAT	480
K128R	NCIMB 3351	ACT-ATC-AGC-TAG-CAA-ACC-CTC-TGA-CTT-GAG-ATA-ACG-TGC-AAT-TGT	491
K131R	NCIMB 3351	ACT-ATC-AGC-TAG-CAA-ACC-CTC-TGA-ACT-GAG-ATA-TTT	492
K282R	NCIMB 3351	GCC-GAA-GTC-AAC-CGT-TAT-TTC-GTC-CGT	493
K338R	NCIMB 3351	GAT-ATC-GAC-ACG-TGT-TTG-TTT-AGC-AGC-TAA-ACG-ACG-GCC-TGA-ACG	494
K343R	NCIMB 3351	GAT-ATC-GAC-ACG-TGT-TTG-ACG-AGC-AGC-TAA-ACG	495
K352R	NCIMB 3351	AAA-CAA-ACA-CGT-GTC-GAT-ATC-GTC-TTC-CGT-GCT-GGT-ACC	496
K454R	NCIMB 3351	ATT-TCA-GCT-GTC-TAC-ACT-GCT-GAT-CGT-GCA-CCA-CTT	497
K461R	NCIMB 3351	TGC-TTC-AGG-TCC-CAT-GGA-ACC-TGA-ACG-GTA-TGT-TTC	498
K5R	NCIMB 3351	ACT-AAC-TAA-TGT-ACG-GAT-ATC-TGC-AAC	500
K5R		GTT-GCA-GAT-ATC-CGT-ACA-TTA-GTT-ACT	501

K182R	NCIMB 3351	CCG-AAG-AGC-TCC-AAC-GTG-ACT-TAG-AA	502
K182R		TTC-TAA-GTC-ACG-TTG-GAG-CTC-TTC-GG	503
A77E	NCIMB 3351	TGA-AAA-GTG-TTC-GAT-GAA-TGC-CTC-GGC	540
A77E		GCC-GAG-GCA-TTC-ATC-GAA-CAC-TTT-TCA	542
A52D	NCIMB 3351	GAT-TTG-ACT-GAT-GAT-GAG-TTC-AAG-CAA	541
A52D		TTG-CTT-GAA-CTC-ATC-ATC-AGT-CAA-ATC	543
A155D/ K162R	NCIMB 3351	TAA-GTC-GTT-TTG-GAG-CTC-TTC-GGC-AGT-GTC-GTA-TGA	544
A155D/ K162R		TCA-TAC-GAC-ACT-GCC-GAA-GAG-CTC-CAA-AAC-GAC-TTA	545
A63K	NCIMB 3351	GTT-CGT-GAA-TCA-ATC-AAG-GAC-TTT-ACT-GAA-G	546
A63K		CTT-CAG-TAA-AGT-CCT-TAA-TTA-ATT-CAC-GAA-C	547
A52C	NCIMB 3351	CAG-CGT-TTG-ACT-GAT-TGT-GAG-TTC-AAG-CAA	606
A52C		TGC-TTG-AAC-TCA-CAA-TCA-GTC-AAA-TC	607
D48A/ A52C	NCIMB 3351	ACA-GCA-CGT-CAG-GCT-TTG-ACT-GAT-TGT-GAC-TTC-AAG-CAA	608
D48A/ A52C		TGC-TTG-AAC-TCA-CAA-TCA-GTC-AAA-GCC-TGA-CGT-GC	609
Q47A/ A48C	ATCC 12291	GGA-ACG-GCC-CGT-GCA-TGC-CTC-AAT-GA	652
Q47A/ A48C		TCA-TTG-AGG-CAT-GCA-CGG-GCC-GTT-CC	653
D52C	ATCC 12291	CAA-TGA-TTG-CAG-GTT-CAA-ACA-A	654
D52C		TTG-TTT-GAA-CTC-GCA-ATC-ATT-G	655
K55C	ATCC 12291	GAT-GAC-GAG-TTC-TGC-CAA-TTG-GTT-CG	658
K55C		CGA-ACC-AAT-TGG-CAG-AAC-TCG-TCA-TC	659
Q56C	ATCC 12291	GAT-GAC-GAG-TTC-AAA-TGC-TTG-GTT-CAT-G	670
Q56C		CAC-GAA-CCA-AGC-ATT-TGA-ACT-CGT-CAT-C	671
E53C	ATCC 12291	CAA-TGA-TGA-CTG-CTT-CAA-ACA	672
E53C		TGT-TTG-AAG-CAG-TCA-TCA-TTG	673

Q47C	ATCC 12291	GGA-ACG-GCC-CGT-TGC-GCC-CTC-AAT-GAT-GAC-GAG-TTC	674
Q47C		GAA-CTC-GTC-ATC-ATT-GAG-GGC-GCA-ACG-GGC-CGT-TCC	675
L49C	ATCC 12291	GGA-ACG-GCC-CGT-CAA-GCC-TGC-AAT-GAT-GAC-GAG-TTC	676
L49C		GAA-CTC-GTC-ATC-ATT-GGA-GGC-TTG-ACG-GGC-CGT-TCC	677
R46C	ATCC 12291	GGA-ACG-GCC-TGC-CAA-GCC-CTC-AAT-GAT-GAC-GAG-TTC	698
R46C		GAA-CTC-GTC-ATC-ATT-GAG-GGC-TTG-GCA-GGC-CGT-TCC	699
N50C	ATCC 12291	CAA-GCC-CTC-TGC-GAT-GAC-GAG-TTC-AAA-CA	700
N50C		TGT-TTG-AAC-TCG-TCA-TCG-CAG-AGG-GCT-TG	701
D51C	ATCC 12291	CAA-GCC-CTC-AAT-TGC-GAC-GAG-TTC-AAA-CA	705
D51C		TGT-TTG-AAC-TCG-TCG-CAA-TTG-AGG-GCT-TG	706
F54C	ATCC 12291	GAT-GAC-GAA-TGC-AAA-CAA-TTG-GTT-CGT-G	707
F54C		CAC-GAA-CCA-ATT-GTT-TGC-ATT-CGT-CAT-C	708
K128C	ATCC 12291	ACA-ATT-GCC-TGC-TAT-CTT-AAG-TCA-GAA-GGT-CTA-CT	709
K128C		AGT-AGA-CCT-TCT-GAC-TTA-AGA-TAG-CAG-GCA-ATT-GT	710
K131C	ATCC 12291	GCC-AAA-TAT-CTT-TGC-TCA-GAA-GGT-CTA-CTA-GCT-G	711
K131C		CAG-CTA-GTA-GAC-CTT-CTG-AGC-AAA-GAT-ATT-TGG-C	712
L57C	ATCC 12291	GAT-GAC-GAG-TTC-AAA-CAA-TGC-GTT-CGT-G	752
L57C		CAC-GAA-CGC-ATT-GTT-TGA-ACT-CGT-CAT-C	753
V58C	ATCC 12291	GAT-GAC-GAG-TTC-AAA-CAA-TTG-TGC-CGT-G	754
V58C		CAC-GGC-ACA-ATT-GTT-TGA-ACT-CGT-CAT-C	755
R59C	ATCC 12291	GAC-GAG-TTC-AAA-CAA-TTG-GTT-TGC-GAT-TCA	756
R59C		TGA-ATC-GAC-AAC-CAA-TTG-TTT-GAA-CTC-GTC	757
D60C	ATCC 12291	GAC-GAG-TTC-AAA-CAA-TTG-GTT-CGT-TGC-TCA-ATT	758
D60C		AAT-TGA-GCA-ACG-AAC-CAA-TTG-TTT-GAA-CTC-GTC	759

a. Sequences are written 5' to 3'.

In the case of the K21R, K19R, K282R, K454R, and K472R mutants in NCIMB 3351 a unique **Bam** HI site approximately 0.3 kb upstream of the **Nco** I site in 3351/pKK233-2 was used to move the entire gene including pKK233-2's

trc promoter and NCIMB 3351 G6PDH gene terminator, on an approximately 2.4 kb **Mlu** I-**Bam** HI fragment from 3351/pKK233-2 into M13um30 (International Biotechnologies, Inc., New Haven, CT.). This construction, called 3351/um30, allowed site-directed mutagenesis by primer extension essentially according to the methods of Zoller, M.J. and Smith, M. in *Methods Enzymol. 100*, 468-500 (1983). Single-stranded DNA template from this 3351/um30 clone corresponded to the sense (coding) strand. For this reason the mutagenic oligodeoxynucleotides used below correspond to the anti-sense strand. Individual K19R, K21R, K282R, K454R and K472R mutants were isolated using the following oligonucleotides (asterisks denote changes from wild type sequence and introduced, or destroyed, restriction sites are noted):

```
                                K21R
                                   ***
K21R:  5'-TGA-AGG-ATA-AAG-TCG-ACG-CTT-TGC-T-3'
                            |_____|
                        destroyed Hind III
                                |______|
                          introduced Sal I
```

Note that K21R 3351/um30 single-stranded template was used for the following K19R mutagenesis to create the double mutant.

```
                                  R21K    K19R
                                  ***      ***
K19R:  5'-TGA-AGG-ATA-AAG-CTT-ACG-ACG-TGC-TAA-ATC-A-
                            |_____|
                          reintroduced Hind III
                                |______|
                                destroyed Sal I

                                 *   * *              ***
K282R:  5'-GGC-ACC-ATA-CTG-AGC-TCT-GAC-GAA-ATA-ACG-GTT-GAC-TTC-G-3'
                                |______|               K282R
                                introduced Sst I

                                 *    *        ***       *
K454R:  5'-CTT-GTA-TGT-TTC-TAG-AGG-TGC-ACG-ATC-TGC-AGT-GTA-GA-3'
                              |______|          K454R   |______|
                          introduced Xba I
                                                  introduced Pst I

                                 ***                      *
K472R: 5'-TTC-GGC-TAA-CAG-ACG-GTC-AGA-TGC-TTC-AGG-GCC-CAT-GGA-AC-3'
                                K472R                  |______|
                                                  introduced Apa I
```

Introduced or destroyed restriction sites were designed to be silent at the protein level except in cases where they also result in the desired K to R or R to K changes (i.e. K21R and K19R oligodeoxynucleotides). The sites are used as an aid in isolating the desired mutant from a mixed mutant and

wild type population. Following mutagenesis in M13, the plaque-purified, sequence verified clone was re-digested with **Mlu** I and **Bam** HI and the 2.4 kb fragment reintroduced into a suitably digested pKK233-2 vector. The introduced or destroyed restriction sites, described above, were used to screen for the mutant 3351/pKK233-2.

The double mutant K19R/K282R was obtained in the following manner. K19R/pKK233-2 was digested with **Nhe** I and **Kpn** I. The large fragment (less approximately 0.65 kb) was isolated. K282R/pKK233-2 was digested with **Nhe** I and **Kpn** I, the approximately 0.65 kb fragment (containing K282R and the **Sst** I site) isolated. These two fragments were ligated and transformed into **E. coli** JM101. Plasmids from the resulting transformants were prepared and screened for K282R's **Sst** I site, indicating the presence of the double mutation.

The double mutant K454R/K472R was obtained in the following manner. K472R/pKK233-2 was digested with **Nco** I and **Bam** HI. The large fragment (less approximately 0.3 kb pKK233-2 **Bam** HI - **Nco** I and approximately 1.4 kb **Nco** I-**Nco** I fragments) isolated. K454R/pKK233-2 was digested with **Kpn** I and **Nco** I, the approximately 0.33 kb fragment (containing K454R and its linked **Xba** I and **Pst** I sites) isolated. Wild type 3351/pKK233-2 was digested with **Bam** HI and **Kpn** I and the approximately 1.4 kb fragment isolated. These three fragments were ligated and transformed into **E. coli** JM101. The resulting transformants screened for K454R's **Xba** I site.

A quadruple mutant, called "4X" or "quad", was constructed from the two double mutants above in the following manner. K19R/K282R/pKK233-2 was digested with **Mlu** I and **Kpn** I, the large fragmented (less approximately 1.2 kb) isolated. K454R/K472R/pKK233-2 was digested with **Mlu** I and **Kpn** I, the approximately 1.2 kb fragment (containing K454R, K472R, **Pst** I, **Xba** I and **Apa** I sites) isolated. These two fragments were ligated and transformed into **E. coli** JM101. The resulting transformants were screened for K472R's **Apa** I site. The resulting "quad" mutant was then K19R/K282R/K454R/K472R.

Contrary to what might have been expected, multiple lysine to arginine substitutions in G6PDH frequently resulted in enzymes, which when conjugated, showed improved specific activity, stability, and better immunoassay performance as compared with precursor enzyme or mutant enzymes containing only individual single mutations. Thus, for example, the quad enzyme performs better, and
is preferred, over precursor G6PDH or mutant G6PDHs containing only each of the single mutations that make it up.

Tables 4 and 5 above describe a number of individual and some multiple mutations that can be made in precursor G6PDH. These mutations may also be made in the quad mutant so as to result in G6PDHs with higher numbers of mutations. For example, using the above described methods, K128R can be introduced into the quad to result in a mutant G6PDH that is K19R/K128R/K282R/K454R/K472R. This mutant is called the "pent" or 5X".

K15R and K461R can be introduced into the quad so as to result in K5R/K19R/K282R/K454R/K461R/K472R. This mutant is called the "hex" or "6X". Further, K128R can be added to the hex to result in a mutant that is K5R/K19R/K128R/K282R/K454R/K461R/K472R. This mutant is called the "sept" or "7X". Additionally, K128R/K131R can be introduced into the hex to result in a mutant G6PDH that is K5R/K19R/K128R/K131R/K282R/K454R/K461R/K472R. This mutant is called the "oct" or "8X", and so on. Multiple lysine to arginine mutations at any two or more naturally occurring lysines are desirable and within the scope of this invention with the exception of positions 21 and 182 (in ATCC 12291 and NCIMB 3351). Mutational substitution of arginine at these latter two positions typically result in lowered specific activity and poor immunoassay performance. In all cases, subsequent DNA sequencing of the G6PDH gene was used to verify the position and nature of all single and multiple mutants.

Isolation and Purification of Recombinant G6PDH

The isolation and purification of G6PDH from **Leuconostoc mesenteroides** was described by Olive, C. and Levy, H.R. *Biochemistry 6*(3), 730-736 (1967). Larger quantities of the protein were accommodated by the modification to this procedure described in Haghighi, B., Flynn, T.G., and Levy, H.R. *Biochemistry 21*(25), 6415-6420 (1982), specifically page 6416 column 1. A more rapid procedure which is useful is that described by Hey, Y. and Dean, P.D.G. *Biochem. J. 209*, 363-371 (1983). The above methods were successful in enabling the isolation and purification of the mutant G6PDHs described in this invention.

EXAMPLE I

The quinidine analog O-carboxypentylcupreidine was activated by reaction with 1 equivalent of N-hydroxysuccinimide (NHS) and 1.1 equivalent of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) in dimethylformamide (DMF). The activation reaction was stirred for 72 hours at 4°C. The concentration of the carboxypentylcupreidine was 0.16 M.

A solution containing 0.5 mg of "8X" G6PDH show with 2 mg glucose-6-phosphate disodium salt and 4 mg of NADH in 100 µl of 0.1 M sodium bicarbonate buffer was prepared. To this was slowly added 25µl of DMF while stirring on ice. A total of 7.5 µl of the activated carboxypentylcupreidine was then added in several aliquots and allowed to react for 1 hour/addition in order to reach the desired endpoint of >70% maximum inhibition.

The enzyme was monitored in accordance with the assay described below for determining % deactivation. The % inhibition was determined by adding an excess of anti-quinidine antibody and assaying the enzyme activity according to the method described below. The conjugate was worked up by chromatography over Bio-Gel P6-DG (Bio Rad) (6 ml column), employing 0.1 M

sodium bicarbonate buffer (pH 8.1) as eluant, collecting 200 µl fractions. These fractions were pooled to give a total volume of 1.2 ml.

The components of enzyme assays used to monitor the progress of the conjugation were as follows:

Samples from the conjugation reaction were diluted into a buffer containing 0.2 M Tris-Cl and 0.1% bovine serum albumin (BSA) at pH 8.0 prior to assay. Dilutions were made such that the rate in the assay described was between 500 and 1000 mΔA/min. The added assay buffer contained 0.055 M Tris-Cl, 0.5% NaCl, and 0.01% v/v Triton X-100 at a pH of 8.1. The substrate solution contained 0.007 M G6P and 0.0028 M NAD^+ at pH 5.5. A Gilford Stasar III microsample spectrophotometer is employed with a Thermocuvette with a flow cell.

The protocol employed for carrying out an assay is as adapted from U.S. Patent 4,238,389, example 7, column 8. A 50 µl aliquot of substrate solution is drawn up into a diluter and dispensed with 250 µl of assay buffer into a 2 ml Croan cup, followed by a 50 µl aliquot of assay buffer (or anti-quinidine antibody solution containing an excess of antibody in cases where % inhibition in being determined) with 250 µl of the assay buffer. A 50 µl aliquot of enzyme solution and 250 µl of assay buffer are then added to the Croan cup. Immediately after the enzyme addition, the entire sample is aspirated into the flow cell. After 15 seconds, a first reading is taken, followed by a second reading, after a 45 second interval from aspiration. The results in terms of deactivation and inhibition are reported in **Table 6**.

The low deactivation achieved in this conjugation (30%) compares very favorably with conjugations of commercial enzyme, in which deactivations of 45-60% are observed. Lower deactivation is often correlated with enhanced thermal stability of the conjugate (see example 3 below).

Table 6

QUINIDINE CONJUGATION RESULTS

Sample	Total Hapten (µl)	% Deactivation	% Inhib.
1	--		
2	5	15	56
3	7.5	30	71

Example II

A preparation of 3',5'-diiodo-4'-hydroxy-3-phenylpropionic acid (DIHPPA) was activated by reaction with 1 equivalent of N-hydroxysuccinimide and 1 equivalent of (EDAC) in dimethylformamide (DMF). The activation reaction was stirred for 24 hours at 4°C. The concentration of the DIHPPA was 0.05 M.

A solution containing 0.25 mg of G6PDH (wild type or variant as listed in **Table 7**) with 4 mg glucose-6-phosphate disodium salt and 4 mg of NADH in 100 µl of 0.55 M Tris-Cl (pH 8.0) buffer was prepared. To this was slowly added 25 µl of DMF while stirring on ice. A total of 11-13 µl of the activated DIHPPA was then added in several aliquots and allowed to react for 1 hour/addition in order to reach the desired endpoint of 65-75% maximum inhibition. The procedures described above were used for monitoring enzyme activity, determining maximum inhibition (using an anti-thyroxine pAb), and working up the conjugate. The final results of these conjugations are reported in **Table 7**.

These conjugates were diluted and used in an immunoassay for thyroxine as described below. The conjugates made from variant G6PDH's showed larger standard curves over the range of thyroxine concentrations tested (See **Table 8**). Larger standard curves often give more reproducible and precise results in immunoassays.

The solutions used for dilution of conjugates and antibodies are:
Antibody Diluent: 0.009 M NAD^+, 0.0011 M PEG 6000, 0.0095 M Glucose-6-phosphate, monosodium, 0.00026 M ANS (8-Analino-1-naphthalene-sulfonic acid), 0.0165 M Tris, 0.1 M NaCl, 0.000038 M Plurafac A39, 0.0013 M EDTA, 0.0000067 M BGG (Bovine gamma globulin), 0.098 M Mannitol, 0.000049 M Thimerosal, 0.053% gentamicin, 0.012% Dow Corning AF silicone, at pH 6.0.
Conjugate Diluent: 0.0189 M glucose-6-phosphate, monosodium, 0.00018 M PEG 6000, 0.073 M Tris, 0.0634 M barbital, sodium, 0.00009 M BSA, 0.0234 M NaCl, 0.053% gentamicin, 0.012% Dow Corning AF silicone, 0.000049 M Thimerosal, 0.0027 M EDTA, at pH 8.0.

The conjugate was diluted to give an appropriate rate (180-190 mΔA/min) in the absence of antibody using the protocol described below. Antibody dilution was optimized to give the largest standard curve in that assay. Calibrators were made by diluting thyroxine into thyroxine-free human serum at concentrations of 0, 2, 4, 8, 12, and 20 µg/dL.

Table 7

DIHPPA CONJUGATION RESULTS

Enzyme	Total Hapten (µl)	Final % Deactivation	Final % Inhib.
wild type 3351 [USB]	11	64	73

3351-K5R	11	63	72
3351-K19R	11	47	75
	13	68	77
3351-K454,472R	11	60	73
3351-K19,282, 454,472R (4X)	13	71	75

Assays were performed on a COBAS MIRA automated analyzer (Roche Diagnostics) with the following protocol (run at 37°C):

Step 1) 4 µl sample + 55 µl water are combined in the instrument cuvette with 150 µl of anti-thyroxine solution

(325 second incubation)

Step 2) 75 µl of conjugate solution + 20 µl of water are added to the cuvette

(25 second incubation)

Step 3) The enzymatic rate (change in absorbance at 340 nm) is measured for 125 seconds

The rates measured for thyroxine samples are shown in **Table 8**.

Table 8

STANDARD CURVES FOR THYROXINE ASSAYS

RATES (mΔA/min)

Sample (µg/dl Thyroxine)	precursor	K5R	K19R#1	K19,282 K454,472R	454,472R
0	143.3	137.2	135.8	136.1	126.9
2	147.0	141.3	139.8	141.4	131.0
4	151.5	144.9	144.9	145.7	136.7
8	159.0	153.1	154.5	154.3	147.2
12	164.6	161.2	160.5	161.2	154.3
20	174.6	174.1	173.8	173.3	167.7
Total mod.	31.3	36.9	38.0	37.2	40.8

Example III

3-ketodigoxigenin O-carboxymethyl oxime was activated by reaction with 1 equivalent of N-hydroxysuccinimide and 1.3 equivalent of dicyclohexylcarbodiimide in tetrahydrofuran (digoxigenin concentration was 0.043 M). The activation reaction was stirred for 24 hours at room temperature, then filtered through glass wool, dried, and stored at -20°C under anhydrous conditions. The crude activated ester was worked up by chromatography over silica gel in 1:1 dichloromethane:acetonitrile. The resultant material was dried under a stream of Ar gas and stored at -20°C under anhydrous conditions. Before use, a 1 mg sample of the activated ester was dissolved in 179 µl of anhydrous DMF to give a 0.01 M solution.

A solution containing 0.25 mg of G6PDH (wild type or variant as listed in **Table 9**) with 0.8 mg glucose-6-phosphate disodium salt in 100 µl of 0.1 M sodium bicarbonate buffer (pH 8.0) was prepared. To this was slowly added 25 µl of DMF while stirring on ice. A total of 8-12 µl of the activated 3-ketodigoxigenin O-carboxymethyl oxime was then added in several aliquots and allowed to react for 1 hour/addition in order to reach the desired endpoint of 50-57% maximum inhibition. The procedures described above were used for monitoring enzyme activity, determining maximum inhibition (using a rabbit antidigoxin antiserum), and working up the conjugate. The final results of the conjugations are shown in **Table 9**.

These conjugates were used in an immunoassay for digoxin as described below. The conjugates made from variant G6PDH's showed larger standard curves than did those made from wild type enzyme over the range of digoxin concentrations tested (See **Table 10**). Larger standard curves often give more reproducible and precise results in immunoassays.

These conjugates were used in a formulation in which the first reagent contained detergent in a low pH buffer for pretreatment of patient samples. This reagent also contained substrates: 0.0101 M NAD^+, 0.0175 M Glucose-6-phosphate, monosodium, 0.176 M NaCl, 0.025 M Glycylglycine, 5.0% Sucrose, 0.025% Proclin 300, 1.6% Triton X-100, 0.004% Silicone, at pH 2.35. Rabbit anti-digoxin antibodies were encapsulated in liposomes as described below. The solution used for dilution of conjugates and liposomes is 0.3 M Tris, 0.1% RSA (Rabbit Serum Albumin), 0.025% EDTA, 0.7 mg/L Pepstatin A, 10 TIU/L Aprotinin, 0.025% Proclin 300, 0.002% Silicone, at pH 8.25. The conjugate was diluted to give an appropriate rate (100 mΔA/min) in the absence of antibody using the protocol described below. The dilution of antibody-containing liposomes was optimized to give the largest standard curve using the digoxin samples described next. Samples consisted of human serum with digoxin added to concentrations of 0, 0.5, 1, 2, 3, and 4 ng/ml.

Liposomes containing antibodies were prepared in the following way. Antibodies from rabbit serum were purified by ammonium sulfate precipitation (0.18 g/ml), reconstitution in 0.1 M Tris, 0.1% EDTA, pH 8.0,

and dialysis against 0.1 M Tris, 0.1% EDTA, pH 8.0. Final protein concentration was 19 mg/ml. 1-Palmitoyl-2-Oleyl Phoshatidylcholine (POPC), 1-Palmitoyl-2-Oleyl Phoshatidylglycerol (POPG), and cholesterol (Avanti Polar Lipids) were combined in a ratio of 76:4:20 (w/w) for a total of 1.8 g. These lipids were dissolved in 13.68 ml chloroform. Using a 60° water bath to speed evaporation, the chloroform was evaporated under a stream of nitrogen gas to leave a thin film of lipids on the inside of the flask. 36 ml of *tert*-butanol was then added and the lipids redissolved at 60°. The lipid/*tert*-butanol solution was divided into aliquots containing 300 mg lipid in 6 ml volume. These aliquots were then frozen, lyophilized, and sealed under vacuum. To 300 mg of dried lipids was added 1.5 ml of antibody solution containing 28.5 mg protein. This solution was agitated on a vortex mixer for 15 minutes, then allowed to stand at room temperature for 30 minutes to fully hydrate the lipids. This suspension was then extruded ("The Extruder", Lipex Biomembranes Inc.) 3 times through stacked polycarbonate filters (pore sizes 1.0, 0.4, and 0.2 μm) using 400 psi of pressure (nitrogen gas). One ml of 0.1 M Tris buffer (pH 8.0) was added, and the extrusion was repeated 4 more times. The sample was then subjected to gel filtration on a Sephacryl 1000 column, and fractions were tested for both cholesterol (using Cholesterol/HP reagents from Boehringer Mannheim according to manufacturers protocol on the COBAS MIRA) and for the ability to inhibit enzymatic rate in the assay system described below. Those fractions containing liposomes were pooled and used in subsequent assays.

Table 9

DIGOXIN CONJUGATION RESULTS

Enzyme	Total Hapten(μl)	Final % Deact.	Final % Inhib.
wild type 3351	9	40	53
3351-K282R	9	21	53
3351-K454R	9	28	54
3351-K454,472R	8	30	56
3351-K19,282, 454,472R (4X)	9	26	56

Assays were performed on a COBAS MIRA automated analyzer (Roche Diagnostics) with the following protocol (run at 37°C):

Step 1) 36 μl sample + 20 μl water are combined in the instrument cuvette with 150 μl of pretreatment/substrate solution

(275 second incubation)

Step 2) 75 μl of conjugate/liposome solution + 20 μl of water are added to the cuvette

(75 second incubation)

Step 3) The enzymatic rate (change in absorbance at 340 nm) is measured for 350 seconds

The rates measured for digoxin samples are shown in **Table 10**.

These conjugates were also subjected to thermal stress (30°C for up to 44 days) to determine their stability. At selected intervals the enzyme activity was measured in assays as described above. The percentage of enzymatic activity remaining at each time point is shown in **Table 11**, from which it can be seen that the conjugates made from variant enzymes were more stable than those made from the wild type enzyme. This enhanced stability may be correlated with the decreased deactivation observed during the conjugation reaction as shown by a comparison of **Tables 9** and **11**.

Table 10

DIGOXIN STANDARD CURVES

Sample (ng/dl digoxin)	precursor	K282R	K454, K454R	472R	K19,282, 454,472R
0	65.0	64.9	64.4	59.3	58.7
0.5	66.1	66.1	65.6	60.7	59.8
1.0	67.5	67.5	66.9	61.8	60.9
2.0	69.7	69.8	69.7	64.3	63.5
3.0	71.7	72.5	71.9	66.8	65.3
4.0	73.4	74.6	74.3	69.2	67.6
Mod.	8.3	9.7	9.9	9.9	8.9

Table 11

DIGOXIN CONJUGATE STABILITY

STUDY 1

Percent Activity Remaining

Conjugate	Day 1	6	3	22	26	44
Wild-type	100	86	78	71	68	58
K282R	100	92	85	78	75	65
K454R	100	90	83	76	73	63

STUDY 2

Percent Activity Remaining

Conjugate	Day 1	7	18
Wild-type	100	85	75
K454,472R	100	91	81
K19,282,454,472R	100	92	83

Example IV

A bromoacetyl derivative of 3-ketodigoxigenin O-carboxymethyl oxime was prepared as described in U.S. Patent 4,727,022 (Example 4, columns 9 and 10). This material was stored either dry or as a 0.037 M solution in anhydrous DMF.

Samples of G6PDH variants 12291-D52C, 12291-E53C, and 12291-Q56C (250 μg in 0.5-2 ml) were dialyzed vs. 4 L of thoroughly degassed buffer containing 50 mM sodium phosphate and 1 mM EDTA at pH 7.0. The enzymes were then concentrated to 100 μl in Centricon-30 concentrators (Amicon). While stirring the enzyme solutions on ice, 5 μl of the bromoacetyl digoxin/DMF solution was added. This addition was followed by a flush with Argon gas, and the reaction container tightly sealed. After stirring the reaction for 24 hours at 4°C in the dark, an additional 3.3 μl of the bromoacetyl digoxin/DMF solution was added. The reaction was stirred once again for 24 hours at 4°C in the dark. Following this second incubation, the conjugate was worked up as described above, except phosphate/EDTA buffer was used in the chromatography.

These conjugates were used in combination with two kinds of antibodies to generate an immunoassay system. One set of antibodies were the anti-G6PDH monoclonal antibodies described in U.S. patent 4,727,022, especially antibody V1A1. The other antibodies used were monoclonal antidigoxin antibodies.

The conjugates were diluted into buffer containing 100 mM Tris-Cl, pH 7.8 at concentrations which would result in rates of 80-110 mΔA/min (in the absence of antibodies) in the assay described below. All antibodies were also diluted into this buffer. For this experiment approximately a 25-fold molar excess of anti-G6PDH and approximately 1 molar equivalent of anti-

digoxin Ab (relative to conjugate) were used. A 0.05 M solution of glucose-6-phosphate was prepared in 0.5% NaCl, and the pH adjusted to 7.0 with NaOH. A 0.053 M solution of NAD^+ was prepared in 0.5% NaCl, and the pH adjusted to 5.9 with NaOH. Human serum samples containing 0, 0.5, 1, 2, 3, and 4 ng/ml of digoxin were used to generate standard curves.

Assays were performed on a COBAS MIRA-S automated analyzer (Roche Diagnostics) with the following protocol (run at 37°C):

Step 1) 12 µl sample + 38 µl water are combined in the instrument cuvette with 100 µl of anti-digoxin mAb
(125 second incubation)
Step 2) 35 µl of conjugate solution + 50 µl of G6P solution (diluent 1) are added to the cuvette
(125 second incubation)
Step 3) 35 µl of anti-G6PDH solution + 50 µl of NAD^+ (diluent 2) solution are added to the cuvette
(100 second incubation)
Step 4) The enzymatic rate (change in absorbance at 340 nm) is measured for 125 seconds

The rates measured for digoxin samples are shown in **Table 12**.

Table 12

DIGOXIN ASSAYS WITH CYSTEINE-LINKED DRUG ANALOG

Digoxin concentration in sample	Rates (mΔA/min) D52C	E53C	Q56C
0	42.1	39.7	56.6
0.5 ng/ml	38.6	36.3	53.1
1 ng/ml	35.6	32.9	49.1
2 ng/ml	29.7	27.9	44.8
3 ng/ml	25.6	22.2	39.8
4 ng/ml	23.5	19.3	36.7
Total modulation	18.6	20.4	19.9

The patents and patent applications referred to in the above description are each incorporated herein by reference in their entirety.

Although the foregoing invention has been described in some detail by way of illustration and example for the purposes of clarity and understanding, it will be obvious that certain changes or modifications may be practiced within the scope of the appended claims. For example, many possible approaches to the cloning, expression, and mutagenesis of the G6PDH genes of the invention could be practiced by one ordinarily skilled in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Syntex (U.S.A.) Inc.
(B) STREET: 3401 Hillview Avenue
(C) CITY: Palo Alto
(D) STATE: California
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 94304
(G) TELEPHONE: 415-852-1356
(H) TELEFAX: 415-496-3529

(i) APPLICANT:
(A) NAME: Genencor International Inc.
(B) STREET: 180 Kimbal Way
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 94080
(G) TELEPHONE: 415-742-7536
(H) TELEFAX: 415-742-7217

(ii) TITLE OF INVENTION: HOMOGENEOUS IMMUNOASSAYS USING MUTANT GLUCOSE-6-PHOSPHATE DEHYDROGENASES

(iii) NUMBER OF SEQUENCES: 124

(iv) COMPUTER READABLE FORM
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1458 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA Genomic

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTTTCAGAAA TCAAAACTTT GGTTACATTC TTCGGCGGCA CTGGTGACTT GGCCAAGCGT 60

AAGCTTTACC CATCAGTTTT CAATCTTTAT AAAAAAGGCT ACTTGCAAAA GCATTTTGCC 120

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TCAATTAAAG ATTTCACTGA CGATCAAGCA CAAGCTGAGG CGTTCACCGA ACATTTCTCA 240

TACCGTGCAC ACGACGTAAC AGATGCTGCT TCATACGCTG TTTTAAAAGA GGCGATTGAA 300

GAAGCTGCCG ACAAATTTGA TATCGATGGC AACCGCATTT TCTATATGTC AGTTGCGCCA 360

CGTTTCTTTG GTACAATTGC CAAATATCTT AAGTCAGAAG GCCTACTAGC TGACACTGGT 420

TACAACCGTT TGATGATTGA AAAGCCTTTC GGTACATCAT ATGACACAGC TGCCGAACTC 480

CAAAATGACT TGGAAAACGC ATTTGATGAT AACCAACTAT TCCGTATTGA CCACTACCTT 540

GGTAAGGAAA TGGTTCAAAA CATTGCTGCC CTTCGCTTTG GTAACCCAAT TTTCGATGCT 600

GCTTGGAACA AGGATTACAT CAAGAACGTT CAAGTAACAT TGTCAGAAGT CTTGGGTGTC 660

GAAGAACGTG CCGGCTACTA TGACACAGCC GGTGCATTGC TCGACATGAT TCAAAACCAC 720

ACCATGCAAA TTGTTGGTTG GTTAGCCATG GAAAAACCAG AATCATTCAC TGACAAAGAC 780

ATTCGTCCGC TAAAAAACGC AGCCTTTAAT GCTTTGAAGA TCTATGATGA AGCAGAAGTT 840

AACAAATACT TTGTTCGTGC ACAATATGGT GCCGGTGATT CAGCTGACTT CAAGCCATAC 900

CTTGAAGAAT TAGACGTACC TGCTGATTCT AAAAACAATA CCTTCATCGC CGGCGAATTG 960

CAATTTGATT TGCCACGTTG GGAGGGTGTC CCATTCTATG TCCGTTCAGG TAAGCGCTTA 1020

GCTGCTAAAC AGACACGGGT TGATATCGTC TTTAAGGCTG GCACGTTTAA CTTTGGTTCA 1080

GAACAAGAAG CACAAGAAGC TGTCTTGTCA ATTATCATTG ATCCAAAGGG TGCTATCGAA 1140

TTGAAGTTGA ACGCTAAGTC AGTTGAAGAT GCTTTCAACA CACGTACAAT TGACTTAGGT 1200

TGGACTGTAT CTGACGAAGA TAAGAAGAAC ACGCCAGAAC CATACGAACG TATGATTCAC 1260

GACACTATGA ATGGTGATGG CTCTAACTTC GCTGACTGGA ATGGCGTTTC AATCGCTTGG 1320

AAGTTCGTTG ATGCTATTTC AGCCGTTTAT ACCGCAGATA AAGCACCACT TGAAACTTAC 1380

AAGTCGGGCT CAATGGGTCC TGAAGCATCC GATAAATTAT TGGCTGCCAA TGGTGATGCT 1440

TGGGTGTTTA AAGGTTAA 1458

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 485 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Val Ser Glu Ile Lys Thr Leu Val Thr Phe Phe Gly Gly Thr Gly Asp
1 5 10 15

Leu Ala Lys Arg Lys Leu Tyr Pro Ser Val Phe Asn Leu Tyr Lys Lys
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Gly Tyr Leu Gln Lys His Phe Ala Ile Val Gly Thr Ala Arg Gln Ala
35 40 45

Leu Asn Asp Asp Glu Phe Lys Gln Leu Val Arg Asp Ser Ile Lys Asp
50 55 60

Phe Thr Asp Asp Gln Ala Gln Ala Glu Ala Phe Ile Glu His Phe Ser
65 70 75 80

Tyr Arg Ala His Asp Val Thr Asp Ala Ala Ser Tyr Ala Val Leu Lys
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Glu Ala Ile Glu Glu Ala Ala Asp Lys Phe Asp Ile Asp Gly Asn Arg
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Ile Phe Tyr Met Ser Val Ala Pro Arg Phe Phe Gly Thr Ile Ala Lys
115 120 125

Tyr Leu Lys Ser Glu Gly Leu Leu Ala Asp Thr Gly Tyr Asn Arg Leu
130 135 140

Met Ile Glu Lys Pro Phe Gly Thr Ser Tyr Asp Thr Ala Ala Glu Leu
145 150 155 160

Gln Asn Asp Leu Glu Asn Ala Phe Asp Asp Asn Gln Leu Phe Arg Ile
165 170 175

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

Phe Gly Asn Pro Ile Phe Asp Ala Ala Trp Asn Lys Asp Tyr Ile Lys
195 200 205

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

Gly Tyr Tyr Asp Thr Ala Gly Ala Leu Leu Asp Met Ile Gln Asn His
225 230 235 240

Thr Met Gln Ile Val Gly Trp Leu Ala Met Glu Lys Pro Glu Ser Phe
245 250 255

Thr Asp Lys Asp Ile Arg Ala Ala Lys Asn Ala Ala Phe Asn Ala Leu
260 265 270

Lys Ile Tyr Asp Glu Ala Glu Val Asn Lys Tyr Phe Val Arg Ala Gln
275 280 285

Tyr Gly Ala Gly Asp Ser Ala Asp Phe Lys Pro Tyr Leu Glu Glu Leu
290 295 300

Asp Val Pro Ala Asp Ser Lys Asn Asn Thr Phe Ile Ala Gly Glu Leu
305 310 315 320

Gln Phe Asp Leu Pro Arg Trp Glu Gly Val Pro Phe Tyr Val Arg Ser
325 330 335

Gly Lys Arg Leu Ala Ala Lys Gln Thr Arg Val Asp Ile Val Phe Lys
340 345 350

Ala Gly Thr Phe Asn Phe Gly Ser Glu Gln Glu Ala Gln Glu Ala Val
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Leu Ser Ile Ile Ile Asp Pro Lys Gly Ala Ile Glu Leu Lys Leu Asn
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Ala Lys Ser Val Glu Asp Ala Phe Asn Thr Arg Thr Ile Asp Leu Gly
385 390 395 400

Trp Thr Val Ser Asp Glu Asp Lys Lys Asn Thr Pro Glu Pro Tyr Glu
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Arg Met Ile His Asp Thr Met Asn Gly Asp Gly Ser Asn Phe Ala Asp
420 425 430

Trp Asn Gly Val Ser Ile Ala Trp Lys Phe Val Asp Ala Ile Ser Ala
435 440 445

Val Tyr Thr Ala Asp Lys Ala Pro Leu Glu Thr Tyr Lys Ser Gly Ser
450 455 460

Met Gly Pro Glu Ala Ser Asp Lys Leu Leu Ala Ala Asn Gly Asp Ala
465 470 475 480

Trp Val Phe Lys Gly
485

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1467 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA genomic

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc citreum
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTGCAGAAA TCAAAACATT AGTTACTTTT TTTGGTGGAA CTGGTGATTT AGCAAAGCGT 60

AAGCTTTATC CTTCAGTTTT TAACCTTTAC AAGAAAGGTT ACTTACAAGA ACATTTTGCC 120

ATTGTTGGTA CAGCACGTCA GGATTTGACT GATGCTGAAT TCAAGCAATT GGTTCGCGAA 180

TCAATCGCTG ACTTTACTGA AGATAAAGCC CAAGCCGAGG CCTTCATCGC ACACTTTTCA 240

TACCGTGCAC ATGATGTAAC CGATGCAGCT TCATACAACA TCTTAAAACA AGCAATTGAA 300

GAAGCAGCCG AAAAGTTCGA TATTCAAGGT AATCGTATTT TCTACATGTC TGTGGCACCA 360

CGATTCTTTG GGACAATTGC AAAATATCTC AAGTCAGAGG GTTTGCTAGC TGATAGTGGT 420

TACAACCGTT TGATGATTGA AAAGCCTTTT GGTACATCAT ACGCCACTGC CGAAGAGCTA 480

CAAAAAGACT TAGAAAACGC TTTTGACGAT AATCAATTAT TCCGTATTGA TCATTATCTT 540

GGTAAAGAAA TGGTCCAAAA TATTGCTGCC CTTCGTTTTG GTAACCCCAT CTTTGATGCC 600

GCTTGGAACA AAGATTACAT TAAAAACGTC CAAGTTACTT TGTCTGAAGT GCTTGGTGTT 660

GAAGAACGTG CCGGTTATTA CGATACAGCC GGTGCATTAT TAGATATGAT TCAAAACCAC 720

ACTATGCAAA TTGTTGGTTG GCTTGCTATG GAAAAGCCAG ATTCATTTAC TGATAAGGAT 780

ATCCGTGCGG CTAAGAATGC GGCTTTTAAT GCTCTTAAAA TTTATGATGA AGCCGAAGTC 840

AACAAGTATT TCGTCCGTGC ACAGTATGGT GCCGGAGACA CTGCTGATTT CAAGCCATAT 900

CTTGAAGAAA TGGACGTACC CGCTGACTCA AAGAACAATA CATTCATCGC TGGTGAATTA 960

CAGTTTGATT TGCCACGTTG GGAAGGTGTG CCATTCTACG TGCGTTCAGG CAAGCGTTTA 1020

GCTGCTAAAC AAACACGTGT CGATATCGTC TTCAAGGCTG GTACCTTTGC CTTTGGTTCT 1080

GAACAAGAAG CGCAAGAAGC TGTGTTATCA ATTTTGATTG ATCCTAAGGG TGGTATCGAA 1140

TTCAAGATTA ATTCAAAGTC AGTTGAAGAT GCTTTCAATA CACGTATGAT TAATCTTGAT 1200

TGGTCAATTT CTGATGAAGA TAACGAAAAT ACACCTGAGC CATACGAACG TATGATTCAC 1260

GACACAATGA ATGGTGACGG ATCAAACTTC GCTGACTGGA ACGGTGTTGC TATTGCTTGG 1320

AAGTTTGTGG ATGCTATTTC AGCTGTCTAC ACTGCTGATA AAGCACCACT TGAAACATAC 1380

AAGTCAGGTT CCATGGGACC TGAAGCATCT GACAAGCTGT TAGCCGAAAA CGGTGACGCT 1440

TGGGTATTTA AGGGCTTAAT AAAATAA 1467

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 485 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc citreum
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Ala Glu Ile Lys Thr Leu Val Thr Phe Phe Gly Gly Thr Gly Asp
1 5 10 15

Leu Ala Lys Arg Lys Leu Tyr Pro Ser Val Phe Asn Leu Tyr Lys Lys
20 25 30

Gly Tyr Leu Gln Glu His Phe Ala Ile Val Gly Thr Ala Arg Gln Asp
35 40 45

Leu Thr Asp Ala Glu Phe Lys Gln Leu Val Arg Glu Ser Ile Ala Asp
50 55 60

Phe Thr Glu Asp Lys Ala Gln Ala Glu Ala Phe Ile Ala His Phe Ser
65 70 75 80

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

Gln Ala Ile Glu Glu Ala Ala Glu Lys Phe Asp Ile Gln Gly Asn Arg
100 105 110

Ile Phe Tyr Met Ser Val Ala Pro Arg Phe Phe Gly Thr Ile Ala Lys
115 120 125

Tyr Leu Lys Ser Glu Gly Leu Leu Ala Asp Ser Gly Tyr Asn Arg Leu
130 135 140

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

Gln Lys Asp Leu Glu Asn Ala Phe Asp Asp Asn Gln Leu Phe Arg Ile
165 170 175

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

Phe Gly Asn Pro Ile Phe Asp Ala Ala Trp Asn Lys Asp Tyr Ile Lys
195 200 205

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

Gly Tyr Tyr Asp Thr Ala Gly Ala Leu Leu Asp Met Ile Gln Asn His
225 230 235 240

Thr Met Gln Ile Val Gly Trp Leu Ala Met Glu Lys Pro Asp Ser Phe
245 250 255

Thr Asp Lys Asp Ile Arg Ala Ala Lys Asn Ala Ala Phe Asn Ala Leu
260 265 270

Lys Ile Tyr Asp Glu Ala Glu Val Asn Lys Tyr Phe Val Arg Ala Gln
275 280 285

Tyr Gly Ala Gly Asp Thr Ala Asp Phe Lys Pro Tyr Leu Glu Glu Met
290 295 300

Asp Val Pro Ala Asp Ser Lys Asn Asn Thr Phe Ile Ala Gly Glu Leu
305 310 315 320

Gln Phe Asp Leu Pro Arg Trp Glu Gly Val Pro Phe Tyr Val Arg Ser
325 330 335

Gly Lys Arg Leu Ala Ala Lys Gln Thr Arg Val Asp Ile Val Phe Lys
340 345 350

Ala Gly Thr Phe Ala Phe Gly Ser Glu Gln Glu Ala Gln Glu Ala Val
355 360 365

Leu Ser Ile Leu Ile Asp Pro Lys Gly Gly Ile Glu Phe Lys Ile Asn
370 375 380

Ser Lys Ser Val Glu Asp Ala Phe Asn Thr Arg Met Ile Asn Leu Asp
385 390 395 400

Trp Ser Ile Ser Asp Glu Asp Lys Gln Asn Thr Pro Glu Pro Tyr Glu
405 410 415

Arg Met Ile His Asp Thr Met Asn Gly Asp Gly Ser Asn Phe Ala Asp
420 425 430

Trp Asn Gly Val Ala Ile Ala Trp Lys Phe Val Asp Ala Ile Ser Ala
435 440 445

Val Tyr Thr Ala Asp Lys Ala Pro Leu Glu Thr Tyr Lys Ser Gly Ser
450 455 460

Met Gly Pro Glu Ala Ser Asp Lys Leu Leu Ala Glu Asn Gly Asp Ala
465 470 475 480

Trp Val Phe Lys Gly
485

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1461 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA genomic

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc lactis
(B) STRAIN: NCDO 546

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGGTTGCAG AAATCAAGAC GTTAGTCACA TTTTTCGGTG CTACTGGTGA TTTGGCAAAG 60

CGTAAGCTTT ACCCATCAGT TTTTAACCTC TTCAAGAAAG GTTATTTGCA AGAACATTTC 120

GCCATTGTTG GAACAGCCCG TCAAGACTTG ACTGAAGATG AATTCAAGCA ACTTGTGCGA 180

GACTCANNNN NNNNNNNNNN NNNNNNNNNN NNNCAAGCCG AAGCATTCAT TGAACACTTC 240

TCATATCGTG CCCATGACGT TACGGATGCA GCGTCATACA GCGTTTTGAA GTCAGCAATC 300

GAAGAAGCTT CTGACAAGTT TGGCATTGAT GGTAACCGTA TCTTCTATAT GTCTGTTGCT 360

CCACGTTTCT TTGGGACGAT TGCAAAGTAT TTGAAGTCAG AAGGTTTGTT GGCCACAACT 420

GGTTACAACC GTTTGATGAT CGAAAAGCCA TTTGGGACAT CATACGAAAC AGCTGAAAAG 480

TTGCAAAACG AATTGGAAAA CGCCTTTGAT GATGACCAAT TGTTCCGTAT TGACCACTAC 540

CTTGGTAAGG AAATGGTCCA AAATATTGCG GCTTTGCGTT TTGGTAACCC AATCTTTGAT 600

GCAGCCTGGA ACAAGGACTA CATCAAGAAC GTGCAAGTGA CATTGTCAGA AGTCTTGGGT 660

GTTGAAGAAC GTGCCGGTTA CTATGATACA GCCGGTGCTT TGCTCGACAT GATTCAAAAC 720

CACACGATGC AAATCGTCGG TTGGTTGGCC ATGGAAAAAC CTGACTCATT CACTGACAAG 780

GATATCCGTG CCGCTAAGAA CGCTGCCTTC AACGCTTTGA AGATTTACAA CGAAGAAGAA 840

GTTAACAAGT ACTTCGTTCG TGGCCAATAT GCAGGTGGTG ATTCTGCTGA ATTCAAGCCA 900

TATCTTGAAG AAATGGACGT ACCTGCTGAC TCAAAGAACA ACACGTACAT CGCTGGTGAA 960

TTGCAATTTG ATTTGCCACG TTGGGAAGGT GTGCCATTCT ACGTGCGTTC AGGTAAGCGC 1020

CTAGCTGCTA AGCAAACACG TATTGATATC NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 1080

NNNNNNNNNG AAGCCCAAGA AGCTATCTTG TCAATTTTGG TTGATCCAAC AGGTGGTATC 1140

GAATTCAAGA TCAATTCAAA GTCAGTTGAA NNNNNNNNNN NNNNNCGTCT CATCGGCCTT 1200

GATTGGCAAG TGTCAGAAGA AGACAAGCTT AACACACCTG AACCATACGA ACGTATGATT 1260

CATGACACGA TGAACGGTGA TGGTTCAAAC TTCGCCGATT GGAACGGTGT TGCCATTGCT 1320

TGGAAGTTCG TTGATGCGAT TTCAGCTGTT TACACCGCTG ATAAGGCACC ACTTGAAACT 1380

TACAAGTCTG GTTCAATGGG ACCAGCCGCA GCTGACAAGT TGTTGGCAAA TAACGGTGAT 1440

GCTTGGGTGT TTAAAGGTTA A 1461

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 485 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc lactis
(B) STRAIN: NCDO 546

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Ala Glu Ile Lys Thr Leu Val Thr Phe Phe Gly Ala Thr Gly Asp
1 5 10 15

Leu Ala Lys Arg Lys Leu Tyr Pro Ser Val Phe Asn Leu Phe Lys Lys
20 25 30

Gly Tyr Leu Gln Glu His Phe Ala Ile Val Gly Thr Ala Arg Gln Asp
35 40 45

Leu Thr Glu Asp Glu Phe Lys Gln Leu Val Arg Asp Ser Ile Ala Asp
50 55 60

Ala Ala Asp Asp Lys Ala Gln Ala Glu Ala Phe Ile Glu His Phe Ser
65 70 75 80

Tyr Arg Ala His Asp Val Thr Asp Ala Ala Ser Tyr Ser Val Leu Lys
85 90 95

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

Ile Phe Tyr Met Ser Val Ala Pro Arg Phe Phe Gly Thr Ile Ala Lys
115 120 125

Tyr Leu Lys Ser Glu Gly Leu Leu Ala Thr Thr Gly Tyr Asn Arg Leu
130 135 140

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

Gln Asn Glu Leu Glu Asn Ala Phe Asp Asp Asp Gln Leu Phe Arg Ile
165 170 175

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

Phe Gly Asn Pro Ile Phe Asp Ala Ala Trp Asn Lys Asp Tyr Ile Lys
195 200 205

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

Gly Tyr Tyr Asp Thr Ala Gly Ala Leu Leu Asp Met Ile Gln Asn His
225 230 235 240

Thr Met Gln Ile Val Gly Trp Leu Ala Met Glu Lys Pro Asp Ser Phe
245 250 255

Thr Asp Lys Asp Ile Arg Ala Ala Lys Asn Ala Ala Phe Asn Ala Leu
260 265 270

Lys Ile Tyr Asn Glu Glu Glu Val Asn Lys Tyr Phe Val Arg Gly Gln
275 280 285

Tyr Ala Gly Gly Asp Ser Ala Glu Phe Lys Pro Tyr Leu Glu Glu Met
290 295 300

Asp Val Pro Ala Asp Ser Lys Asn Asn Thr Tyr Ile Ala Gly Glu Leu
305 310 315 320

Gln Phe Asp Leu Pro Arg Trp Glu Gly Val Pro Phe Tyr Val Arg Ser
325 330 335

Gly Lys Arg Leu Ala Ala Lys Gln Thr Arg Ile Asp Ile Val Phe Lys
340 345 350

Ala Gly Thr Phe Gln Phe Gly Ala Ala Gln Glu Ala Gln Glu Ala Ile
355 360 365

Leu Ser Ile Leu Val Asp Pro Thr Gly Gly Ile Glu Phe Lys Ile Asn
370 375 380

Ser Lys Ser Val Glu Asp Asp Phe Asn Thr Arg Leu Ile Gly Leu Asp
385 390 395 400

Trp Gln Val Ser Glu Glu Asp Lys Leu Asn Thr Pro Glu Pro Tyr Glu
405 410 415

Arg Met Ile His Asp Thr Met Asn Gly Asp Gly Ser Asn Phe Ala Asp
420 425 430

Trp Asn Gly Val Ala Ile Ala Trp Lys Phe Val Asp Ala Ile Ser Ala
435 440 445

Val Tyr Thr Ala Asp Lys Ala Pro Leu Glu Thr Tyr Lys Ser Gly Ser
450 455 460

Met Gly Pro Ala Ala Ala Asp Lys Leu Leu Ala Asn Asn Gly Asp Ala
465 470 475 480

Trp Val Phe Lys Gly
485

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1455 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA genomic

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc dextranicum
(B) STRAIN: ATCC 19255

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTTTCAGAAA TCAAAACGTT GGTAACTTTC TTTGGCGGAA CTGGTGATTT AGCAAAGCGT 60

AAGCTTTACC CATCAGTTTT CAACCTCTAC AAAAAAGGAT ACTTACAAGA ACACTTTGCC 120

ATTGTTGGGA CAGCACGTCA ACAATTAAGT GATGACGAGT TTAAGCAATT GGTTCGTGAT 180

TCAATTAAAG ACTTTACTGA AGATCAAGCA CAAGCCGAAG CGTTTATTGC GCATTTTTCT 240

TACCGTGCGC ACGATGTCAC AGATGCCGCT TCTTATGGTA TCTTGAAGTC AGCGATCGAA 300

GAAGCAGCAA CCAAATTTGA CATTGATGGC AATCGTATTT TCTATATGTC AGTTGCCCCT 360

CGTTTCTTCG GTACAATCGC TAAATATTTG AAATCAGAAG GTTTGCTAGC TGAGACTGGC 420

TACAATCGTT TGATGATTGA AAAGCCTTTT GGTACATCAT ACGCCACCGC AGAAGAATTG 480

CAAAGTGATT TGGAAAATGC ATTTGATGAT GACCAACTGT TCCGTATTGA CCACTATCTT 540

GGAAAAGAAA TGGTACAAAA TATTGCAGCA TTACGTTTTG GTAACCCAAT CTTTGATGCC 600

GCTTGGAATA AGGACTATAT CAAAAACGTA CAAGTAACTT TGGCTGAAGT TCTAGGTGTT 660

GAAGAGCGTG CTGGTTACTA CGATACCACT GGCGCCCTTT TGGATATGAT TCAAAACCAC 720

ACAATGCAAA TTGTTGGTTG GTTAGCAATG GAAAAACCTG AATCATTCAA TGATAAGGAT 780

ATCCGTGCAG CTAAAAACGC CGCCTTCAAT GCATTAAAGA TTTATAACGA AGAAGAAGTG 840

AATAAGTACT TCGTTCGTGC ACAATATGGT GCTGGTGATA CAGCTGATTA CAAGCCATAT 900

TTGGAAGAAG CAGATGTCCC TGCTGACTCA AAGAACAACA CATTCATTGC TGGTGAATTG 960

CAGTTCGATT TGCCACGTTG GGAAGGTGTT CCTTTCTATG TTCGTTCAGG TAAGCGTTTG 1020

GCTGCCAAGC AAACACGTGT TGATATTGTA TTTAAGGCTG GCACATTCAA CTTTGGTTCA 1080

GAACAAGAAG CACAAGAATC AGTACTCTCA ATCATCATTG ATCCAAAGGG TGCTATTGAA 1140

TTGAAGCTTA ACGCTAAGTC AGTTGAAGAT GCCTTCAACA CCCGCACAAT CAACTTGGAT 1200

TGGGCAGTAT CTGATGAAGA CAAGAAGAAC ACACCAGAAC CATACGAACG TATGATTCAC 1260

GATACAATGA ATGGTGACGG ATCAAACTTT GCTGATTGGA ACGGTGTATC AATTGCTTGG 1320

AAGTTTGTTG ACGCAATTAC TGCCGTTTAC GATGCAGATA AAGCACCATT GGAGACATAT 1380

AAGTCAGGTT CAATGGGTCC TGAAGCATCA GACAAGCTAT TAGCTGAAAA TGGCGATGCT 1440

TGGGTATTTA AAGGA 1455

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 485 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc dextranicum
(B) STRAIN: ATCC 19255

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Ser Glu Ile Lys Thr Leu Val Thr Phe Phe Gly Gly Thr Gly Asp
1 5 10 15

Leu Ala Lys Arg Lys Leu Tyr Pro Ser Val Phe Asn Leu Tyr Lys Lys
20 25 30

Gly Tyr Leu Gln Glu His Phe Ala Ile Val Gly Thr Ala Arg Gln Gln
35 40 45

Leu Ser Asp Asp Glu Phe Lys Gln Leu Val Arg Asp Ser Ile Lys Asp
50 55 60

Phe Thr Glu Asp Gln Ala Gln Ala Glu Ala Phe Ile Ala His Phe Ser
65 70 75 80

Tyr Arg Ala His Asp Val Thr Asp Ala Ala Ser Tyr Gly Ile Leu Lys
85 90 95

Ser Ala Ile Glu Glu Ala Ala Thr Lys Phe Asp Ile Asp Gly Asn Arg
100 105 110

Ile Phe Tyr Met Ser Val Ala Pro Arg Phe Phe Gly Thr Ile Ala Lys
115 120 125

Tyr Leu Lys Ser Glu Gly Leu Leu Ala Glu Thr Gly Tyr Asn Arg Leu
130 135 140

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

Gln Ser Asp Leu Glu Asn Ala Phe Asp Asp Asp Gln Leu Phe Arg Ile
165 170 175

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

Phe Gly Asn Pro Ile Phe Asp Ala Ala Trp Asn Lys Asp Tyr Ile Lys
195 200 205

Asn Val Gln Val Thr Leu Ala Glu Val Leu Gly Val Glu Glu Arg Ala
210 215 220

Gly Tyr Tyr Asp Thr Thr Gly Ala Leu Leu Asp Met Ile Gln Asn His
225 230 235 240

Thr Met Gln Ile Val Gly Trp Leu Ala Met Glu Lys Pro Glu Ser Phe
245 250 255

Asn Asp Lys Asp Ile Arg Ala Ala Lys Asn Ala Ala Phe Asn Ala Leu
260 265 270

Lys Ile Tyr Asn Glu Glu Glu Val Asn Lys Tyr Phe Val Arg Ala Gln
275 280 285

Tyr Gly Ala Gly Asp Thr Ala Asp Tyr Lys Pro Tyr Leu Glu Glu Ala
290 295 300

Asp Val Pro Ala Asp Ser Lys Asn Asn Thr Phe Ile Ala Gly Glu Leu
305 310 315 320

Gln Phe Asp Leu Pro Arg Trp Glu Gly Val Pro Phe Tyr Val Arg Ser
325 330 335

Gly Lys Arg Leu Ala Ala Lys Gln Thr Arg Val Asp Ile Val Phe Lys
340 345 350

Ala Gly Thr Phe Asn Phe Gly Ser Glu Gln Glu Ala Gln Glu Ser Val
355 360 365

Leu Ser Ile Ile Ile Asp Pro Lys Gly Ala Ile Glu Leu Lys Leu Asn
370 375 380

Ala Lys Ser Val Glu Asp Ala Phe Asn Thr Arg Thr Ile Asn Leu Asp
385 390 395 400

Trp Ala Val Ser Asp Glu Asp Lys Lys Asn Thr Pro Glu Pro Tyr Glu
405 410 415

Arg Met Ile His Asp Thr Met Asn Gly Asp Gly Ser Asn Phe Ala Asp
420 425 430

Trp Asn Gly Val Ser Ile Ala Trp Lys Phe Val Asp Ala Ile Thr Ala
435 440 445

Val Tyr Asp Ala Asp Lys Ala Pro Leu Glu Thr Tyr Lys Ser Gly Ser
450 455 460

Met Gly Pro Glu Ala Ser Asp Lys Leu Leu Ala Glu Asn Gly Asp Ala
465 470 475 480

Trp Val Phe Lys Gly
485

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTAGTGGT CAATACGGAA TAGTTGGTTA TCATCAAATG CGTTTT 46

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc citreum
(B) STRAIN: ATCC 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGGTTGCA GAAATCAAAA CATTAGTTAC TTTTTTTGGT GGAACTGGTG ATTTAGCAAA 60

GCGTA 65

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc citreum
(B) STRAIN: ATCC 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCTTACGCT TTGCTAAATC ACCAGTTCCA CCAAAAAAAG TAACTAATGT TTTGATTTCT 60

GCAAC 65

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTTTTGGGAT CCATCCATGG TTTCAGAAAT CAAGACGTTA GTAACTTTCT TTGG 54

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTTTCTAG ATTAAGTTAA CCTTTAAACA CCCAAGCATC ACCATTGGCA GCCAATAATT 60

TATCGGATG 69

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAACGCCTCA GCTTGTGC 18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GACACAGCCG GTGCATTG 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTAGTAGGC CTTCTGAC 18

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTGGTGCTTT ATCTGCGG 18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGTCAGATAC AGTCCAACC 19

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CCGCAGATAA AGCACCAC 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CACGTTCTTC GACACCCA 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCGCTTACCT GAACGGAC 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCACCATATT GTGCACGAAC 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCTTCATCGC CGGCGAATT 19

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCAAAGGGTG CTATCGAA 18

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GACACAGCTG CCGAACTC 18

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GACGTAACAG ATGCTGCT 18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGTGGCACTG GTGACTTG 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAAAACTGAT GGGTAAAGC 19

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TCAGTTGCGC CACGTTTC 18

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGCAGCCTTT AATGCTTTG 19

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCTGACTGGA ATGGCGTT 18

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACGCCAGAAC CATACGAA 18

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTTAGCTGCT CGTCAGACAC G 21

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGTTGATATC GTCTTTAAGG CTGGTACCTT TAACTTTG 38

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTCCCATTCT ACGTACGTTC AGGTCGTCGC TTAGCTG 37

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAAGTTAACC GTTACTTTGT TAGAGCTCAA TATGGTG 37

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AAGCACCACT CGAGACTTAC CGTTCGGGCT CAA 33

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCATCCGATC GTTTATTGGC TG 22

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTTCGAGAAA TCCGTACACT AGTTACTTT 29

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGTTTATACT GCAGATCGTG CACCACTTG 29

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTACGTGGGT TGCGAAATGG TTC 23

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GATCATTACC TGGGTTGCGA AATGGTCC 28

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTATTCCGTA TTGACCACTA CTGTGGTAAG GAAATGGTTC AAAACATTGC TGCCC 55

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTTGCTATGG AACGTCCAGA TTCA 24

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CTTGCTATGG AATGCCCAGA TTCA 24

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATTTGACTGA TAAGGAATTC AAGCA 25

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CTGTGGCACC GCGGTTCTTT GGGACAATTG CACGTTATCT CAAG 44

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GACAATTGCA CGTTATCTCC GTTCAGAGGG T 31

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TGAAAAGTGT TCGATGAATG CCTCGGC 27

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATTC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TATAAAAAAG GATATCTGCA ATGCCATTTT GCCATT 36

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GACCATGGTT TGCGAAATCA AAACATTAG 29

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AGCTAGTAGG CCTTCTGACT TAAGATAACG GGCAATTGTA CC 42

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AGCTAGTAGG CCTTCTGAAC GAAGATATTT GGC 33

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GAATGATTCT GGACGTTCCA TCGCTAA 27

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

TTAGCGATGG AACGTCCAGA ATCATTC 27

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GAGGTACCGA AAGGACGTTC AATC 24

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GATTGAACGT CCTTTCGGTA CCTC 24

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

TCATTCACTG ACCGTGATAT CCGTGCC 27

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGCACGGATA TCACGGTCAG TGAATGA 27

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATTCGTGCCG CGCGCAACGC AGCCTTT 27

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

AAAGGCTGCG TTGCGCGCGG CACGAAT 27

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ACTATCAGCT AGCAAACCCT CTGACTTGAG ATAACGTGCA ATTGT 45

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

ACTATCAGCT AGCAAACCCT CTGAACTGAG ATATTT 36

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GCCGAAGTCA ACCGTTATTT CCTCCGT 27

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GATATCGACA CGTGTTTGTT TAGCAGCTAA ACGACGGCCT GAACG 45

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GATATCGACA CGTGTTTGAC GAGCAGCTAA ACG 33

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

AAACAAACAC GTGTCGATAT CGTCTTCCGT GCTGGTACC 39

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

ATTTCAGCTG TCTACACTGC TGATCGTGCA CCACTT 36

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

TGCTTCAGGT CCCATGGAAC CTGAACGGTA TGTTTC 36

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

ACTAACTAAT GTACGGATAT CTGCAAC 27

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GTTGCAGATA TCCGTACATT AGTTACT 27

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CCGAAGAGCT CCAACGTGAC TTAGAA 26

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

TTCTAACTCA CGTTGGAGCT CTTCGG 26

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

TGAAAAGTGT TCGATGAATG CCTCGGC 27

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GCCGAGGCAT TCATCGAACA CTTTTCA 27

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GATTTGACTG ATGATGAGTT CAAGCAA 27

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

TTGCTTGAAC TCATCATCAG TCAAATC 27

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TAACTCGTTT TGGAGCTCTT CGGCAGTGTC GTATGA 36

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

TCATACGACA CTGCCGAAGA GCTCCAAAAC GACTTA 36

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GTTCGTGAAT CAATCAAGGA CTTTACTGAA G 31

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CTTCAGTAAA GTCCTTAATT AATTCACGAA C 31

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CAGCGTTTGA CTGATTGTGA GTTCAAGCAA 30

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

TGCTTGAACT CACAATCAGT CAAATC 26

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

ACAGCACGTC AGGCTTTGAC TGATTGTGAC TTCAAGCAA 39

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TGCTTGAACT CACAATCAGT CAAAGCCTGA CGTGC 35

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GGAACGGCCC GTGCATGCCT CAATCA 26

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TCATTGAGGC ATGCACGGGC CGTTCC 26

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CAATGATTGC AGGTTCAAAC AA 22

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

TTGTTTGAAC TCGCAATCAT TG 22

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GATGACGAGT TCTGCCAATT GGTTCG 26

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CGAACCAATT GGCAGAACTC GTCATC 26

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATTC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GATGACGAGT TCAAATGCTT GGTTCATG 28

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

CACGAACCAA GCATTTGAAC TCGTCATC 28

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

CAATGATGAC TGCTTCAAAC A 21

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

TGTTTGAAGC AGTCATCATT G 21

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GGAACGGCCC GTTGCGCCCT CAATGATGAC GAGTTC 36

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GAACTCGTCA TCATTGAGGG CGCAACGGGC CGTTCC 36

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GGAACGGCCC GTCAAGCCTG CAATGATGAC GAGTTC 36

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

GAACTCGTCA TCATTGGAGG CTTGACGGGC CGTTCC 36

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GGAACGGCCT GCCAAGCCCT CAATGATGAC GAGTTC 36

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GAACTCGTCA TCATTGAGGG CTTGGCAGGC CGTTCC 36

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

CAAGCCCTCT GCGATGACGA GTTCAAACA 29

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TGTTTGAACT CGTCATCGCA GAGGGCTTG 29

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

CAAGCCCTCA ATTGCGACGA GTTCAAACA 29

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

TGTTTGAACT CGTCGCAATT GAGGGCTTG 29

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

GATGACGAAT GCAAACAATT GGTTCGTG 28

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

CACGAACCAA TTGTTTGCAT TCGTCATC 28

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

ACAATTGCCT GCTATCTTAA GTCAGAAGGT CTACT 35

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

AGTAGACCTT CTGACTTAAG ATAGCAGGCA ATTGT 35

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GCCAAATATC TTTGCTCAGA AGGTCTACTA GCTG 34

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

CAGCTAGTAG ACCTTCTGAG CAAAGATATT TGGC 34

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GATGACGAGT TCAAACAATG CGTTCGTG 28

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

CACGAACGCA TTGTTTGAAC TCGTCATC 28

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

GATGACGAGT TCAAACAATT GTGCCGTG 28

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

CACGGCACAA TTGTTTGAAC TCGTCATC 28

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GACGAGTTCA AACAATTGGT TTGCGATTCA 30

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

TGAATCGACA ACCAATTGTT TGAACTCGTC 30

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

GACGAGTTCA AACAATTGGT TCGTTGCTCA ATT 33

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: ATCC 12291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

AATTGAGCAA CGAACCAATT GTTTGAACTC GTC 33

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

TGAAGGATAA AGTCGACGCT TTGCT 25

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

TGAAGGATAA AGCTTACGAC GTGCTAAATC A 31

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GGCACCATAC TGAGCTCTGA CGAAATAACG GTTGACTTCG 40

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

CTTGTATGTT TCTAGAGGTG CACGATCTGC AGTGTAGA 38

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Leuconostoc mesenteroides
(B) STRAIN: NCIMB 3351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

TTCGGCTAAC AGACGGTCAG ATGCTTCAGG GCCCATGGAA C 41

WHAT IS CLAIMED IS:

1. A method for homogeneous immunoassay of an analyte in a sample suspected of containing said analyte which comprises: (1) combining in a liquid medium: (a) said sample, (b) a conjugate of an analog of said analyte with a mutant NAD^+ dependent G6PDH differing from any precursor G6PDH by the deletion, substitution, or insertion or any combination thereof of at least one amino acid per subunit, (c) a receptor for said analyte, and (d) substrates for said G6PDH; (2) determining the enzymatic activity of said G6PDH in said medium; and (3) comparing said activity to the enzymatic activity observed with a sample containing said analyte.

2. The method of Claim 1 wherein said G6PDH differs by at least two amino acids per subunit from any precursor G6PDH.

3. The method of Claim 1 wherein said amino acid is selected from a group consisting of amino acid residues 5, 19, 128, 131, 282, 454, 461 and 472.

4. The method of Claim 3 wherein said G6PDH differs by at least four amino acids per subunit from any precursor G6PDH.

5. The method of Claim 4 wherein said group consists of amino acid residues 19, 282, 454, and 472.

6. The method of Claim 2 wherein at least two of said amino acids per subunit are replaced with arginine.

7. The method of Claim 4 wherein at least four of said amino acids per subunit are replaced by arginine.

8. The method of Claim 1 wherein said G6PDH has at least one cysteine per subunit to which said analyte is conjugated.

9. The method of Claim 8 which further comprises combining in said medium an inhibitory antibody to said G6PDH.

10. The method of Claim 1 wherein said receptor is an antibody for said analyte and said analyte is a drug.

11. The method of Claim 10 wherein said drug is selected from the group consisting of digoxin, vancomycin, thyroxine, cyclosporin, folate, rapamycin, B12, FK506 and tetrahydrocannabinol.

12. The method of Claim 1 wherein said mutation comprises introduction of at least one cysteine residue per subunit, by insertion or

substitution, proximate to an epitopic site recognized by an anti-G6PDH antibody capable of simultaneously binding to two of said subunits within the same G6PDH molecule and capable of inhibiting the activity of said mutant G6PDH.

13. The method of Claim 1 wherein said G6PDH is derived from an organism selected from the group consisting of *Leuconostoc mesenteroides*, *Leuconostoc citreum*, *Leuconostoc lactis*, and *Leuconostoc dextranicus*.

14. A composition which comprises a specific binding pair member conjugated to a mutant NAD^+ dependent bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one amino acid mutation per subunit as compared to precursor G6PDH.

15. A kit for conducting an assay for the determination of a specific binding pair (sbp) member, said kit comprising in packaged combination an sbp partner of said sbp member and a composition which comprises said sbp member or an analog of said sbp member conjugated to a mutant NAD^+ dependent bacterial glucose-6-phosphate dehydrogenase (G6PDH) having at least one amino acid mutation per subunit as compared to precursor G6PDH.

16. A kit for conducting an immunoassay for a ligand comprising in packaged combination: an analog of said ligand conjugated to a cysteine in a mutant recombinant bacterial G6PDH; an inhibitory antibody to said G6PDH capable of binding simultaneously to separate subunits if said G6PDH; and an antibody capable of binding said ligand.

17. A mutant glucose-6-phosphate dehydrogenase (G6PDH) that is the expression product of a mutated DNA sequence encoding a G6PDH, the mutant G6PDH being derived from a precursor G6PDH by the deletion, insertion or substitution of at least one amino acid per subunit as compared to the precursor G6PDH.

18. The mutant G6PDH of Claim 17 comprising the deletion or substitution of said amino acid wherein said amino acid is any amino acid that upon conjugation of said precursor G6PDH with an sbp member results in a conjugate wherein binding of said sbp member with an sbp partner does not substantially inhibit the activity of said conjugate.

19. The mutant G6PDH of Claim 17 comprising the deletion of at least one lysine amino acid residue per subunit.

20. The mutant G6PDH of Claim 17 wherein the substituent amino acid is arginine or histidine.

* * * * *

```
        10          20          30          40          50          60
         *           *           *           *           *           *
GCGCTATAATGA AAAGTGAATTTA ACTAAAAATAAG GGGTACATCATG GTTTCAGAAATC

        70          80          90         100         110         120
         *           *           *           *           *           *
AAGACGTTAGTA ACTTTCTTTGGT GGCACTGGTGAC TTGGCCAAGCGT AAGCTTTACCCA

       130         140         150         160         170         180
         *           *           *           *           *           *
TCAGTTTTCAAT CTTTATAAAAAA GGCTACTTGCAA AAGCATTTTGCC ATTGTTGGAACG

       190         200         210         220         230         240
         *           *           *           *           *           *
GCCCGTCAAGCC CTCAATGATGAC GAATTCAAACAA TTGGTTCGTGAT TCAATTAAAGAT

       250         260         270         280         290         300
         *           *           *           *           *           *
TTCACTGACGAT CAAGCACAAGCT GAGGCGTTCATC GAACATTTCTCA TACCGTGCACAC

       310         320         330         340         350         360
         *           *           *           *           *           *
GACGTAACAGAT GCTGCTTCATAC GCTGTTTTAAAA GAGGCGATTGAA GAAGCTGCCGAC

       370         380         390         400         410         420
         *           *           *           *           *           *
AAATTTGATATC GATGGCAACCGC ATTTTCTATATG TCAGTTGCGCCA CGTTTCTTTGGT

       430         440         450         460         470         480
         *           *           *           *           *           *
ACAATTGCCAAA TATCTTAAGTCA GAAGGCCTACTA GCTGACACTGGT TACAACCGTTTG

       490         500         510         520         530         540
         *           *           *           *           *           *
ATGATTGAAAAG CCTTTCGGTACA TCATATGACACA GCTGCCGAACTC CAAAATGACTTG

       550         560         570         580         590         600
         *           *           *           *           *           *
GAAAACGCATTT GATGATAACCAA CTATTCCGTATT GACCACTACCTT GGTAAGGAAATG

       610         620         630         640         650         660
         *           *           *           *           *           *
GTTCAAAACATT GCTGCCCTTCGC TTTGGTAACCCA ATTTTCGATGCT GCTTGGAACAAG

       670         680         690         700         710         720
         *           *           *           *           *           *
GATTACATCAAG AACGTTCAAGTA ACATTGTCAGAA GTCTTGGGTGTC GAAGAACGTGCC

       730         740         750         760         770         780
         *           *           *           *           *           *
GGCTACTATGAC ACAGCCGGTGCA TTGCTCGACATG ATTCAAAACCAC ACCATGCAAATT

       790         800         810         820         830         840
         *           *           *           *           *           *
GTTGGTTGGTTA GCCATGGAAAAA CCAGAATCATTC ACTGACAAAGAC ATTCGTGCCGCT

       850         860         870         880         890         900
         *           *           *           *           *           *
AAAAACGCAGCC TTTAATGCTTTG AAGATCTATGAT GAAGCAGAAGTT AACAAATACTTT
```

FIG.1A

```
      910         920         930         940         950         960
       *           *           *           *           *           *
GTTCGTGCACAA TATGGTGCCGGT GATTCAGCTGAC TTCAAGCCATAC CTTGAAGAATTA

      970         980         990        1000        1010        1020
       *           *           *           *           *           *
GACGTACCTGCT GATTCTAAAAAC AATACCTTCATC GCCGGCGAATTG CAATTTGATTTG

     1030        1040        1050        1060        1070        1080
       *           *           *           *           *           *
CCACGTTGGGAG GGTGTCCCATTC TATGTCCGTTCA GGTAAGCGCTTA GCTGCTAAACAG

     1090        1100        1110        1120        1130        1140
       *           *           *           *           *           *
ACACGGGTTGAT ATCGTCTTTAAG GCTGGCACGTTT AACTTTGGTTCA GAACAAGAAGCA

     1150        1160        1170        1180        1190        1200
       *           *           *           *           *           *
CAAGAAGCTGTC TTGTCAATTATC ATTGATCCAAAG GGTGCTATCGAA TTGAAGTTGAAC

     1210        1220        1230        1240        1250        1260
       *           *           *           *           *           *
GCTAAGTCAGTT GAAGATGCTTTC AACACACGTACA ATTGACTTAGGT TGGACTGTATCT

     1270        1280        1290        1300        1310        1320
       *           *           *           *           *           *
GACGAAGATAAG AAGAACACGCCA GAACCATACGAA CGTATGATTCAC GACACTATGAAT

     1330        1340        1350        1360        1370        1380
       *           *           *           *           *           *
GGTGATGGCTCT AACTTCGCTGAC TGGAATGGCGTT TCAATCGCTTGG AAGTTCGTTGAT

     1390        1400        1410        1420        1430        1440
       *           *           *           *           *           *
GCTATTTCAGCC GTTTATACCGCA GATAAAGCACCA CTTGAAACTTAC AAGTCGGGCTCA

     1450        1460        1470        1480        1490        1500
       *           *           *           *           *           *
ATGGGTCCTGAA GCATCCGATAAA TTATTGGCTGCC AATGGTGATGCT TGGGTGTTTAAA

GGTTAA
```

FIG.1B

```
          10         20         30         40         50         60
           *          *          *          *          *          *
VSEIKTLVTFFG GTGDLAKRKLYP SVFNLYKKGYLQ KHFAIVGTARQA LNDDEFKQLVRD

          70         80         90        100        110        120
           *          *          *          *          *          *
SIKDFTDDQAQA EAFIEHFSYRAH DVTDAASYAVLK EAIEEAADKFDI DGNRIFYMSVAP

         130        140        150        160        170        180
           *          *          *          *          *          *
RFFGTIAKYLKS EGLLADTGYNRL MIEKPFGTSYDT AAELQNDLENAF DDNQLFRIDHYL

         190        200        210        220        230        240
           *          *          *          *          *          *
GKEMVQNIAALR FGNPIFDAAWNK DYIKNVQVTLSE VLGVEERAGYYD TAGALLDMIQNH

         250        260        270        280        290        300
           *          *          *          *          *          *
TMQIVGWLAMEK PESFTDKDIRAA KNAAFNALKIYD EAEVNKYFVRAQ YGAGDSADFKPY

         310        320        330        340        350        360
           *          *          *          *          *          *
LEELDVPADSKN NTFIAGELQFDL PRWEGVPFYVRS GKRLAAKQTRVD IVFKAGTFNFGS

         370        380        390        400        410        420
           *          *          *          *          *          *
EQEAQEAVLSII IDPKGAIELKLN AKSVEDAFNTRT IDLGWTVSDEDK KNTPEPYERMIH

         430        440        450        460        470        480
           *          *          *          *          *          *
DTMNGDGSNFAD WNGVSIAWKFVD AISAVYTADKAP LETYKSGSMGPE ASDKLLAANGDA

WVFKG
```

FIG.2

```
          10          20          30          40          50          60
           *           *           *           *           *           *
AATCCGTTTGTG AAAATAGGTACT TTTTAAATGATT TTGCGTTATAAT GGTAAATGAATT

          70          80          90         100         110         120
           *           *           *           *           *           *
TAATTATAGTAT TAAGGGGAACAT CATGGTTGCAGA AATCAAAACATT AGTTACTTTTTT

         130         140         150         160         170         180
           *           *           *           *           *           *
TGGTGGAACTGG TGATTTAGCAAA GCGTAAGCTTTA TCCTTCAGTTTT TAACCTTTACAA

         190         200         210         220         230         240
           *           *           *           *           *           *
GAAAGGTTACTT ACAAGAACATTT TGCCATTGTTGG TACAGCACGTCA GGATTTGACTGA

         250         260         270         280         290         300
           *           *           *           *           *           *
TGCTGAATTCAA GCAATTGGTTCG CGAATCAATCGC TGACTTTACTGA AGATAAAGCCCA

         310         320         330         340         350         360
           *           *           *           *           *           *
AGCCGAGGCCTT CATCGCACACTT TTCATACCGTGC ACATGATGTAAC CGATGCAGCTTC

         370         380         390         400         410         420
           *           *           *           *           *           *
ATACAACATCTT AAAACAAGCAAT TGAAGAAGCAGC CGAAAAGTTCGA TATTCAAGGTAA

         430         440         450         460         470         480
           *           *           *           *           *           *
TCGTATTTTCTA CATGTCTGTGGC ACCACGATTCTT TGGGACAATTGC AAAATATCTCAA

         490         500         510         520         530         540
           *           *           *           *           *           *
GTCAGAGGGTTT GCTAGCTGATAG TGGTTACAACCG TTTGATGATTGA AAAGCCTTTTGG

         550         560         570         580         590         600
           *           *           *           *           *           *
TACATCATACGC CACTGCCGAAGA GCTACAAAAAGA CTTAGAAAACGC TTTTGACGATAA

         610         620         630         640         650         660
           *           *           *           *           *           *
TCAATTATTCCG TATTGATCATTA TCTTGGTAAAGA AATGGTCCAAAA TATTGCTGCCCT

         670         680         690         700         710         720
           *           *           *           *           *           *
TCGTTTTGGTAA CCCCATCTTTGA TGCCGCTTGGAA CAAAGATTACAT TAAAAACGTCCA

         730         740         750         760         770         780
           *           *           *           *           *           *
AGTTACTTTGTC TGAAGTGCTTGG TGTTGAAGAACG TGCCGGTTATTA CGATACAGCCGG

         790         800         810         820         830         840
           *           *           *           *           *           *
TGCATTATTAGA TATGATTCAAAA CCACACTATGCA AATTGTTGGTTG GCTTGCTATGGA

         850         860         870         880         890         900
           *           *           *           *           *           *
AAAGCCAGATTC ATTTACTGATAA GGATATCCGTGC GGCTAAGAATGC GGCTTTTAATGC
```

FIG.3A

```
      910         920         930         940         950         960
       *           *           *           *           *           *
TCTTAAAATTTA TGATGAAGCCGA AGTCAACAAGTA TTTCGTCCGTGC ACAGTATGGTGC

      970         980         990        1000        1010        1020
       *           *           *           *           *           *
CGGAGACACTGC TGATTTCAAGCC ATATCTTGAAGA AATGGACGTACC CGCTGACTCAAA

     1030        1040        1050        1060        1070        1080
       *           *           *           *           *           *
GAACAATACATT CATCGCTGGTGA ATTACAGTTTGA TTTGCCACGTTG GGAAGGTGTGCC

     1090        1100        1110        1120        1130        1140
       *           *           *           *           *           *
ATTCTACGTGCG TTCAGGCAAGCG TTTAGCTGCTAA ACAAACACGTGT CGATATCGTCTT

     1150        1160        1170        1180        1190        1200
       *           *           *           *           *           *
CAAGGCTGGTAC CTTTGCCTTTGG TTCTGAACAAGA AGCGCAAGAAGC TGTGTTATCAAT

     1210        1220        1230        1240        1250        1260
       *           *           *           *           *           *
TTTGATTGATCC TAAGGGTGGTAT CGAATTCAAGAT TAATTCAAAGTC AGTTGAAGATGC

     1270        1280        1290        1300        1310        1320
       *           *           *           *           *           *
TTTCAATACACG TATGATTAATCT TGATTGGTCAAT TTCTGATGAAGA TAAGCAAAATAC

     1330        1340        1350        1360        1370        1380
       *           *           *           *           *           *
ACCTGAGCCATA CGAACGTATGAT TCACGACACAAT GAATGGTGACGG ATCAAACTTCGC

     1390        1400        1410        1420        1430        1440
       *           *           *           *           *           *
TGACTGGAACGG TGTTGCTATTGC TTGGAAGTTTGT GGATGCTATTTC AGCTGTCTACAC

     1450        1460        1470        1480        1490        1500
       *           *           *           *           *           *
TGCTGATAAAGC ACCACTTGAAAC ATACAAGTCAGG TTCCATGGGACC TGAAGCATCTGA

     1510        1520        1530        1540        1550        1560
       *           *           *           *           *           *
CAAGCTGTTAGC CGAAAACGGTGA CGCTTGGGTATT TAAGGGTTAAT AAAATAAAAAAAG

     1570
       *
AAGACTAGCTT
```

FIG.3B

```
        10         20         30         40         50         60
         *          *          *          *          *          *
VAEIKTLVTFFG GTGDLAKRKLYP SVFNLYKKGYLQ EHFAIVGTARQD LTDAEFKQLVRE

        70         80         90        100        110        120
         *          *          *          *          *          *
SIADFTEDKAQA EAFIAHFSYRAH DVTDAASYNILK QAIEEAAEKFDI QGNRIFYMSVAP

       130        140        150        160        170        180
         *          *          *          *          *          *
RFFGTIAKYLKS EGLLADSGYNRL MIEKPFGTSYAT AEELQKDLENAF DDNQLFRIDHYL

       190        200        210        220        230        240
         *          *          *          *          *          *
GKEMVQNIAALR FGNPIFDAAWNK DYIKNVQVTLSE VLGVEERAGYYD TAGALLDMIQNH

       250        260        270        280        290        300
         *          *          *          *          *          *
TMQIVGWLAMEK PDSFTDKDIRAA KNAAFNALKIYD EAEVNKYFVRAQ YGAGDTADFKPY

       310        320        330        340        350        360
         *          *          *          *          *          *
LEEMDVPADSKN NTFIAGELQFDL PRWEGVPFYVRS GKRLAAKQTRVD IVFKAGTFAFGS

       370        380        390        400        410        420
         *          *          *          *          *          *
EQEAQEAVLSIL IDPKGGIEFKIN SKSVEDAFNTRM INLDWSISDEDK QNTPEPYERMIH

       430        440        450        460        470        480
         *          *          *          *          *          *
DTMNGDGSNFAD WNGVAIAWKFVD AISAVYTADKAP LETYKSGSMGPE ASDKLLAENGDA

WVFKG
```

FIG.4

```
        10          20          30          40          50          60
         *           *           *           *           *           *
ATGGTTGCAGAA ATCAAGACGTTA GTCACATTTTTC GGTGCTACTGGT GATTTGGCAAAG

        70          80          90         100         110         120
         *           *           *           *           *           *
CGTAAGCTTTAC CCATCAGTTTTT AACCTCTTCAAG AAAGGTTATTTG CAAGAACATTTC

       130         140         150         160         170         180
         *           *           *           *           *           *
GCCATTGTTGGA ACAGCCCGTCAA GACTTGACTGAA GATGAATTCAAG CAACTTGTGCGA

       190         200         210         220         230         240
         *           *           *           *           *           *
GACTCANNNNNN NNNNNNNNNNNN NNNNNNNNNCAA GCCGAAGCATTC ATTGAACACTTC

       250         260         270         280         290         300
         *           *           *           *           *           *
TCATATCGTGCC CATGACGTTACG GATGCAGCGTCA TACAGCGTTTTG AAGTCAGCAATC

       310         320         330         340         350         360
         *           *           *           *           *           *
GAAGAAGCTTCT GACAAGTTTGGC ATTGATGGTAAC CGTATCTTCTAT ATGTCTGTTGCT

       370         380         390         400         410         420
         *           *           *           *           *           *
CCACGTTTCTTT GGGACGATTGCA AAGTATTTGAAG TCAGAAGGTTTG TTGGCCACAACT

       430         440         450         460         470         480
         *           *           *           *           *           *
GGTTACAACCGT TTGATGATCGAA AAGCCATTTGGG ACATCATACGAA ACAGCTGAAAAG

       490         500         510         520         530         540
         *           *           *           *           *           *
TTGCAAAACGAA TTGGAAAACGCC TTTGATGATGAC CAATTGTTCCGT ATTGACCACTAC

       550         560         570         580         590         600
         *           *           *           *           *           *
CTTGGTAAGGAA ATGGTCCAAAAT ATTGCGGCTTTG CGTTTTGGTAAC CCAATCTTTGAT

       610         620         630         640         650         660
         *           *           *           *           *           *
GCAGCCTGGAAC AAGGACTACATC AAGAACGTGCAA GTGACATTGTCA GAAGTCTTGGGT

       670         680         690         700         710         720
         *           *           *           *           *           *
GTTGAAGAACGT GCCGGTTACTAT GATACAGCCGGT GCTTTGCTCGAC ATGATTCAAAAC

       730         740         750         760         770         780
         *           *           *           *           *           *
CACACGATGCAA ATCGTCGGTTGG TTGGCCATGGAA AAACCTGACTCA TTCACTGACAAG

       790         800         810         820         830         840
         *           *           *           *           *           *
GATATCCGTGCC GCTAAGAACGCT GCCTTCAACGCT TTGAAGATTTAC AACGAAGAAGAA

       850         860         870         880         890         900
         *           *           *           *           *           *
GTTAACAAGTAC TTCGTTCGTGGC CAATATGCAGGT GGTGATTCTGCT GAATTCAAGCCA
```

FIG.5A

```
      910         920         930         940         950         960
       *           *           *           *           *           *
TATCTTGAAGAA ATGGACGTACCT GCTGACTCAAAG AACAACACGTAC ATCGCTGGTGAA

      970         980         990        1000        1010        1020
       *           *           *           *           *           *
TTGCAATTTGAT TTGCCACGTTGG GAAGGTGTGCCA TTCTACGTGCGT TCAGGTAAGCGC

     1030        1040        1050        1060        1070        1080
       *           *           *           *           *           *
CTAGCTGCTAAG CAAACACGTATT GATATCNNNNNN NNNNNNNNNNN NNNNNNNNNNN

     1090        1100        1110        1120        1130        1140
       *           *           *           *           *           *
NNNNNNNNNGAA GCCCAAGAAGCT ATCTTGTCAATT TTGGTTGATCCA ACAGGTGGTATC

     1150        1160        1170        1180        1190        1200
       *           *           *           *           *           *
GAATTCAAGATC AATTCAAAGTCA GTTGAANNNNNN NNNNNNNNCGT CTCATCGGCCTT

     1210        1220        1230        1240        1250        1260
       *           *           *           *           *           *
GATTGGCAAGTG TCAGAAGAAGAC AAGCTTAACACA CCTGAACCATAC GAACGTATGATT

     1270        1280        1290        1300        1310        1320
       *           *           *           *           *           *
CATGACACGATG AACGGTGATGGT TCAAACTTCGCC GATTGGAACGGT GTTGCCATTGCT

     1330        1340        1350        1360        1370        1380
       *           *           *           *           *           *
TGGAAGTTCGTT GATGCGATTTCA GCTGTTTACACC GCTGATAAGGCA CCACTTGAAACT

     1390        1400        1410        1420        1430        1440
       *           *           *           *           *           *
TACAAGTCTGGT TCAATGGGACCA GCCGCAGCTGAC AAGTTGTTGGCA AATAACGGTGAT

     1450        1460
       *           *
GCTTGGGTGTTT AAAGGTTAA
```

FIG.5B

```
        10         20         30         40         50         60
         *          *          *          *          *          *
VAEIKTLVTFFG ATGDLAKRKLYP SVFNLFKKGYLQ EHFAIVGTARQD LTEDEFKQLVRD

        70         80         90        100        110        120
         *          *          *          *          *          *
SIADAADDKAQA EAFIEHFSYRAH DVTDAASYSVLK SAIEEASDKFGI DGNRIFYMSVAP

       130        140        150        160        170        180
         *          *          *          *          *          *
RFFGTIAKYLKS EGLLATTGYNRL MIEKPFGTSYET AEKLQNELENAF DDDQLFRIDHYL

       190        200        210        220        230        240
         *          *          *          *          *          *
GKEMVQNIAALR FGNPIFDAAWNK DYIKNVQVTLSE VLGVEERAGYYD TAGALLDMIQNH

       250        260        270        280        290        300
         *          *          *          *          *          *
TMQIVGWLAMEK PDSFTDKDIRAA KNAAFNALKIYN EEEVNKYFVRGQ YAGGDSAEFKPY

       310        320        330        340        350        360
         *          *          *          *          *          *
LEEMDVPADSKN NTYIAGELQFDL PRWEGVPFYVRS GKRLAAKQTRID IVFKAGTFQFGA

       370        380        390        400        410        420
         *          *          *          *          *          *
AQEAQEAILSIL VDPTGGIEFKIN SKSVEDDFNTRL IGLDWQVSEEDK LNTPEPYERMIH

       430        440        450        460        470        480
         *          *          *          *          *          *
DTMNGDGSNFAD WNGVAIAWKFVD AISAVYTADKAP LETYKSGSMGPA AADKLLANNGDA

WVFKG
```

FIG.6

```
        10          20          30          40          50          60
         *           *           *           *           *           *
TCTGATCGTTTA ATCAATTTTTGA CTTGTTCAACGC TTAATATGTTTG TGAATCCCGTAC

        70          80          90         100         110         120
         *           *           *           *           *           *
TTTTCCAGACCT TTTTGCGTTATA ATGGAGAGTGAA TTTAATTATAAT ATAAGGGGAACA

       130         140         150         160         170         180
         *           *           *           *           *           *
TCATGGTTTCAG AAATCAAAACGT TGGTAACTTTCT TTGGCGGAACTG GTGATTTAGCAA

       190         200         210         220         230         240
         *           *           *           *           *           *
AGCGTAAGCTTT ACCCATCAGTTT TCAACCTCTACA AAAAAGGATACT TACAAGAACACT

       250         260         270         280         290         300
         *           *           *           *           *           *
TTGCCATTGTTG GGACAGTACGTC AACAATTAAGTG ATGACGAGTTTA AGCAATTGGTTC

       310         320         330         340         350         360
         *           *           *           *           *           *
GTGATTCAATTA AAGACTTTACTG AAGATCAAGCAC AAGCCGAAGCGT TTATTGCGCATT

       370         380         390         400         410         420
         *           *           *           *           *           *
TTTCTTACCGTG CGCACGATGTCA CAGATGCCGCTT CTTATGGTATCT TGAAGTCAGCGA

       430         440         450         460         470         480
         *           *           *           *           *           *
TCGAAGAAGCAG CAACCAAATTTG ACATTGATGGCA ATCGTATTTTCT ATATGTCAGTTG

       490         500         510         520         530         540
         *           *           *           *           *           *
CCCCTCGTTTCT TCGGTACAATCG CTAAATATTTGA AATCAGAAGGTT TGCTAGCTGAGA

       550         560         570         580         590         600
         *           *           *           *           *           *
CTGGCTACAATC GTTTGATGATTG AAAAGCCTTTTG GTACATCATACG CCACCGCAGAAG

       610         620         630         640         650         660
         *           *           *           *           *           *
AATTGCAAAGTG ATTTGGAAAATG CATTTGATGATG ACCAACTGTTCC GTATTGACCACT

       670         680         690         700         710         720
         *           *           *           *           *           *
ATCTTGGAAAAG AAATGGTACAAA ATATTGCAGCAT TACGTTTTGGTA ACCCAATCTTTG

       730         740         750         760         770         780
         *           *           *           *           *           *
ATGCCGCTTGGA ATAAGGACTATA TCAAAAACGTAC AAGTAACTTTGG CTGAAGTTCTAG

       790         800         810         820         830         840
         *           *           *           *           *           *
GTGTTGAAGAGC GTGCTGGTTACT ACGATACCACTG GCGCCCTTTTGG ATATGATTCAAA

       850         860         870         880         890         900
         *           *           *           *           *           *
ACCACACAATGC AAATTGTTGGTT GGTTAGCAATGG AAAAACCTGAAT CATTCAATGATA
```

FIG.7A

```
      910         920         930         940         950         960
       *           *           *           *           *           *
AGGATATCCGTG CAGCTAAAAACG CCGCCTTCAATG CATTAAAGATTT ATAACGAAGAAG

      970         980         990        1000        1010        1020
       *           *           *           *           *           *
AAGTGAATAAGT ACTTCGTTCGTG CACAATATGGTG CTGGTGATACAG CTGATTACAAGC

     1030        1040        1050        1060        1070        1080
       *           *           *           *           *           *
CATATTTGGAAG AAGCAGATGTCC CTGCTGACTCAA AGAACAACACAT TCATTGCTGGTG

     1090        1100        1110        1120        1130        1140
       *           *           *           *           *           *
AATTGCAGTTCG ATTTGCCACGTT GGGAAGGTGTTC CTTTCTATGTTC GTTCAGGTAAGC

     1150        1160        1170        1180        1190        1200
       *           *           *           *           *           *
GTTTGGCTGCCA AGCAAACACGTG TTGATATTGTAT TTAAGGCTGGCA CATTCAACTTTG

     1210        1220        1230        1240        1250        1260
       *           *           *           *           *           *
GTTCAGAACAAG AAGCACAAGAAT CAGTACTCTCAA TCATCATTGATC CAAAGGGTGCTA

     1270        1280        1290        1300        1310        1320
       *           *           *           *           *           *
TTGAATTGAAGC TTAACGCTAAGT CAGTTGAAGATG CCTTCAACACCC GCACAATCAACT

     1330        1340        1350        1360        1370        1380
       *           *           *           *           *           *
TGGATTGGGCAG TATCTGATGAAG ACAAGAAGAACA CACCAGAACCAT ACGAACGTATGA

     1390        1400        1410        1420        1430        1440
       *           *           *           *           *           *
TTCACGATACAA TGAATGGTGACG GATCAAACTTTG CTGATTGGAACG GTGTATCAATTG

     1450        1460        1470        1480        1490        1500
       *           *           *           *           *           *
CTTGGAAGTTTG TTGACGCAATTA CTGCCGTTTACG ATGCAGATAAAG CACCATTGGAGA

     1510        1520        1530        1540        1550        1560
       *           *           *           *           *           *
CATATAAGTCAG GTTCAATGGGTC CTGAAGCATCAG ACAAGCTATTAG CTGAAAATGGCG

     1570        1580        1590        1600        1610        1620
       *           *           *           *           *           *
ATGCTTGGGTAT TTAAAGGATAAG CACATTTAAAAA GACCATCAAACA AATCTTTGTTTG

     1630        1640        1650        1660        1670        1680
       *           *           *           *           *           *
ACGGTCTTTTTA TATTGTCTGATT TAAGATGCGTTT GGTTTCACGGAA AACGGCTGACAA

     1690
       *
ATTGGTGTATTG ATCC
```

FIG.7B

```
          10         20         30         40
          *          *          *          *
MVSEIKTLVTFF GGTGDLAKRKLY PSVFNLYKKGYL QEHFAIVGTVRQ

50         60         70         80         90
*          *          *          *          *
QLSDDEFKQLVR DSIKDFTEDQAQ AEAFIAHFSYRA HDVTDAASYGIL

 100        110        120        130        140
  *          *          *          *          *
KSAIEEAATKFD IDGNRIFYMSVA PRFFGTIAKYLK SEGLLAETGYNR

   150        160        170        180        190
    *          *          *          *          *
LMIEKPFGTSYA TAEELQSDLENA FDDDQLFRIDHY LGKEMVQNIAAL

     200        210        220        230        240
      *          *          *          *          *
RFGNPIFDAAWN KDYIKNVQVTLA EVLGVEERAGYY DTTGALLDMIQN

       250        260        270        280
        *          *          *          *
HTMQIVGWLAME KPESFNDKDIRA AKNAAFNALKIY NEEEVNKYFVRA

290        300        310        320        330
 *          *          *          *          *
QYGAGDTADYKP YLEEADVPADSK NNTFIAGELQFD LPRWEGVPFYVR

 340        350        360        370        380
  *          *          *          *          *
SGKRLAAKQTRV DIVFKAGTFNFG SEQEAQESVLSI IIDPKGAIELKL

   390        400        410        420        430
    *          *          *          *          *
NAKSVEDAFNTR TINLDWAVSDED KKNTPEPYERMI HDTMNGDGSNFA

     440        450        460        470        480
      *          *          *          *          *
DWNGVSIAWKFV DAITAVYDADKA PLETYKSGSMGP EASDKLLAENGD

AWVFKG
```

FIG.8

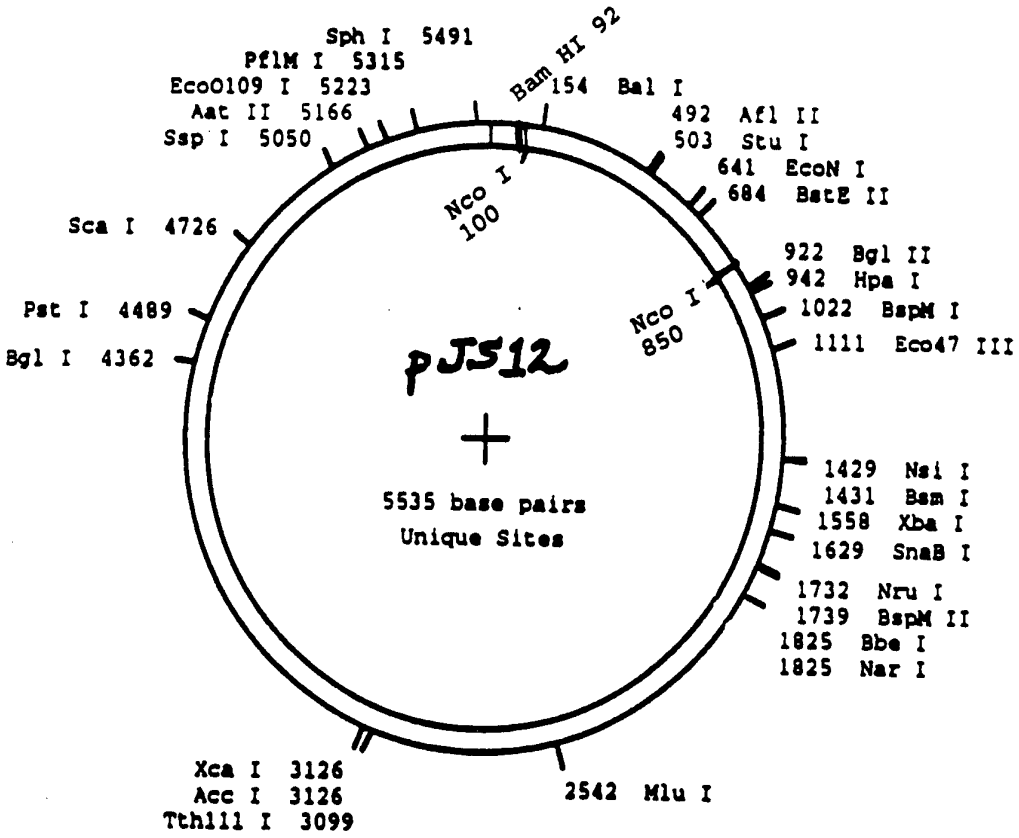
Sph I 5491
PflM I 5315
Eco0109 I 5223
Aat II 5166
Ssp I 5050
Bam HI 92
154 Bal I
492 Afl II
503 Stu I
641 EcoN I
684 BstE II
Nco I 100
Sca I 4726
Pst I 4489
Bgl I 4362
922 Bgl II
942 Hpa I
1022 BspM I
1111 Eco47 III
Nco I 850
pJ512
5535 base pairs
Unique Sites
1429 Nsi I
1431 Bsm I
1558 Xba I
1629 SnaB I
1732 Nru I
1739 BspM II
1825 Bbe I
1825 Nar I
Xca I 3126
Acc I 3126
Tth111 I 3099
2542 Mlu I

FIG.9

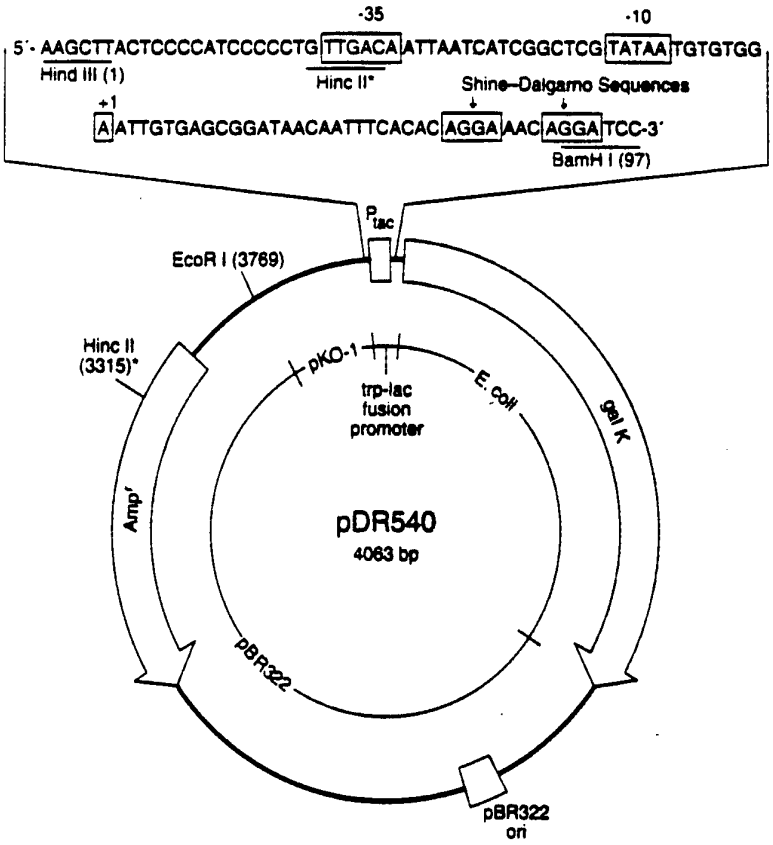
-35
-10
5'- AAGCTTACTCCCCATCCCCCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGG
Hind III (1)
Hinc II*
Shine-Dalgarno Sequences
+1
A ATTGTGAGCGGATAACAATTTCACACAGGAAACAGGATCC-3'
BamH I (97)
P tac
EcoR I (3769)
Hinc II (3315)*
pKO-1
trp-lac fusion promoter
E. coli
gal K
Amp'
pDR540
4063 bp
pBR322
pBR322 ori

FIG.10

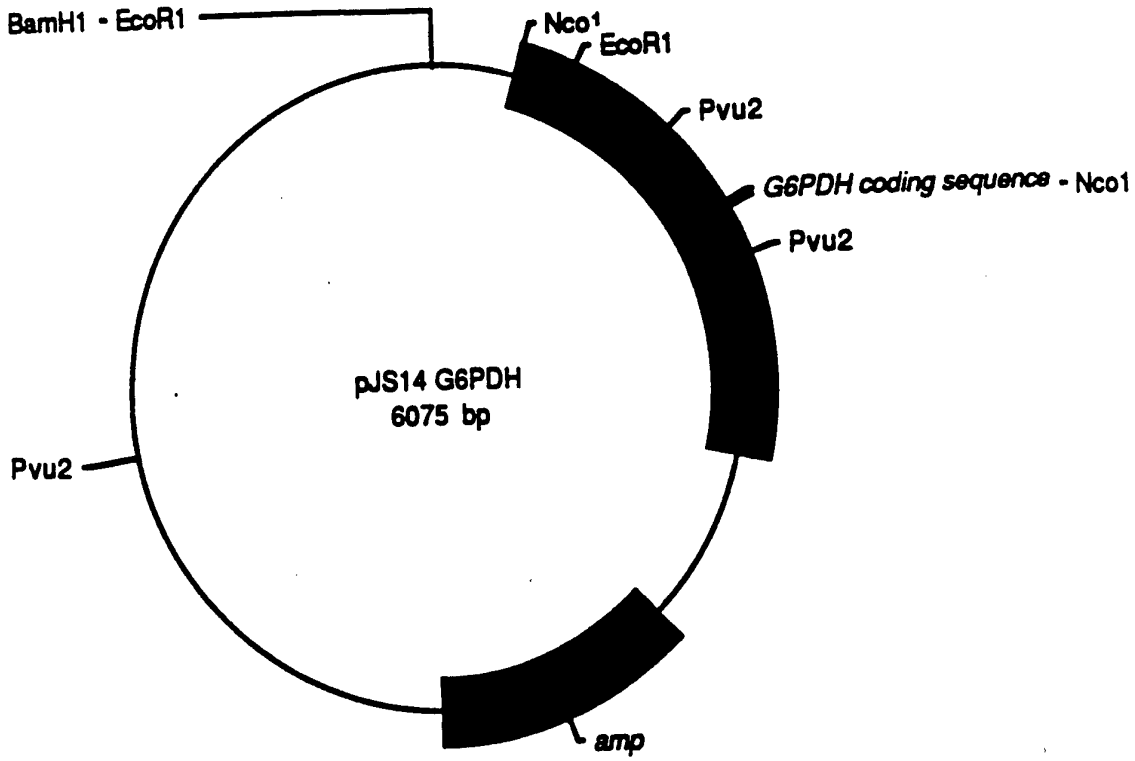
BamH1 - EcoR1
Nco1
EcoR1
Pvu2
G6PDH coding sequence - Nco1
Pvu2
pJS14 G6PDH
6075 bp
Pvu2
amp

FIG.11

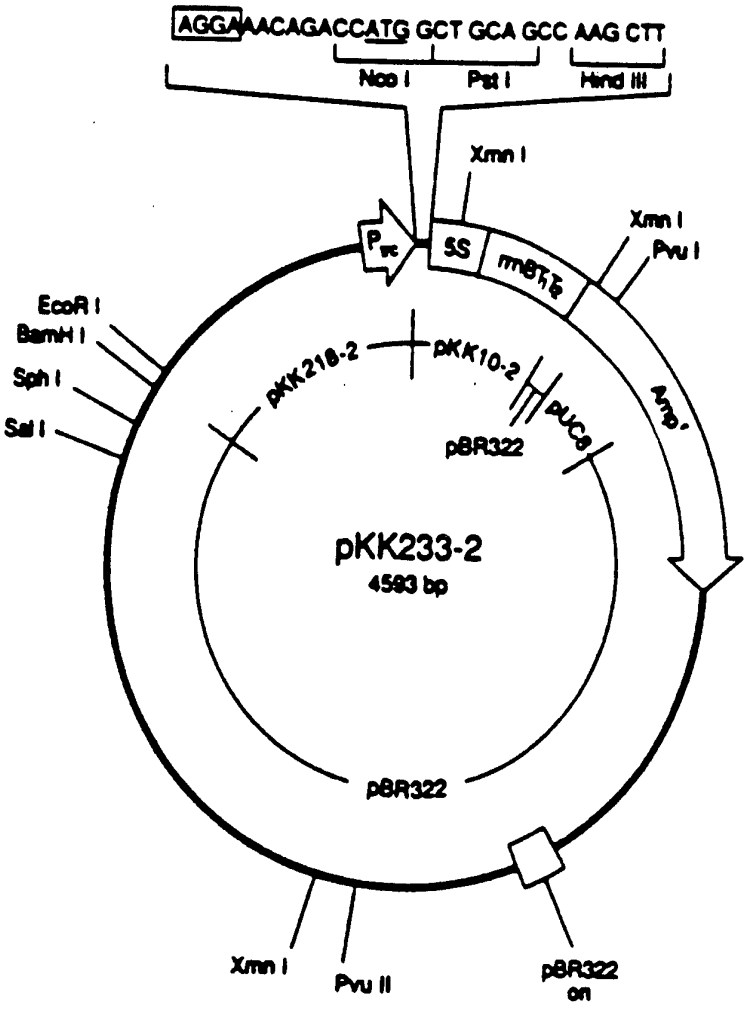
AGGAAACAGACCATG GCT GCA GCC AAG CTT
Nco I
Pst I
Hind III
Xmn I
Xmn I
Pvu I
SS
EcoR I
BamH I
Sph I
Sal I
pKK218-2
pKK10-2
pUC8
pBR322
Amp'
pKK233-2
4583 bp
pBR322
Xmn I
Pvu II
pBR322
ori

FIG.12