DESCRIPTION

METHOD FOR IMPROVED HIGH-LEVEL SECRETORY PRODUCTION OF PROTEIN

Technical Field

[0001]

The present invention relates to a method for high-level secretory production of a protein in yeast.

Background Art

[0002]

The market for protein pharmaceuticals such as therapeutic proteins and antibody drugs is rapidly expanding due to the development of genetic engineering techniques. Animal cells such as CHO or NSO, insects such as silkworms, insect cells such as SF9, and microorganisms such as *E. coli* or yeast have been used as hosts in which protein pharmaceuticals are to be produced. In particular, yeast systems are capable of high-density culture and thus they are extensively used as systems that are capable of secretory production of useful proteins in relatively inexpensive media.

[0003]

Secretory proteins pass through the translocon and enter into the endoplasmic reticulum when the amino acid regions of signal sequences at their N terminuses are recognized by signal recognition particles (SRPs). When secretory proteins pass through the translocon, the higher-order structures thereof are loosened, and the proteins are folded in the endoplasmic reticulum. While secretory protein folding is able to spontaneously occur, various molecular chaperones assist such folding. A native conformation formed in the endoplasmic reticulum is critical for secretion, and misfolded proteins cannot enter the secretory pathway located downstream. Thus, proteins having abnormal higher-order structures are disadvantageously accumulated therein. Such disturbance in modification that takes place in the endoplasmic reticulum (i.e., addition of a sugar chain or a disulfide bond) and deteriorated transportation from the endoplasmic reticulum causes "endoplasmic reticulum stress." As a means for dealing with such endoplasmic reticulum stress, a stress

response referred to as "unfolded protein response (UPR)" is induced in eukaryotic cells. Transcription induction and translation regulation of UPR are responses that restore accumulated abnormal proteins. There is also a mechanism referred to as "ER-associated degradation (ERAD)" that degrades and eliminates abnormal proteins so as to maintain homeostasis in the endoplasmic reticulum. Further, molecular chaperones that loosen the aggregated proteins for the purpose of folding are known, as are molecular chaperones that assist protein folding in the endoplasmic reticulum. For example, HSP104 can perform a reaction that cannot be performed with the aid of other chaperones that cooperate with HSP70 and solubilizes proteins from the aggregates (Non-Patent Document 1).

[0004]

Meanwhile, a variety of interactions, such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions, occur between amino acids inside a protein steric structure. In particular, covalent bonds between sulfur atoms that are formed upon two-electron oxidation of two cysteines (which are referred to as "disulfide bonds") play very important roles in stabilizing protein steric structure because of their strong properties. In fact, many secretory proteins that are secreted extracellularly have disulfide bonds. This is presumed to be the case because of the necessity of strengthening of protein structure, so that a protein can function outside a cell, where the environment is physically and chemically more severe than that in the environment inside the cell, which is enveloped by a membrane. In the case of eukaryotic cells such as yeast cells, introduction of a disulfide bond via protein oxidative folding is carried out by the oxidative protein disulfide isomerase (PDI) in the endoplasmic reticulum (Non-Patent Document 2). PDI that is reduced via oxidization of substrate proteins is reoxidized by oxidative ERO1 localized in the vicinity of the membrane (Non-Patent Documents 3 and 4). In yeast endoplasmic reticulum, there are 5 types of PDI families (i.e., PDI1, EUG1, MPD1, MPD2, and EPS1) (Non-Patent Document 5). Among such PDI families, those that are confirmed to form an intramolecular disulfide bond with ERO1 are limited to PDI1 and MPD2. It is also reported that the efficiency of protein oxidative folding is improved with BiP/Kar2, which functions in conjunction with PDI (Non-Patent Document 6). BiP/Kar2 is also associated with induction by active HAC1 of various genes associated with the aforementioned UPR. Active HAC1 is activated by the splicing of

HAC1 via the IRE1 transmembrane kinase/nuclease. IRE1 to which BiP/Kar2 is bound is dissociated when BiP/Kar2 acts on a protein having an abnormal structure in the endoplasmic reticulum, it exhibits nuclease activity through the formation of a dimmer, and it produces active HAC1 through the splicing of HAC1 (Non-Patent Documents 7 and 8). Also, Bip/Kar2 is associated with protein folding in the endoplasmic reticulum in conjunction with SCJ1 located in the endoplasmic reticulum (Non-Patent Document 9).

[0005]

Thus, it has been demonstrated that various molecular chaperones are associated with the correct folding of secretory proteins. It has been reported that one or more types of genes encoding molecular chaperone proteins, such as PDI1, ERO1, or Kar2, are co-expressed in the presence of a target protein to be expressed in yeast, so as to improve the secretory productivity of a target protein having a complicated steric structure (Patent Document 1).

[0006]

Even if the productivity of target protein secretion into media is improved with co-expression of genes encoding chaperone proteins, such as PDI1, ERO1, or Kar2, some target proteins may occasionally rapidly degrade in media. In particular, a protease existing in a vacuole that is known as a protein-degrading organelle of yeast is reported to be associated with secretory protein degradation (Non-Patent Document 10). Many proteolytic enzymes are present in vacuoles, such as vacuolar trehalase, aminopeptidase I, vacuolar alkaline phosphatase, and vacuolar RNase, in addition to proteinase A, proteinase B, and carboxypeptidase Y, and activity thereof is regulated such that it is exerted in vacuoles. In particular, proteinase A and proteinase B function as key proteases that activate themselves or carboxypeptidase Y, and they play key roles in a proteolytic system (Non-Patent Documents 11 and 12). It has been reported that an acidic protease (i.e., proteinase A) exerts strong activity under acidic conditions, but such activity is attenuated as pH increases (Non-Patent Document 13). Thus, a culture method in which the pH of a culture medium is adjusted so as to inhibit protease activity has been studied, although such method may affect the proliferation of host cells.

[0007]

In methhylotrophic yeast, methanol metabolism is initiated upon oxidation of methanol by alcohol oxidase (AOX), the generated formaldehyde is fixed to xylose 5-phosphate with the aid of a dihydroxyacetone synthase (DAS), it is used as a cell constituent in a glycolysis system, and it is also oxidized to CO_2 with the aid of glutathione-dependent formaldehyde dehydrogenase (FLD) and formate dehydrogenase (FDH) in the cytoplasm (Non-Patent Document 14). Many gene promoters encoding enzymes associated with methanol metabolism, such as pmp20- and pmp47- promoters, have been known as gene promoters the expression of which is regulated by methanol, and examples thereof include alcohol oxidase (aox1, aox2) promoter, dihydroxyacetone synthase (das1) promoter, formate dehydrogenase (fdh1) promoter, and methanol oxidase (mox) promoter (Non-Patent Document 15). Promoters that regulate the expression of enzymes associated with methanol metabolism are very strong. Thus, such promoters are generally used to achieve secretory production of various target proteins in methhylotrophic yeast. In particular, an aox1 promoter of *Pichia pastoris* is known as a very strong promoter induced by methanol.

[0008]

When target proteins are to be secreted and produced under the control of a promoter that regulates the expression of enzymes associated with methanol metabolism, methanol induction is considered to be necessary. Methanol is a deleterious substance classified as a Class 2 Flammable Liquid, the use thereof in an amount exceeding the designated level is regulated under the Fire Defense Law, and explosion-proof factories and facilities are required. If secretory production of target proteins equivalent to that induced to express with the aid of a large quantity of methanol can be achieved with the use of methanol in as small an amount as possible, accordingly, industrial values thereof are significant. In addition, various positive transcription factors used as methanol-metabolizing enzyme promoters of methhylotrophic yeast are used to induce the expression of methanol-metabolizing enzymes inherent to yeast, as well as the expression of target proteins that have been newly introduced. In fact, expression of various oxidases, such as D-amino acid oxidase, fructosyl amino acid oxidase, and peroxisome/acetyl spermidine oxidase, in addition to the hepatitis B surface antigen gene, has been attempted under the control of the aox1 promoter with the use of a methhylotrophic yeast (*Candida boidinii*) in which the aox1 gene inherent thereto has been

disrupted, and the target protein expression level is enhanced in a strain in which the aox1 gene inherent thereto has been disrupted, compared with the original parent strain (Patent Document 2 and Non-Patent Documents 16, 17, and 18).

[0009]

As described above, the use of a wide variety of methods has been demonstrated regarding high-level secretory expression of proteins in yeast. When host cells are transformed via gene introduction, gene disruption, or other means, in general, cells receive some stress. Thus, synergistic or additive effects cannot always be attained merely by employing several conventional techniques in combination. In addition, there have been no reports concerning high-level secretory expression of target proteins with the use of the various chaperones in combination with the various techniques described above.

Prior Art Documents

Patent Documents

[0010]

[Patent Document 1] WO 2009/057813

[Patent Document 2] JP S62-104585 A (1987)

[Non-Patent Document]

[0011]

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[Non-Patent Document 8] Sidrauski C. and Walter P., The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell (1997) 90(6): 1031-1039

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[Non-Patent Document 11] H. Bart van den HAZEL, Morten C. KIELLAND-BRANDT, and Jakob R. WINTHER, Autoactivation of proteinase A initiates activation of yeast vacuolar zymogens. Eur. J. Biochem. (1992) 207: 277-283

[Non-Patent Document 12] Vicki L. Nebes and Elizabeth W. Jones, Activation of the proteinase B precursor of the yeast Saccharomyces cerevisiae by autocatalysis and by an internal sequence. J. Biol. Chem. (1991) 266(34): 22851-22857

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[Non-Patent Document 14] Ida J. van der Kleia, Yurimotob H, Sakaib Y, Venhuisa M, The significance of peroxisomes in methanol metabolism in methylotrophic yeast. Biochim. Biophys. Acta. (2006) 1763: 1453-1462

[Non-Patent Document 15] Yurimoto H, Komeda T, Lim CR, Nakagawa T, Kato N, Sakai Y, Regulation and evaluation of five methanol-inducible promoters in methylotrophic yeast Candida boidinii. Biochim. Biophys. Acta. (2000) 1493(1-2): 56-63

[Non-Patent Document 16] Yurimoto H, Hasegawa T, Sakai Y, Kato N, Characterization and High-level production of D-amino acid oxidase in Candida boidinii. Biosci. Biotechnol. Biochem. (2001) 65(3), 627-633

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Summary of the Invention

Problems to be Solved by the Invention

[0012]

It is an object of the present invention to provide a production system that is capable of high-level secretory production of a protein (and in particular, a protein with a complicated structure, such as a structure with S-S bonds) in a host cell such as yeast, is suitable for industrial production with high safety, and does not require explosion-proof facilities.

Means for Solving the Problem

[0013]

The present inventors have conducted concentrated studies in order to attain the above objects. As a result, they discovered that a yeast strain into which a chaperone gene has been introduced and in which the aox1 gene encoding alcohol oxidase has been disrupted may be used, so that high-level secretory production of a target protein induced by low-concentration methanol would become possible under the control of the aox1 promoter. The present inventors also discovered that acidic proteases, such as proteinase B (PRB1) and proteinase A (PEP4), were significantly associated with degradation of the target protein expressed in yeast, and they confirmed that regulation of the pH level of a medium aimed at

disruption of the prb1 gene encoding proteinase B and/or suppression of activity of acidic protease such as proteinase A in yeast would lead to significant improvement in the secretory production amount of the target protein.

[0014]

The high-level protein secretory production system comprising the above described features in combination enables a significant reduction in the amount of methanol to be added. Thus, such system can be used as a highly safe production system that is suitable for industrial production. The present invention has been completed on the basis of such findings.

[0015]

Specifically, the present invention includes the following.

(1) A transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene has been disrupted.

(2) The transformed yeast according to (1), wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (d) below:

(a) a gene encoding PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1, or HSP104 derived from *Ogataea minuta* (*O. minuta*);

(b) a gene encoding PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5, or EUG1 derived from *Saccharomyces cerevisiae* (*S. cerevisia*);

(c) a gene encoding PDI, ERO1-Lα, ERO1-Lβ, or GRP78 derived from a human; and

(d) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (c).

(3) The transformed yeast according to (1), wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (g) below:

(a) a gene encoding PDI1 derived from *O. minuta*;

(b) a gene encoding ERO1 derived from *O. minuta*;

(c) a gene encoding Kar2 derived from *O. minuta*;

(d) a gene encoding PDI1 derived from *S. cerevisiae*;

(e) a gene encoding PDI derived from a human;

(f) a gene encoding ERO1 derived from a human; and

(g) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (f).

(4) The transformed yeast according to (1), wherein the chaperone gene is any of the chaperone genes (a) to (g) below:

(a) a combination of a gene encoding PDI1, a gene encoding ERO1, and a gene encoding Kar2 derived from *O. minuta*;

(b) a combination of a gene encoding PDI1 and a gene encoding Kar2 derived from *O. minuta*;

(c) a combination of a gene encoding PDI derived from a human and a gene encoding ERO1 derived from *O. minuta*;

(d) a combination of a gene encoding PDI1 and a gene encoding ERO1 derived from *O. minuta*;

(e) a combination of a gene encoding PDI derived from a human, a gene encoding ERO1-Lβ derived from a human, and a gene encoding GRP78 derived from a human;

(f) a combination of a gene encoding PDI derived from a human, a gene encoding ERO1 derived from *O. minuta*, and a gene encoding GRP78 derived from a human; and

(g) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (f).

(5) The transformed yeast according to any of (1) to (4), wherein the protease gene has been disrupted.

(6) The transformed yeast according to (5), wherein the protease is a prb1 gene.

(7) A transformed yeast into which a chaperone gene has been introduced and in which a protease gene has been disrupted.

(8) The transformed yeast according to (7), wherein the protease is a prb1 gene.

(9) The transformed yeast according to any of (1) to (8), wherein the yeast is a methhylotrophic yeast.

(10) The transformed yeast according to any of (1) to (9), which comprises a gene encoding a target protein introduced thereinto.

(11) Use of the transformed yeast according to any of (1) to (10) for the production of a target protein.

(12) A method for producing a protein comprising culturing the transformed yeast according to (10) in a medium and sampling a target protein from the culture product.

(13) The method for producing a protein according to (12), wherein culture is conducted under conditions in which protease activity is inhibited.

(14) The method for producing a protein according to (12) or (13), wherein culture is conducted in a medium with a pH of 6.0 to 7.5.

(15) The method for producing a protein according to any of (12) to (14), wherein a nitrogen source is added to the medium.

(16) The method for producing a protein according to any of (12) to (15), wherein the amount of methanol added to the medium is 2% (v/v) or less.

(17) A target protein produced by the method according to any of (12) to (16).

(18) A method for producing a transformed yeast comprising step (i) in addition to either or both step (ii) and/or (iii):

(i) a step of introducing a chaperone gene into yeast; and

(ii) a step of disrupting the aox1 gene in yeast; and/or

(iii) a step of disrupting the prb1 gene in yeast.

(19) The method of production according to (18), which further comprises a step of introducing a gene encoding a target protein.

Effects of the Invention

[0016]

The present invention enables high-level secretory production of a protein having a complicated structure, such as a structure with S-S bonds, as well as a normal protein, in a correctly folded form in a transformed yeast resulting from the introduction of a chaperone gene, the disruption of the aox1 gene, and/or the disruption of the protease gene. In addition, long-term culture (mass production) can be performed by culturing such transformed yeast under conditions in which protease activity is inhibited. In the protein production system involving the use of the transformed yeast according to the present invention, the amount of methanol used can be reduced to a significant extent. Thus, such system can be used as a

highly safe protein production system that is suitable for industrial production (mass production).

[0017]

This patent application claims priority from Japanese Patent Application No. 2014-155272 filed on July 30, 2014, and it includes part or all of the contents as disclosed in the description thereof.

Brief Description of the Drawings

[0018]

[Fig. 1] Fig. 1 shows a method for producing a strain in which the ura3 gene has been disrupted.

[Fig. 2] Fig. 2 shows a method for producing a strain in which the aox1 gene has been disrupted.

[Fig. 3] Fig. 3 shows the structure of a chaperone gene expression vector (onaP11007: OmPDI1+OmERO1+OmKar2 expression vector).

[Fig. 4] Fig. 4 shows a method for producing a strain in which the prb1 gene has been interrupted.

[Fig. 5] Fig. 5 shows a method for producing a kex2 expression plasmid (kex2 expression plasmid: pOMEA-Z1-KEX2, kex2 expression cassette).

[Fig. 6-1] Fig. 6-1 shows a comparison of the secretory production amount of a KEX2 protein induced by methanol (1: the NBRC 10746 strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced (a KEX2-producing strain derived from the NBRC10746+PEK strain); 2: a strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced and in which the aox1 gene has been disrupted (a KEX2-producing strain derived from the Δaox1+PEK strain); 3: a strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced and in which the prb1 gene has been interrupted (a KEX2-producing strain derived from the NBRC10746+PEK dprb1 strain); and 4: a strain in which the prb1 gene has been interrupted, into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced, and in which the aox1 gene has been disrupted (a KEX2-producing strain derived from the Δaox1+PEK dprb1 strain).

[Fig. 6-2] Fig. 6-2 shows a comparison of enzymatic activity of the KEX2 protein (1: the NBRC 10746 into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced (the KEX2-producing strain derived from the NBRC10746+PEK strain); 2: a strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced and in which the aox1 gene has been disrupted (the KEX2-producing strain derived from the Δaox1+PEK strain); 3: a strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced and in which the prb1 gene has been interrupted (the KEX2-producing strain derived from the NBRC10746+PEK dprb1 strain); and 4: a strain in which the prb1 gene has been interrupted, into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced, and in which the aox1 gene has been disrupted (the KEX2-producing strain derived from the Δaox1+PEK dprb1 strain); (white bar: third quartile-median; black bar: median-first quartile).

[Fig. 7] Fig. 7 shows a method for producing the hsa expression plasmid (hsa expression plasmid: pOMEA-Z1-HSA; hsa gene expression cassette).

[Fig. 8] Fig. 8 shows the secretory production amount of the HSA protein induced by methanol (deep well plate scale) (1: the NBRC 10746 strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced (the HSA-producing strain derived from the NBRC10746+PEK strain); 2: a strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced and in which the aox1 gene has been disrupted (the HSA-producing strain derived from the Δaox1+PEK strain); 3: a strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced and in which the prb1 gene has been interrupted (the HSA-producing strain derived from the NBRC10746+PEK dprb1 strain); and 4: a strain in which the prb1 gene has been interrupted, into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced, and in which the aox1 gene has been disrupted (the HSA-producing strain derived from the Δaox1+PEK dprb1 strain).

[Fig. 9] Fig. 9 shows the secretory production amount of the HSA protein induced by methanol (3L Jar scale) [Jar1: the NBRC 10746 strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced (the HSA-producing strain derived from the NBRC10746+PEK strain); Jar2: the NBRC 10746 strain into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced (the HSA-producing strain derived from the NBRC10746+PEK strain) (nitrogen source fed-batch culture; pH 7 control); Jar3: a strain

in which the prb1 gene has been interrupted, into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced, and in which the aox1 gene has been disrupted (the HSA-producing strain derived from the Δaox1+PEK dprb1 strain) (nitrogen source fed-batch culture; pH 7 control).

[Fig. 10] Fig. 10 shows the secretory production amount of the HSA protein achieved by carbon source starvation-induced culture (3L Jar scale) [Jar1: a strain in which the prb1 gene has been interrupted, into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced, and in which the aox1 gene has been disrupted (the HSA-producing strain derived from the Δaox1+PEK dprb1 strain) (low methanol-induced culture); Jar2: a strain in which the prb1 gene has been interrupted, into which a chaperone (OmPDI1/OmERO1/OmKar2) has been introduced, and in which the aox1 gene has been disrupted (the HSA-producing strain derived from the Δaox1+PEK dprb1 strain) (carbon source starvation-induced culture).

Embodiments for Carrying out the Invention

[0019]

1. Transformed yeast

The transformed yeast of the present invention is obtained by introduction of a chaperone gene, disruption of the aox1 gene, and/or disruption of the protease gene. Specifically, a transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene has been disrupted, a transformed yeast into which a chaperone gene has been introduced and in which the protease gene has been disrupted, and a transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene and a protease gene have been disrupted are within the scope of the transformed yeast of the present invention.

(Host cells)

[0020]

Host cells to be transformed are preferably yeast strains. Examples of yeast strains include methhylotrophic yeast strains such as *Ogataea minuta*, *Pichia lindneri*, *Pichia pastoris*, *Hansenulla polymorpha* (*Pichia angusta*), and *Candida boidinii* and yeast strains such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Yarowia lipolytica*, and

Shizosaccharomyces pombe, with methhylotrophic yeast strains being preferable. A specific example of the *Ogataea minuta* strain is the *Ogataea minuta* YK3 strain (Δoch1Δpep4Δprb1Δyps1Δura3Δade1), and a specific example of the *Saccharomyces cerevisiae* strain is the *Saccharomyces cerevisiae* BY4741 strain (MATa Δhis3Δleu2Δmet15Δura3), although yeast strains are not limited thereto.

(Introduction of chaperone gene)

[0021]

Examples of chaperone genes used in the present invention include genes encoding PDI1 (SEQ ID NO: 35 (the base sequence); SEQ ID NO: 36 (the amino acid sequence)), ERO1 (SEQ ID NO: 43 (the base sequence); SEQ ID NO: 44 (the amino acid sequence)), Kar2 (SEQ ID NO: 47 (the base sequence); SEQ ID NO: 48 (the amino acid sequence)), MPD1 (SEQ ID NO: 37 (the base sequence); SEQ ID NO: 38 (the amino acid sequence)), SCJ1 (SEQ ID NO: 39 (the base sequence); SEQ ID NO: 40 (the amino acid sequence)), EUG1 (SEQ ID NO: 41 (the base sequence); SEQ ID NO: 42 (the amino acid sequence)), and HSP104 (SEQ ID NO: 45 (the base sequence); SEQ ID NO: 46 (the amino acid sequence)) derived from *Ogataea minuta* (*O. minuta*).

[0022]

The chaperone gene used in the present invention may be a chaperone gene derived from another organism species, such as other types of yeast, mold, or a human.

[0023]

As a chaperone gene derived from another type of yeast, for example, a chaperone gene derived from *Saccharomyces cerevisiae* can be used. Specific examples include genes encoding PDI1 (Primary SGDID: S000000548; SEQ ID NO: 49 (the base sequence); SEQ ID NO: 50 (the amino acid sequence)), MPD1 (Primary SGDID: S000005814; SEQ ID NO: 51 (the base sequence); SEQ ID NO: 52 (the amino acid sequence)), SCJ1 (Primary SGDID: S000004827; SEQ ID NO: 53 (the base sequence); SEQ ID NO: 54 (the amino acid sequence)), ERO1 (Primary SGDID: S000004599; SEQ ID NO: 55 (the base sequence); SEQ ID NO: 56 (the amino acid sequence)), FKB2 (Primary SGDID: S000002927; SEQ ID NO: 57 (the base sequence); SEQ ID NO: 58 (the amino acid sequence)), JEM1 (Primary SGDID: S000003609; SEQ ID NO: 59 (the base sequence); SEQ ID NO: 60 (the amino acid sequence)), LHS1 (Primary SGDID: S000001556; SEQ ID NO: 61 (the base sequence); SEQ ID NO: 62 (the amino acid sequence)), MPD2 (Primary SGDID: S000005448; SEQ ID NO:

63 (the base sequence); SEQ ID NO: 64 (the amino acid sequence)), ERJ5 (Primary SGDID: S000001937; SEQ ID NO: 65 (the base sequence); SEQ ID NO: 66 (the amino acid sequence)), and EUG1 (Primary SGDID: S000002926; SEQ ID NO: 67 (the base sequence); SEQ ID NO: 68 (the amino acid sequence)). Sequence information regarding genes derived from *Saccharomyces cerevisiae* is available from SGD (*Saccharomyces* genome database: http://www.yeastgenome.org/).

[0024]

Examples of chaperone genes derived from a human include genes encoding PDI (GenBank Accession No. BC010859; SEQ ID NO: 69 (the base sequence); SEQ ID NO: 70 (the amino acid sequence)), ERO1-Lα (GenBank Accession No. AF081886; SEQ ID NO: 71 (the base sequence); SEQ ID NO: 72 (the amino acid sequence)), ERO1-Lβ (GenBank Accession No. BC044573; SEQ ID NO: 73 (the base sequence); SEQ ID NO: 74 (the amino acid sequence)), and GRP78 (GenBank Accession No. AL354710; SEQ ID NO: 75 (the base sequence); SEQ ID NO: 76 (the amino acid sequence)).

[0025]

The chaperone gene used in the present invention may be a gene encoding a protein that consists of an amino acid sequence derived from any of the amino acid sequences described above by deletion, substitution, and/or addition of one or several amino acids, provided that such gene has activity of promoting foreign protein secretion. The number of amino acids that may be deleted, substituted, and/or added is preferably 1 to several. The number represented by the term "several" is not particularly limited. For example, such number may be 50 or less, 40 or less, 30 or less, 25 or less, 20 or less, 15 or less, 12 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2. The term "mutation" used herein primarily refers to a mutation that is artificially introduced via a known method for preparing a mutant protein, and the term may refer to a mutation that is similar to one existing in nature. The term "foreign protein" is used in the same sense as the term "target protein" herein.

[0026]

Also, the chaperone gene used in the present invention may be a gene encoding a protein that consists of an amino acid sequence having at least 80% sequence identity with any of the amino acid sequences described above and has activity of promoting foreign

protein secretion. Specific examples include a gene that consists of a base sequence having at least 80% sequence identity with the base sequence as shown in SEQ ID NO: 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, or 75 and encodes a protein having activity of promoting foreign protein secretion; and a gene encoding a protein that consists of an amino acid sequence having at least 80% sequence identity with the amino acid sequence as shown in SEQ ID NO: 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, or 76 and has activity of promoting foreign protein secretion. The term "at least 80% sequence identity" preferably refers to at least 85% sequence identity, more preferably at least 90% sequence identity, further preferably at least 95%, and most preferably at least 99% sequence identity. A protein homology search can be carried out with the use of, for example, the DNA Databank of Japan (DDBJ) via FASTA, BLAST, or another program.

[0027]

The chaperone gene used in the present invention may be a gene that hybridizes under stringent conditions to DNA consisting of a base sequence complementary to DNA consisting of any of the base sequences described above and encodes a protein having activity of promoting foreign protein secretion. Under the aforementioned "stringent conditions," a so-called specific hybrid is formed, but a non-specific hybrid is not formed. Under such conditions, for example, complementary strands of a nucleic acid exhibiting a high degree of sequence identity, i.e., a nucleic acid consisting of a base sequence having at least 80%, preferably at least 85%, more preferably at least 90%, further preferably at least 95%, and most preferably at least 99% sequence identity with any of the base sequences above, undergo hybridization, but complementary strands of a nucleic acid having lesser degrees of sequence identity do not undergo hybridization. More specifically, the sodium salt concentration is 15 to 750 mM, preferably 50 to 750 mM, and more preferably 300 to 750 mM, the temperature is 25°C to 70°C, preferably 50°C to 70°C, and more preferably 55°C to 65°C, and the formamide concentration is 0% to 50%, preferably 20% to 50%, and more preferably 35% to 45%. Under stringent conditions, further, a filter is generally washed at a sodium salt concentration of 15 to 600 mM, preferably 50 to 600 mM, and more preferably 300 to 600 mM, and a temperature of 50°C to 70°C, preferably 55°C to 70°C, and more preferably 60°C to 65°C, after hybridization.

[0028]

A person skilled in the art can easily obtain such homologous genes by referring to, for example, Molecular Cloning (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual 2nd ed., Cold Spring Harbor Laboratory Press, 10 Skyline Drive Plainview, N.Y.,

1989). Also, a base sequence identity search can be carried out via FASTA, BLAST, or other programs.

[0029]

The amino acid mutation mentioned above, such as deletion, substitution, and/or addition, can be introduced via a technique known in the art, such as the Kunkel method or the Gapped duplex method, or via a technique in accordance therewith. For example, mutagenesis kits utilizing site-directed mutagenesis, such as a Mutant-K (Takara Bio Inc.), Mutant-G (Takara Bio Inc.), or LA PCR in vitro Mutagenesis series kit (Takara Bio Inc.), can be used.

[0030]

Also, chaperone genes derived from other organism species may be codon-modified genes that are modified so as to improve translation efficiency via substitution of a base sequence with a codon that is frequently used in a host cell. A specific example is a codon-modified gene of a gene encoding PDI derived from a human. DNA having a modified base sequence can be artificially synthesized. In the case of a long DNA sequence, the sequence is first divided into several fragments, fragments are synthesized in advance, and the resultants are then bound to each other at the end.

[0031]

In the present invention, one or more types of the aforementioned chaperone genes are used in combination. When two or more genes are used in combination, such genes may be derived from the same or different organism species.

[0032]

Preferable examples of the chaperone genes that are used in the present invention include the pdi1 gene derived from *O. minuta*, the ero1 gene derived from *O. minuta*, the kar2 gene derived from *O. minuta*, the pdi1 gene derived from *S. cerevisiae*, the pdi gene derived from a human, the ero1 gene derived from a human, and a gene encoding a protein that consists of an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, further preferably at least 95%, and most preferably at least 99% sequence identity with any of the amino acid sequences above and has activity of promoting foreign protein secretion.

[0033]

Further preferable examples of the chaperone genes that are used in the present invention include a combination of the pdi1 gene, the ero1 gene, and the kar2 gene derived from *O. minuta*, a combination of the pdi1 gene and the ero1 gene derived from *O. minuta*, a

combination of the pdi1 gene and the kar2 gene derived from *O. minuta*, a combination of the pdi gene derived from a human and the ero1 gene derived from *O. minuta*, a combination of the pdi gene, the ero1-Lβ gene, and the grp78 gene derived from a human, a combination of the pdi gene derived from a human, the ero1 gene derived from *O. minuta*, and the grp78 gene derived from a human, and a combination of genes each encoding a protein that consists of an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, further preferably at least 95%, and most preferably at least 99% sequence identity with any of the amino acid sequences of the combinations of the chaperones described above and has activity of promoting foreign protein secretion.

[0034]

The most preferable examples of the chaperone genes that are used in the present invention include a combination of the pdi1 gene, the ero1 gene, and the kar2 gene derived from *O. minuta*, or a combination of genes each encoding a protein that consists of an amino acid sequence having at least 80% sequence identity with any of the amino acid sequences of PDI1, ERO1, and Kar2 derived from *O. minuta* and has activity of promoting foreign protein secretion (e.g., a combination of genes each that consists of the base sequence having at least 80%, preferably at least 85%, more preferably at least 90%, further preferably at least 95%, and most preferably at least 99% sequence identity with the base sequence as shown in SEQ ID NO: 35, 43, or 47 and encodes a protein having activity of promoting foreign protein secretion; or a combination of genes each encoding a protein that consists of an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, further preferably at least 95%, and most preferably at least 99% sequence identity with the amino acid sequence as shown in SEQ ID NO: 36, 44, or 48 and has activity of promoting foreign protein secretion). Regarding the gene-related notation used herein, for example, the term "a gene encoding PDI1" is used in the same sense as the term "the pdi1 gene."

[0035]

The chaperone gene is introduced into yeast, which is a host cell, with the use of an expression vector. In the present invention, an expression vector can be introduced into a host cell by any method, provided that the introduced gene is stably present and adequately expressed in a yeast host. Examples of methods that are generally employed include the calcium phosphate method (Ito et al., Agric. Biol. Chem., 48, 341, 1984), electroporation (Becker, D. M. et al., 1990; Methods. Enzymol., 194, 182-187), use of spheroplasts (Creggh et al., Mol. Cell. Biol., 5, 3376, 1985), the lithium acetate method (Itoh, H., 1983; J. Bacteriol. 153, 163-168), and lipofection.

(Disruption of aox1 gene and/or disruption of protease gene)

[0036]

The transformed yeast of the present invention is obtained by, in addition to the introduction of a chaperone gene, disruption of the aox1 gene endogenous in the host genome, disruption of the protease gene endogenous in the host genome, or disruption of both the aox1 gene and the protease gene endogenous in the host genome.

[0037]

Examples of the aox1 genes include a gene encoding AOX1 derived from *O. minuta* (SEQ ID NO: 27 (the base sequence); SEQ ID NO: 28 (the amino acid sequence)), a gene encoding AOX1 derived from *Pichia pastoris* (GenBank accession number: U96967), and a gene encoding AOX1 derived from *Candida boidinii* (GenBank accession number: Q00922). The aox1 gene is not limited thereto, provided that the gene encodes AOX1 derived from yeast.

[0038]

Examples of the protease genes include the prb1 gene (SEQ ID NO: 31 (the base sequence); SEQ ID NO: 32 (the amino acid sequence)) and the pep4 gene (GenBank accession number: AB236164) derived from *O. minuta*, the prb1 gene (GenBank accession number: AB060541) and the pep4 gene (JP 2000-078978 A) derived from *Candida boidinii*, the prb1 gene and the pep4 gene derived from *Pichia pastoris*, the prb1 gene (GenBank accession number: M18097) derived from *Saccharomyces cerevisiae*, and the prb1 gene (GenBank accession number: A75534) derived from *Kluyveromyces lactis*. The protease gene is not limited thereto, provided that the gene is derived from yeast.

[0039]

Accordingly, the transformed yeast of the present invention is preferably obtained by, in addition to the introduction of a chaperone gene, disruption of the aox1 gene endogenous in the host genome or disruption of the prb1 gene endogenous in the host genome. More preferably, the transformed yeast is obtained by, in addition to the introduction of a chaperone gene, disruption of both the aox1 gene and the prb1 gene endogenous in the host genome. It is most preferable that, in addition to the introduction of, as the the chaperon gene, a combination of the pdi1 gene, the ero1 gene, and the kar2 gene derived from *O. minuta* or a combination of genes each encoding a protein that consists of an amino acid sequence having at least 80%, preferably at least 85%, more preferably at least 90%, further preferably at least 95%, and most preferably at least 99% sequence identity with any of the amino acid

sequences of PDI1, ERO1, and Kar2 and has activity of promoting foreign protein secretion, both the aox1 gene and the prb1 gene endogenous in the host genome are disrupted.

[0040]

In the present invention, the term "gene disruption" refers to "gene deletion" whereby all or a part of the target gene is deleted from the chromosome, substitution of the target gene, and "gene interruption" that inhibits the expression of a functional protein encoded by a target gene by interruption of the target gene without deleting such gene. From the viewpoint of disruption of functions of the target gene, gene disruption may take the form of mutagenesis or expression inhibition of a gene causing functional deficiency. A means for gene disruption is not particularly limited, provided that the expression or functions of a protein encoded by the target gene is/are inhibited or deleted.

[0041]

Typically, the target gene can be disrupted via homologous recombination. At the outset, the target gene is interrupted or partially deleted, an adequate selection marker gene is inserted thereinto, and a DNA construct comprising a selection marker flanked by the upstream region and the downstream region of the target gene is prepared. Subsequently, this construct is introduced into a yeast strain, so as to perform recombination in homologous regions at both ends of the introduced fragment (a DNA construct comprising a selection marker) and the target gene in the chromosome, and the target gene in the chromosome is then substituted with the introduced fragment. In such a case, a selection marker used for gene disruption can be an auxotrophic marker or a drug-tolerant marker, as described below.

[0042]

An embodiment involving the use of the ura3 gene as a selection marker is specifically described. A plasmid comprising the ura3 gene having repeat structures before and after the structural gene is constructed, the gene cassette is cleaved with a restriction enzyme, and the resultant is inserted into the target gene of a plasmid, so as to construct the disrupted alleles. This plasmid is substituted with the target gene of the chromosome, so as to obtain a gene-disrupted strain. The ura3 gene inserted into the chromosome has repeat structures before and after the ura3 gene, homologous recombination takes place between repeat sequences, and the ura3 gene is thus deleted from the chromosome. The deleted strain can be selected with the use of 5-fluoroorotic acid (5-FOA). The ura3 variant is resistant to 5-FOA (Boeke et al., Mol. Gen. Genet., 197, 345-346, 1984; Boeke et al., Methods Enzymol., 154, 165-174, 1987), and strains having URA+ phenotypes cannot grow in a 5-FOA medium. If a strain exhibiting tolerance is separated with the use of a medium supplemented with 5-

FOA, accordingly, the use of the ura3 gene marker becomes possible again. In general, use of a selection marker is necessary in order to disrupt a gene. With the use of the ura3 gene, however, ura3 traits can be efficiently reproduced.

[0043]

A mutation aimed at causing functional defects can be introduced into a gene by modifying a gene via mutagenesis, such as site-directed mutagenesis. Specifically, a gene mutation aimed at causing functional defects at a particular site is, for example, a mutation that has been caused by frame-shift or amino acid substitution at the active center resulting from insertion or deletion of nucleotides into or from ORF, and the gene is mutated to encode a protein that has been inactivated. When gene expression is suppressed, the expression level of the relevant gene is lowered or lost. Examples of methods for suppressing gene expression include a method involving the use of antisense RNA or RNAi and a method comprising attenuating a promoter.

(Expression vector)

[0044]

The chaperone gene is introduced into yeast with the use of an expression vector. Examples of such expression vector include a vector comprising a single type of chaperone gene, a vector comprising two or more copies of a single type of chaperone gene, and a vector comprising a combination of two or more types of chaperone genes. In order to express the chaperone gene in yeast, a vector comprising a single gene may be used to carry out transformation. Alternatively, a vector comprising a plurality of genes may be used to carry out transformation. Also, such expression vector may comprise a gene encoding a foreign protein. Alternatively, aiming high expression and secretion, expression vectors comprising a gene encoding a foreign protein may be prepared separately. In such a case, vectors are cotransfected into a host cell.

[0045]

A gene encoding a foreign protein is not particularly limited. Examples include: various enzyme genes, such as the lysozyme gene, the α-amylase gene, and the α-galactosidase gene, and in particular, glycosyltransferase genes that are necessary for production of pharmaceutically useful glycoproteins, such as the erythropoietin (EPO) gene and granulocyte-colony stimulating factor (G-CSF) genes; various interferon genes that are pharmaceutically useful and physiologically active proteins, such as interferon α and interferon γ genes; various interleukin genes, such as IL1 and IL2 genes; various cytokine genes, such as the erythropoietin (EPO) gene and the granulocyte-colony stimulating factor

(G-CSF) gene; growth factor genes; and various vaccine antigens such as influenza. Such genes may be obtained via any means.

[0046]

The present invention is particularly effective on a protein that is highly hydrophobic and a protein whose secretory production is insufficient due to composite formation. Thus, the aforementioned foreign protein includes a multimeric protein, such as an antibody or a functional fragment thereof; i.e., a heteromultimer.

[0047]

An expression regulation region may be adequately added to the chaperone gene or a gene encoding a foreign protein to constitute an expression vector as a protein expression unit. A protein expression unit comprises, in the direction of a transcription reading frame, at least a promoter region, the above gene, and a transcription terminator region. A promoter that can be used herein may be an inducible expression promoter or constitutive expression promoter. Examples of inducible expression promoters include a promoter of a gene encoding alcohol oxidase (AOX), a promoter of a gene encoding dihydroxyacetone synthase (DAS), and a promoter of a gene encoding formate dehydrogenase (FDH) involved in the methanol metabolism of methhylotrophic yeast. An example of another inducible promoter that can be used is a copper-inducible promoter (CUP). Examples of constitutive expression promoters include promoters of the genes encoding glyceraldehyde-3-phosphate dehydrogenase (TDH, GAP), phosphoglycerokinase (PGK), phosphotriose isomerase (TPI), enolase (ENO), actin (ACT), cytochrome c (CYC), trehalose synthase (TPS), and alcohol dehydrogenase (ADH). Also, a transcription terminator may be a sequence having activity of terminating transcription from a promoter. It may have the same or a different sequence as the gene of the promoter.

[0048]

Also, an expression vector may comprise DNA encoding a secretory signal sequence that functions in a yeast cell added to a gene encoding a foreign protein. Thus, secretory production becomes possible, and a foreign protein of interest can be easily isolated and purified. Examples of secretory signal sequences include a secretory signal sequence of an *S. cerevisiae*-derived α-mating factor (αMF), a secretory signal sequence of *S. cerevisiae*-derived invertase (SUC2), and a secretory signal sequence of human-derived α-galactosidase.

[0049]

The expression vector can comprise a selection marker for selecting a transformant. For example, yeast expression vectors can comprise auxotrophic marker genes selected from

among his1, his2, his3, his4, his5, his6, leu2, arg1, arg2, arg3, trp1, lys2, ade1, ade2, ura3, and ura5 genes.

[0050]

As selection markers, drug-resistant markers that impart resistance to drugs such as cerulenin, aureobasidin, Zeocin, canavanine, cycloheximide, hygromycin, blasticidin, tetracycline, kanamycin, ampicillin, tetracycline, and neomycin can be used, in addition to the aforementioned auxotrophic markers. Thus, transformants can be selected. Also, genes that impart solvent resistance to ethanol, osmotic resistance to glycerol or salt, metal ion resistance to copper, and the like may be used as markers, so that transformants can be selected.

[0051]

2. Method for producing protein

In the present invention, proteins can be produced by culturing the transformed yeast obtained in 1. above via a conventional technique and sampling the proteins from the culture product, followed by purification. The term "culture product" used herein refers to culture cells, cultured strains, or disrupted cells or strains, in addition to a culture supernatant.

[0052]

When the host cell is yeast, either a natural or synthetic medium may be used for culture, provided that it contains carbon sources, nitrogen sources, and inorganic salts assimilable by the yeast and permits efficient culture of the transformed yeast. Examples of carbon sources that can be used include: carbohydrates such as glucose, fructose, sucrose, and starch; organic acids such as acetic acid, lactic acid, citric acid, and propionic acid; and alcohols such as methanol, ethanol, propanol, and glycerol. Examples of nitrogen sources include: ammonia; ammonium salts of inorganic or organic acids such as ammonium chloride, ammonium sulfate, ammonium acetate, ammonium phosphate, and ammonium carbonate; other nitrogen-containing compounds such as urea; nitrogenous organic substances such as amino acids, yeast extracts, peptone, meat extracts, corn steep liquor, casein hydrolysate, soybean cake, and soybean cake hydrolysate. Examples of inorganic salts include: monopotassium phosphate, dipotassium phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, iron(I) sulfate, manganese sulfate, copper sulfate, and calcium carbonate. In the case of an auxotrophic yeast, nutritive substances necessary for the growth thereof may be added to a medium. Examples of such nutritive substances include amino acids, vitamins, nucleic acids, and salts. In accordance with the type of selection marker, an antibiotic agent, such as aureobasidin, ampicillin, or tetracycline, may be adequately added to a medium.

Alternatively, an amino acid that can be supplied by a gene complementing auxotrophy (e.g., leu, ura, or trp) may be removed.

[0053]

When culturing yeast transformed with the use of an expression vector comprising an inducible promoter, an inducer may be added to the medium, according to need. When culturing yeast transformed with the use of an expression vector comprising a methanol-inducible promoter (e.g., aox, das, or mox), for example, methanol is added to the medium. When culturing yeast transformed with the use of an expression vector comprising a GAL promoter, galactose is added to the medium.

[0054]

During culture, an inhibitor of PMT activity may be added to the medium, so as to inhibit addition of an O-sugar chain peculiar to yeast. Examples of inhibitors of PMT activity include the rhodanine-3-acetic acid derivative (5-[[3,4-(1-phenylmethoxy)phenyl]methylene]-4-oxo-2-thioxo-3-thiazolidineacetic acid, compound (1c) described in Bioorganic & Medicinal Chemistry Letters, Vol. 14, p. 3975, 2004) and {(5Z)-4-oxo-5-[3-(1-phenylethoxy)-4-(2-phenylethoxy)benzylidene]-2-thioxo-1,3-thiazolidin-3-yl}acetic acid (compound (5a) described in Bioorganic & Medicinal Chemistry Letters, Vol. 14, p. 3975, 2004).

[0055]

Culture is carried out at about 20°C to 30°C for 24 to 1,000 hours. Culture can be carried out via batch culture or continuous culture, such as static, shake, agitation, or aeration culture.

[0056]

When the transformed yeast of the present invention has been transformed with the use of an expression vector comprising a methanol-inducible promoter, it is cultured in a medium supplemented with methanol. Methanol may be added to the medium with the use of a pump or by other means while observing the growth of yeast. Such addition may be continuously or intermittently carried out, and it is not necessary to perform addition over the entire period of culture. When culturing a transformed yeast strain (i.e., a strain in which the aox1 gene has been disrupted), for example, methanol-induced culture is initiated in a medium supplemented with methanol at a concentration of preferably 0.3% to 2% (v/v) and more preferably 0.5% to 1% (v/v). When the methanol concentration is reduced to about 0.2% to 0.3% (v/v), methanol may be intermittently added, preferably at a final concentration

of 0.1% to 0.5% (v/v), and more preferably at a final concentration of 0.2% to 0.4% (v/v) per day.

[0057]

When carbon sources are depleted from the medium used for the growth of the transformed yeast and the medium is brought to a carbon-starved state, a methanol-inducible promoter is strongly activated. After the medium has been depleted of the carbon sources, accordingly, the carbon source content in the medium may be adequately adjusted. Thus, culture can be conducted without the addition of methanol or at a low methanol concentration of 0.2% (v/v) or lower. Examples of "carbon sources" include glycerin, alanine, mannitol, sorbitol, trehalose, and lactose. When "the carbon source content in the medium may be adequately adjusted," the carbon sources may be added to the medium at the lowest concentration necessary for the growth of the transformed yeast of the present invention and protein expression. Specifically, culture may be initiated under carbon source-depleted conditions (carbon source-starvation conditions) when the target cell density is attained. When culturing a transformed yeast strain (a strain in which the aox gene has been disrupted), for example, whether or not the cell density has reached the target level and carbon sources have been depleted is confirmed. At the same time, glycerin and sorbitol may be continuously added at a concentration of preferably 0.5% to 6% (w/v), and more preferably 1% to 4% (w/v) per day.

[0058]

It is preferable that culture be carried out under conditions in which protease activity is inhibited. Thus, degradation of the target protein that is secreted and produced by yeast is inhibited, and the secretory production amount significantly increases. Protease activity can be inhibited by disrupting the proteinase B gene of yeast as described above, and it can be inhibited by regulating the pH level of the medium. The pH level of the medium is preferably 6.0 to 7.5, so that activity of acidic protease such as proteinase A in the medium is inhibited and the growth of the yeast is not affected. The pH level is regulated with the use of, for example, inorganic acid, organic acid, or an alkaline solution.

[0059]

Further, culture is preferably carried out under conditions in which nitrogen sources are continuously added. By supplying nitrogen sources during culture, secretory production of proteins and maintenance thereof are remarkably improved. The final concentration of the nitrogen sources to be added per day is preferably 0.1% to 0.75% (w/v) in the case of a yeast extract and 0.05% to 0.15% (w/v) in the case of L-histidine monohydrochloride monohydrate,

and it is more preferably 0.3% to 0.5% (w/v) in the case of a yeast extract and 0.1% to 0.13% (w/v) in the case of L-histidine monohydrochloride monohydrate. Nitrogen sources can be added with the use of a mixture of a yeast extract and L-histidine monohydrochloride monohydrate.

[0060]

A transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene and the protease gene have been disrupted, which is an embodiment of the transformed yeast of the present invention, is cultured in a medium with the pH level within the aforementioned range supplemented with nitrogen sources. Thus, the amount of methanol to be added can be reduced to 4% to 7% of the amount of methanol added, compared with the case of a transformed yeast into which only the chaperone gene has been introduced.

[0061]

The expression product of a gene of a foreign protein from the culture product (i.e., a culture solution or culture cells) can be identified via SDS-PAGE, Western blotting, ELISA, or the like. The produced proteins may be isolated and purified via conventional techniques for protein isolation and purification. When target proteins are produced in the cells after culture, the cells may be pulverized using, for example, an ultrasonic pulverizer, a French press, a Manton-Gaulin homogenizer, or a Dyno-mil, to obtain target proteins. When the target proteins are produced outside the cells, the culture solution is used as it is, or the cells are removed via centrifugation or the like. Thereafter, the target proteins are collected via extraction using an organic solvent, subjected to various chromatography techniques (e.g., hydrophobic, reversed-phase, affinity, or ion-exchange chromatography), gel filtration using molecular sieves, electrophoresis using polyacrylamide gel, or the like, according to need. These techniques may be employed solely or in combinations of two or more.

[0062]

The above culture and purification techniques are examples, and methods are not limited thereto. The amino acid sequence of the purified gene product can be confirmed by a conventional amino acid analysis method, such as automated amino acid sequencing via the Edman degradation technique.

Examples

[0063]

Hereafter, the present invention is described in detail with reference to the examples, although the technical scope of the present invention is not limited to the examples. Plasmids, restriction enzymes, DNA modifying enzymes, and the like that are used in the examples of the present invention are commercially available products, and these products can be used in accordance with conventional techniques. Also, procedures of DNA cloning, nucleotide sequencing, yeast transformation, culture of transformed yeast, and the like are well-known in the art or can be learned through existing publications.

[0064]

[Example 1] Construction of vector for foreign gene expression

(1) Construction of vector for foreign gene introduction carrying a Zeocin-resistant gene as a selection marker and comprising the aox1 gene promoter of NBRC 10746 (*O. minuta*, Biological Resource Center, NITE) and the terminator cassette

NBRC 10746 AOX1 (GenBank Accession Number AB242209) comprises an amino acid sequence of 663 amino acids encoded by a 1,992-bp base sequence (SEQ ID NO: 27, SEQ ID NO: 28). PCR was carried out using the genomic DNA of NBRC 10746 prepared with the use of the Y-DER Yeast DNA Extraction Reagent (78870, PIERCE) as a template, the Hd AOXp Fw primer (5'-GCAAGCTTTCTTTCGCAAACAGCTCTTTG-3': SEQ ID NO: 1), the AOXp rv primer (5'-GAACCCGGGAACAGAATCTAGATTTTTTCGTAAGTCGTAAG-3': SEQ ID NO: 2), and the PrimeSTAR Max DNA Polymerase (RO45A, Takara Bio Inc.) at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 15 seconds, and this cycle was repeated 30 times. Thus, an aox1 promoter region-contaning DNA fragment comprising the aox1 promoter region of about 2.4 kbp and a spacer region of 22 bp was amplified. Also, PCR was carried out using DNA of NBRC 10746 as a template, the AOXt fw primer (5'-CTGTTCCCGGGTTCCTGGATCCGAGACGGTGCCCGACTC-3': SEQ ID NO: 3), and the Kp AOXt Rv primer (5'-GCGGTACCGTTAGTGGTACGGGCAG-3': SEQ ID NO: 4) at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 5 seconds, and this cycle was repeated 30 times. Thus, an aox1 terminator region-contaning DNA fragment comprising the aox1 terminator region of about 0.8 kbp and a spacer region of 22 bp was amplified. PCR was carried out using these DNA fragments as templates, the Hd AOXp Fw primer, and the Kp AOXt Rv primer at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 15 seconds, and this cycle was repeated 30 times. Thus, the aox1 promoter region of about 2.4 kbp was ligated to the terminator region of about 0.8 kbp, and the resultant was then amplified. The ligated DNA fragment was subjected to agarose electrophoresis, recovered, and then cloned into pCR-Blunt II-TOPO. The cloned plasmid was subjected to double digestion with the

restriction enzymes *Hin*dIII and *Kpn*I to obtain a DNA fragment comprising the aox1 gene promoter and the terminator cassette.

[0065]

The pOMexGP1Z plasmid described in WO 2009/057813 (i.e., a vector for foreign gene expression carrying a Zeocin-resistant gene as a selection marker and comprising the gap gene promoter and the terminator cassette) was subjected to double digestion with the restriction enzymes *Hin*dIII and *Kpn*I to obtain a DNA fragment comprising a Zeocin-resistant gene marker. The DNA fragment comprising the aox1 gene promoter and the terminator cassette was introduced into the fragment obtained. Thus, the pOMEA-Z1 plasmid was obtained.

[0066]

[Example 2] Preparation of strain in which the ura3 gene has been disrupted

(1) Preparation of DNA fragment for ura3 gene disruption

Fig. 1 shows forms of gene disruption using a DNA fragment comprising the ura3 ORF promoter and the terminator. NBRC 10746 URA3 (GenBank Accession Number AB242207) comprises an amino acid sequence of 265 amino acids encoded by a 798-bp base sequence (SEQ ID NO: 29, SEQ ID NO: 30). PCR was carried out using the genomic DNA of NBRC 10746 prepared with the use of the Y-DER Yeast DNA Extraction Reagent (78870, PIERCE) as a template, the dURA Fw primer (5'-GGTACCAGTACTGGAAA-3': SEQ ID NO: 5), the dURA rv primer (5'-CAGATAAACAGGCGACT TTTCGGGTCACGTGACT-3': SEQ ID NO: 6), and the PrimeSTAR Max DNA Polymerase (RO45A, Takara Bio Inc.) at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 5 seconds, and this cycle was repeated 30 times. Thus, a ura3 terminator region-contaning DNA fragment comprising the ura3 terminator region of about 0.5 kbp and a ura3 promoter region of 17 bp was amplified. Also, PCR was carried out using DNA of NBRC 10746 as a template, the dURA fw primer (5'-AGTCACGTGACCCGAAA AGTCGCCTGTTTATCTG-3': SEQ ID NO: 7), and the dURA Rv primer (5'-CCAAGGAGGAAGAAATT-3': SEQ ID NO: 8) at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 5 seconds, and this cycle was repeated 30 times. Thus, a ura3 promotor region- -contaning DNA fragment comprising the ura3 promoter region of about 1.2 kbp and a ura3 terminator region of 17 bp was amplified. PCR was carried out using these DNA fragments as templates, the dURA Fw primer, and the dURA Rv primer at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 5 seconds, and this cycle was repeated 30 times. Thus, the ura3 promoter region of about 1.2 kbp was ligated to the terminator region of about 0.5 kbp, and the resultant was then amplified. The ligated DNA fragment was subjected to agarose electrophoresis, recovered, and then designated as a fragment for ura3 gene disruption.

[0067]

(2) Preparation of a strain in which the ura3 gene has been disrupted

A fragment for ura3 gene disruption was introduced into the NBRC 10746 strain via electroporation. The resultant was inoculated into 5 ml of YPD medium and cultured at 28°C for 12 to 14 hours up to the logarithmic growth phase (OD_{600} = about 0.5 to 4). The strains were recovered via centrifugation at 1400 × g for 5 minutes and washed once with 10 ml of ice-cooled sterile water and then washed once with 4 ml of ice-cooled sterile water. The strains were suspended in 2 ml of LC buffer (100 mM LiCl, 50 mM potassium phosphate buffer, pH 7.5), the suspension was shaken at 28°C for 45 minutes, 0.05 ml of 1 M DTT was added thereto, and the resultant was shaken for an additional 15 minutes. The strains were recovered via centrifugation at 1400 × g for 5 minutes, the recovered strains were washed with 8 ml of ice-cooled STM buffer (270 mM sucrose, 10 mM Tris-HCl buffer, pH 7.5, 1 mM $MgCl_2$) and then with 1 ml of STM buffer, and the resultants were suspended in 0.05 ml of ice-cooled STM buffer. Transformation experiment via an electric pulse method was carried out using the Electro Cell Manipulator ECM 600 (BTX). After 0.05 ml of the cell suspension was mixed with 0.005 ml (3 μg) of a DNA sample of a fragment for ura3 gene disruption, the mixture was introduced into a 0.2-cm disposable cuvette, and electric pulses were applied under adequate conditions (voltage: 1.5 kV, 100-200 Ω). Immediately thereafter, YPD medium containing 1 ml of ice-cooled 1 M sorbitol was added, and shake culture was conducted at 28°C for 4 to 6 hours. After culture, the strains were applied to YPD selection medium containing an adequate amount of antibiotics, and the plate was subjected to culture at 28°C to obtain transformed colonies. After electroporation, the resultant was applied to a YPAD agar medium (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, 40 mg/l adenine-HCl, and 20 g/l agar) containing 5-FOA (5-fluoroorotic acid) at a final concentration of 0.1% (w/v), and the transformants were allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on YPAD agar medium containing 5-FOA (5-fluoroorotic acid) at a final concentration of 0.1% (w/v). Transformants in which the URA3 gene has been disrupted were selected via colony PCR. Some yeast strains that had proliferated on the YPAD agar medium containing 5-FOA at a final concentration of 0.1% (w/v) were suspended in 10 μl of a 0.25% SDS solution, 90 μl of sterile water was added, and strains were removed via centrifugation at 3,100 × g and 4°C for 5 minutes. The resulting supernatant as a DNA solution was inspected with the use of the dURA check -1.5 kbp primer designed in the upstream sequence of the ura3 promoter (5'-ATCACAGGAAAGCGCAT-3': SEQ ID NO: 9) and the dURA check +1kbp primer designed in the downstream sequence of the ura3 terminator (5'-ATTCGAGCATCGCCGTG-3': SEQ ID NO: 10), and a strain in

which a region of about 2.7 kbp without the ura3 coding region has been amplified was designated as the strain in which the ura3 gene has been disrupted (Δura3 strain).

[0068]

[Example 3] Preparation of a strain in which the aox1 gene has been disrupted

(1) Preparation of vector for aox1 gene disruption

Fig. 2 shows forms of gene disruption using a DNA fragment comprising the aox1 ORF promoter and the terminator region. pOMEA-Z1 constructed in Example 1 was subjected to double digestion with *Hin*dIII and *Kpn*I to obtain a DNA fragment comprising the aox1 gene promoter and the terminator cassette. The DNA fragment comprising the aox1 gene promoter and the terminator cassette was introduced into the DNA fragment obtained via double digestion of the onaP09007 plasmid described in WO 2009/057813 (i.e., a constant expression vector for a gene encoding OmKar2) with *Hin*dIII and *Kpn*I to obtain the pOMEU1 plasmid.

[0069]

(2) Preparation of a strain in which the aox1 gene has been disrupted

The pOMEU1 plasmid was introduced into the strain in which the ura3 gene has been disrupted (Δura3 strain) described in Example 2 via electroporation under the conditions described in Example 2. The pOMEU1 was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (3 μg) was used. After electroporation, the resultant was applied onto a casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar), and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium to obtain a strain into which the aox1 gene-disrupting plasmid had been introduced.

[0070]

Subsequently, the strain into which the aox1 gene-disrupting plasmid had been introduced was inoculated into a 50-ml polypropyrene centrifuge tube (227241, Greiner) containing 5 ml of YPAD medium (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, and 40 mg/l adenine-HCl), and the upper part of the centrifuge tube was sealed with CO_2 permeable plate seal (676051, Greiner). After the reciprocal shake culture was conducted at an agitation speed of 250 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, the culture product was applied onto a YPAD medium (1 g/l yeast extract, 2 g/l peptone, 20 g/l glucose, 40 mg/l adenine-HCl, and 20 g/l agar) containing 5-FOA at a final concentration of 0.1% (w/v), and it was then allowed to proliferate at 28°C for about 3 days. The proliferated

transformants were allowed to proliferate again on the YPAD medium containing 5-FOA at a final concentration of 0.1% (w/v), and transformants in which the aox1 gene has been disrupted were selected via colony PCR. Some yeast strains that had proliferated on the YPAD medium containing 5-FOA at a final concentration of 0.1% (w/v) were suspended in 10 μl of a 0.25% SDS solution, 90 μl of sterile water was added thereto, and strains were then removed via centrifugation at 3,100 × g and 4°C for 5 minutes. The resulting supernatant as a DNA solution was inspected with the use of the AOX ORF fw primer (5'-ATGGCTATTCCTGACGAATT-3': SEQ ID NO: 11) and the AOX ORF rv primer (5'-TTAGAATCTAGCCAGACCCTTC-3': SEQ ID NO: 12) designed within the aox1 ORF sequence, and a strain in which DNA amplification was not observed was designated as a strain in which the aox1 gene has been disrupted (Δaox1 strain).

[0071]

[Example 4] Preparation of strain into which OmPDI1, OmERO1, and OmKar2 chaperones have been introduced

The onaP11007 coexpression vector for OmPDI1, OmERO1, and OmKar2 described in WO 2009/057813 was used (Fig. 3). The onaP11007 plasmid was introduced into the NBRC 10746 strain via electroporation. The onaP11007 plasmid was digested with the restriction enzyme *Not*I, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. The constructed onaP11007 was digested with the restriction enzyme *Not*I and then introduced via electroporation described in Example 2. After electroporation, the resultant was applied onto a casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar), and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium, and transformants into which a chaperone has been introduced were selected via colony PCR. Some yeast strains that had proliferated on the casamino acid-U-A agar medium were suspended in 10 μl of a 0.25% SDS solution, 90 μl of sterile water was added thereto, and strains were then removed via centrifugation at 3,100 × g and 4°C for 5 minutes. The resulting supernatant as a DNA solution was inspected in the manner described below. That is, introduction of the pdi1 gene was confirmed by detecting amplification of a DNA fragment of about 1.6 kbp with the use of the GAPpforS-F primer (5'-GATCTCAGGCCGAGTCAAGAC-3': SEQ ID NO: 13) and the OmPDI-END Rv primer (5'-TTACAACTCGTCGTGAGCC-3': SEQ ID NO: 14) designed within the gap promoter sequence. Introduction of the ero1 gene was confirmed by detecting amplification of a DNA fragment of about 1.6 kbp with the use of the GAPpforS-F primer (5'-GATCTCAGGCCGAGTCAAGAC-3': SEQ ID NO: 13) and the OmERO-END Rv

primer (5'-TTATAGCTCCAAACGATACAG-3': SEQ ID NO: 15) designed within the gap promoter sequence. Introduction of the kar2 gene was confirmed by detecting amplification of a DNA fragment of about 2 kbp with the use of the PGKp-END Fw primer (5'-TAAACACTAACGCCGCAT-3': SEQ ID NO: 16) and the OmKar-END Rv primer (5'-TCACAGCTCATCATGATCC-3': SEQ ID NO: 17) designed within the pgk promoter sequence. PCR was carried out using TaKaRa LA Taq™ with GC Buffer (RR02AG, TaKaRa Bio) to amplify a target fragment (a cycle of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 120 seconds was repeated 30 times). A transformant in which amplification of interest was observed was designated as a strain into which a chaperone gene had been introduced (ura3: pdi1/ero1/kar2 strain, NBRC10746+PEK strain).

[0072]

[Example 5] Preparation of a strain in which the prb1 gene has been interrupted

(1) Preparation of a vector for interrupting the prb1 gene

Fig. 4 shows forms of gene disruption using an internal sequence of prb1 ORF. NBRC 10746 PRB1 comprises an amino acid sequence of 539 amino acids encoded by a 1,620-bp base sequence (SEQ ID NO: 31, SEQ ID NO: 32). The pOMexPGHy plasmid described in WO 2009/057813 (a vector for foreign gene expression carrying a hygromycin B-resistant gene as a selection marker and comprising a phosphoglycerin kinase (PGK1) promoter and a terminator) was subjected to double digestion with the restriction enzymes *Hin*dIII and *Kpn*I to obtain a DNA fragment comprising the hygromycin-resistant gene marker. PCR was carried out using the genomic DNA of NBRC 10746 prepared with the use of the Y-DER Yeast DNA Extraction Reagent (78870, PIERCE) as a template, the Doprb1F primer (5'-CAAGCTTCGTTGGCAGCAGTGGAG-3': SEQ ID NO: 18) and the Doprb1R primer (5'-CGGTACCCGATGGAATCTCAGACA-3': SEQ ID NO: 19) designed within PRB1 ORF, and PrimeStarmax Polymerase (12344-024, TaKaRa Bio) at 98°C for 10 seconds, 55°C for 5 seconds, and 72°C for 5 seconds, and this cycle was repeated 30 times. Thus, a target DNA fragment of about 1.4 kbp as shown in SEQ ID NO: 20 was amplified and then cloned into pCR2.1-TOPO. The base sequence of the inserted DNA fragment was inspected, and it was confirmed to have a prb1 internal sequence. A DNA fragment comprising the prb1 internal sequence was recovered via *Hin*dIII-*Kpn*I digestion from the plasmid carrying the DNA fragment in which the base sequence had been inspected with the use of the restriction enzyme *Hin*dIII site that had been introduced into the Doprb1F primer and the restriction enzyme *Kpn*I site that had been introduced into the Doprb1R primer. The DNA fragment comprising the prb1 internal sequence was introduced into the DNA fragment comprising the hygromycin-resistant gene marker to obtain the pdPRB1 plasmid.

[0073]

(2) Preparation of a strain in which the prb1 gene has been interrupted

The pdPRB1 plasmid was introduced into the NBRC 10746 strain via electroporation. The pdPRB1 was digested with the restriction enzyme *Age*I, followed by ethanol precipitation, and the resultant DNA (5 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing hygromycin B at a final concentration of 200 μg/ml, and transformants into which the pdPRB1 plasmid had been introduced were selected via colony PCR. Some yeast strains that had proliferated on the casamino acid-U-A agar medium containing hygromycin B at a final concentration of 200 μg/ml were suspended in 10 μl of a 0.25% (w/v) SDS solution, 90 μl of sterile water was added thereto, and strains were then removed via centrifugation at 3,100 × g and 4°C for 5 minutes. The resulting supernatant as a DNA solution was inspected with the use of the M13 RV primer (5'-CAGGAAACAGCTATGAC-3': SEQ ID NO: 21) designed within the PRB1 ORF sequence and the dprb1 check rv primer (5'-CTAATCGAACAAATCAGCAACC-3': SEQ ID NO: 22) designed within the pdPRB1 sequence, and a strain in which DNA amplification of about 1.5 kbp was observed was identified. Also, the DNA solution was inspected with the use of the dprb1 check fw primer (5'-ATGAAGTTATCCCAGTCTGCTG-3': SEQ ID NO: 23) designed within the prb1 ORF sequence and the Hyg-t primer (5'-CAAAGGAATAGATCCCCCAT-3': SEQ ID NO: 24) designed in the hygromycin-resistant gene within the pdPRB1 sequence, and a strain in which DNA amplification of about 2 kbp was observed was identified. These identified strains were designated as strains in which the prb1 gene had been interrupted (prb1::hyg, dprb1 strain).

[0074]

[Example 6] Preparation of a strain into which a chaperone gene has been introduced and in which the prb1 gene has been interrupted

The pdPRB1 plasmid was introduced into the NBRC10746+PEK strain described in Example 4 via electroporation. The pdPRB1 was digested with the restriction enzyme *Age*I, followed by ethanol precipitation, and the resultant DNA (5 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing

hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing hygromycin B at a final concentration of 200 μg/ml, and transformants into which the pdPRB1 plasmid had been introduced were selected via colony PCR. Some yeast strains that had proliferated on the casamino acid-U-A agar medium containing hygromycin B at a final concentration of 200 μg/ml were suspended in 10 μl of a 0.25% (w/v) SDS solution, 90 μl of sterile water was added thereto, and strains were then removed via centrifugation at 3,100 × g and 4°C for 5 minutes. The resulting supernatant as a DNA solution was inspected with the use of the M13 RV primer (5'-CAGGAAACAGCTATGAC-3': SEQ ID NO: 21) designed within the prb1 ORF sequence and the dprb1 check rv primer (5'-CTAATCGAACAAATCAGCAACC-3': SEQ ID NO: 22) designed within the pdPRB1 sequence, and a strain in which DNA amplification of about 1.5 kbp was observed was identified. Also, the DNA solution was inspected with the use of the dprb1 check fw primer (5'-ATGAAGTTATCCCAGTCTGCTG-3': SEQ ID NO: 23) designed within the prb1 ORF sequence and the Hyg-t primer (5'-CAAAGGAATAGATCCCCCAT-3': SEQ ID NO: 24) designed in the hygromycin-resistant gene within the pdPRB1 sequence, and a strain in which DNA amplification of about 2 kbp was observed was identified. These identified strains were designated as strains into which a chaperone had been introduced and the prb1 gene had been interrupted (ura3::pdi1/ero1/kar2 prb1::hyg, NBRC10746+PEK dprb1 strains).

[0075]

[Example 7] Preparation of a strain in which the aox1 gene has been disrupted and the chaperone has been introduced

The onaP11007 plasmid was introduced into the Δaox1 strain described in Example 3 via electroporation. The onaP11007 was digested with the restriction enzyme *Not*I, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar), and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium, and transformants into which a chaperone gene had been introduced were selected via colony PCR. Some yeast strains that had proliferated on the casamino acid-U-A agar medium were suspended in 10 μl of a 0.25% SDS solution, 90 μl of sterile water was added thereto, and strains were then removed via centrifugation at 3,100 × g and 4°C for 5 minutes.

[0076]

The resulting supernatant as a DNA solution was inspected in the manner described below. That is, introduction of the pdi1 gene was confirmed by detecting amplification of a DNA fragment of about 1.6 kbp with the use of the GAPpforS-F primer (5'-GATCTCAGGCCGAGTCAAGAC-3': SEQ ID NO: 13) and the OmPDI-END Rv primer (5'-TTACAACTCGTCGTGAGCC-3': SEQ ID NO: 14) designed within the gap promoter sequence. Introduction of the ero1 gene was confirmed by detecting amplification of a DNA fragment of about 1.6 kbp with the use of the GAPpforS-F primer (5'-GATCTCAGGCCGAGTCAAGAC-3': SEQ ID NO: 13) and the OmERO-END Rv primer (5'-TTATAGCTCCAAACGATACAG-3': SEQ ID NO: 15) designed within the gap promoter sequence. Introduction of the kar2 gene was confirmed by detecting amplification of a DNA fragment of about 2 kbp with the use of the PGKp-END Fw primer (5'-TAAACACTAACGCCGCAT-3': SEQ ID NO: 16) and the OmKar-END Rv primer (5'-TCACAGCTCATCATGATCC-3': SEQ ID NO: 17) designed within the pgk promoter sequence. PCR was carried out using TaKaRa LA Taq™ with GC Buffer (RR02AG, TaKaRa Bio) to amplify a target fragment (a cycle of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 120 seconds was repeated 30 times). Transformants in which amplification of interest was observed were designated as strains in which the aox1 gene had been disrupted and the chaperone gene had been introduced (Δaox1, ura3::pdi1/ero1/kar2, Δaox1+PEK strains).

[0077]

[Example 8] Preparation of a strain in which the aox1 gene has been disrupted, the chaperone has been introduced, and the prb1 gene has been interrupted

The pdPRB1 plasmid was introduced into the Δaox1+PEK strain described in Example 7 via electroporation. The pdPRB1 plasmid was digested with the restriction enzyme *Age*I, followed by ethanol precipitation, and the resultant DNA (5 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing hygromycin B at a final concentration of 200 μg/ml, and transformants into which the pdPRB1 plasmid had been introduced were selected via colony PCR. Some yeast strains that had proliferated on the casamino acid-U-A agar medium containing hygromycin B at a final concentration of 200

μg/ml were suspended in 10 μl of a 0.25% (w/v) SDS solution, 90 μl of sterile water was added thereto, and strains were then removed via centrifugation at 3,100 × g and 4°C for 5 minutes.

[0078]

The resulting supernatant as a DNA solution was inspected with the use of the M13 RV primer (5'-CAGGAAACAGCTATGAC-3': SEQ ID NO: 21) designed within the prb1 ORF sequence and the dprb1 check rv primer (5'-CTAATCGAACAAATCAGCAACC-3': SEQ ID NO: 22) designed within the pdPRB1 sequence, and a strain in which DNA amplification of about 1.5 kbp was observed was identified. Also, the DNA solution was inspected with the use of the dprb1 check fw primer (5'-ATGAAGTTATCCCAGTCTGCTG-3': SEQ ID NO: 23) designed within the prb1 ORF sequence and the Hyg-t primer (5'-CAAAGGAATAGATCCCCCAT-3': SEQ ID NO: 24) designed in the hygromycin-resistant gene within the pdPRB1 sequence, and a strain in which DNA amplification of about 2 kbp was observed was identified. These identified strains were designated as strains in which the aox1 gene had been disrupted, into which the chaperone had been introduced, and in which the prb1 gene had been interrupted (Δaox1, ura3::pdi1/ero1/kar2, prb1::hyg, Δaox1+PEK dprb1 strains).

[0079]

[Example 9] Amount of KEX2 protein secretion induced by methanol at deep well plate scale

(1) Preparation of kex2 expression plasmid

The KEX2 protein derived from *S. cerevisiae* (GenBank Accession Number M22870.1) comprises an amino acid sequence of 814 amino acid residues (SEQ ID NO: 33). In order to express an α-factor pre-sequence, an α-factor pro-sequence, KEX2 (amino acids 24 to 660 of SEQ ID NO: 33), and His9-tag with 9 His tags in the form of a fusion protein, the base sequence as shown in SEQ ID NO: 25 was artificially synthesized at Life technologies in accordance with the codon usage frequency of *P. pastoris*. A vector comprising the artificially synthesized base sequence was digested with the restriction enzymes *Xba*I and *Bam*HI, and the resultant was introduced into a fragment obtained by digesting pOMEA-Z1 prepared in Example 1 with the restriction enzymes *Xba*I and *Bam*HI. The resulting plasmid was designated as the kex2 expression plasmid (pOMEA-Z1-KEX2) (Fig. 5).

[0080]

(2) Preparation of KEX2-producing strain

(2-1) Preparation of KEX2-producing strain derived from chaperone-introduced strain

The kex2 expression plasmid was introduced into the NBRC10746+PEK strain described in Example 4 via electroporation. The kex2 expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base w/o amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing Zeocin at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml.

[0081]

Strains capable of high levels of production of KEX2 were selected by conducting culture in the manner described below. A 2xYP-P6-dp medium [wherein the 2xYP-P6-dp medium was prepared by dissolving 20 g of a yeast extract and 40 g of peptone in 900 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 100 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH), 10 ml of a separately sterilized 50% glucose solution, and 6.25 ml of separately sterilized 80% glycerin] was used. The 2xYP-P6-dpn medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol [wherein the 2xYP-P6-dpn medium was prepared by dissolving 20 g of a yeast extract and 40 g of peptone in 900 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 100 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH)] was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a KEX2-producing sample. Quantitative assays of the KEX2 that had been secreted and produced were performed in accordance with the dot blot technique. An SDS-PAGE buffer (SDS/β-mercaptoethanol) was added, the reaction was allowed to proceed at 100°C for 5 minutes, 1 μl of the resulting culture supernatant was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the KEX2-producing strain was selected via luminescence detection using the ECL

Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled penta-His-specific antibody (Penta-His HRP Conjugate Kit, 34460, QIAGEN). The selected KEX2-producing strain was designated as the KEX2-producing strain derived from the chaperone-introduced strain (ura3::pdi1/ero1/kar2, NBRC10746+PEK strain).

[0082]

(2-2) Preparation of KEX2-producing strain derived from a strain into which a chaperone had been introduced and in which the prb1 gene had been interrupted

The kex2 expression plasmid was introduced into the NBRC10746+PEK dprb1 strain described in Example 6 via electroporation. The kex2 expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml.

[0083]

Strains capable of high levels of production of KEX2 were selected by conducting culture in the manner described below. The 2xYP-P6-dpn medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a KEX2-producing sample. Quantitative assays of the KEX2 that had been secreted and produced were performed in accordance with the dot blot technique. An SDS-PAGE buffer (SDS/β-mercaptoethanol) was added, the reaction was allowed to proceed at 100°C for 5 minutes, 1 μl of the resulting culture supernatant was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the KEX2-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled penta-His-specific antibody (Penta-

His HRP Conjugate Kit, 34460, QIAGEN). The selected KEX2-producing strain was designated as the KEX2-producing strain derived from the strain into which a chaperone had been introduced and in which the prb1 gene had been interrupted (ura3::pdi1/ero1/kar2, prb1::hyg, NBRC10746+PEK dprb1).

[0084]

(2-3) Preparation of KEX2-producing strain derived from a strain in which the aox1 gene had been disrupted and into which the chaperone had been introduced

The kex2 expression plasmid was introduced into the Δaox1+PEK strain described in Example 7 via electroporation. The kex2 expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml.

[0085]

Strains capable of high levels of production of KEX2 were selected by conducting culture in the manner described below. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 5%(w/v) glycerin was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a KEX2-producing sample. Quantitative assays of the KEX2 that had been secreted and produced were performed in accordance with the dot blot technique. The Tris-SDS β-ME sample treatment solution (5 μl, 423437, Cosmo Bio, Co., Ltd.) was added to 5 μl of the culture supernatant, 1 μl of the culture supernatant resulting from a reaction conducted at 100℃ for 5 minutes was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the KEX2-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled penta-His-specific antibody (Penta-His HRP Conjugate Kit, 34460, QIAGEN). The selected KEX2-producing strain was designated as the

KEX2-producing strain derived from the strain in which the aox1 gene had been disrupted and the chaperone had been introduced (Δaox1, ura3::pdi1/ero1/kar2, Δaox1+PEK strain).
[0086]

(2-4) Preparation of KEX2-producing strain derived from a strain in which the aox1 gene had been disrupted, into which the chaperone had been introduced, and in which the prb1 gene had been interrupted

The kex2 expression plasmid was introduced into the Δaox1+PEK dprb1 strain described in Example 8 via electroporation. The kex2 expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml.
[0087]

Strains capable of high levels of production of KEX2 were selected by conducting culture in the manner described below. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 5% glycerin was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a KEX2-producing sample. Quantitative assays of the KEX2 that had been secreted and produced were performed in accordance with the dot blot technique. The Tris-SDS β-ME sample treatment solution (5 μl, 423437, Cosmo Bio, Co., Ltd.) was added to 5 μl of the culture supernatant, 1 μl of the culture supernatant resulting from a reaction conducted at 100℃ for 5 minutes was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the KEX2-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled penta-His-specific antibody (Penta-His HRP

Conjugate Kit, 34460, QIAGEN). The selected KEX2-producing strain was designated as the KEX2-producing strain derived from the strain in which the aox1 gene had been disrupted, into which the chaperone had been introduced, and in which the prb1 gene had been interrupted (Δaox1, ura3::pdi1/ero1/kar2, prb1::hyg, Δaox1+PEK dprb1 strain).

[0088]

(3) Comparison of secretory production amounts of KEX2

The KEX2-secreting and producing strains obtained above were applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml (in the case of dprb1-derived strain, contaning Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml), and the strains were allowed to proliferate at 28°C for about 2 days. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days. In the case of the NBRC10746+PEK- and NBRC10746+PEK dprb1-derived strains, thereafter, 100 μl of the 2xYP-P6-dpn medium containing 40 μM of rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM of rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was further added 3 days after the initiation of culture. In the case of the Δaox1+PEK- and Δaox1+PEK dprb1-derived strains, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 5%(w/v) glycerin was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a KEX2-producing sample.

[0089]

The KEX2-producing samples were compared by removing the N-linked sugar chain added to the produced KEX2 with the aid of a sugar chain cleavage enzyme (Endo H, P0702S, NEW ENGLAND Bio Labs) and observing the band intensities of the samples via SDS-PAGE/CBB staining. Band intensity was determined by photographing the SDS-PAGE gel using a Light-Capture (ATTO) and analyzing the photographs using analytical software (Cool Saver 2, Version 1.01.1058, ATTO). As shown in Fig. 6-1, the control sample (NBRC10746+PEK strain) exhibited a band intensity of 81178, and the NBRC10746+PEK dprb1 strain exhibited a band intensity of 93277. That is, the NBRC10746+PEK dprb1 strain exhibited improvement in secretory production that was about 1.1 times greater than that of

the control sample. Meanwhile, the Δaox1+PEK dprb1 strain exhibited a band intensity of 143819 with the addition of methanol at a lower concentration than that in the case of the NBRC10746+PEK strain and the NBRC10746+PEK dprb1 strain. That is, the Δaox1+PEK dprb1 strain exhibited improvement in secretory production that was about 1.8 times greater than that of the control sample. In particular, the Δaox1+PEK dprb1 strain exhibited high-level secretory productivity with the addition of methanol at a lower concentration than that in the case of the control sample.

[0090]

[Example 10] Comparison of KEX2 protein enzyme activity

Fig. 6-2 shows enzyme activity of KEX2 secreted and produced in Example 9. Enzyme activity was evaluated by fluorescence detection of AMC (7-amino-4-methylcoumarin) released upon the reaction of KEX2 with Boc-Leu-Arg-Arg-MCA (4-methylcoumaryl-7-amide) (#3140-v, Peptide Institute, Inc.) as a substrate. The strains were removed from the culture supernatant via centrifugation at 3,100 × g and 4°C for 5 minutes, and the resulting culture supernatant was designated as a KEX2-producing sample. After the sample had been adequately diluted with 100 mM Tris (pH 7.0), 100 μl of the diluted sample was mixed with 100 μl of the substrate solution (400 mM Tris (pH 7.0), 2 mM $CaCl_2$, 0.2% lubrol, 100 μM BOC-Leu-Arg-Arg-MCA), the mixture was subjected to reaction at 28°C for 30 minutes, and 50 μl of a reaction terminator (5 mM EGTA (Na)) was added to terminate the reaction. Thereafter, AMC was quantified using a fluorescence plate reader (excitation wavelength: 355 nm; measurement wavelength: 460 nm). The AMC standard sample (#3099-v, manufactured by Peptide Institute, Inc.) was diluted and subjected to detection in the same manner, so as to prepare a calibration curve, and the KEX2 protease activity in the sample was determined. A unit of KEX2 activity was defined as the KEX2 content that releases 1 pmol of AMC every minute under the reaction conditions described above. The Δaox1+PEK dprb1 strain exhibited improvement in enzyme activity that was ▪BR>▪.8 times greater than that of the control (the NBRC10746+PEK strain).

[0091]

[Example 11] Amount of HSA protein secretion induced by methanol at deep well plate scale

(1) Preparation of hsa expression plasmid

HSA (human serum albumin) (GenBank Accession Number NP000468) comprises an amino acid sequence of 609 amino acids (SEQ ID NO: 34). In order to express an α-factor pre-sequence and an α-factor pro-sequence derived from *S. cerevisiae* and HSA in the form of a fusion protein, DNA as shown in SEQ ID NO: 26 was artificially synthesized at Life technologies in accordance with the codon usage frequency of *P. pastoris*. A vector comprising the artificially synthesized DNA was digested with the restriction enzymes *Xba*I

and *Bam*HI, and the resultant was introduced into a fragment obtained by digesting pOMEA-Z1 with the restriction enzymes *Xba*I and *Bam*HI. The resulting plasmid was designated as the hsa expression plasmid (pOMEA-Z1-HSA) (Fig. 7).

[0092]

(2) Preparation of HSA-producing strain

(2-1) Preparation of HSA-producing strain derived from chaperone-introduced strain

The hsa expression plasmid was introduced into the NBRC10746+PEK strain described in Example 4 via electroporation. The hsa expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base W/O amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing Zeocin at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml.

[0093]

Strains capable of high levels of production of HSA were selected by conducting culture in the manner described below. A 2xYP-P6-dp medium [wherein the 2xYP-P6-dp medium was prepared by dissolving 20 g of a yeast extract and 40 g of peptone in 900 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 100 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH), 10 ml of a separately sterilized 50% glucose solution, and 6.25 ml of separately sterilized 80% glycerin] was used. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol [wherein the 2xYP-P6-dp medium was prepared by dissolving 20 g of a yeast extract and 40 g of peptone in 900 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 100 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH)] was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g

and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a HSA-producing sample. Quantitative assays of the HSA that had been secreted and produced were performed in accordance with the dot blot technique. The Tris-SDS b-ME sample treatment solution (5 μl, 423437, Cosmo Bio, Co., Ltd.) was added to 5 μl of the culture supernatant, 1 μl of the culture supernatant resulting from a reaction conducted at 100℃ for 5 minutes was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the HSA-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled human albumin-specific antibody (Goat anti-Human Albumin-HRP Conjugated) (A80-129P, BETHYL). The selected HSA-producing strain was designated as the HSA-producing strain derived from the chaperone-introduced strain (ura3::pdi1/ero1/kar2, NBRC10746+PEK strain).

[0094]

(2-2) Preparation of HSA-producing strain derived from a strain into which a chaperone had been introduced and in which the prb1 gene had been interrupted

The hsa expression plasmid was introduced into the NBRC10746+PEK dprb1 strain described in Example 6 via electroporation. The hsa expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml.

[0095]

Strains capable of high levels of production of HSA were selected by conducting culture in the manner described below. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was further added 3 days after the initiation of culture. The strains were removed

from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a HSA-producing sample. Quantitative assays of the HSA that had been secreted and produced were performed in accordance with the dot blot technique. An SDS-PAGE buffer (SDS/β-mercaptoethanol) was added, the reaction was allowed to proceed at 100°C for 5 minutes, 1 μl of the resulting culture supernatant was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the HSA-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled human albumin-specific antibody (Goat anti-Human Albumin-HRP Conjugated) (A80-129P, BETHYL). The selected HSA-producing strain was designated as the HSA-producing strain derived from the strain into which a chaperone had been introduced and the prb1 gene had been interrupted (ura3::pdi1/ero1/kar2, prb1::hyg, NBRC10746+PEK dprb1).

[0096]

(2-3) Preparation of HSA-producing strain derived from a strain in which the aox1 gene had been disrupted and into which the chaperone had been introduced

The hsa expression plasmid was introduced into the Δaox1+PEK strain described in Example 7 via electroporation. The hsa expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml.

[0097]

Strains capable of high levels of production of HSA were selected by conducting culture in the manner described below. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 5%(w/v) glycerin was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes

4 days after the initiation of culture, and the resulting culture supernatant was designated as a HSA-producing sample. Quantitative assays of the HSA that had been secreted and produced were performed in accordance with the dot blot technique. The Tris-SDS β-ME sample treatment solution (5 μl, 423437, Cosmo Bio, Co., Ltd.) was added to 5 μl of the culture supernatant, 1 μl of the culture supernatant resulting from a reaction conducted at 100℃ for 5 minutes was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the HSA-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled human albumin-specific antibody (Goat anti-Human Albumin-HRP Conjugated) (A80-129P, BETHYL). The selected HSA-producing strain was designated as the HSA-producing strain derived from the strain in which the aox1 gene had been disrupted and into which the chaperone had been introduced (Δaox1, ura3::pdi1/ero1/kar2, Δaox1+PEK strain).

[0098]

(2-4) Preparation of HSA-producing strain derived from a strain in which the aox1 gene had been disrupted, into which the chaperone had been introduced, and in which the prb1 gene had been interrupted

The hsa expression plasmid was introduced into the Δaox1+PEK dprb1 strain described in Example 8 via electroporation. The hsa expression plasmid was digested with the restriction enzyme *Bgl*II, followed by ethanol precipitation, and the resultant DNA (1 μg) was used. After electroporation had been conducted under the conditions described in Example 2, the resultant was applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml, and it was then allowed to proliferate at 28°C for about 2 or 3 days. The proliferated transformants were allowed to proliferate again on the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml.

[0099]

Strains capable of high levels of production of HSA were selected by conducting culture in the manner described below. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added,

and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 5% glycerin was further added 3 days after the initiation of culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a HSA-producing sample. Quantitative assays of the HSA that had been secreted and produced were performed in accordance with the dot blot technique. An SDS-PAGE buffer (SDS/β-mercaptoethanol) was added, the reaction was allowed to proceed at 100°C for 5 minutes, 1 μl of the resulting culture supernatant was added dropwise to a nitrocellulose membrane to adsorb the proteins in the culture supernatant, and the HSA-producing strain was selected via luminescence detection using the ECL Select™ Western Blotting Detection Reagent (RPN2235, GE Healthcare) and the peroxidase-labeled human albumin-specific antibody (Goat anti-Human Albumin-HRP Conjugated) (A80-129P, BETHYL). The selected HSA-producing strain was designated as the HSA-producing strain derived from the strain in which the aox1 gene had been disrupted, into which the chaperone had been introduced, and in which the prb1 gene had been interrupted (Δaox1, ura3::pdi1/ero1/kar2, prb1::hyg, Δaox1+PEK dprb1 strain).

[0100]

(3) Comparison of secretory production amounts of HSA

The HSA-secreting and producing strains obtained above were applied onto the casamino acid-U-A agar medium containing Zeocin at a final concentration of 200 μg/ml (in the case of the dprb1-derived strain, contaning Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml), and the strains were allowed to proliferate at 28°C for about 2 days. The 2xYP-P6-dp medium (800 μl) was introduced into a 96-deep well plate (780271, Greiner), the strains were introduced thereinto using a toothpick, and the upper part of the plate was sealed with CO_2 permeable plate seal (676051, Greiner). Culture was conducted at an agitation speed of 310 rpm, an amplitude of 25 mm, and a temperature of 28°C for 2 days. In the case of the NBRC10746+PEK- and NBRC10746+PEK dprb1-derived strains, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was further added 3 days after the initiation of culture. In the case of theΔaox1+PEK- and Δaox1+PEK dprb1-derived strains, 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added, and 100 μl of the 2xYP-P6-dpn medium containing 40 μM rhodanine-3-acetic acid derivative 1c and 5%(w/v) glycerin was further added 3 days after the initiation of

culture. The strains were removed from the culture solution via centrifugation at 3,100 × g and 4°C for 5 minutes 4 days after the initiation of culture, and the resulting culture supernatant was designated as a HSA-producing sample. The samples were compared based on the band intensity obtained from SDS-PAGE/CBB staining. Band intensity was determined by photographing the SDS-PAGE gel using a Light-Capture (ATTO) and analyzing the photographs using analytical software (Cool Saver 2, Version 1.01.1058, ATTO). As shown in Fig. 8, the control sample (NBRC10746+PEK strain) exhibited a band intensity of 1585061, and the NBRC10746+PEK dprb1 strain exhibited a band intensity of 2882752. That is, the NBRC10746+PEK dprb1 strain exhibited improvement in secretory production that was about 1.8 times greater than that of the control sample. The Δaox1+PEK strain exhibited a band intensity of 2111007, which is 1.3 times greater than that of the control sample. The Δaox1+PEK dprb1 strain exhibited a band intensity of 3041627, which is 1.9 times greater than that of the control sample and the greatest improvement among these strains. The Δaox1+PEK strain, especially, the Δaox1+PEK dprb1 strain exhibited high secretory productivity with the addition of methanol at a lower concentration than the case of the control sample (i.e., the NBRC10746+PEK strain).

[0101]

[Example 12] Amount of secretion and production of HSA protein induced by methanol at 3-L aeration-agitation culture scale

The HSA-producing strains obtained in Example 11 (NBRC10746+PEK strain and Δaox1+PEK dprb1 strain) were compared in terms of the secretory production amount of the HSA protein induced by methanol at 3-L aeration-agitation culture scale. 3-L aeration-agitation culture was conducted in the manner described below. The HSA-producing strains obtained in Example 11 (NBRC10746+PEK strain and Δaox1+PEK dprb1 strain) were applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base W/O amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing Zeocin at a final concentration of 200 μg/ml (in the case of the dprb1-derived strain, containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml), and the strains were allowed to proliferate at 28°C for about 2 days. The proliferated strains were inoculated into a 50-ml polypropyrene centrifuge tube (227241, Greiner) containing 5 ml of the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base W/O amino acids, 0.5 g/l casamino acid, 20 g/l glucose, and 20 mg/l L-tryptophan) containing Zeocin at a final concentration of 25 μg/ml (in the case of the dprb1-derived strain, contaning Zeocin at a final concentration of 25 μg/ml and hygromycin B at a final concentration of 50 μg/ml), and the upper part of the centrifuge tube was sealed with

plate seal (676051, Greiner). The reciprocal shake culture was conducted at an agitation speed of 250 rpm, an amplitude of 25 mm, and a temperature of 28°C for 24 hours, and the resulting culture solution was designated as the first-type culture solution.

[0102]

Subsequently, 10 ml of the first-type culture solution was inoculated into a 500-ml baffle flask (355123, BD Falcon) containing 40 ml of the 2x YP-P6 seed medium containing Zeocin at a final concentration of 25 μg/ml (in the case of the dprb1-derived strain, containing Zeocin at a final concentration of 25 μg/ml and hygromycin B at a final concentration of 50 μg/ml) [wherein the 2x YP-P6 seed medium was prepared by dissolving 20 g of a yeast extract and 40 g of peptone in 900 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 100 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH), 12.5 ml of a separately sterilized 50% glucose solution, and 62.5 ml of separately sterilized 80% glycerin] was applied onto the medium containing Zeocin at a final concentration of 25 μg/ml and hygromycin B at a final concentration of 50 μg/ml), and the upper part was sealed with plate seal (676051, Greiner). The reciprocal shake culture was conducted at an agitation speed of 180 rpm, an amplitude of 50 mm, and a temperature of 28°C for 24 hours, and the resulting culture solution was designated as the second-type culture solution.

[0103]

Subsequently, 60 ml of the second-type culture solution was inoculated into a 3-liter jar fermentor (BMS 03PI and BMS-03PII; ABLE Corporation) containing the 3x YP-P6 medium, which was prepared by dissolving 36 g of a yeast extract and 72 g of peptone in 1080 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 120 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH), 12 ml of a separately sterilized 50%(w/v) glucose solution, 60 ml of separately sterilized 80%(w/v) glycerin, and 100 μg of a defoaming agent (CB-442) (1.2 ml of 50 mg/ml hygromycin B was added in the case of the dprb1-derived strain). Culture was conducted at the culture temperature of 28°C , the internal pressure of 0.1 MPa, and DO of 2 ppm (automatically regulated via agitation). The feeding of an aqueous solution of 50%(w/v) glycerin was initiated 8 hours after the initiation of culture and the feeding was continued at 3m/hr up to 24 hours after the initiation of culture. After the depletion of glycerin from the medium was confirmed, 780 μl of the 40 mM rhodanine-3-acetic acid derivative 1c was added.

[0104]

Subsequently, methanol-induced culture was conducted in the manner described below. The control sample (NBRC10746+PEK strain: Jar1) was subjected to methanol-induced culture in a medium prepared by adding 32 ml of sterile water and 12 ml of 100% methanol to the medium that was confirmed to have been deprived of glycerin. The addition of 20 ml of sterile water and 86 ml of 100% methanol per day was initiated 2 hours after the initiation of methanol-induced culture and continued until the end of the culture period. The NBRC10746+PEK strain (Jar2) and the Δaox1+PEK dprb1 strain (Jar3) were subjected to pH-controlled, nitrogen-source-fed, and methanol-induced culture in the manner described below. The NBRC10746+PEK strain (Jar2) was subjected to methanol-induced culture in the medium that was confirmed to have been deprived of glycerin by adding 32 ml of 10%(w/v) L-histidine monohydrochloride monohydrate, initiating automatic control of the pH level with 14% (v/v) ammonia water, and adding 12 ml of 100% methanol. The Δaox1+PEK dprb1 strain (Jar3) was subjected to methanol-induced culture in the medium that was confirmed to have been deprived of glycerin by adding 32 ml of 10%(w/v) L-histidine monohydrochloride monohydrate and 1.2 ml of 50 mg/ml hygromycin B, initiating automatic control of the pH level with 14% (v/v) ammonia water, and adding 6.5 ml of 100% methanol. In the case of the NBRC10746+PEK strain (Jar2), the pH level of the culture solution was adjusted to 6.75 three hours after the initiation of methanol-induced culture and it was adjusted to 7 six hours after the initiation of culture and thereafter. Immediately after the initiation of methanol-induced culture, the feeding of 20 ml of the nitrogen source feeding solution (300 g/l yeast extract and 80 g/l L-histidine monohydrochloride monohydrate) and 86 ml of 100% methanol per day was initiated and it was continued until the end of the culture period. In the case of the Δaox1+PEK dprb1 strain (Jar3), the pH level of the culture solution was adjusted to 6.75 three hours after the initiation of methanol-induced culture and it was adjusted to 7 six hours after the initiation of culture and thereafter. Further, the feeding of 20 ml of a nitrogen source feeding solution, 30 ml of 80%(w/v) glycerin, and 56 ml of sterile water per day was initiated immediately after the initiation of methanol-induced culture and it was continued until the end of the culture period. Also, 1.2 ml of 50 mg/ml hygromycin B and methanol at a final concentration of 0.2-0.5%(v/v) were intermittently fed per day from 24 hours after the initiation of methanol-induced culture. From 9 days after the initiation of methanol-induced culture, 100 ml of the culture was allowed to overflow every day.

[0105]

The culture solutions were subjected to centrifugation at 3,000 × g and 4°C for 5 minutes to remove the strains, and the resulting culture supernatant was designated as a HSA-producing sample. Quantitative assays of HSA secreted and produced were carried out by

comparing the samples based on the band intensity obtained by SDS-PAGE/CBB staining. Band intensity was determined by photographing the SDS-PAGE gel using a Light-Capture (ATTO) and analyzing the photographs using analytical software (Cool Saver 2, Version 1.01.1058, ATTO). HSA was quantified on the basis of the calibration curve indicating the band intensity of the Albumin Standard (232209, Thermo scientific) as the standard sample.
[0106]

Fig. 9 shows the results of quantification of the secretory production amount of HSA. The control sample (NBRC10746+PEK strain: Jar1) exhibited the productivity peak 4 or 5 days after the initiation of methanol-induced culture. In the case of pH-controlled and nitrogen source fed-batch culture, the productivity peak of the NBRC10746+PEK strain (Jar2) was observed 2 or 3 days after the initiation of methanol-induced culture, and degradation of the secreted and produced HSA became significant 4 days after the initiation of culture and thereafter. In contrast, productivity of the Δaox1+PEK dprb1 strain (Jar3) was maintained up to 21 days after the initiation of culture, degradation was not substantially observed, and it was thus found to be a protein production system capable of long-term culture. In the case of pH-controlled and nitrogen source fed-batch culture, the amount of HSA secreted and produced by the NBRC10746+PEK strain (Jar2) was improved by about 1.4 times compared with the control sample (NBRC10746+PEK strain: Jar1) (improved from 2486 mg/l to 3594 mg/l). Secretory productivity was improved by about 2.9 times in the case of the Δaox1+PEK dprb1 strain (Jar3) (improved from 2486 mg/l to 7104 mg/l). The amount of methanol added when culturing the Δaox1+PEK dprb1 strain (Jar3) was reduced to about 1/17 (5.8%) that of the control sample (NBRC10746+PEK strain: Jar1) and the NBRC10746+PEK strain (Jar2) up to 7 days after the initiation of methanol-induced culture. Accordingly, the Δ aox1+PEK dprb1 strain (Jar3) that had been subjected to pH-controlled and nitrogen source fed-batch culture was found to have realized a significant reduction of the amount of methanol added and high-level secretory productivity, compared with the control sample (NBRC10746+PEK strain: Jar1) and the NBRC10746+PEK strain (Jar2).
[0107]

[Example13] Secretory productivity of HSA protein under carbon source-starvation-induction at 3-L aeration-agitation culture scale

The amount of the HSA protein secreted and produced by the HSA-producing strain derived from the Δaox1+PEK dprb1 strain obtained in Example 11 under carbon source-starvation-induction was compared with that under methanol-induction at the 3-L aeration-agitation culture scale. 3-L aeration-agitation culture was conducted in the manner described below. The HSA-producing strain derived from the Δaox1+PEK dprb1 strain

obtained in Example 11 was applied onto the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base W/O amino acids, 0.5 g/l casamino acid, 20 g/l glucose, 20 mg/l L-tryptophan, and 20 g/l agar) containing Zeocin at a final concentration of 200 μg/ml and hygromycin B at a final concentration of 200 μg/ml, and the strains were allowed to proliferate at 28°C for about 2 days. The proliferated strains were inoculated into a 50-ml polypropyrene centrifuge tube (227241, Greiner) containing 5 ml of the casamino acid-U-A agar medium (6.7 g/l yeast nitrogen base W/O amino acids, 0.5 g/l casamino acid, 20 g/l glucose, and 20 mg/l L-tryptophan) containing Zeocin at a final concentration of 25 μg/ml and hygromycin B at a final concentration of 50 μg/ml, and the upper part was sealed with plate seal (676051, Greiner). The reciprocal shake culture was conducted at an agitation speed of 250 rpm, an amplitude of 25 mm, and a temperature of 28°C for 24 hours, and the resulting culture solution was designated as the first-type culture solution.

[0108]

Subsequently, 10 ml of the first-type culture solution was inoculated into a 500-ml baffle flask (355123, BD Falcon) containing 40 ml of the 2x YP-P6 seed medium containing Zeocin at a final concentration of 31.25 μg/ml and hygromycin B at a final concentration of 62.5 μg/ml [wherein the 2x YP-P6 seed medium was prepared by dissolving 20 g of a yeast extract and 40 g of peptone in 900 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 100 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH), 12.5 ml of a separately sterilized 50% glucose solution, and 62.5 ml of separately sterilized 80% glycerin], and the upper part was sealed with plate seal (676051, Greiner). The reciprocal shake culture was conducted at an agitation speed of 180 rpm, an amplitude of 50 mm, and a temperature of 28°C for 24 hours, and the resulting culture solution was designated as the second-type culture solution.

[0109]

Subsequently, 75 ml of the second-type culture solution was inoculated into a 3-liter jar fermentor (BMS 03PI and BMS-03PII; ABLE Corporation) containing the 3x YP-P6 medium, which was prepared by dissolving 45 g of a yeast extract and 90 g of peptone in 1350 ml of pure water, subjecting the solution to high-pressure steam sterilization, and adding 150 ml of a separately sterilized 10x phosphate buffer (pH 6.0) (1 M KH_2PO_4, 0.15 M $(NH_4)_2SO_4$, 0.355 N KOH), 15 ml of a separately sterilized 50%(w/v) glucose solution, 75 ml of separately sterilized 80%(w/v) glycerin, 1.5 ml of 50 mg/ml hygromycin B, and 100 μg of a defoaming agent (CB-442). Culture was conducted at the culture temperature of 28°C , the internal pressure of 0.1 MPa, and DO of 2 ppm (automatically regulated via agitation). The feeding of an aqueous solution of 50%(w/v) glycerin was initiated 8 hours after the initiation

of culture and the feeding was continued at 4.3 ml/hr up to 22 hours after the initiation of culture.

[0110]

After the depletion of glycerin from the medium was confirmed, subsequently, methanol-induced culture, carbon source-starvation culture, and induction culture from the aox promoter were conducted in the manner described below. Methanol-induced culture was initiated in the medium that was confirmed to have been deprived of glycerin by adding 54 ml of sterile water, 40 ml of 10%(w/v) L-histidine monohydrochloride monohydrate, and 1.5 ml of 50 mg/ml of hygromycin B, regulating the pH level of the culture solution to 6.5 with 14%(v/v) ammonia water, and adding 8.5 ml of 100% methanol. Thereafter, the pH level was adjusted to 6.75 three hours after the initiation of methanol-induced culture and it was adjusted to 7 six and a half hours after the initiation of culture and thereafter. Immediately after the initiation of methanol-induced culture, the feeding of 12.5 ml of the nitrogen source feeding solution (300 g/l yeast extract and 80 g/l L-histidine monohydrochloride monohydrate) and 37.5 ml of 80%(w/v) glycerin per day was initiated, it was continued until the end of the culture period, and 5.1 to 5.7 ml of 100% methanol (final concentration: 0.25-0.35%(v/v)) was intermittently fed per day from 24 hours after the initiation of methanol-induced culture and thereafter. Further, 1.5 ml of 50 mg/ml hygromycin B was added 48, 96, and 144 hours after the initiation of methanol-induced culture.

[0111]

Meanwhile, starvation-induced culture comprising carbon source depletion was initiated in the medium that was confirmed to have been deprived of glycerin by adding 40 ml of 10%(w/v) L-histidine monohydrochloride monohydrate and 1.5 ml of 50 mg/ml hygromycin B, and regulating the pH level to 6.5 with 14%(v/v) ammonia water (hereafter, it is referred to as "carbon source starvation-induced culture"). The pH level was adjusted to 6.75 three hours after the initiation of carbon source starvation-induced culture and it was adjusted to 7 six and a half hours after the initiation of culture and thereafter. Immediately after the initiation of carbon source starvation-induced culture, continuous feeding of 12.5 ml of the nitrogen source feeding solution (300 g/l yeast extract and 80 g/l L-histidine monohydrochloride monohydrate) and 92 ml of 65%(w/v) sorbitol per day was initiated. The nitrogen source feeding solution (12.5 ml) and 37.5 ml of 80%(w/v) glycerin were continuously fed per day from 24 hours after the initiation of methanol-induced culture up to the end of the culture period. Further, 1.5 ml of 50 mg/ml hygromycin B was intermittently fed per day 48, 96, and 144 hours after the initiation of carbon source starvation-induced culture.

[0112]

The culture solutions were subjected to centrifugation at 3,000 × g and 4°C for 5 minutes to remove the strains, and the resulting culture supernatant was designated as a HSA-producing sample. Quantitative comparison assays of HSA secreted and produced were carried out by comparing the samples based on the SDS-PAGE/CBB staining intensity. Fig. 10 shows the results of quantification of the secretory production amount of HSA. It was found that the productivity attained via carbon source starvation-induced culture was substantially equivalent to that attained via methanol-induced culture.

Industrial Applicability

[0113]

The present invention is applicable to the field of production of protein pharmaceuticals, such as antigen or antibody.

[0114]

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

CLAIMS

1. A transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene has been disrupted.

2. The transformed yeast according to claim 1, wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (d) below:

(a) a gene encoding PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1, or HSP104 derived from *Ogataea minuta* (*O. minuta*);

(b) a gene encoding PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5, or EUG1 derived from *Saccharomyces cerevisiae* (*S. cerevisia*);

(c) a gene encoding PDI, ERO1-Lα, ERO1-Lβ, or GRP78 derived from a human; and

(d) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (c).

3. The transformed yeast according to claim 1, wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (g) below:

(a) a gene encoding PDI1 derived from *O. minuta*;

(b) a gene encoding ERO1 derived from *O. minuta*;

(c) a gene encoding Kar2 derived from *O. minuta*;

(d) a gene encoding PDI1 derived from *S. cerevisiae*;

(e) a gene encoding PDI derived from a human;

(f) a gene encoding ERO1 derived from a human; and

(g) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (f).

4. The transformed yeast according to claim 1, wherein the chaperone gene is any of the chaperone genes (a) to (g) below:

(a) a combination of a gene encoding PDI1, a gene encoding ERO1, and a gene encoding Kar2 derived from *O. minuta*;

(b) a combination of a gene encoding PDI1 and a gene encoding Kar2 derived from *O. minuta*;

(c) a combination of a gene encoding PDI derived from a human and a gene encoding ERO1 derived from *O. minuta*;

(d) a combination of a gene encoding PDI1 and a gene encoding ERO1 derived from *O. minuta*;

(e) a combination of a gene encoding PDI derived from a human, a gene encoding ERO1-Lβ derived from a human, and a gene encoding GRP78 derived from a human;

(f) a combination of a gene encoding PDI derived from a human, a gene encoding ERO1 derived from *O. minuta*, and a gene encoding GRP78 derived from a human; and

(g) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (f).

5. The transformed yeast according to any of claims 1 to 4, wherein the protease gene has been disrupted.

6. The transformed yeast according to claim 5, wherein the protease is a prb1 gene.

7. A transformed yeast into which a chaperone gene has been introduced and in which a protease gene has been disrupted.

8. The transformed yeast according to claim 7, wherein the protease is a prb1 gene.

9. The transformed yeast according to any of claims 1 to 8, wherein the yeast is a methhylotrophic yeast.

10. The transformed yeast according to any of claims 1 to 9, which comprises a gene encoding a target protein introduced thereinto.

11. Use of the transformed yeast according to any of claims 1 to 10 for the production of a target protein.

12. A method for producing a protein comprising culturing the transformed yeast according to claim 10 in a medium and sampling a target protein from the culture product.

13. The method for producing a protein according to claim 12, wherein culture is conducted under conditions in which protease activity is inhibited.

14. The method for producing a protein according to claim 12 or 13, wherein culture is conducted in a medium with a pH of 6.0 to 7.5.

15. The method for producing a protein according to any of claims 12 to 14, wherein a nitrogen source is added to the medium.

16. The method for producing a protein according to any of claims 12 to 15, wherein the amount of methanol added to the medium is 2% (v/v) or less.

17. A target protein produced by the method according to any of claims 12 to 16.

18. A method for producing a transformed yeast comprising step (i) in addition to either or both step (ii) and/or (iii):

(i) a step of introducing a chaperone gene into yeast; and

(ii) a step of disrupting the aox1 gene in yeast; and/or

(iii) a step of disrupting the prb1 gene in yeast.

19. The method of production according to claim 18, which further comprises a step of introducing a gene encoding a target protein.

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National Institute of Advanced Industrial Science and Technology

<120> An improved method for high-yield secretory production of proteins

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gagtccatcg actcctccaa gaccgagacc ttcgttttcg gtaacgacaa ggaggaggtt 2040

gagccagctg ctaccgagtc caccgtttcc caatactccg cttcctccac ctccatctcc 2100

atctccgcta cctccacctc ctccatctcc atcggtgttg agacctccgc tatcccacaa 2160

accaccaccg cttccaccga cccacaccac caccaccacc accaccacca ctaaggatcc 2220

<210> 26

<211> 2040

<212> DNA

<213> Artificial

<220>

<223> synthetic nucleotide

<400> 26

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gctgctccag ttaacactac tactgaggac gagactgctc agattccagc tgaagctgtt 120

attggttact ccgacttgga aggtgacttc gacgttgctg ttttgccatt ctccaactcc 180

actaacaacg gtttgttgtt catcaacact acaatcgctt ccattgctgc taaagaagag 240

ggagtttcct tggagaagag agaagctgag gctgacgctc acaagtctga agttgctcac 300

agattcaagg acttgggtga agagaacttc aaggctttgg ttttgatcgc tttcgctcag 360

tacttgcagc agtgtccatt cgaggatcac gttaagttgg ttaacgaggt tactgagttc 420

gctaagactt gtgttgctga cgaatccgct gagaactgtg ataagtcctt gcacactttg 480

ttcggtgaca agttgtgtac tgttgctact ttgagagaaa cttacggtga gatggctgac 540

tgttgtgcta agcaagagcc agaaagaaac gagtgtttct tgcagcacaa ggacgacaac 600

ccaaacttgc caagattggt tagaccagag gttgacgtta tgtgtactgc tttccacgac 660

aacgaagaga ctttcttgaa gaagtacttg tacgagatcg ctagaagaca cccatacttc 720

tacgctccag agttgttgtt ctttgctaag agatacaagg ctgctttcac tgagtgttgt 780

caggctgctg acaaagctgc ttgtttgttg ccaaagttgg acgagttgag agatgagggt 840

aaggcttctt ctgctaagca gagattgaag tgtgcttcct tgcagaagtt cggagagaga 900

gcttttaagg cttgggctgt tgctagattg tcccagagat ttccaaaggc tgaattcgct 960

gaggtttcca aattggttac agacttgact aaggttcaca cagaatgttg tcacggtgac 1020

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tccatctcct ccaagttgaa agaatgttgt gagaagcctt tgttggagaa gtcccactgt 1140

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gttgaatcca aggacgtttg taagaactac gctgaggcta aggatgtttt cttgggtatg 1260

ttcttgtatg aatatgctag aagacatcca gactactccg ttgttttgtt gttgagattg 1320

gctaaaactt acgagactac tttggaaaag tgttgtgctg ctgctgaccc acacgaatgt 1380

tacgctaagg ttttcgacga gttcaagcca ttggttgagg aaccacagaa cttgattaag 1440

caaaactgtg agttgttcga gcagttgggt gagtacaagt tccagaacgc tttgttggtt 1500

agatacacta agaaggttcc acaggtttcc actcctactt tggttgaagt ttccagaaac 1560

ttgggtaagg ttggttccaa gtgttgtaag cacccagagg ctaagagaat gccatgtgct 1620

gaagattact tgtctgttgt tttgaaccag ttgtgtgttt tgcacgaaaa gactccagtt 1680

tccgacagag ttacaaaatg ttgtactgag tccttggtta acagaagacc ttgtttctct 1740

gctttggaag ttgacgagac ttacgttcca aaagagttca acgctgagac tttcactttc 1800

cacgctgaca tctgtacttt gtccgagaaa gagagacaga tcaagaagca gactgctttg 1860

gttgagttgg ttaagcacaa gccaaaggct acaaaagagc aattgaaggc tgttatggac 1920

gactttgctg ctttcgttga gaaatgttgt aaggctgacg acaaagagac ttgtttcgct 1980

gaagagggta agaagttggt tgctgcttct caagctgctt tgggtttgta gtaaggatcc 2040

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<211> 1992
<212> DNA
<213> Ogataea minuta

<220>
<221> CDS
<222> (1)..(1992)

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1 5 10 15

tgc ggc tgc gcc atc gcc ggt aga ctc ggt aac ctc gac ccg gac gtt 96
Cys Gly Cys Ala Ile Ala Gly Arg Leu Gly Asn Leu Asp Pro Asp Val
20 25 30

act gtg gct ctc atc gag ggt ggt gag aac aac atc aat aac cca tgg 144
Thr Val Ala Leu Ile Glu Gly Gly Glu Asn Asn Ile Asn Asn Pro Trp
35 40 45

gtc tac ctt cct ggt gtc tat cca aga aac atg aga ctc gac tcc aag 192
Val Tyr Leu Pro Gly Val Tyr Pro Arg Asn Met Arg Leu Asp Ser Lys
50 55 60

acg gct acc ttc tac aac tcg aga cca tcc aag cac ctg aac ggc aga 240
Thr Ala Thr Phe Tyr Asn Ser Arg Pro Ser Lys His Leu Asn Gly Arg
65 70 75 80

agg gcc att gtc ccc tgc gct aac att ctt ggt gga ggt tcc tcc atc 288
Arg Ala Ile Val Pro Cys Ala Asn Ile Leu Gly Gly Gly Ser Ser Ile
85 90 95

aac ttc ctc atg tac acc aga gcc tcg gcc tcc gac tac gac gac tgg 336
Asn Phe Leu Met Tyr Thr Arg Ala Ser Ala Ser Asp Tyr Asp Asp Trp
100 105 110

gag caa gag gga tgg acc acc gac gag ctg ctt ccg ctc atg aag aag 384
Glu Gln Glu Gly Trp Thr Thr Asp Glu Leu Leu Pro Leu Met Lys Lys
115 120 125

ctc gag acg tat caa cgt cct tgc aac aac agg gag gtg cac ggt ttc 432
Leu Glu Thr Tyr Gln Arg Pro Cys Asn Asn Arg Glu Val His Gly Phe
130 135 140

gac ggt ccg atc aag gtc tcc ttc ggt aac tac acc tac cca act gcc 480
Asp Gly Pro Ile Lys Val Ser Phe Gly Asn Tyr Thr Tyr Pro Thr Ala
145 150 155 160

caa gac ttc ctg aga gcc tgc gag tcg cag ggt att cct ttc aac gac 528

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

gat ctt gaa gac ctc aag gcc tcg cac gga gct gag tac tgg ctc aag 576
Asp Leu Glu Asp Leu Lys Ala Ser His Gly Ala Glu Tyr Trp Leu Lys
180 185 190

tgg atc aac agg gat ctc ggt aga aga tcg gac tcg gca cac gcc tac 624
Trp Ile Asn Arg Asp Leu Gly Arg Arg Ser Asp Ser Ala His Ala Tyr
195 200 205

atc cac cct acc atg aga aac aag agc aat ctg ttc ctc att acg tcc 672
Ile His Pro Thr Met Arg Asn Lys Ser Asn Leu Phe Leu Ile Thr Ser
210 215 220

acc aag gct gac aag gtg atc att gag aac ggc gtt gct gtc ggt gtc 720
Thr Lys Ala Asp Lys Val Ile Ile Glu Asn Gly Val Ala Val Gly Val
225 230 235 240

agg acc gtt cca atg aag ccg gtc gag acc aaa aac cct cca agc agg 768
Arg Thr Val Pro Met Lys Pro Val Glu Thr Lys Asn Pro Pro Ser Arg
245 250 255

atc ttc aag gcc aga aag caa att gtg gtt tcg tgc ggt acg atc tcc 816
Ile Phe Lys Ala Arg Lys Gln Ile Val Val Ser Cys Gly Thr Ile Ser
260 265 270

tct cca ttg gtg ctg caa aga tct ggt atc ggt gcg gcc cac aag ctg 864
Ser Pro Leu Val Leu Gln Arg Ser Gly Ile Gly Ala Ala His Lys Leu
275 280 285

aga caa gcg ggc atc aag ccg atc gtc gac ttg cct ggt gtc ggt gag 912
Arg Gln Ala Gly Ile Lys Pro Ile Val Asp Leu Pro Gly Val Gly Glu

290 295 300

aac ttc cag gac cac tac tgc ttc ttc acc cca tac tat tcc aag cca 960
Asn Phe Gln Asp His Tyr Cys Phe Phe Thr Pro Tyr Tyr Ser Lys Pro
305 310 315 320

gag gtt cca acc ttt gac gac ttt gtc aga ggt gac cca gtc gct caa 1008
Glu Val Pro Thr Phe Asp Asp Phe Val Arg Gly Asp Pro Val Ala Gln
325 330 335

aag tcc gcc ttt gac cag tgg tac tcc aac aag gac ggt cct ctt acc 1056
Lys Ser Ala Phe Asp Gln Trp Tyr Ser Asn Lys Asp Gly Pro Leu Thr
340 345 350

acc aac ggt atc gag gct ggt gtc aag atc aga cca acc gac gag gag 1104
Thr Asn Gly Ile Glu Ala Gly Val Lys Ile Arg Pro Thr Asp Glu Glu
355 360 365

ttg gcc acg gct gac gat gac ttc atc caa ggg tac cac gag tac ttt 1152
Leu Ala Thr Ala Asp Asp Asp Phe Ile Gln Gly Tyr His Glu Tyr Phe
370 375 380

gac aac aag cca gac aag cca ctg atg cat tac tct gtc att tcc ggt 1200
Asp Asn Lys Pro Asp Lys Pro Leu Met His Tyr Ser Val Ile Ser Gly
385 390 395 400

ttc ttc ggt gac cac acc aag att cca aac ggc aag ttc ttc acc atg 1248
Phe Phe Gly Asp His Thr Lys Ile Pro Asn Gly Lys Phe Phe Thr Met
405 410 415

ttc cac ttt ttg gag tac cca ttt tcg aga ggt ttc gtt tat gct gtt 1296
Phe His Phe Leu Glu Tyr Pro Phe Ser Arg Gly Phe Val Tyr Ala Val
420 425 430

tcc cca gac cca tac gaa gct cca gac ttt gat cca ggt ttc ctg aac 1344
Ser Pro Asp Pro Tyr Glu Ala Pro Asp Phe Asp Pro Gly Phe Leu Asn
435 440 445

gat tcc aga gac atg tgg cct atg gtt tgg tct tac aag aag tcg aga 1392
Asp Ser Arg Asp Met Trp Pro Met Val Trp Ser Tyr Lys Lys Ser Arg
450 455 460

cag aca gcc aga aga atg gag tcg ttt gct ggt gaa gtc acc tcg cac 1440
Gln Thr Ala Arg Arg Met Glu Ser Phe Ala Gly Glu Val Thr Ser His
465 470 475 480

cac cca ctc tac ccg gtt gac tct cca gcc cgt gcc aag gac ttg gat 1488
His Pro Leu Tyr Pro Val Asp Ser Pro Ala Arg Ala Lys Asp Leu Asp
485 490 495

ctc gag aca tgc aag gca ttt gct gga cca aac cac ttc acc gcc aac 1536
Leu Glu Thr Cys Lys Ala Phe Ala Gly Pro Asn His Phe Thr Ala Asn
500 505 510

ttg tac cac ggt tcc tgg act gtt cca att gag aag cca acg cca aag 1584
Leu Tyr His Gly Ser Trp Thr Val Pro Ile Glu Lys Pro Thr Pro Lys
515 520 525

aac gac tcg cac gtg acc tgc aac cag gtc gag atc ttc tcc gac att 1632
Asn Asp Ser His Val Thr Cys Asn Gln Val Glu Ile Phe Ser Asp Ile
530 535 540

gac tac tct gcc gag gac gat gag gct att gtc aag tac atc aag gag 1680
Asp Tyr Ser Ala Glu Asp Asp Glu Ala Ile Val Lys Tyr Ile Lys Glu
545 550 555 560

cac act gag acc acc tgg cac tgt ttg gga acc tgt tcg atg gct cca 1728
His Thr Glu Thr Thr Trp His Cys Leu Gly Thr Cys Ser Met Ala Pro
565 570 575

caa gaa ggt agc aag atc gct cca aag ggt ggt gtt gtc gat gcc aga 1776
Gln Glu Gly Ser Lys Ile Ala Pro Lys Gly Gly Val Val Asp Ala Arg
580 585 590

ttg aac gtg tac gaa gtg aag aac ctc aag gtt gcc gac ctg tcg atc 1824
Leu Asn Val Tyr Glu Val Lys Asn Leu Lys Val Ala Asp Leu Ser Ile
595 600 605

tgc cca gat aac gtt gga tgt aat act tac tcc act gct ctt ctg att 1872
Cys Pro Asp Asn Val Gly Cys Asn Thr Tyr Ser Thr Ala Leu Leu Ile
610 615 620

ggt gag aag gct gcc act ttg gtc gcc gag gac ctg gga tac tca gga 1920
Gly Glu Lys Ala Ala Thr Leu Val Ala Glu Asp Leu Gly Tyr Ser Gly
625 630 635 640

tct gat ctc gcc atg acc att cca aac ttc aag cta ggt act tac gag 1968
Ser Asp Leu Ala Met Thr Ile Pro Asn Phe Lys Leu Gly Thr Tyr Glu
645 650 655

gag aag ggt ctg gct aga ttc taa 1992
Glu Lys Gly Leu Ala Arg Phe
660

<210> 28
<211> 663
<212> PRT
<213> Ogataea minuta

〈400〉 28

Met Ala Ile Pro Asp Glu Phe Asp Ile Ile Val Val Gly Gly Gly Ser
1 5 10 15

Cys Gly Cys Ala Ile Ala Gly Arg Leu Gly Asn Leu Asp Pro Asp Val
20 25 30

Thr Val Ala Leu Ile Glu Gly Gly Glu Asn Asn Ile Asn Asn Pro Trp
35 40 45

Val Tyr Leu Pro Gly Val Tyr Pro Arg Asn Met Arg Leu Asp Ser Lys
50 55 60

Thr Ala Thr Phe Tyr Asn Ser Arg Pro Ser Lys His Leu Asn Gly Arg
65 70 75 80

Arg Ala Ile Val Pro Cys Ala Asn Ile Leu Gly Gly Gly Ser Ser Ile
85 90 95

Asn Phe Leu Met Tyr Thr Arg Ala Ser Ala Ser Asp Tyr Asp Asp Trp
100 105 110

Glu Gln Glu Gly Trp Thr Thr Asp Glu Leu Leu Pro Leu Met Lys Lys
115 120 125

Leu Glu Thr Tyr Gln Arg Pro Cys Asn Asn Arg Glu Val His Gly Phe
130 135 140

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

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

Asp Leu Glu Asp Leu Lys Ala Ser His Gly Ala Glu Tyr Trp Leu Lys
180 185 190

Trp Ile Asn Arg Asp Leu Gly Arg Arg Ser Asp Ser Ala His Ala Tyr
195 200 205

Ile His Pro Thr Met Arg Asn Lys Ser Asn Leu Phe Leu Ile Thr Ser
210 215 220

Thr Lys Ala Asp Lys Val Ile Ile Glu Asn Gly Val Ala Val Gly Val
225 230 235 240

Arg Thr Val Pro Met Lys Pro Val Glu Thr Lys Asn Pro Pro Ser Arg
245 250 255

Ile Phe Lys Ala Arg Lys Gln Ile Val Val Ser Cys Gly Thr Ile Ser
260 265 270

Ser Pro Leu Val Leu Gln Arg Ser Gly Ile Gly Ala Ala His Lys Leu
275 280 285

Arg Gln Ala Gly Ile Lys Pro Ile Val Asp Leu Pro Gly Val Gly Glu
290 295 300

Asn Phe Gln Asp His Tyr Cys Phe Phe Thr Pro Tyr Tyr Ser Lys Pro
305 310 315 320

Glu Val Pro Thr Phe Asp Asp Phe Val Arg Gly Asp Pro Val Ala Gln
325 330 335

Lys Ser Ala Phe Asp Gln Trp Tyr Ser Asn Lys Asp Gly Pro Leu Thr
340 345 350

Thr Asn Gly Ile Glu Ala Gly Val Lys Ile Arg Pro Thr Asp Glu Glu
355 360 365

Leu Ala Thr Ala Asp Asp Asp Phe Ile Gln Gly Tyr His Glu Tyr Phe
370 375 380

Asp Asn Lys Pro Asp Lys Pro Leu Met His Tyr Ser Val Ile Ser Gly
385 390 395 400

Phe Phe Gly Asp His Thr Lys Ile Pro Asn Gly Lys Phe Phe Thr Met
405 410 415

Phe His Phe Leu Glu Tyr Pro Phe Ser Arg Gly Phe Val Tyr Ala Val
420 425 430

Ser Pro Asp Pro Tyr Glu Ala Pro Asp Phe Asp Pro Gly Phe Leu Asn
435 440 445

Asp Ser Arg Asp Met Trp Pro Met Val Trp Ser Tyr Lys Lys Ser Arg
450 455 460

Gln Thr Ala Arg Arg Met Glu Ser Phe Ala Gly Glu Val Thr Ser His
465 470 475 480

His Pro Leu Tyr Pro Val Asp Ser Pro Ala Arg Ala Lys Asp Leu Asp
485 490 495

Leu Glu Thr Cys Lys Ala Phe Ala Gly Pro Asn His Phe Thr Ala Asn
500 505 510

Leu Tyr His Gly Ser Trp Thr Val Pro Ile Glu Lys Pro Thr Pro Lys

515 520 525

Asn Asp Ser His Val Thr Cys Asn Gln Val Glu Ile Phe Ser Asp Ile
530 535 540

Asp Tyr Ser Ala Glu Asp Asp Glu Ala Ile Val Lys Tyr Ile Lys Glu
545 550 555 560

His Thr Glu Thr Thr Trp His Cys Leu Gly Thr Cys Ser Met Ala Pro
565 570 575

Gln Glu Gly Ser Lys Ile Ala Pro Lys Gly Gly Val Val Asp Ala Arg
580 585 590

Leu Asn Val Tyr Glu Val Lys Asn Leu Lys Val Ala Asp Leu Ser Ile
595 600 605

Cys Pro Asp Asn Val Gly Cys Asn Thr Tyr Ser Thr Ala Leu Leu Ile
610 615 620

Gly Glu Lys Ala Ala Thr Leu Val Ala Glu Asp Leu Gly Tyr Ser Gly
625 630 635 640

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

Glu Lys Gly Leu Ala Arg Phe
660

<210> 29
<211> 798
<212> DNA
<213> Ogataea minuta

<220>
<221> CDS
<222> (1)..(798)

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Met Ser Ser Thr Lys Thr Tyr Ala Gln Arg Ala Ala Ala His Pro Ser
1 5 10 15

cct gtg gcc aga aga ctg ctg aac ttg atg gaa tcc aag aag acg aac 96
Pro Val Ala Arg Arg Leu Leu Asn Leu Met Glu Ser Lys Lys Thr Asn
20 25 30

ttg tgt gcc tcg gtc gat ctc acc tct aca aag gac ctt ttg gag ctg 144
Leu Cys Ala Ser Val Asp Leu Thr Ser Thr Lys Asp Leu Leu Glu Leu
35 40 45

ttg gac aag ctg gga ccg ttc att tgt ctg gtc aag aca cac atc gac 192
Leu Asp Lys Leu Gly Pro Phe Ile Cys Leu Val Lys Thr His Ile Asp
50 55 60

att gtg gaa gac ttt tcg tac gaa aac acc gtg gtg ccg ctg ctg aaa 240
Ile Val Glu Asp Phe Ser Tyr Glu Asn Thr Val Val Pro Leu Leu Lys
65 70 75 80

ctg gcc aag aaa cac aac ttc atg atc ttc gag gac cga aaa ttt gcc 288
Leu Ala Lys Lys His Asn Phe Met Ile Phe Glu Asp Arg Lys Phe Ala
85 90 95

gat ata ggc aac acc gtc aaa ctc cag tac aag gga gga gtt tac caa 336
Asp Ile Gly Asn Thr Val Lys Leu Gln Tyr Lys Gly Gly Val Tyr Gln
100 105 110

atc gca aag tgg gcc gat atc acc aac gcc cac gga gtg acc ggc tcg 384
Ile Ala Lys Trp Ala Asp Ile Thr Asn Ala His Gly Val Thr Gly Ser
115 120 125

cga att gtc tcg ggt ctc aga cag gct gcc cag gag acc acc gac gag 432
Arg Ile Val Ser Gly Leu Arg Gln Ala Ala Gln Glu Thr Thr Asp Glu
130 135 140

cca aga ggt ctg ctc atg ctg gct gag ctg tcg tct gaa ggc tcg ctc 480
Pro Arg Gly Leu Leu Met Leu Ala Glu Leu Ser Ser Glu Gly Ser Leu
145 150 155 160

gcg tac gga gag tac acc aaa aag acg gtt gaa atc gca aag tcc gac 528
Ala Tyr Gly Glu Tyr Thr Lys Lys Thr Val Glu Ile Ala Lys Ser Asp
165 170 175

aga gat ttt gtg atc ggt ttc att gcg caa aac gac atg ggt ggc cgc 576
Arg Asp Phe Val Ile Gly Phe Ile Ala Gln Asn Asp Met Gly Gly Arg
180 185 190

gat gag ggc ttc gac tgg ctc atc atg acc cca ggt gtc gga ctc gac 624

Asp Glu Gly Phe Asp Trp Leu Ile Met Thr Pro Gly Val Gly Leu Asp
195 200 205

gac acc ggt gac gct ctg ggc cag cag tac cgc acg gtc agc gcc gtt 672
Asp Thr Gly Asp Ala Leu Gly Gln Gln Tyr Arg Thr Val Ser Ala Val
210 215 220

atg aag acg gga act gac atc ata atc gtg ggc agg gga ctg ttc ggc 720
Met Lys Thr Gly Thr Asp Ile Ile Ile Val Gly Arg Gly Leu Phe Gly
225 230 235 240

aag gga aga gac cct gtc gtg gaa ggc gaa aga tac aga aag gct gga 768
Lys Gly Arg Asp Pro Val Val Glu Gly Glu Arg Tyr Arg Lys Ala Gly
245 250 255

tgg gac gct tat ttg agt cgt gtc gca tga 798
Trp Asp Ala Tyr Leu Ser Arg Val Ala
260 265

<210> 30
<211> 265
<212> PRT
<213> Ogataea minuta

<400> 30

Met Ser Ser Thr Lys Thr Tyr Ala Gln Arg Ala Ala Ala His Pro Ser
1 5 10 15

Pro Val Ala Arg Arg Leu Leu Asn Leu Met Glu Ser Lys Lys Thr Asn
20 25 30

Leu Cys Ala Ser Val Asp Leu Thr Ser Thr Lys Asp Leu Leu Glu Leu
35 40 45

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

Ile Val Glu Asp Phe Ser Tyr Glu Asn Thr Val Val Pro Leu Leu Lys
65 70 75 80

Leu Ala Lys Lys His Asn Phe Met Ile Phe Glu Asp Arg Lys Phe Ala
85 90 95

Asp Ile Gly Asn Thr Val Lys Leu Gln Tyr Lys Gly Gly Val Tyr Gln
100 105 110

Ile Ala Lys Trp Ala Asp Ile Thr Asn Ala His Gly Val Thr Gly Ser
115 120 125

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

Pro Arg Gly Leu Leu Met Leu Ala Glu Leu Ser Ser Glu Gly Ser Leu
145 150 155 160

Ala Tyr Gly Glu Tyr Thr Lys Lys Thr Val Glu Ile Ala Lys Ser Asp
165 170 175

Arg Asp Phe Val Ile Gly Phe Ile Ala Gln Asn Asp Met Gly Gly Arg
180 185 190

Asp Glu Gly Phe Asp Trp Leu Ile Met Thr Pro Gly Val Gly Leu Asp
195 200 205

Asp Thr Gly Asp Ala Leu Gly Gln Gln Tyr Arg Thr Val Ser Ala Val
210 215 220

Met Lys Thr Gly Thr Asp Ile Ile Ile Val Gly Arg Gly Leu Phe Gly
225 230 235 240

Lys Gly Arg Asp Pro Val Val Glu Gly Glu Arg Tyr Arg Lys Ala Gly
245 250 255

Trp Asp Ala Tyr Leu Ser Arg Val Ala
260 265

<210> 31
<211> 1620
<212> DNA
<213> Ogataea minuta

<220>

<221> CDS

<222> (1)..(1620)

<400> 31

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1 5 10 15

gca gtg gag gcc ttg gtc atc ccg tta ttt gac gac ttg cca gca gag 96
Ala Val Glu Ala Leu Val Ile Pro Leu Phe Asp Asp Leu Pro Ala Glu
20 25 30

ttt gcc ctt gtt cca atg gat gcg aaa gcg gaa gtc att tct gac gtt 144
Phe Ala Leu Val Pro Met Asp Ala Lys Ala Glu Val Ile Ser Asp Val
35 40 45

cct gtc gac tcg gcc att agt gat gct cct atc gcg gca cta aat gat 192
Pro Val Asp Ser Ala Ile Ser Asp Ala Pro Ile Ala Ala Leu Asn Asp
50 55 60

gct cca agc cct ctc gtc aca tcg ctg atc gca tct caa aat ttg att 240
Ala Pro Ser Pro Leu Val Thr Ser Leu Ile Ala Ser Gln Asn Leu Ile
65 70 75 80

cca aac tct tat att gtc gtt ttc aag aat ggc cta gct tcc ggg gca 288
Pro Asn Ser Tyr Ile Val Val Phe Lys Asn Gly Leu Ala Ser Gly Ala
85 90 95

gtt gac ttc cac atg gag tgg ctc aag gaa acg cac tcc caa acc ctg 336
Val Asp Phe His Met Glu Trp Leu Lys Glu Thr His Ser Gln Thr Leu

100 105 110

gct gct ttg tct aag gac atg cca gca gaa gaa ttg gcc gcc gaa ggt 384
Ala Ala Leu Ser Lys Asp Met Pro Ala Glu Glu Leu Ala Ala Glu Gly
115 120 125

ttc gtt tcc gaa agc att gat ctt act gag gtg ttt agc atc tcc gat 432
Phe Val Ser Glu Ser Ile Asp Leu Thr Glu Val Phe Ser Ile Ser Asp
130 135 140

ttg ttc agt gga tat acc gga tac ttc ccg gag aag gtg gtt gac ctc 480
Leu Phe Ser Gly Tyr Thr Gly Tyr Phe Pro Glu Lys Val Val Asp Leu
145 150 155 160

atc aga aga cac cct gac gtg gcg ttc gtt gag cag gac tcg aga gtt 528
Ile Arg Arg His Pro Asp Val Ala Phe Val Glu Gln Asp Ser Arg Val
165 170 175

ttc gcc gat aag tcg tct act caa aac ggt gct cct tgg ggt ttg tct 576
Phe Ala Asp Lys Ser Ser Thr Gln Asn Gly Ala Pro Trp Gly Leu Ser
180 185 190

aga atc tct cac aga gag cct ctc agt ctc ggc aat ttc aac gag tac 624
Arg Ile Ser His Arg Glu Pro Leu Ser Leu Gly Asn Phe Asn Glu Tyr
195 200 205

gtt tac gac gat ctt gct gga gat ggc gtc acg gct tat gtc att gat 672
Val Tyr Asp Asp Leu Ala Gly Asp Gly Val Thr Ala Tyr Val Ile Asp
210 215 220

acc ggt atc aat gtg aag cac gag cag ttc ggt ggc aga gca gag tgg 720
Thr Gly Ile Asn Val Lys His Glu Gln Phe Gly Gly Arg Ala Glu Trp
225 230 235 240

ggt aag acc atc cca acc ggt gat gat gat att gac gga aac ggt cac 768
Gly Lys Thr Ile Pro Thr Gly Asp Asp Asp Ile Asp Gly Asn Gly His
245 250 255

ggt act cac tgc gct ggt aca att ggc tcg gaa gat tat gga gtt tct 816
Gly Thr His Cys Ala Gly Thr Ile Gly Ser Glu Asp Tyr Gly Val Ser
260 265 270

aag aac tcc aaa att gtc gca gtg aag gtt ttg aga tct aac ggt tct 864
Lys Asn Ser Lys Ile Val Ala Val Lys Val Leu Arg Ser Asn Gly Ser
275 280 285

ggt tcc atg tct gac gtg atc aag ggt gtt gaa ttc gct gca aat gat 912
Gly Ser Met Ser Asp Val Ile Lys Gly Val Glu Phe Ala Ala Asn Asp
290 295 300

cac gtt gcc aag tct aaa gcc aag aag gac ggt ttc aag gga tcg act 960
His Val Ala Lys Ser Lys Ala Lys Lys Asp Gly Phe Lys Gly Ser Thr
305 310 315 320

gcc aac atg tct ttg gga ggt ggc aag tct cct gct ctt gac ttg gct 1008
Ala Asn Met Ser Leu Gly Gly Gly Lys Ser Pro Ala Leu Asp Leu Ala
325 330 335

gtc aat gcc gct gtc aaa gct ggt tta cac ttt gct gtt gcc gct ggt 1056
Val Asn Ala Ala Val Lys Ala Gly Leu His Phe Ala Val Ala Ala Gly
340 345 350

aac gac aat gct gac gca tgc aac tat tct cct gct gct gca gag aac 1104
Asn Asp Asn Ala Asp Ala Cys Asn Tyr Ser Pro Ala Ala Ala Glu Asn
355 360 365

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gca gtc act gtt ggt gcg tcc act ttg tct gac tct aga gct tac ttt      1152
Ala Val Thr Val Gly Ala Ser Thr Leu Ser Asp Ser Arg Ala Tyr Phe
    370                 375                 380

tcc aac tat ggt aaa tgt gtt gac att ttt gct ccg ggc ttg aac atc      1200
Ser Asn Tyr Gly Lys Cys Val Asp Ile Phe Ala Pro Gly Leu Asn Ile
385                 390                 395                 400

ctt tcc acc tac ata ggt tct gac act gcc acc gcc act ctt tct ggt      1248
Leu Ser Thr Tyr Ile Gly Ser Asp Thr Ala Thr Ala Thr Leu Ser Gly
                405                 410                 415

aca tcg atg gcc tcc cct cac gtt tgt ggt ctg ttg acc tac ttt ttg      1296
Thr Ser Met Ala Ser Pro His Val Cys Gly Leu Leu Thr Tyr Phe Leu
            420                 425                 430

agc ttg caa cca gaa tcg tcg tcg ttg ttt tct tcg gca gct atc tcc      1344
Ser Leu Gln Pro Glu Ser Ser Ser Leu Phe Ser Ser Ala Ala Ile Ser
        435                 440                 445

cct gct cag ctg aag aag aac ctg atc aag ttt ggt acg aag aac gtt      1392
Pro Ala Gln Leu Lys Lys Asn Leu Ile Lys Phe Gly Thr Lys Asn Val
    450                 455                 460

ttg tct gag att cca tcg gac gga acc cca aat att ctc att tac aac      1440
Leu Ser Glu Ile Pro Ser Asp Gly Thr Pro Asn Ile Leu Ile Tyr Asn
465                 470                 475                 480

ggt gct ggc aag aac atc agt gac ttc tgg gcg ttt gaa gac gag gcc      1488
Gly Ala Gly Lys Asn Ile Ser Asp Phe Trp Ala Phe Glu Asp Glu Ala
                485                 490                 495

tcg gcc aag tcc gac ttg aag aag gct gtc gat att gcc aca agt gtt      1536
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Ser Ala Lys Ser Asp Leu Lys Lys Ala Val Asp Ile Ala Thr Ser Val
500 505 510

gac tta gac ctg caa gat atc aag gag aag ttc aac cat att ttg gag 1584
Asp Leu Asp Leu Gln Asp Ile Lys Glu Lys Phe Asn His Ile Leu Glu
515 520 525

gag gtc gcc gaa gag gtt gct gat ttg ttc gat tag 1620
Glu Val Ala Glu Glu Val Ala Asp Leu Phe Asp
530 535

<210> 32
<211> 539
<212> PRT
<213> Ogataea minuta

<400> 32

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

Ala Val Glu Ala Leu Val Ile Pro Leu Phe Asp Asp Leu Pro Ala Glu
20 25 30

Phe Ala Leu Val Pro Met Asp Ala Lys Ala Glu Val Ile Ser Asp Val
35 40 45

Pro Val Asp Ser Ala Ile Ser Asp Ala Pro Ile Ala Ala Leu Asn Asp
50 55 60

Ala Pro Ser Pro Leu Val Thr Ser Leu Ile Ala Ser Gln Asn Leu Ile
65 70 75 80

Pro Asn Ser Tyr Ile Val Val Phe Lys Asn Gly Leu Ala Ser Gly Ala
85 90 95

Val Asp Phe His Met Glu Trp Leu Lys Glu Thr His Ser Gln Thr Leu
100 105 110

Ala Ala Leu Ser Lys Asp Met Pro Ala Glu Glu Leu Ala Ala Glu Gly
115 120 125

Phe Val Ser Glu Ser Ile Asp Leu Thr Glu Val Phe Ser Ile Ser Asp
130 135 140

Leu Phe Ser Gly Tyr Thr Gly Tyr Phe Pro Glu Lys Val Val Asp Leu
145 150 155 160

Ile Arg Arg His Pro Asp Val Ala Phe Val Glu Gln Asp Ser Arg Val
165 170 175

Phe Ala Asp Lys Ser Ser Thr Gln Asn Gly Ala Pro Trp Gly Leu Ser
180 185 190

Arg Ile Ser His Arg Glu Pro Leu Ser Leu Gly Asn Phe Asn Glu Tyr
195 200 205

Val Tyr Asp Asp Leu Ala Gly Asp Gly Val Thr Ala Tyr Val Ile Asp
210 215 220

Thr Gly Ile Asn Val Lys His Glu Gln Phe Gly Gly Arg Ala Glu Trp
225 230 235 240

Gly Lys Thr Ile Pro Thr Gly Asp Asp Asp Ile Asp Gly Asn Gly His
245 250 255

Gly Thr His Cys Ala Gly Thr Ile Gly Ser Glu Asp Tyr Gly Val Ser
260 265 270

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

Gly Ser Met Ser Asp Val Ile Lys Gly Val Glu Phe Ala Ala Asn Asp
290 295 300

His Val Ala Lys Ser Lys Ala Lys Lys Asp Gly Phe Lys Gly Ser Thr
305 310 315 320

Ala Asn Met Ser Leu Gly Gly Gly Lys Ser Pro Ala Leu Asp Leu Ala
325 330 335

Val Asn Ala Ala Val Lys Ala Gly Leu His Phe Ala Val Ala Ala Gly
340 345 350

Asn Asp Asn Ala Asp Ala Cys Asn Tyr Ser Pro Ala Ala Ala Glu Asn
355 360 365

Ala Val Thr Val Gly Ala Ser Thr Leu Ser Asp Ser Arg Ala Tyr Phe
370 375 380

Ser Asn Tyr Gly Lys Cys Val Asp Ile Phe Ala Pro Gly Leu Asn Ile
385 390 395 400

Leu Ser Thr Tyr Ile Gly Ser Asp Thr Ala Thr Ala Thr Leu Ser Gly
405 410 415

Thr Ser Met Ala Ser Pro His Val Cys Gly Leu Leu Thr Tyr Phe Leu
420 425 430

Ser Leu Gln Pro Glu Ser Ser Ser Leu Phe Ser Ser Ala Ala Ile Ser
435 440 445

Pro Ala Gln Leu Lys Lys Asn Leu Ile Lys Phe Gly Thr Lys Asn Val

450 455 460

Leu Ser Glu Ile Pro Ser Asp Gly Thr Pro Asn Ile Leu Ile Tyr Asn
465 470 475 480

Gly Ala Gly Lys Asn Ile Ser Asp Phe Trp Ala Phe Glu Asp Glu Ala
485 490 495

Ser Ala Lys Ser Asp Leu Lys Lys Ala Val Asp Ile Ala Thr Ser Val
500 505 510

Asp Leu Asp Leu Gln Asp Ile Lys Glu Lys Phe Asn His Ile Leu Glu
515 520 525

Glu Val Ala Glu Glu Val Ala Asp Leu Phe Asp
530 535

<210> 33
<211> 814
<212> PRT
<213> Saccharomyces cerevisiae

<400> 33

Met Lys Val Arg Lys Tyr Ile Thr Leu Cys Phe Trp Trp Ala Phe Ser
1 5 10 15

Thr Ser Ala Leu Val Ser Ser Gln Gln Ile Pro Leu Lys Asp His Thr
20 25 30

Ser Arg Gln Tyr Phe Ala Val Glu Ser Asn Glu Thr Leu Ser Arg Leu
35 40 45

Glu Glu Met His Pro Asn Trp Lys Tyr Glu His Asp Val Arg Gly Leu
50 55 60

Pro Asn His Tyr Val Phe Ser Lys Glu Leu Leu Lys Leu Gly Lys Arg
65 70 75 80

Ser Ser Leu Glu Glu Leu Gln Gly Asp Asn Asn Asp His Ile Leu Ser
85 90 95

Val His Asp Leu Phe Pro Arg Asn Asp Leu Phe Lys Arg Leu Pro Val
100 105 110

Pro Ala Pro Pro Met Asp Ser Ser Leu Leu Pro Val Lys Glu Ala Glu
115 120 125

Asp Lys Leu Ser Ile Asn Asp Pro Leu Phe Glu Arg Gln Trp His Leu
130 135 140

Val Asn Pro Ser Phe Pro Gly Ser Asp Ile Asn Val Leu Asp Leu Trp
145 150 155 160

Tyr Asn Asn Ile Thr Gly Ala Gly Val Val Ala Ala Ile Val Asp Asp
165 170 175

Gly Leu Asp Tyr Glu Asn Glu Asp Leu Lys Asp Asn Phe Cys Ala Glu
180 185 190

Gly Ser Trp Asp Phe Asn Asp Asn Thr Asn Leu Pro Lys Pro Arg Leu
195 200 205

Ser Asp Asp Tyr His Gly Thr Arg Cys Ala Gly Glu Ile Ala Ala Lys
210 215 220

Lys Gly Asn Asn Phe Cys Gly Val Gly Val Gly Tyr Asn Ala Lys Ile
225 230 235 240

Ser Gly Ile Arg Ile Leu Ser Gly Asp Ile Thr Thr Glu Asp Glu Ala
245 250 255

Ala Ser Leu Ile Tyr Gly Leu Asp Val Asn Asp Ile Tyr Ser Cys Ser
260 265 270

Trp Gly Pro Ala Asp Asp Gly Arg His Leu Gln Gly Pro Ser Asp Leu

275 280 285

Val Lys Lys Ala Leu Val Lys Gly Val Thr Glu Gly Arg Asp Ser Lys
290 295 300

Gly Ala Ile Tyr Val Phe Ala Ser Gly Asn Gly Gly Thr Arg Gly Asp
305 310 315 320

Asn Cys Asn Tyr Asp Gly Tyr Thr Asn Ser Ile Tyr Ser Ile Thr Ile
325 330 335

Gly Ala Ile Asp His Lys Asp Leu His Pro Pro Tyr Ser Glu Gly Cys
340 345 350

Ser Ala Val Met Ala Val Thr Tyr Ser Ser Gly Ser Gly Glu Tyr Ile
355 360 365

His Ser Ser Asp Ile Asn Gly Arg Cys Ser Asn Ser His Gly Gly Thr
370 375 380

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

Ala Asn Pro Asn Leu Thr Trp Arg Asp Val Gln Tyr Leu Ser Ile Leu
405 410 415

Ser Ala Val Gly Leu Glu Lys Asn Ala Asp Gly Asp Trp Arg Asp Ser
420 425 430

Ala Met Gly Lys Lys Tyr Ser His Arg Tyr Gly Phe Gly Lys Ile Asp
435 440 445

Ala His Lys Leu Ile Glu Met Ser Lys Thr Trp Glu Asn Val Asn Ala
450 455 460

Gln Thr Trp Phe Tyr Leu Pro Thr Leu Tyr Val Ser Gln Ser Thr Asn
465 470 475 480

Ser Thr Glu Glu Thr Leu Glu Ser Val Ile Thr Ile Ser Glu Lys Ser
485 490 495

Leu Gln Asp Ala Asn Phe Lys Arg Ile Glu His Val Thr Val Thr Val
500 505 510

Asp Ile Asp Thr Glu Ile Arg Gly Thr Thr Thr Val Asp Leu Ile Ser
515 520 525

Pro Ala Gly Ile Ile Ser Asn Leu Gly Val Val Arg Pro Arg Asp Val
530 535 540

Ser Ser Glu Gly Phe Lys Asp Trp Thr Phe Met Ser Val Ala His Trp
545 550 555 560

Gly Glu Asn Gly Val Gly Asp Trp Lys Ile Lys Val Lys Thr Thr Glu
565 570 575

Asn Gly His Arg Ile Asp Phe His Ser Trp Arg Leu Lys Leu Phe Gly
580 585 590

Glu Ser Ile Asp Ser Ser Lys Thr Glu Thr Phe Val Phe Gly Asn Asp
595 600 605

Lys Glu Glu Val Glu Pro Ala Ala Thr Glu Ser Thr Val Ser Gln Tyr
610 615 620

Ser Ala Ser Ser Thr Ser Ile Ser Ile Ser Ala Thr Ser Thr Ser Ser
625 630 635 640

Ile Ser Ile Gly Val Glu Thr Ser Ala Ile Pro Gln Thr Thr Thr Ala
645 650 655

Ser Thr Asp Pro Asp Ser Asp Pro Asn Thr Pro Lys Lys Leu Ser Ser
660 665 670

Pro Arg Gln Ala Met His Tyr Phe Leu Thr Ile Phe Leu Ile Gly Ala
675 680 685

Thr Phe Leu Val Leu Tyr Phe Met Phe Phe Met Lys Ser Arg Arg Arg
690 695 700

Ile Arg Arg Ser Arg Ala Glu Thr Tyr Glu Phe Asp Ile Ile Asp Thr
705 710 715 720

Asp Ser Glu Tyr Asp Ser Thr Leu Asp Asn Gly Thr Ser Gly Ile Thr
725 730 735

Glu Pro Glu Glu Val Glu Asp Phe Asp Phe Asp Leu Ser Asp Glu Asp
740 745 750

His Leu Ala Ser Leu Ser Ser Ser Glu Asn Gly Asp Ala Glu His Thr
755 760 765

Ile Asp Ser Val Leu Thr Asn Glu Asn Pro Phe Ser Asp Pro Ile Lys
770 775 780

Gln Lys Phe Pro Asn Asp Ala Asn Ala Glu Ser Ala Ser Asn Lys Leu
785 790 795 800

Gln Glu Leu Gln Pro Asp Val Pro Pro Ser Ser Gly Arg Ser

805 810

<210> 34
<211> 609
<212> PRT
<213> Homo sapiens

<400> 34

Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val

355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu

<210> 35

<211> 1551

<212> DNA

<213> Ogataea minuta

<220>

<221> CDS

<222> (1)..(1551)

<400> 35

atg aag tta ttt gga ttg act aca ttg acc agc atc ctg gcc gcc ctc 48
Met Lys Leu Phe Gly Leu Thr Thr Leu Thr Ser Ile Leu Ala Ala Leu
1 5 10 15

aca gtg gtg agc gcc acc gaa gag cca gca gtt gcc tcg cca gac tcg 96
Thr Val Val Ser Ala Thr Glu Glu Pro Ala Val Ala Ser Pro Asp Ser
20 25 30

gcg gtg gtg aag ctg acg gcc gac act ttc gag agc ttc atc aag gag 144
Ala Val Val Lys Leu Thr Ala Asp Thr Phe Glu Ser Phe Ile Lys Glu
35 40 45

aac cca ttg gtt ctg gcg gag ttt ttt gcg cca tgg tgt ggc cac tgc 192
Asn Pro Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys
50 55 60

aag aag ctg ggt cca gaa ttc agc gca gcc gca gac cag ctg gtg gag 240
Lys Lys Leu Gly Pro Glu Phe Ser Ala Ala Ala Asp Gln Leu Val Glu
65 70 75 80

aag aac atc aag ctt gca cag atc gac tgt acc gag gaa aga gat ctg 288

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Lys Asn Ile Lys Leu Ala Gln Ile Asp Cys Thr Glu Glu Arg Asp Leu
                85                  90                  95

tgt tcg tcg cac gga atc aga gga tac cca act ttg aag gtg ttc agg      336
Cys Ser Ser His Gly Ile Arg Gly Tyr Pro Thr Leu Lys Val Phe Arg
            100                 105                 110

ggc gct agt gag cct gct gac tac caa ggc gcc aga gaa cag gaa gct      384
Gly Ala Ser Glu Pro Ala Asp Tyr Gln Gly Ala Arg Glu Gln Glu Ala
        115                 120                 125

att gtc agt caa atg atc aag ctt tct tta cct gct gtt tcc gtc att      432
Ile Val Ser Gln Met Ile Lys Leu Ser Leu Pro Ala Val Ser Val Ile
    130                 135                 140

gag gat tct gcc gac ctg ttt gat acc att gca gaa gtc tcc gac gcc      480
Glu Asp Ser Ala Asp Leu Phe Asp Thr Ile Ala Glu Val Ser Asp Ala
145                 150                 155                 160

ctc att gtg caa gtg ttt cct gcg gga gct gct cag tct tcc aac gag      528
Leu Ile Val Gln Val Phe Pro Ala Gly Ala Ala Gln Ser Ser Asn Glu
                165                 170                 175

acg ttc tac gaa gtc gcc aac gaa ctg aga aac gac ttt gtt ttt gtc      576
Thr Phe Tyr Glu Val Ala Asn Glu Leu Arg Asn Asp Phe Val Phe Val
            180                 185                 190

tcc acc act aac gag ggg tac gtg aaa aag tac gcg aag gac tca aag      624
Ser Thr Thr Asn Glu Gly Tyr Val Lys Lys Tyr Ala Lys Asp Ser Lys
        195                 200                 205

tca cct gct tat gtc atc ttc agg caa gga gaa aag gtt gaa gat gcg      672
Ser Pro Ala Tyr Val Ile Phe Arg Gln Gly Glu Lys Val Glu Asp Ala
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210 215 220

tcc aca tac acc gga aag act gtt gac gac act cac ttg aag cag ttc 720
Ser Thr Tyr Thr Gly Lys Thr Val Asp Asp Thr His Leu Lys Gln Phe
225 230 235 240

atc aat acc gaa acc aaa cct ctg ttt ggt gaa atc acc ggc aac act 768
Ile Asn Thr Glu Thr Lys Pro Leu Phe Gly Glu Ile Thr Gly Asn Thr
245 250 255

ttc aag acc tac atg gag gcc gag ctt cct ttg gcg tac ttt ttc tgg 816
Phe Lys Thr Tyr Met Glu Ala Glu Leu Pro Leu Ala Tyr Phe Phe Trp
260 265 270

gac gaa gag tct caa agg gcc gag gtc gct gac atc atc acc gag ctg 864
Asp Glu Glu Ser Gln Arg Ala Glu Val Ala Asp Ile Ile Thr Glu Leu
275 280 285

gcc aag aag ttt aga gga gag atg aac ttt gtt ggt ttg gaa gcc aag 912
Ala Lys Lys Phe Arg Gly Glu Met Asn Phe Val Gly Leu Glu Ala Lys
290 295 300

aga tac ggt atg cat gcc aag aac ctc aac atg gag gaa aag ttc ccc 960
Arg Tyr Gly Met His Ala Lys Asn Leu Asn Met Glu Glu Lys Phe Pro
305 310 315 320

ttg ttc gcc atc cac gat ttg acc gga aac ctc aag tac ggt att tct 1008
Leu Phe Ala Ile His Asp Leu Thr Gly Asn Leu Lys Tyr Gly Ile Ser
325 330 335

caa gag tct gat ctt gac gtc aag gaa atc cct aag ttc gtt gag gat 1056
Gln Glu Ser Asp Leu Asp Val Lys Glu Ile Pro Lys Phe Val Glu Asp
340 345 350

ttc aag aag ggc aag ttg caa gca att gtc aag tct gag cca att cca 1104
Phe Lys Lys Gly Lys Leu Gln Ala Ile Val Lys Ser Glu Pro Ile Pro
355 360 365

gaa gtc caa gag gag tcc gtg tac cac ctg gtt gga cac gag cac gac 1152
Glu Val Gln Glu Glu Ser Val Tyr His Leu Val Gly His Glu His Asp
370 375 380

gcc atc acc aag cag aag aag gac gtt ttg gtt gag tac tac gcc cca 1200
Ala Ile Thr Lys Gln Lys Lys Asp Val Leu Val Glu Tyr Tyr Ala Pro
385 390 395 400

tgg tgt gga cac tgc aag aag ctg gct cca act tac gaa att ttg gcc 1248
Trp Cys Gly His Cys Lys Lys Leu Ala Pro Thr Tyr Glu Ile Leu Ala
405 410 415

agc atc tac cag aac gac act gat gcc aag gaa aag gtt gtg att gcc 1296
Ser Ile Tyr Gln Asn Asp Thr Asp Ala Lys Glu Lys Val Val Ile Ala
420 425 430

aag att gac cac act gcc aac gat gtt gcc ggt gtc gac atc gcc ggt 1344
Lys Ile Asp His Thr Ala Asn Asp Val Ala Gly Val Asp Ile Ala Gly
435 440 445

tat cca acc atc atc ttg tat cct ggt gac gaa tct gag ccg gtt gtg 1392
Tyr Pro Thr Ile Ile Leu Tyr Pro Gly Asp Glu Ser Glu Pro Val Val
450 455 460

tac gag ggt tct aga act cta gag gct ctc agt tca ttc atc aag gag 1440
Tyr Glu Gly Ser Arg Thr Leu Glu Ala Leu Ser Ser Phe Ile Lys Glu
465 470 475 480

aag ggt tcg aac ggc gtt gac gct ttg tcc atc aag gaa tcg cgt gtt 1488
Lys Gly Ser Asn Gly Val Asp Ala Leu Ser Ile Lys Glu Ser Arg Val
485 490 495

gaa aaa gaa gcc gat gct caa gcc gat gct cct gac gct ggc gtg gct 1536
Glu Lys Glu Ala Asp Ala Gln Ala Asp Ala Pro Asp Ala Gly Val Ala
500 505 510

cac gac gag ttg taa 1551
His Asp Glu Leu
515

<210> 36
<211> 516
<212> PRT
<213> Ogataea minuta

<400> 36

Met Lys Leu Phe Gly Leu Thr Thr Leu Thr Ser Ile Leu Ala Ala Leu
1 5 10 15

Thr Val Val Ser Ala Thr Glu Glu Pro Ala Val Ala Ser Pro Asp Ser
20 25 30

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

Asn Pro Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys

50 55 60

Lys Lys Leu Gly Pro Glu Phe Ser Ala Ala Ala Asp Gln Leu Val Glu
65 70 75 80

Lys Asn Ile Lys Leu Ala Gln Ile Asp Cys Thr Glu Glu Arg Asp Leu
85 90 95

Cys Ser Ser His Gly Ile Arg Gly Tyr Pro Thr Leu Lys Val Phe Arg
100 105 110

Gly Ala Ser Glu Pro Ala Asp Tyr Gln Gly Ala Arg Glu Gln Glu Ala
115 120 125

Ile Val Ser Gln Met Ile Lys Leu Ser Leu Pro Ala Val Ser Val Ile
130 135 140

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

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

Thr Phe Tyr Glu Val Ala Asn Glu Leu Arg Asn Asp Phe Val Phe Val
180 185 190

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

Ser Pro Ala Tyr Val Ile Phe Arg Gln Gly Glu Lys Val Glu Asp Ala
210 215 220

Ser Thr Tyr Thr Gly Lys Thr Val Asp Asp Thr His Leu Lys Gln Phe
225 230 235 240

Ile Asn Thr Glu Thr Lys Pro Leu Phe Gly Glu Ile Thr Gly Asn Thr
245 250 255

Phe Lys Thr Tyr Met Glu Ala Glu Leu Pro Leu Ala Tyr Phe Phe Trp
260 265 270

Asp Glu Glu Ser Gln Arg Ala Glu Val Ala Asp Ile Ile Thr Glu Leu
275 280 285

Ala Lys Lys Phe Arg Gly Glu Met Asn Phe Val Gly Leu Glu Ala Lys
290 295 300

Arg Tyr Gly Met His Ala Lys Asn Leu Asn Met Glu Glu Lys Phe Pro
305 310 315 320

Leu Phe Ala Ile His Asp Leu Thr Gly Asn Leu Lys Tyr Gly Ile Ser
325 330 335

Gln Glu Ser Asp Leu Asp Val Lys Glu Ile Pro Lys Phe Val Glu Asp
340 345 350

Phe Lys Lys Gly Lys Leu Gln Ala Ile Val Lys Ser Glu Pro Ile Pro
355 360 365

Glu Val Gln Glu Glu Ser Val Tyr His Leu Val Gly His Glu His Asp
370 375 380

Ala Ile Thr Lys Gln Lys Lys Asp Val Leu Val Glu Tyr Tyr Ala Pro
385 390 395 400

Trp Cys Gly His Cys Lys Lys Leu Ala Pro Thr Tyr Glu Ile Leu Ala
405 410 415

Ser Ile Tyr Gln Asn Asp Thr Asp Ala Lys Glu Lys Val Val Ile Ala
420 425 430

Lys Ile Asp His Thr Ala Asn Asp Val Ala Gly Val Asp Ile Ala Gly
435 440 445

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

Tyr Glu Gly Ser Arg Thr Leu Glu Ala Leu Ser Ser Phe Ile Lys Glu
465 470 475 480

Lys Gly Ser Asn Gly Val Asp Ala Leu Ser Ile Lys Glu Ser Arg Val
485 490 495

Glu Lys Glu Ala Asp Ala Gln Ala Asp Ala Pro Asp Ala Gly Val Ala
500 505 510

His Asp Glu Leu
515

<210> 37
<211> 936
<212> DNA
<213> Ogataea minuta

<220>
<221> CDS
<222> (1)..(936)

<400> 37
atg aaa gtg gca agt ttg ata gca ttg gtc gtt acg ccg att att gct 48
Met Lys Val Ala Ser Leu Ile Ala Leu Val Val Thr Pro Ile Ile Ala

1 5 10 15

gcc act ggg gtg gta gca gat ccg cag cag cag gcc aaa aga cct ggt 96
Ala Thr Gly Val Val Ala Asp Pro Gln Gln Gln Ala Lys Arg Pro Gly
20 25 30

ttt tac aag aat tca aag cat atc tac aat ctc act ccc cag aac ttt 144
Phe Tyr Lys Asn Ser Lys His Ile Tyr Asn Leu Thr Pro Gln Asn Phe
35 40 45

gac gac gtg gtc ctg caa acc aac cat acg tct gtc gtg gag ttc tat 192
Asp Asp Val Val Leu Gln Thr Asn His Thr Ser Val Val Glu Phe Tyr
50 55 60

gcg cca tgg tgt ggc tat tgc gca gag ttt gag agc cag tac cgc aaa 240
Ala Pro Trp Cys Gly Tyr Cys Ala Glu Phe Glu Ser Gln Tyr Arg Lys
65 70 75 80

gca gca aag atc gga tcg gag ttc gtg aat ttt gcg gcc gtt aac tgc 288
Ala Ala Lys Ile Gly Ser Glu Phe Val Asn Phe Ala Ala Val Asn Cys
85 90 95

gac gaa gac aag aac aaa cca ttg tgc aac aag tac cgc gtc gaa ggg 336
Asp Glu Asp Lys Asn Lys Pro Leu Cys Asn Lys Tyr Arg Val Glu Gly
100 105 110

ttc ccg acg gtg atg gtt ttc cgt cca gcg aag gtc aac tcg gcg gga 384
Phe Pro Thr Val Met Val Phe Arg Pro Ala Lys Val Asn Ser Ala Gly
115 120 125

tcc aac ggc aac agg cct cat tcc tcc gaa acg tat cgg ggc gag aga 432
Ser Asn Gly Asn Arg Pro His Ser Ser Glu Thr Tyr Arg Gly Glu Arg
130 135 140

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acg gcg gct aag ttg ctc gag cat gtc aag ggc cgt gtg gtg aat tac      480
Thr Ala Ala Lys Leu Leu Glu His Val Lys Gly Arg Val Val Asn Tyr
145                 150                 155                 160

gtg aag aga atc aag ctc aac aaa ctt gat gaa ttt ctc aaa ccg aat      528
Val Lys Arg Ile Lys Leu Asn Lys Leu Asp Glu Phe Leu Lys Pro Asn
                165                 170                 175

gaa aag agc aga gtc ttg ctg gtg act tca aaa agc act ctt tcg ccg      576
Glu Lys Ser Arg Val Leu Leu Val Thr Ser Lys Ser Thr Leu Ser Pro
            180                 185                 190

gtt ttc aag agc ctg tcg atc gat ttt ctc gac tca gtc acg ttg gca      624
Val Phe Lys Ser Leu Ser Ile Asp Phe Leu Asp Ser Val Thr Leu Ala
        195                 200                 205

tac ctc act ctg agc gaa aac gac tcc gaa ggt aga gac aaa ctg ctg      672
Tyr Leu Thr Leu Ser Glu Asn Asp Ser Glu Gly Arg Asp Lys Leu Leu
    210                 215                 220

gaa aag att cct gcc ctc aaa gcg gac ttc aaa gtc ccg act tta ctc      720
Glu Lys Ile Pro Ala Leu Lys Ala Asp Phe Lys Val Pro Thr Leu Leu
225                 230                 235                 240

gcc atc gac aag gga acg aaa aat gtg acg gtt tat gat tcc gaa tcg      768
Ala Ile Asp Lys Gly Thr Lys Asn Val Thr Val Tyr Asp Ser Glu Ser
                245                 250                 255

atg tcg aaa aaa gag ctg acg aag ttc atg tct aag ttc ggc cag cca      816
Met Ser Lys Lys Glu Leu Thr Lys Phe Met Ser Lys Phe Gly Gln Pro
            260                 265                 270
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caa gag ggg gca atg agc gaa aga ggg ggc atc ttg aaa gga atc aag 864
Gln Glu Gly Ala Met Ser Glu Arg Gly Gly Ile Leu Lys Gly Ile Lys
275 280 285

aag ggt gct tac aag agc ttc aaa gat tac aaa aag aag atg caa caa 912
Lys Gly Ala Tyr Lys Ser Phe Lys Asp Tyr Lys Lys Lys Met Gln Gln
290 295 300

gct ctt gaa aaa gat gag cta tga 936
Ala Leu Glu Lys Asp Glu Leu
305 310

<210> 38
<211> 311
<212> PRT
<213> Ogataea minuta

<400> 38

Met Lys Val Ala Ser Leu Ile Ala Leu Val Val Thr Pro Ile Ile Ala
1 5 10 15

Ala Thr Gly Val Val Ala Asp Pro Gln Gln Gln Ala Lys Arg Pro Gly
20 25 30

Phe Tyr Lys Asn Ser Lys His Ile Tyr Asn Leu Thr Pro Gln Asn Phe
35 40 45

Asp Asp Val Val Leu Gln Thr Asn His Thr Ser Val Val Glu Phe Tyr

50 55 60

Ala Pro Trp Cys Gly Tyr Cys Ala Glu Phe Glu Ser Gln Tyr Arg Lys
65 70 75 80

Ala Ala Lys Ile Gly Ser Glu Phe Val Asn Phe Ala Ala Val Asn Cys
85 90 95

Asp Glu Asp Lys Asn Lys Pro Leu Cys Asn Lys Tyr Arg Val Glu Gly
100 105 110

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

Ser Asn Gly Asn Arg Pro His Ser Ser Glu Thr Tyr Arg Gly Glu Arg
130 135 140

Thr Ala Ala Lys Leu Leu Glu His Val Lys Gly Arg Val Val Asn Tyr
145 150 155 160

Val Lys Arg Ile Lys Leu Asn Lys Leu Asp Glu Phe Leu Lys Pro Asn
165 170 175

Glu Lys Ser Arg Val Leu Leu Val Thr Ser Lys Ser Thr Leu Ser Pro
180 185 190

Val Phe Lys Ser Leu Ser Ile Asp Phe Leu Asp Ser Val Thr Leu Ala
195 200 205

Tyr Leu Thr Leu Ser Glu Asn Asp Ser Glu Gly Arg Asp Lys Leu Leu
210 215 220

Glu Lys Ile Pro Ala Leu Lys Ala Asp Phe Lys Val Pro Thr Leu Leu
225 230 235 240

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

Met Ser Lys Lys Glu Leu Thr Lys Phe Met Ser Lys Phe Gly Gln Pro
260 265 270

Gln Glu Gly Ala Met Ser Glu Arg Gly Gly Ile Leu Lys Gly Ile Lys
275 280 285

Lys Gly Ala Tyr Lys Ser Phe Lys Asp Tyr Lys Lys Lys Met Gln Gln
290 295 300

Ala Leu Glu Lys Asp Glu Leu
305 310

<210> 39

<211> 930

<212> DNA

<213> Ogataea minuta

<220>

<221> CDS

<222> (1)..(930)

<400> 39

atg ttt atg gag atc gga gag gcg tac gag gtg ctg atg gat tca gaa 48
Met Phe Met Glu Ile Gly Glu Ala Tyr Glu Val Leu Met Asp Ser Glu
1 5 10 15

aag aga tcc ata tat gac agg tac gga gag gaa ggc ttg aaa ggc ggt 96
Lys Arg Ser Ile Tyr Asp Arg Tyr Gly Glu Glu Gly Leu Lys Gly Gly
20 25 30

gca ggg ggc gga gga gga gga caa cac cac gat ccg ttc gac atg ttt 144
Ala Gly Gly Gly Gly Gly Gly Gln His His Asp Pro Phe Asp Met Phe
35 40 45

gcc aac ttt ttc ggc ggc gcc ggt ggg cgt caa caa gca cgg gga gtt 192
Ala Asn Phe Phe Gly Gly Ala Gly Gly Arg Gln Gln Ala Arg Gly Val
50 55 60

cca aga ggg tcg gac att gcc acg gag atg gag ttt acc ttg aaa gag 240
Pro Arg Gly Ser Asp Ile Ala Thr Glu Met Glu Phe Thr Leu Lys Glu
65 70 75 80

ttt tac aac gga gtg aat agc gac ttt tca ctc gaa ctg caa gac atc 288

Phe Tyr Asn Gly Val Asn Ser Asp Phe Ser Leu Glu Leu Gln Asp Ile
85 90 95

tgt gac cgt tgt gac gga agc ggg tcg cag gac ggg aaa gtg cac aag 336
Cys Asp Arg Cys Asp Gly Ser Gly Ser Gln Asp Gly Lys Val His Lys
100 105 110

tgt tct cga tgc aat ggt cgt ggc cgg gtg tta gtg aag aga cag ttg 384
Cys Ser Arg Cys Asn Gly Arg Gly Arg Val Leu Val Lys Arg Gln Leu
115 120 125

ggt cct ggc atg ttc cag cag atg gag tcg gcg tgt ccc gac tgt cgt 432
Gly Pro Gly Met Phe Gln Gln Met Glu Ser Ala Cys Pro Asp Cys Arg
130 135 140

gga gca gga aaa cag att act cac cat tgc aag aag tgt cgg ggt ggc 480
Gly Ala Gly Lys Gln Ile Thr His His Cys Lys Lys Cys Arg Gly Gly
145 150 155 160

ggg gtt gtc cgt gga att cgc aac ttc aac atc cac ctt gag cca gga 528
Gly Val Val Arg Gly Ile Arg Asn Phe Asn Ile His Leu Glu Pro Gly
165 170 175

act ccg cgc gac cac gtc gaa gtg tac gag ggt cag tcc gac agg tct 576
Thr Pro Arg Asp His Val Glu Val Tyr Glu Gly Gln Ser Asp Arg Ser
180 185 190

ccg gag tgg gag gct ggt aac tta cgt ctg agc gtc aga gag aag aaa 624
Pro Glu Trp Glu Ala Gly Asn Leu Arg Leu Ser Val Arg Glu Lys Lys
195 200 205

agc gga aac ctt ggg tat cgt cgg atc gga aac aat ctg tac cgc aca 672
Ser Gly Asn Leu Gly Tyr Arg Arg Ile Gly Asn Asn Leu Tyr Arg Thr

210 215 220

gag atc ttg acg ctg agc gag tct ctg aag ggt ggt tgg gtc cgc gag 720
Glu Ile Leu Thr Leu Ser Glu Ser Leu Lys Gly Gly Trp Val Arg Glu
225 230 235 240

atc ccg ttt ctg gac aac tac gac gcc gtc tta aag ctg gaa aga cca 768
Ile Pro Phe Leu Asp Asn Tyr Asp Ala Val Leu Lys Leu Glu Arg Pro
245 250 255

ctc gga agt gtt gtt acc agc ggg gaa gtg cag gtg gtg aaa gga aag 816
Leu Gly Ser Val Val Thr Ser Gly Glu Val Gln Val Val Lys Gly Lys
260 265 270

ggt atg ccg att gcc aac tcc gtg gat cag ttt ggc gat ctg tat gtg 864
Gly Met Pro Ile Ala Asn Ser Val Asp Gln Phe Gly Asp Leu Tyr Val
275 280 285

gag tat gtg gtg ttg tat ccg gga gga agc ccg aag aag gtg agc aag 912
Glu Tyr Val Val Leu Tyr Pro Gly Gly Ser Pro Lys Lys Val Ser Lys
290 295 300

ttg cac gac gag ctg tga 930
Leu His Asp Glu Leu
305

<210> 40
<211> 309
<212> PRT
<213> Ogataea minuta

<400> 40

Met Phe Met Glu Ile Gly Glu Ala Tyr Glu Val Leu Met Asp Ser Glu
1 5 10 15

Lys Arg Ser Ile Tyr Asp Arg Tyr Gly Glu Glu Gly Leu Lys Gly Gly
20 25 30

Ala Gly Gly Gly Gly Gly Gly Gln His His Asp Pro Phe Asp Met Phe
35 40 45

Ala Asn Phe Phe Gly Gly Ala Gly Gly Arg Gln Gln Ala Arg Gly Val
50 55 60

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

Phe Tyr Asn Gly Val Asn Ser Asp Phe Ser Leu Glu Leu Gln Asp Ile
85 90 95

Cys Asp Arg Cys Asp Gly Ser Gly Ser Gln Asp Gly Lys Val His Lys
100 105 110

Cys Ser Arg Cys Asn Gly Arg Gly Arg Val Leu Val Lys Arg Gln Leu
115 120 125

Gly Pro Gly Met Phe Gln Gln Met Glu Ser Ala Cys Pro Asp Cys Arg
130 135 140

Gly Ala Gly Lys Gln Ile Thr His His Cys Lys Lys Cys Arg Gly Gly
145 150 155 160

Gly Val Val Arg Gly Ile Arg Asn Phe Asn Ile His Leu Glu Pro Gly
165 170 175

Thr Pro Arg Asp His Val Glu Val Tyr Glu Gly Gln Ser Asp Arg Ser
180 185 190

Pro Glu Trp Glu Ala Gly Asn Leu Arg Leu Ser Val Arg Glu Lys Lys
195 200 205

Ser Gly Asn Leu Gly Tyr Arg Arg Ile Gly Asn Asn Leu Tyr Arg Thr
210 215 220

Glu Ile Leu Thr Leu Ser Glu Ser Leu Lys Gly Gly Trp Val Arg Glu
225 230 235 240

Ile Pro Phe Leu Asp Asn Tyr Asp Ala Val Leu Lys Leu Glu Arg Pro
245 250 255

Leu Gly Ser Val Val Thr Ser Gly Glu Val Gln Val Val Lys Gly Lys

260 265 270

Gly Met Pro Ile Ala Asn Ser Val Asp Gln Phe Gly Asp Leu Tyr Val
275 280 285

Glu Tyr Val Val Leu Tyr Pro Gly Gly Ser Pro Lys Lys Val Ser Lys
290 295 300

Leu His Asp Glu Leu
305

<210> 41
<211> 1137
<212> DNA
<213> Ogataea minuta

<220>
<221> CDS
<222> (1)..(1137)

<400> 41
atg aaa gtc acg tct atc tgg caa gtc ctg ttt atg ttg atc gcc ggc 48
Met Lys Val Thr Ser Ile Trp Gln Val Leu Phe Met Leu Ile Ala Gly
1 5 10 15

gtt atg gcg tca gcg tcc aaa gtg atg gag ctg aac gac aag aac ttt 96
Val Met Ala Ser Ala Ser Lys Val Met Glu Leu Asn Asp Lys Asn Phe
20 25 30

gac gag gtg gtt ctc aac tcc gga aag acc tcg cta gtg gaa ttc tac 144
Asp Glu Val Val Leu Asn Ser Gly Lys Thr Ser Leu Val Glu Phe Tyr
35 40 45

gcg tcg tgg tgc agt cac tgc aag aag ttg gag cct act tgg gaa gag 192
Ala Ser Trp Cys Ser His Cys Lys Lys Leu Glu Pro Thr Trp Glu Glu
50 55 60

ctg gcc tcg gcg tac gga aac aag aac gat atc cag atc gtc aag atc 240
Leu Ala Ser Ala Tyr Gly Asn Lys Asn Asp Ile Gln Ile Val Lys Ile
65 70 75 80

gac gct gac gaa aac gga aac gtg gga aga aaa ttc gga atc aag gga 288
Asp Ala Asp Glu Asn Gly Asn Val Gly Arg Lys Phe Gly Ile Lys Gly
85 90 95

ttt ccc acg ctg aaa ctg ttc aaa aaa gat gat ctc aac aac cca gtg 336
Phe Pro Thr Leu Lys Leu Phe Lys Lys Asp Asp Leu Asn Asn Pro Val
100 105 110

gaa ttt gaa ggc tcc agg gac ttc cat tct ttc acc aac ttc att gct 384
Glu Phe Glu Gly Ser Arg Asp Phe His Ser Phe Thr Asn Phe Ile Ala
115 120 125

gca cac acg ggt atc aag gct gcc aac gcg gtt ccc act gag ccg tcc 432
Ala His Thr Gly Ile Lys Ala Ala Asn Ala Val Pro Thr Glu Pro Ser
130 135 140

aaa gtg gtg gaa ctg cac gat gga aat ttg gag gag ctt gtt aag gag 480
Lys Val Val Glu Leu His Asp Gly Asn Leu Glu Glu Leu Val Lys Glu
145 150 155 160

cag gga aaa aat gct ctt ttt gca atc acc gca gag tgg tgt ggt tac 528
Gln Gly Lys Asn Ala Leu Phe Ala Ile Thr Ala Glu Trp Cys Gly Tyr
165 170 175

tgc aag aag ctc aag cct aca tgg gag cag ctg gct gcc gtt ttc caa 576
Cys Lys Lys Leu Lys Pro Thr Trp Glu Gln Leu Ala Ala Val Phe Gln
180 185 190

ggc gac gag gaa aac atc ttg att gga cag gtc caa acc acc ggc gat 624
Gly Asp Glu Glu Asn Ile Leu Ile Gly Gln Val Gln Thr Thr Gly Asp
195 200 205

aac cca aca gaa tgg atc cag gag aaa tac aac ctc cag tcg ttc ccc 672
Asn Pro Thr Glu Trp Ile Gln Glu Lys Tyr Asn Leu Gln Ser Phe Pro
210 215 220

aca ata gtc ttc atc gag aag ggc aac ctg gac gag cct gtg ttc tat 720
Thr Ile Val Phe Ile Glu Lys Gly Asn Leu Asp Glu Pro Val Phe Tyr
225 230 235 240

ccg tac gga aga gag ctg gga gac ctg gtt gag ttc gtt aac acc cag 768
Pro Tyr Gly Arg Glu Leu Gly Asp Leu Val Glu Phe Val Asn Thr Gln
245 250 255

gcc gga act cac cgg aac gag aaa ggc gag ctg gac tcc gag gcc ggg 816
Ala Gly Thr His Arg Asn Glu Lys Gly Glu Leu Asp Ser Glu Ala Gly
260 265 270

ctg ata cac gca gtc gac gag ctg gtt gag cag ttt gtc ggt tcc tcg 864
Leu Ile His Ala Val Asp Glu Leu Val Glu Gln Phe Val Gly Ser Ser
275 280 285

agc agc ggc aga aaa aat ctg gtt ccg aaa ttc ttg gaa gct ttg aaa 912

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

tcg gct gac acc gac aat gca ttg tcg aaa gaa gtg aaa tac tac aac 960
Ser Ala Asp Thr Asp Asn Ala Leu Ser Lys Glu Val Lys Tyr Tyr Asn
305 310 315 320

aag atc atc cat acg atg gtc aac ggt ccc ttt gac ttc gtc gcg aaa 1008
Lys Ile Ile His Thr Met Val Asn Gly Pro Phe Asp Phe Val Ala Lys
325 330 335

gaa acc gct aga ctg gag tcg cta ctg aag tcg gat ctg tct tcc cga 1056
Glu Thr Ala Arg Leu Glu Ser Leu Leu Lys Ser Asp Leu Ser Ser Arg
340 345 350

gcc aga gac tca gct tcc ttt aga ctc aac atc ctc aag ttt ttc agc 1104
Ala Arg Asp Ser Ala Ser Phe Arg Leu Asn Ile Leu Lys Phe Phe Ser
355 360 365

gat cct gcc cct cca gcc aag gat gag ctg tga 1137
Asp Pro Ala Pro Pro Ala Lys Asp Glu Leu
370 375

<210> 42
<211> 378
<212> PRT
<213> Ogataea minuta

<400> 42

Met Lys Val Thr Ser Ile Trp Gln Val Leu Phe Met Leu Ile Ala Gly
1 5 10 15

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

Asp Glu Val Val Leu Asn Ser Gly Lys Thr Ser Leu Val Glu Phe Tyr
35 40 45

Ala Ser Trp Cys Ser His Cys Lys Lys Leu Glu Pro Thr Trp Glu Glu
50 55 60

Leu Ala Ser Ala Tyr Gly Asn Lys Asn Asp Ile Gln Ile Val Lys Ile
65 70 75 80

Asp Ala Asp Glu Asn Gly Asn Val Gly Arg Lys Phe Gly Ile Lys Gly
85 90 95

Phe Pro Thr Leu Lys Leu Phe Lys Lys Asp Asp Leu Asn Asn Pro Val
100 105 110

Glu Phe Glu Gly Ser Arg Asp Phe His Ser Phe Thr Asn Phe Ile Ala
115 120 125

Ala His Thr Gly Ile Lys Ala Ala Asn Ala Val Pro Thr Glu Pro Ser
130 135 140

Lys Val Val Glu Leu His Asp Gly Asn Leu Glu Glu Leu Val Lys Glu
145 150 155 160

Gln Gly Lys Asn Ala Leu Phe Ala Ile Thr Ala Glu Trp Cys Gly Tyr
165 170 175

Cys Lys Lys Leu Lys Pro Thr Trp Glu Gln Leu Ala Ala Val Phe Gln
180 185 190

Gly Asp Glu Glu Asn Ile Leu Ile Gly Gln Val Gln Thr Thr Gly Asp
195 200 205

Asn Pro Thr Glu Trp Ile Gln Glu Lys Tyr Asn Leu Gln Ser Phe Pro
210 215 220

Thr Ile Val Phe Ile Glu Lys Gly Asn Leu Asp Glu Pro Val Phe Tyr
225 230 235 240

Pro Tyr Gly Arg Glu Leu Gly Asp Leu Val Glu Phe Val Asn Thr Gln
245 250 255

Ala Gly Thr His Arg Asn Glu Lys Gly Glu Leu Asp Ser Glu Ala Gly
260 265 270

Leu Ile His Ala Val Asp Glu Leu Val Glu Gln Phe Val Gly Ser Ser
275 280 285

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

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

Lys Ile Ile His Thr Met Val Asn Gly Pro Phe Asp Phe Val Ala Lys
325 330 335

Glu Thr Ala Arg Leu Glu Ser Leu Leu Lys Ser Asp Leu Ser Ser Arg
340 345 350

Ala Arg Asp Ser Ala Ser Phe Arg Leu Asn Ile Leu Lys Phe Phe Ser
355 360 365

Asp Pro Ala Pro Pro Ala Lys Asp Glu Leu
370 375

<210> 43
<211> 1728
<212> DNA
<213> Ogataea minuta

<220>

<221> CDS

<222> (1)..(1728)

<400> 43

atg aag cac gtg ata agt ggc cga tcc cgg gtg gcg ttg ggc tgg att 48
Met Lys His Val Ile Ser Gly Arg Ser Arg Val Ala Leu Gly Trp Ile
1 5 10 15

ctg acg tgg ttg atc tgt gcg att tgc gct gtt ccc gga gct ggg ttg 96
Leu Thr Trp Leu Ile Cys Ala Ile Cys Ala Val Pro Gly Ala Gly Leu
20 25 30

cag gag att tcg tcc ttg gag agg aaa ccg gct tac ctg tcg ccg cag 144
Gln Glu Ile Ser Ser Leu Glu Arg Lys Pro Ala Tyr Leu Ser Pro Gln
35 40 45

tat gag tac gac aac att cac gag ttt gag tcg acg cct ttc cgc gat 192
Tyr Glu Tyr Asp Asn Ile His Glu Phe Glu Ser Thr Pro Phe Arg Asp
50 55 60

tac gag acg ttc acg ggg tcc aag gtg agt gag agt tcg aac gtg act 240
Tyr Glu Thr Phe Thr Gly Ser Lys Val Ser Glu Ser Ser Asn Val Thr
65 70 75 80

ttt ggc cag atc aac gcg ctc aac aac gaa atc aga ccg gtt ttg cac 288
Phe Gly Gln Ile Asn Ala Leu Asn Asn Glu Ile Arg Pro Val Leu His
85 90 95

gat ctg att aac gag aac ttc ttc aaa atc ttt cga ctc aac ctg tac 336
Asp Leu Ile Asn Glu Asn Phe Phe Lys Ile Phe Arg Leu Asn Leu Tyr
100 105 110

aag gag tgt ccg ttc tgg tcg agt tcg gag gga ttt tgc atg cac aag 384
Lys Glu Cys Pro Phe Trp Ser Ser Ser Glu Gly Phe Cys Met His Lys
115 120 125

agc tgt gcc gtg gac acc att gac gac tgg aaa gat ctt ccg gag ata 432
Ser Cys Ala Val Asp Thr Ile Asp Asp Trp Lys Asp Leu Pro Glu Ile
130 135 140

tgg cag ccc gag gct ctg ggt cgg atc gag tcg ttg acg cga gaa ccg 480
Trp Gln Pro Glu Ala Leu Gly Arg Ile Glu Ser Leu Thr Arg Glu Pro
145 150 155 160

cct acg acg ata tct gac gcg gga aat ggc tcg tgt gtc gct gca ggc 528
Pro Thr Thr Ile Ser Asp Ala Gly Asn Gly Ser Cys Val Ala Ala Gly
165 170 175

gga cgg agc acg cgg gat tac tgc gaa ctg gac gag gtc aac gag gac 576
Gly Arg Ser Thr Arg Asp Tyr Cys Glu Leu Asp Glu Val Asn Glu Asp
180 185 190

tcg gta tac gtg aat ctg gtg gac aat ccc gag aga ttc acg ggg tac 624
Ser Val Tyr Val Asn Leu Val Asp Asn Pro Glu Arg Phe Thr Gly Tyr
195 200 205

gga gga gat cag tcg ttc caa att tgg cgc agc att tac aac gag aac 672
Gly Gly Asp Gln Ser Phe Gln Ile Trp Arg Ser Ile Tyr Asn Glu Asn
210 215 220

tgt ttc aat ttg ggc tcg gat caa tgt ctc gag aag aac ttt ttc tac 720
Cys Phe Asn Leu Gly Ser Asp Gln Cys Leu Glu Lys Asn Phe Phe Tyr
225 230 235 240

aag ttg atc agt gga atg cac tcg tcg atc tcg act cat ctg acc aac 768
Lys Leu Ile Ser Gly Met His Ser Ser Ile Ser Thr His Leu Thr Asn
245 250 255

gag tac ctg aac ttc aag acc aag cag tat gga cag gat ctc aag cag 816
Glu Tyr Leu Asn Phe Lys Thr Lys Gln Tyr Gly Gln Asp Leu Lys Gln
260 265 270

ttc atg atc cgg gtg ggg gac ttc cct gac cgg ttc gag aac ttg tat 864
Phe Met Ile Arg Val Gly Asp Phe Pro Asp Arg Phe Glu Asn Leu Tyr
275 280 285

ctg aac tac gtt ctg gtg gtg aag tcc ttg atc aag ctg gag cag tcg 912
Leu Asn Tyr Val Leu Val Val Lys Ser Leu Ile Lys Leu Glu Gln Ser
290 295 300

ggt gtg ctg gac aac ctc cag ttc tgt gac gag gag gtg ttt cag acg 960
Gly Val Leu Asp Asn Leu Gln Phe Cys Asp Glu Glu Val Phe Gln Thr
305 310 315 320

aag gag aag gag cta aaa cgc gag ctg aag gag atg att tct ccg ttc 1008
Lys Glu Lys Glu Leu Lys Arg Glu Leu Lys Glu Met Ile Ser Pro Phe
325 330 335

tac cag ctg gcg gaa ggt ggg aaa gtg gac gag tgt ctg ttc aac gag 1056
Tyr Gln Leu Ala Glu Gly Gly Lys Val Asp Glu Cys Leu Phe Asn Glu
340 345 350

cac tcg ctg ttc cag agc gag gac tcg acg tat ctg aag gac gag ttc 1104
His Ser Leu Phe Gln Ser Glu Asp Ser Thr Tyr Leu Lys Asp Glu Phe
355 360 365

agt gag aac ttc agg aac gtg tcg cgg atc atg gat tgt gtc cac tgc 1152

Ser Glu Asn Phe Arg Asn Val Ser Arg Ile Met Asp Cys Val His Cys
370 375 380

gac agg tgc aga ctg tgg ggg aag gtg cag acc acc ggg tac gga act 1200
Asp Arg Cys Arg Leu Trp Gly Lys Val Gln Thr Thr Gly Tyr Gly Thr
385 390 395 400

gcg ctg aag att ttg ttt gag ctg gat gca agc gac agc cac gag ctg 1248
Ala Leu Lys Ile Leu Phe Glu Leu Asp Ala Ser Asp Ser His Glu Leu
405 410 415

ggc aag aat ttc cag atc tcc aaa atc gag ctg gtc gcc ctg atc aac 1296
Gly Lys Asn Phe Gln Ile Ser Lys Ile Glu Leu Val Ala Leu Ile Asn
420 425 430

acg ttt gac agg ctg tcc aag agc gtg cac gcc atc gga aac ttc aaa 1344
Thr Phe Asp Arg Leu Ser Lys Ser Val His Ala Ile Gly Asn Phe Lys
435 440 445

caa ctg tac gat ctg aga atg aaa cag gag gaa gaa ggg ggg tcc atg 1392
Gln Leu Tyr Asp Leu Arg Met Lys Gln Glu Glu Glu Gly Gly Ser Met
450 455 460

att act gca gac acg ttt gac ttg gag caa ttg ctg ttg aca gac cag 1440
Ile Thr Ala Asp Thr Phe Asp Leu Glu Gln Leu Leu Leu Thr Asp Gln
465 470 475 480

acg gtg gac gta ttc ggc cag agc act tct gag cca gaa acc ccg tcg 1488
Thr Val Asp Val Phe Gly Gln Ser Thr Ser Glu Pro Glu Thr Pro Ser
485 490 495

gac gtt aga tat ccc gac aga aca cgg ggc tcg ctc gtt ccc gag ggg 1536
Asp Val Arg Tyr Pro Asp Arg Thr Arg Gly Ser Leu Val Pro Glu Gly

500 505 510

ctc ggc gag gcg ttc aag aca gag ctg tac agc gtt tat cag gcg ttc 1584
Leu Gly Glu Ala Phe Lys Thr Glu Leu Tyr Ser Val Tyr Gln Ala Phe
515 520 525

tac ttt gtc gtg acc agc tac acc atg ttc ccc aag ctg atc tac aac 1632
Tyr Phe Val Val Thr Ser Tyr Thr Met Phe Pro Lys Leu Ile Tyr Asn
530 535 540

tac ctg ctg atc cgg gtg gtg tac tgg tgg aac atc ttt gtg ggt cat 1680
Tyr Leu Leu Ile Arg Val Val Tyr Trp Trp Asn Ile Phe Val Gly His
545 550 555 560

gta cac gag gac ttt gac gtg gat cgc ctg tat cgt ttg gag cta taa 1728
Val His Glu Asp Phe Asp Val Asp Arg Leu Tyr Arg Leu Glu Leu
565 570 575

<210> 44
<211> 575
<212> PRT
<213> Ogataea minuta

<400> 44

Met Lys His Val Ile Ser Gly Arg Ser Arg Val Ala Leu Gly Trp Ile
1 5 10 15

Leu Thr Trp Leu Ile Cys Ala Ile Cys Ala Val Pro Gly Ala Gly Leu
20 25 30

Gln Glu Ile Ser Ser Leu Glu Arg Lys Pro Ala Tyr Leu Ser Pro Gln
35 40 45

Tyr Glu Tyr Asp Asn Ile His Glu Phe Glu Ser Thr Pro Phe Arg Asp
50 55 60

Tyr Glu Thr Phe Thr Gly Ser Lys Val Ser Glu Ser Ser Asn Val Thr
65 70 75 80

Phe Gly Gln Ile Asn Ala Leu Asn Asn Glu Ile Arg Pro Val Leu His
85 90 95

Asp Leu Ile Asn Glu Asn Phe Phe Lys Ile Phe Arg Leu Asn Leu Tyr
100 105 110

Lys Glu Cys Pro Phe Trp Ser Ser Ser Glu Gly Phe Cys Met His Lys
115 120 125

Ser Cys Ala Val Asp Thr Ile Asp Asp Trp Lys Asp Leu Pro Glu Ile
130 135 140

Trp Gln Pro Glu Ala Leu Gly Arg Ile Glu Ser Leu Thr Arg Glu Pro
145 150 155 160

Pro Thr Thr Ile Ser Asp Ala Gly Asn Gly Ser Cys Val Ala Ala Gly
165 170 175

Gly Arg Ser Thr Arg Asp Tyr Cys Glu Leu Asp Glu Val Asn Glu Asp
180 185 190

Ser Val Tyr Val Asn Leu Val Asp Asn Pro Glu Arg Phe Thr Gly Tyr
195 200 205

Gly Gly Asp Gln Ser Phe Gln Ile Trp Arg Ser Ile Tyr Asn Glu Asn
210 215 220

Cys Phe Asn Leu Gly Ser Asp Gln Cys Leu Glu Lys Asn Phe Phe Tyr
225 230 235 240

Lys Leu Ile Ser Gly Met His Ser Ser Ile Ser Thr His Leu Thr Asn
245 250 255

Glu Tyr Leu Asn Phe Lys Thr Lys Gln Tyr Gly Gln Asp Leu Lys Gln
260 265 270

Phe Met Ile Arg Val Gly Asp Phe Pro Asp Arg Phe Glu Asn Leu Tyr
275 280 285

Leu Asn Tyr Val Leu Val Val Lys Ser Leu Ile Lys Leu Glu Gln Ser

290 295 300

Gly Val Leu Asp Asn Leu Gln Phe Cys Asp Glu Glu Val Phe Gln Thr
305 310 315 320

Lys Glu Lys Glu Leu Lys Arg Glu Leu Lys Glu Met Ile Ser Pro Phe
325 330 335

Tyr Gln Leu Ala Glu Gly Gly Lys Val Asp Glu Cys Leu Phe Asn Glu
340 345 350

His Ser Leu Phe Gln Ser Glu Asp Ser Thr Tyr Leu Lys Asp Glu Phe
355 360 365

Ser Glu Asn Phe Arg Asn Val Ser Arg Ile Met Asp Cys Val His Cys
370 375 380

Asp Arg Cys Arg Leu Trp Gly Lys Val Gln Thr Thr Gly Tyr Gly Thr
385 390 395 400

Ala Leu Lys Ile Leu Phe Glu Leu Asp Ala Ser Asp Ser His Glu Leu
405 410 415

Gly Lys Asn Phe Gln Ile Ser Lys Ile Glu Leu Val Ala Leu Ile Asn
420 425 430

Thr Phe Asp Arg Leu Ser Lys Ser Val His Ala Ile Gly Asn Phe Lys
435 440 445

Gln Leu Tyr Asp Leu Arg Met Lys Gln Glu Glu Glu Gly Gly Ser Met
450 455 460

Ile Thr Ala Asp Thr Phe Asp Leu Glu Gln Leu Leu Leu Thr Asp Gln
465 470 475 480

Thr Val Asp Val Phe Gly Gln Ser Thr Ser Glu Pro Glu Thr Pro Ser
485 490 495

Asp Val Arg Tyr Pro Asp Arg Thr Arg Gly Ser Leu Val Pro Glu Gly
500 505 510

Leu Gly Glu Ala Phe Lys Thr Glu Leu Tyr Ser Val Tyr Gln Ala Phe
515 520 525

Tyr Phe Val Val Thr Ser Tyr Thr Met Phe Pro Lys Leu Ile Tyr Asn
530 535 540

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gct cag aag cta tgt cag cag aac tca aac gca cag ata gtg cca ttg 96
Ala Gln Lys Leu Cys Gln Gln Asn Ser Asn Ala Gln Ile Val Pro Leu
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cac ttt ctg gcg gca atg act ccg acc tcc agt gag ggg gag gca atc 144
His Phe Leu Ala Ala Met Thr Pro Thr Ser Ser Glu Gly Glu Ala Ile
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tat ctc aag acg ttg att gag aga ggt cga ttt gac tgg act gct ttt 192
Tyr Leu Lys Thr Leu Ile Glu Arg Gly Arg Phe Asp Trp Thr Ala Phe
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gaa aga gcc gtt aat aaa gca gtg gtg cgg ctc cca agc gtg gcc ggt 240

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

aac gca tcc aag atc aag gca cag cag aaa gac tcg tac atc ggc caa 336
Asn Ala Ser Lys Ile Lys Ala Gln Gln Lys Asp Ser Tyr Ile Gly Gln
100 105 110

gac cac att ctc tcg gcc ctt ttg gac gat tct agt atc cag gct gtg 384
Asp His Ile Leu Ser Ala Leu Leu Asp Asp Ser Ser Ile Gln Ala Val
115 120 125

ctg aag gaa gcg aac gtc aag ccc gac gca ttg aag aca cag atc gtt 432
Leu Lys Glu Ala Asn Val Lys Pro Asp Ala Leu Lys Thr Gln Ile Val
130 135 140

gaa ctc aga ggc aat cag aga att gat tct cgt caa gct gat tcg tct 480
Glu Leu Arg Gly Asn Gln Arg Ile Asp Ser Arg Gln Ala Asp Ser Ser
145 150 155 160

cag aag ttt gag ttt ctg tcc aag tac gcc ctt gat ctt acc gag cag 528
Gln Lys Phe Glu Phe Leu Ser Lys Tyr Ala Leu Asp Leu Thr Glu Gln
165 170 175

gcg ttg cag ggt aag att gac cct gtg ata ggc aga gag gag gag atc 576
Ala Leu Gln Gly Lys Ile Asp Pro Val Ile Gly Arg Glu Glu Glu Ile
180 185 190

aga agg gcc att cgg gtt ctt tcg aga cgg gcc aag tcg aac ccg tgt 624
Arg Arg Ala Ile Arg Val Leu Ser Arg Arg Ala Lys Ser Asn Pro Cys

195 200 205

ctg att gga gat cct ggt gtt ggt aag act agt att gtt gag gga gtc 672
Leu Ile Gly Asp Pro Gly Val Gly Lys Thr Ser Ile Val Glu Gly Val
210 215 220

gca cag agg ata gtg gac aac gat gtt cct acc gtt tta cag ggg tgc 720
Ala Gln Arg Ile Val Asp Asn Asp Val Pro Thr Val Leu Gln Gly Cys
225 230 235 240

aag ctg tac tcg ctg gac ttg ggt gcg ttg aaa gcg gga gcc aag tac 768
Lys Leu Tyr Ser Leu Asp Leu Gly Ala Leu Lys Ala Gly Ala Lys Tyr
245 250 255

cag ggt gag ttc gag gaa aga ttg aag gga gtt ttg agc gac atc gaa 816
Gln Gly Glu Phe Glu Glu Arg Leu Lys Gly Val Leu Ser Asp Ile Glu
260 265 270

agc tcg aac tcg atg atc atc ttg ttc atc gac gag atc cac atg ttg 864
Ser Ser Asn Ser Met Ile Ile Leu Phe Ile Asp Glu Ile His Met Leu
275 280 285

atg ggc gat gga aag tcg gac gct gcc aac ttg ctg aag cct gct ctg 912
Met Gly Asp Gly Lys Ser Asp Ala Ala Asn Leu Leu Lys Pro Ala Leu
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gcg aga ggt cag ttc cac tgc atc ggt gcg act acc gtg acc gag tac 960
Ala Arg Gly Gln Phe His Cys Ile Gly Ala Thr Thr Val Thr Glu Tyr
305 310 315 320

aga aag cac atc gag aag gac ggt gct ttc gag aga aga ttt cag aga 1008
Arg Lys His Ile Glu Lys Asp Gly Ala Phe Glu Arg Arg Phe Gln Arg
325 330 335

atc gac gtg aga gag ccg acc atc aga gaa acc gtt gcc att ctc aga 1056
Ile Asp Val Arg Glu Pro Thr Ile Arg Glu Thr Val Ala Ile Leu Arg
340 345 350

gga ctg cag cca agg tac gag atc cac cac ggt gtc cga atc ctg gac 1104
Gly Leu Gln Pro Arg Tyr Glu Ile His His Gly Val Arg Ile Leu Asp
355 360 365

agt gcc ctg gtg acg gct gct caa ctg gcc tcc aga tac ctc acc tac 1152
Ser Ala Leu Val Thr Ala Ala Gln Leu Ala Ser Arg Tyr Leu Thr Tyr
370 375 380

aga aag ctt ccg gac tct gca gtg gat ctg att gac gag tcc gcc gcg 1200
Arg Lys Leu Pro Asp Ser Ala Val Asp Leu Ile Asp Glu Ser Ala Ala
385 390 395 400

gga gtc gct gtt gcc agg gac tcc aag ccg gag gag ctg gac tcc aag 1248
Gly Val Ala Val Ala Arg Asp Ser Lys Pro Glu Glu Leu Asp Ser Lys
405 410 415

gag aga cag cta cag ctg atc gag gtt gag atc aat gct ctg gaa aga 1296
Glu Arg Gln Leu Gln Leu Ile Glu Val Glu Ile Asn Ala Leu Glu Arg
420 425 430

gac cag gac gcc gac acg tcc acc aag gag aga ctg gag cag gcc aga 1344
Asp Gln Asp Ala Asp Thr Ser Thr Lys Glu Arg Leu Glu Gln Ala Arg
435 440 445

cag aga aga cag aac ctg gag gaa gag ctg gct cca ttg agg gag aag 1392
Gln Arg Arg Gln Asn Leu Glu Glu Glu Leu Ala Pro Leu Arg Glu Lys
450 455 460

tac cag cag gaa aga gcg ggc cac gag gag ctg act gct gcc aag aga 1440
Tyr Gln Gln Glu Arg Ala Gly His Glu Glu Leu Thr Ala Ala Lys Arg
465 470 475 480

aag tta gac gac ctc gaa gtt aag gcg caa gat gcg gag aga aga cac 1488
Lys Leu Asp Asp Leu Glu Val Lys Ala Gln Asp Ala Glu Arg Arg His
485 490 495

gac tct cag acc att gcg gac ctg cgg atg ttt gcc att ccg gac gtg 1536
Asp Ser Gln Thr Ile Ala Asp Leu Arg Met Phe Ala Ile Pro Asp Val
500 505 510

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Lys Arg Arg Ile Glu Glu Leu Glu Gln Lys Val Val Glu Glu Glu Ala
515 520 525

act tct gaa gat ttc atg gtg aag aac gtt gtt ggt tcg gag caa gtt 1632
Thr Ser Glu Asp Phe Met Val Lys Asn Val Val Gly Ser Glu Gln Val
530 535 540

tcc gag acc gcg gcc agg ttg acc ggt att ccg gtc agc aag ctg acg 1680
Ser Glu Thr Ala Ala Arg Leu Thr Gly Ile Pro Val Ser Lys Leu Thr
545 550 555 560

cag gca gaa aat gct aag ctg atc acc atg gag aag gag ctg tcc gct 1728
Gln Ala Glu Asn Ala Lys Leu Ile Thr Met Glu Lys Glu Leu Ser Ala
565 570 575

gcc gtt gtc gga cag ggc gag gct gtc aag gct gtt tcg aac tcg atc 1776
Ala Val Val Gly Gln Gly Glu Ala Val Lys Ala Val Ser Asn Ser Ile
580 585 590

aga ttg tcg aga tct ggt ctg gcc aat cct aac cag ccg gcc tcg ttc 1824

Arg Leu Ser Arg Ser Gly Leu Ala Asn Pro Asn Gln Pro Ala Ser Phe
595 600 605

ctg ttc ctg gga ctc tcg ggt tcc ggt aag acg gag ctg gcc aaa aag 1872
Leu Phe Leu Gly Leu Ser Gly Ser Gly Lys Thr Glu Leu Ala Lys Lys
610 615 620

ctg gca ggg ttc ctg ttc tcg gat gag aag gcc atg atc aga atc gac 1920
Leu Ala Gly Phe Leu Phe Ser Asp Glu Lys Ala Met Ile Arg Ile Asp
625 630 635 640

tgt tca gaa ctg atg gag aag tac tcg gtt tcc aag ctg ctg ggc tcc 1968
Cys Ser Glu Leu Met Glu Lys Tyr Ser Val Ser Lys Leu Leu Gly Ser
645 650 655

acc gcc ggt tac gtt ggc tac gag gag ggt ggt atg ttg acc aac cag 2016
Thr Ala Gly Tyr Val Gly Tyr Glu Glu Gly Gly Met Leu Thr Asn Gln
660 665 670

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Leu Leu Arg Arg Pro Tyr Ser Val Val Leu Phe Asp Glu Val Glu Lys
675 680 685

gcc gct ccc gag gtg ctg aac att ctc ctg cag atg ttg gac gac gga 2112
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690 695 700

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Arg Ile Thr Ala Ala Asn Gly Thr Leu Val Asn Cys Ser Asn Ala Ile
705 710 715 720

gtg atc atg acg tcc aac ctc ggc gcg gag tac atc aac gca tca aag 2208
Val Ile Met Thr Ser Asn Leu Gly Ala Glu Tyr Ile Asn Ala Ser Lys

725 730 735

gga acg aag gtg acc gac gag gtc aga agc ctg gtg atg aac act gtc 2256
Gly Thr Lys Val Thr Asp Glu Val Arg Ser Leu Val Met Asn Thr Val
740 745 750

aag gga cat ttc aga ccg gag ttc ctc aac aga atc tcg gcc acc gtg 2304
Lys Gly His Phe Arg Pro Glu Phe Leu Asn Arg Ile Ser Ala Thr Val
755 760 765

gtg ttc aac aga ctc tcc aga cat gcc atc gcc aag atc gtg cgg ctg 2352
Val Phe Asn Arg Leu Ser Arg His Ala Ile Ala Lys Ile Val Arg Leu
770 775 780

aga ctg aag gaa atc gaa gag agg ttc gag gcc aac ggc aag tcg atc 2400
Arg Leu Lys Glu Ile Glu Glu Arg Phe Glu Ala Asn Gly Lys Ser Ile
785 790 795 800

aaa ctg aac gtg gac gat ggg gca ctc gag tac ctg tgc aaa aag gga 2448
Lys Leu Asn Val Asp Asp Gly Ala Leu Glu Tyr Leu Cys Lys Lys Gly
805 810 815

tac tct ccc gac ttg ggc gct aga ccg ctg aac aga ctg atc cag agt 2496
Tyr Ser Pro Asp Leu Gly Ala Arg Pro Leu Asn Arg Leu Ile Gln Ser
820 825 830

gaa atc ctg aac cat ctg gcg gtg atg gtg ttg aac gga cag gtg ttg 2544
Glu Ile Leu Asn His Leu Ala Val Met Val Leu Asn Gly Gln Val Leu
835 840 845

gac aaa gag gaa gtt cag att acg acg ggc agc aag gga ctg tct gtg 2592
Asp Lys Glu Glu Val Gln Ile Thr Thr Gly Ser Lys Gly Leu Ser Val
850 855 860

gtt cct aac cat gac atc gag gac gaa gct atg gac gtg gac gtg gac 2640
Val Pro Asn His Asp Ile Glu Asp Glu Ala Met Asp Val Asp Val Asp
865 870 875 880

gag tgg acc gac gcc gcc gac gac gac gac tcg ggc tac ggc agt cct 2688
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885 890 895

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Asp Leu Asp

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His Phe Leu Ala Ala Met Thr Pro Thr Ser Ser Glu Gly Glu Ala Ile
35 40 45

Tyr Leu Lys Thr Leu Ile Glu Arg Gly Arg Phe Asp Trp Thr Ala Phe
50 55 60

Glu Arg Ala Val Asn Lys Ala Val Val Arg Leu Pro Ser Val Ala Gly
65 70 75 80

Ser Asn Thr Glu Pro Ser Ile Ser Ala Ser Ala Ala Ser Ile Ile Thr
85 90 95

Asn Ala Ser Lys Ile Lys Ala Gln Gln Lys Asp Ser Tyr Ile Gly Gln
100 105 110

Asp His Ile Leu Ser Ala Leu Leu Asp Asp Ser Ser Ile Gln Ala Val
115 120 125

Leu Lys Glu Ala Asn Val Lys Pro Asp Ala Leu Lys Thr Gln Ile Val
130 135 140

Glu Leu Arg Gly Asn Gln Arg Ile Asp Ser Arg Gln Ala Asp Ser Ser
145 150 155 160

Gln Lys Phe Glu Phe Leu Ser Lys Tyr Ala Leu Asp Leu Thr Glu Gln
165 170 175

Ala Leu Gln Gly Lys Ile Asp Pro Val Ile Gly Arg Glu Glu Glu Ile

180 185 190

Arg Arg Ala Ile Arg Val Leu Ser Arg Arg Ala Lys Ser Asn Pro Cys
195 200 205

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

Ala Gln Arg Ile Val Asp Asn Asp Val Pro Thr Val Leu Gln Gly Cys
225 230 235 240

Lys Leu Tyr Ser Leu Asp Leu Gly Ala Leu Lys Ala Gly Ala Lys Tyr
245 250 255

Gln Gly Glu Phe Glu Glu Arg Leu Lys Gly Val Leu Ser Asp Ile Glu
260 265 270

Ser Ser Asn Ser Met Ile Ile Leu Phe Ile Asp Glu Ile His Met Leu
275 280 285

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

Ala Arg Gly Gln Phe His Cys Ile Gly Ala Thr Thr Val Thr Glu Tyr
305 310 315 320

Arg Lys His Ile Glu Lys Asp Gly Ala Phe Glu Arg Arg Phe Gln Arg
325 330 335

Ile Asp Val Arg Glu Pro Thr Ile Arg Glu Thr Val Ala Ile Leu Arg
340 345 350

Gly Leu Gln Pro Arg Tyr Glu Ile His His Gly Val Arg Ile Leu Asp
355 360 365

Ser Ala Leu Val Thr Ala Ala Gln Leu Ala Ser Arg Tyr Leu Thr Tyr
370 375 380

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385 390 395 400

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405 410 415

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

Asp Gln Asp Ala Asp Thr Ser Thr Lys Glu Arg Leu Glu Gln Ala Arg
435 440 445

Gln Arg Arg Gln Asn Leu Glu Glu Glu Leu Ala Pro Leu Arg Glu Lys
450 455 460

Tyr Gln Gln Glu Arg Ala Gly His Glu Glu Leu Thr Ala Ala Lys Arg
465 470 475 480

Lys Leu Asp Asp Leu Glu Val Lys Ala Gln Asp Ala Glu Arg Arg His
485 490 495

Asp Ser Gln Thr Ile Ala Asp Leu Arg Met Phe Ala Ile Pro Asp Val
500 505 510

Lys Arg Arg Ile Glu Glu Leu Glu Gln Lys Val Val Glu Glu Glu Ala
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565 570 575

Ala Val Val Gly Gln Gly Glu Ala Val Lys Ala Val Ser Asn Ser Ile
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625 630 635 640

Cys Ser Glu Leu Met Glu Lys Tyr Ser Val Ser Lys Leu Leu Gly Ser
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660 665 670

Leu Leu Arg Arg Pro Tyr Ser Val Val Leu Phe Asp Glu Val Glu Lys
675 680 685

Ala Ala Pro Glu Val Leu Asn Ile Leu Leu Gln Met Leu Asp Asp Gly
690 695 700

Arg Ile Thr Ala Ala Asn Gly Thr Leu Val Asn Cys Ser Asn Ala Ile

705 710 715 720

Val Ile Met Thr Ser Asn Leu Gly Ala Glu Tyr Ile Asn Ala Ser Lys
725 730 735

Gly Thr Lys Val Thr Asp Glu Val Arg Ser Leu Val Met Asn Thr Val
740 745 750

Lys Gly His Phe Arg Pro Glu Phe Leu Asn Arg Ile Ser Ala Thr Val
755 760 765

Val Phe Asn Arg Leu Ser Arg His Ala Ile Ala Lys Ile Val Arg Leu
770 775 780

Arg Leu Lys Glu Ile Glu Glu Arg Phe Glu Ala Asn Gly Lys Ser Ile
785 790 795 800

Lys Leu Asn Val Asp Asp Gly Ala Leu Glu Tyr Leu Cys Lys Lys Gly
805 810 815

Tyr Ser Pro Asp Leu Gly Ala Arg Pro Leu Asn Arg Leu Ile Gln Ser
820 825 830

Glu Ile Leu Asn His Leu Ala Val Met Val Leu Asn Gly Gln Val Leu
835 840 845

Asp Lys Glu Glu Val Gln Ile Thr Thr Gly Ser Lys Gly Leu Ser Val
850 855 860

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Ala Val Leu Leu Val Val Leu Pro Leu Ala Ser Gln Gln Phe Val Glu
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gca gag gcg aac gac aac tat ggt act gtt atc ggt atc gat ttg gga       144
Ala Glu Ala Asn Asp Asn Tyr Gly Thr Val Ile Gly Ile Asp Leu Gly
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acc act tac tca tgt gtg ggt gtg atg aaa gct ggt aga gtt gag atc       192
Thr Thr Tyr Ser Cys Val Gly Val Met Lys Ala Gly Arg Val Glu Ile
    50                  55                  60

ctt gcc aat gac cag ggt aac aga att act cca tct tat gtg gca ttt       240
Leu Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser Tyr Val Ala Phe
65                  70                  75                  80

act gat gaa gag aga ctt gtc gga gat gcc gca aag aac cag att gcc       288
Thr Asp Glu Glu Arg Leu Val Gly Asp Ala Ala Lys Asn Gln Ile Ala
                85                  90                  95

tcc aac cca agc aac aca atc ttc gat atc aag aga ctc ata gga cac       336
Ser Asn Pro Ser Asn Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly His
            100                 105                 110

aga ttt gac gat aag gtt gtg caa aaa gag att gca cac ctc cct tac       384
Arg Phe Asp Asp Lys Val Val Gln Lys Glu Ile Ala His Leu Pro Tyr
        115                 120                 125

aag atc aga aac caa gag ggc aga ccg gtc gtt gag gcc act gtc aat       432
Lys Ile Arg Asn Gln Glu Gly Arg Pro Val Val Glu Ala Thr Val Asn
    130                 135                 140

gga gag gtg acc acg ttc acg gcc gaa gaa gtt tcg gcc atg atc ttg       480
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Gly Glu Val Thr Thr Phe Thr Ala Glu Glu Val Ser Ala Met Ile Leu
145 150 155 160

gga aag atg aag caa att gct gaa gat tat ctc gga aag aag gtt acc 528
Gly Lys Met Lys Gln Ile Ala Glu Asp Tyr Leu Gly Lys Lys Val Thr
165 170 175

cat gct gtt gtc acg gtt cct gca tac ttt aac gac gcc caa aga cag 576
His Ala Val Val Thr Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln
180 185 190

gcc act aag gat gct ggt acc att gcc ggt ctg gaa gtt ttg aga att 624
Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Glu Val Leu Arg Ile
195 200 205

gtt aac gag cct act gcc gct gca att gct tac ggt ctc gac aag acg 672
Val Asn Glu Pro Thr Ala Ala Ala Ile Ala Tyr Gly Leu Asp Lys Thr
210 215 220

gac gaa gag aag cat atc att gtt tac gat ttg ggt gga gga act ttt 720
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225 230 235 240

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245 250 255

acc gct ggt gat acc cat ctt ggt ggt gag gac ttt gat tac aga gtt 816
Thr Ala Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Tyr Arg Val
260 265 270

gtc aga cat ttc atc aag gtg ttt aag aag aag cat ggc att gat atc 864
Val Arg His Phe Ile Lys Val Phe Lys Lys Lys His Gly Ile Asp Ile

275 280 285

agt gat aat cca aag gct ctt gct aaa ttg aag aga gaa gtt gaa aaa 912
Ser Asp Asn Pro Lys Ala Leu Ala Lys Leu Lys Arg Glu Val Glu Lys
290 295 300

gct aag aga acc ttg tct tct caa atg tcc acc aga att gag att gac 960
Ala Lys Arg Thr Leu Ser Ser Gln Met Ser Thr Arg Ile Glu Ile Asp
305 310 315 320

tcg ttc gct gac ggt att gac ttc tcc gag tcc tta tcc agg gcc aag 1008
Ser Phe Ala Asp Gly Ile Asp Phe Ser Glu Ser Leu Ser Arg Ala Lys
325 330 335

ttc gag gaa ttg aac att gag ttg ttc aaa aag acc ttg aag cct gtt 1056
Phe Glu Glu Leu Asn Ile Glu Leu Phe Lys Lys Thr Leu Lys Pro Val
340 345 350

caa cgt gtt ctt gaa gac gcc aaa ttc aag gtt tca gaa att gat gac 1104
Gln Arg Val Leu Glu Asp Ala Lys Phe Lys Val Ser Glu Ile Asp Asp
355 360 365

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Ile Val Leu Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Glu Leu
370 375 380

ctg gaa agt tac ttc aac ggc aag caa gtg tcc aag gga att aac cca 1200
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385 390 395 400

gat gaa gct gtt gct tac ggt gcg gct gtt caa gct ggt gtc ctc tct 1248
Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser
405 410 415

ggt gaa gaa ggc gtt gaa gac att gtt ttg att gat gtg aat cca tta 1296
Gly Glu Glu Gly Val Glu Asp Ile Val Leu Ile Asp Val Asn Pro Leu
420 425 430

act ttg ggt atc gag acc tcc ggc ggt gtc atg acc act ttg att aag 1344
Thr Leu Gly Ile Glu Thr Ser Gly Gly Val Met Thr Thr Leu Ile Lys
435 440 445

aga aac act gca att cca acc aag aag tct caa att ttc tct act gct 1392
Arg Asn Thr Ala Ile Pro Thr Lys Lys Ser Gln Ile Phe Ser Thr Ala
450 455 460

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Ala Asp Asn Gln Pro Val Val Leu Ile Gln Val Tyr Glu Gly Glu Arg
465 470 475 480

gcc atg gca aag gat aac aat ttg cta gga aag ttc gag ttg aag gat 1488
Ala Met Ala Lys Asp Asn Asn Leu Leu Gly Lys Phe Glu Leu Lys Asp
485 490 495

att cct cca gcc cca aga ggt acc cca caa att gag gtg act ttc act 1536
Ile Pro Pro Ala Pro Arg Gly Thr Pro Gln Ile Glu Val Thr Phe Thr
500 505 510

ctg gac tcc aac gga atc ctg aag gtt gct gcc act gat aaa ggt act 1584
Leu Asp Ser Asn Gly Ile Leu Lys Val Ala Ala Thr Asp Lys Gly Thr
515 520 525

ggt aag tct aac tct atc aca atc aca aac gac aag ggt aga ctt tcg 1632
Gly Lys Ser Asn Ser Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser
530 535 540

aag gag gag att gag aag aag gtt gag gag gcc gaa cag tat gct caa 1680
Lys Glu Glu Ile Glu Lys Lys Val Glu Glu Ala Glu Gln Tyr Ala Gln
545 550 555 560

caa gat aag gag gtc aga gag aag atc gag agc aga aac gga ctt gag 1728
Gln Asp Lys Glu Val Arg Glu Lys Ile Glu Ser Arg Asn Gly Leu Glu
565 570 575

aac tac gcc cac tcg ttg aaa aac caa gtg aac gat gag acc gga ttc 1776
Asn Tyr Ala His Ser Leu Lys Asn Gln Val Asn Asp Glu Thr Gly Phe
580 585 590

ggc tcc aag ctt gat gag gat gac aag gaa act ttg ttg gat gcc atc 1824
Gly Ser Lys Leu Asp Glu Asp Asp Lys Glu Thr Leu Leu Asp Ala Ile
595 600 605

aac gag gca ttg gag tac ttg gac gac aac ttt gag acc gca aca aag 1872
Asn Glu Ala Leu Glu Tyr Leu Asp Asp Asn Phe Glu Thr Ala Thr Lys
610 615 620

caa gac ttt gag gat cag aag gaa aaa ttg agt aag gtt gct tac cca 1920
Gln Asp Phe Glu Asp Gln Lys Glu Lys Leu Ser Lys Val Ala Tyr Pro
625 630 635 640

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Ile Thr Ser Lys Leu Tyr Asp Thr Pro Pro Thr Ser Asp Glu Asp Asp
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<400> 48

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

Thr Thr Tyr Ser Cys Val Gly Val Met Lys Ala Gly Arg Val Glu Ile
50 55 60

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

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

Ser Asn Pro Ser Asn Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly His
100 105 110

Arg Phe Asp Asp Lys Val Val Gln Lys Glu Ile Ala His Leu Pro Tyr
115 120 125

Lys Ile Arg Asn Gln Glu Gly Arg Pro Val Val Glu Ala Thr Val Asn
130 135 140

Gly Glu Val Thr Thr Phe Thr Ala Glu Glu Val Ser Ala Met Ile Leu
145 150 155 160

Gly Lys Met Lys Gln Ile Ala Glu Asp Tyr Leu Gly Lys Lys Val Thr
165 170 175

His Ala Val Val Thr Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln
180 185 190

Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Glu Val Leu Arg Ile
195 200 205

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

Asp Glu Glu Lys His Ile Ile Val Tyr Asp Leu Gly Gly Gly Thr Phe
225 230 235 240

Asp Val Ser Leu Leu Thr Ile Ala Gly Gly Ala Phe Glu Val Arg Ala
245 250 255

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

Val Arg His Phe Ile Lys Val Phe Lys Lys Lys His Gly Ile Asp Ile
275 280 285

Ser Asp Asn Pro Lys Ala Leu Ala Lys Leu Lys Arg Glu Val Glu Lys
290 295 300

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

Ser Phe Ala Asp Gly Ile Asp Phe Ser Glu Ser Leu Ser Arg Ala Lys
325 330 335

Phe Glu Glu Leu Asn Ile Glu Leu Phe Lys Lys Thr Leu Lys Pro Val
340 345 350

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

Ile Val Leu Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Glu Leu
370 375 380

Leu Glu Ser Tyr Phe Asn Gly Lys Gln Val Ser Lys Gly Ile Asn Pro
385 390 395 400

Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser
405 410 415

Gly Glu Glu Gly Val Glu Asp Ile Val Leu Ile Asp Val Asn Pro Leu
420 425 430

Thr Leu Gly Ile Glu Thr Ser Gly Gly Val Met Thr Thr Leu Ile Lys
435 440 445

Arg Asn Thr Ala Ile Pro Thr Lys Lys Ser Gln Ile Phe Ser Thr Ala
450 455 460

Ala Asp Asn Gln Pro Val Val Leu Ile Gln Val Tyr Glu Gly Glu Arg
465 470 475 480

Ala Met Ala Lys Asp Asn Asn Leu Leu Gly Lys Phe Glu Leu Lys Asp
485 490 495

Ile Pro Pro Ala Pro Arg Gly Thr Pro Gln Ile Glu Val Thr Phe Thr

500 505 510

Leu Asp Ser Asn Gly Ile Leu Lys Val Ala Ala Thr Asp Lys Gly Thr
515 520 525

Gly Lys Ser Asn Ser Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser
530 535 540

Lys Glu Glu Ile Glu Lys Lys Val Glu Glu Ala Glu Gln Tyr Ala Gln
545 550 555 560

Gln Asp Lys Glu Val Arg Glu Lys Ile Glu Ser Arg Asn Gly Leu Glu
565 570 575

Asn Tyr Ala His Ser Leu Lys Asn Gln Val Asn Asp Glu Thr Gly Phe
580 585 590

Gly Ser Lys Leu Asp Glu Asp Asp Lys Glu Thr Leu Leu Asp Ala Ile
595 600 605

Asn Glu Ala Leu Glu Tyr Leu Asp Asp Asn Phe Glu Thr Ala Thr Lys
610 615 620

Gln Asp Phe Glu Asp Gln Lys Glu Lys Leu Ser Lys Val Ala Tyr Pro
625 630 635 640

Ile Thr Ser Lys Leu Tyr Asp Thr Pro Pro Thr Ser Asp Glu Asp Asp
645 650 655

Glu Asp Asp Trp Asp His Asp Glu Leu
660 665

<210> 49
<211> 1569
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS
<222> (1)..(1569)

<400> 49
atg aag ttt tct gct ggt gcc gtc ctg tca tgg tcc tcc ctg ctg ctc 48
Met Lys Phe Ser Ala Gly Ala Val Leu Ser Trp Ser Ser Leu Leu Leu
1 5 10 15

gcc tcc tct gtt ttc gcc caa caa gag gct gtg gcc cct gaa gac tcc 96
Ala Ser Ser Val Phe Ala Gln Gln Glu Ala Val Ala Pro Glu Asp Ser
20 25 30

gct gtc gtt aag ttg gcc acc gac tcc ttc aat gag tac att cag tcg 144
Ala Val Val Lys Leu Ala Thr Asp Ser Phe Asn Glu Tyr Ile Gln Ser
35 40 45

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cac gac ttg gtg ctt gcg gag ttt ttt gct cca tgg tgt ggc cac tgt      192
His Asp Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys
    50                  55                  60

aag aac atg gct cct gaa tac gtt aaa gcc gcc gag act tta gtt gag      240
Lys Asn Met Ala Pro Glu Tyr Val Lys Ala Ala Glu Thr Leu Val Glu
65                  70                  75                  80

aaa aac att acc ttg gcc cag atc gac tgt act gaa aac cag gat ctg      288
Lys Asn Ile Thr Leu Ala Gln Ile Asp Cys Thr Glu Asn Gln Asp Leu
                85                  90                  95

tgt atg gaa cac aac att cca ggg ttc cca agc ttg aag att ttc aaa      336
Cys Met Glu His Asn Ile Pro Gly Phe Pro Ser Leu Lys Ile Phe Lys
            100                 105                 110

aac agc gat gtt aac aac tcg atc gat tac gag gga cct aga act gcc      384
Asn Ser Asp Val Asn Asn Ser Ile Asp Tyr Glu Gly Pro Arg Thr Ala
        115                 120                 125

gag gcc att gtc caa ttc atg atc aag caa agc caa ccg gct gtc gcc      432
Glu Ala Ile Val Gln Phe Met Ile Lys Gln Ser Gln Pro Ala Val Ala
    130                 135                 140

gtt gtt gct gat cta cca gct tac ctt gct aac gag act ttt gtc act      480
Val Val Ala Asp Leu Pro Ala Tyr Leu Ala Asn Glu Thr Phe Val Thr
145                 150                 155                 160

cca gtt atc gtc caa tcc ggt aag att gac gcc gac ttc aac gcc acc      528
Pro Val Ile Val Gln Ser Gly Lys Ile Asp Ala Asp Phe Asn Ala Thr
                165                 170                 175

ttt tac tcc atg gcc aac aaa cac ttc aac gac tac gac ttt gtc tcc      576
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Phe Tyr Ser Met Ala Asn Lys His Phe Asn Asp Tyr Asp Phe Val Ser
180 185 190

gct gaa aac gca gac gat gat ttc aag ctt tct att tac ttg ccc tcc 624
Ala Glu Asn Ala Asp Asp Asp Phe Lys Leu Ser Ile Tyr Leu Pro Ser
195 200 205

gcc atg gac gag cct gta gta tac aac ggt aag aaa gcc gat atc gct 672
Ala Met Asp Glu Pro Val Val Tyr Asn Gly Lys Lys Ala Asp Ile Ala
210 215 220

gac gct gat gtt ttt gaa aaa tgg ttg caa gtg gaa gcc ttg ccc tac 720
Asp Ala Asp Val Phe Glu Lys Trp Leu Gln Val Glu Ala Leu Pro Tyr
225 230 235 240

ttt ggt gaa atc gac ggt tcc gtt ttc gcc caa tac gtc gaa agc ggt 768
Phe Gly Glu Ile Asp Gly Ser Val Phe Ala Gln Tyr Val Glu Ser Gly
245 250 255

ttg cct ttg ggt tac tta ttc tac aat gac gag gaa gaa ttg gaa gaa 816
Leu Pro Leu Gly Tyr Leu Phe Tyr Asn Asp Glu Glu Glu Leu Glu Glu
260 265 270

tac aag cct ctc ttt acc gag ttg gcc aaa aag aac aga ggt cta atg 864
Tyr Lys Pro Leu Phe Thr Glu Leu Ala Lys Lys Asn Arg Gly Leu Met
275 280 285

aac ttt gtt agc atc gat gcc aga aaa ttc ggc aga cac gcc ggc aac 912
Asn Phe Val Ser Ile Asp Ala Arg Lys Phe Gly Arg His Ala Gly Asn
290 295 300

ttg aac atg aag gaa caa ttc cct cta ttt gcc atc cac gac atg act 960
Leu Asn Met Lys Glu Gln Phe Pro Leu Phe Ala Ile His Asp Met Thr

305 310 315 320

gaa gac ttg aag tac ggt ttg cct caa ctc tct gaa gag gcg ttt gac 1008
Glu Asp Leu Lys Tyr Gly Leu Pro Gln Leu Ser Glu Glu Ala Phe Asp
325 330 335

gaa ttg agc gac aag atc gtg ttg gag tct aag gct att gaa tct ttg 1056
Glu Leu Ser Asp Lys Ile Val Leu Glu Ser Lys Ala Ile Glu Ser Leu
340 345 350

gtt aag gac ttc ttg aaa ggt gat gcc tcc cca atc gtg aag tcc caa 1104
Val Lys Asp Phe Leu Lys Gly Asp Ala Ser Pro Ile Val Lys Ser Gln
355 360 365

gag atc ttc gag aac caa gat tcc tct gtc ttc caa ttg gtc ggt aag 1152
Glu Ile Phe Glu Asn Gln Asp Ser Ser Val Phe Gln Leu Val Gly Lys
370 375 380

aac cat gac gaa atc gtc aac gac cca aag aag gac gtt ctt gtt ttg 1200
Asn His Asp Glu Ile Val Asn Asp Pro Lys Lys Asp Val Leu Val Leu
385 390 395 400

tac tat gcc cca tgg tgt ggt cac tgt aag aga ttg gcc cca act tac 1248
Tyr Tyr Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala Pro Thr Tyr
405 410 415

caa gaa cta gct gat acc tac gcc aac gcc aca tcc gac gtt ttg att 1296
Gln Glu Leu Ala Asp Thr Tyr Ala Asn Ala Thr Ser Asp Val Leu Ile
420 425 430

gct aaa cta gac cac act gaa aac gat gtc aga ggc gtc gta att gaa 1344
Ala Lys Leu Asp His Thr Glu Asn Asp Val Arg Gly Val Val Ile Glu
435 440 445

ggt tac cca aca atc gtc tta tac cca ggt ggt aag aag tcc gaa tct 1392
Gly Tyr Pro Thr Ile Val Leu Tyr Pro Gly Gly Lys Lys Ser Glu Ser
450 455 460

gtt gtg tac caa ggt tca aga tcc ttg gac tct tta ttc gac ttc atc 1440
Val Val Tyr Gln Gly Ser Arg Ser Leu Asp Ser Leu Phe Asp Phe Ile
465 470 475 480

aag gaa aac ggt cac ttc gac gtc gac ggt aag gcc ttg tac gaa gaa 1488
Lys Glu Asn Gly His Phe Asp Val Asp Gly Lys Ala Leu Tyr Glu Glu
485 490 495

gcc cag gaa aaa gct gct gag gaa gcc gat gct gac gct gaa ttg gct 1536
Ala Gln Glu Lys Ala Ala Glu Glu Ala Asp Ala Asp Ala Glu Leu Ala
500 505 510

gac gaa gaa gat gcc att cac gat gaa ttg taa 1569
Asp Glu Glu Asp Ala Ile His Asp Glu Leu
515 520

<210> 50
<211> 522
<212> PRT
<213> Saccharomyces cerevisiae

<400> 50

Met Lys Phe Ser Ala Gly Ala Val Leu Ser Trp Ser Ser Leu Leu Leu
1 5 10 15

Ala Ser Ser Val Phe Ala Gln Gln Glu Ala Val Ala Pro Glu Asp Ser
20 25 30

Ala Val Val Lys Leu Ala Thr Asp Ser Phe Asn Glu Tyr Ile Gln Ser
35 40 45

His Asp Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys
50 55 60

Lys Asn Met Ala Pro Glu Tyr Val Lys Ala Ala Glu Thr Leu Val Glu
65 70 75 80

Lys Asn Ile Thr Leu Ala Gln Ile Asp Cys Thr Glu Asn Gln Asp Leu
85 90 95

Cys Met Glu His Asn Ile Pro Gly Phe Pro Ser Leu Lys Ile Phe Lys
100 105 110

Asn Ser Asp Val Asn Asn Ser Ile Asp Tyr Glu Gly Pro Arg Thr Ala
115 120 125

Glu Ala Ile Val Gln Phe Met Ile Lys Gln Ser Gln Pro Ala Val Ala
130 135 140

Val Val Ala Asp Leu Pro Ala Tyr Leu Ala Asn Glu Thr Phe Val Thr

145 150 155 160

Pro Val Ile Val Gln Ser Gly Lys Ile Asp Ala Asp Phe Asn Ala Thr
165 170 175

Phe Tyr Ser Met Ala Asn Lys His Phe Asn Asp Tyr Asp Phe Val Ser
180 185 190

Ala Glu Asn Ala Asp Asp Asp Phe Lys Leu Ser Ile Tyr Leu Pro Ser
195 200 205

Ala Met Asp Glu Pro Val Val Tyr Asn Gly Lys Lys Ala Asp Ile Ala
210 215 220

Asp Ala Asp Val Phe Glu Lys Trp Leu Gln Val Glu Ala Leu Pro Tyr
225 230 235 240

Phe Gly Glu Ile Asp Gly Ser Val Phe Ala Gln Tyr Val Glu Ser Gly
245 250 255

Leu Pro Leu Gly Tyr Leu Phe Tyr Asn Asp Glu Glu Glu Leu Glu Glu
260 265 270

Tyr Lys Pro Leu Phe Thr Glu Leu Ala Lys Lys Asn Arg Gly Leu Met
275 280 285

Asn Phe Val Ser Ile Asp Ala Arg Lys Phe Gly Arg His Ala Gly Asn
290 295 300

Leu Asn Met Lys Glu Gln Phe Pro Leu Phe Ala Ile His Asp Met Thr
305 310 315 320

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

Glu Leu Ser Asp Lys Ile Val Leu Glu Ser Lys Ala Ile Glu Ser Leu
340 345 350

Val Lys Asp Phe Leu Lys Gly Asp Ala Ser Pro Ile Val Lys Ser Gln
355 360 365

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

Asn His Asp Glu Ile Val Asn Asp Pro Lys Lys Asp Val Leu Val Leu
385 390 395 400

Tyr Tyr Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala Pro Thr Tyr
405 410 415

Gln Glu Leu Ala Asp Thr Tyr Ala Asn Ala Thr Ser Asp Val Leu Ile
420 425 430

Ala Lys Leu Asp His Thr Glu Asn Asp Val Arg Gly Val Val Ile Glu
435 440 445

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

Val Val Tyr Gln Gly Ser Arg Ser Leu Asp Ser Leu Phe Asp Phe Ile
465 470 475 480

Lys Glu Asn Gly His Phe Asp Val Asp Gly Lys Ala Leu Tyr Glu Glu
485 490 495

Ala Gln Glu Lys Ala Ala Glu Glu Ala Asp Ala Asp Ala Glu Leu Ala
500 505 510

Asp Glu Glu Asp Ala Ile His Asp Glu Leu
515 520

<210> 51
<211> 957
<212> DNA
<213> Saccharomyces cerevisiae

<220>

<221> CDS

<222> (1)..(957)

<400> 51

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Met Leu Phe Leu Asn Ile Ile Lys Leu Leu Leu Gly Leu Phe Ile Met
1 5 10 15

aat gaa gta aag gcg caa aac ttt tac gat tcc gat cct cat ata tca 96
Asn Glu Val Lys Ala Gln Asn Phe Tyr Asp Ser Asp Pro His Ile Ser
20 25 30

gag tta acg cca aaa agc ttc gat aaa gcg atc cat aac aca aat tac 144
Glu Leu Thr Pro Lys Ser Phe Asp Lys Ala Ile His Asn Thr Asn Tyr
35 40 45

aca tca tta gtg gaa ttt tat gct ccg tgg tgc ggc cat tgt aag aag 192
Thr Ser Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys
50 55 60

ctc tct agt acg ttc cgc aag gca gca aaa aga ttg gat ggt gta gtc 240
Leu Ser Ser Thr Phe Arg Lys Ala Ala Lys Arg Leu Asp Gly Val Val
65 70 75 80

caa gtt gct gct gta aac tgt gac ctt aac aag aat aag gct ttg tgt 288
Gln Val Ala Ala Val Asn Cys Asp Leu Asn Lys Asn Lys Ala Leu Cys
85 90 95

gct aaa tac gac gta aac gga ttt ccc acg tta atg gta ttt agg ccc 336
Ala Lys Tyr Asp Val Asn Gly Phe Pro Thr Leu Met Val Phe Arg Pro

100 105 110

cca aaa att gac cta tct aag cca ata gat aac gcc aaa aaa agt ttc 384
Pro Lys Ile Asp Leu Ser Lys Pro Ile Asp Asn Ala Lys Lys Ser Phe
115 120 125

agc gct cat gcc aat gaa gtg tac tca ggt gca aga act ctc gcg cct 432
Ser Ala His Ala Asn Glu Val Tyr Ser Gly Ala Arg Thr Leu Ala Pro
130 135 140

att gtt gat ttt tct ctt tca aga ata agg tca tat gtc aaa aag ttt 480
Ile Val Asp Phe Ser Leu Ser Arg Ile Arg Ser Tyr Val Lys Lys Phe
145 150 155 160

gtc cgt ata gat aca ctt ggc tct tta ctt aga aag tca ccc aaa ctt 528
Val Arg Ile Asp Thr Leu Gly Ser Leu Leu Arg Lys Ser Pro Lys Leu
165 170 175

tcc gtg gtg ttg ttt tcc aaa caa gac aaa att tca ccg gtt tat aaa 576
Ser Val Val Leu Phe Ser Lys Gln Asp Lys Ile Ser Pro Val Tyr Lys
180 185 190

agc att gcc ctt gat tgg tta gga aag ttc gat ttt tat tca att tca 624
Ser Ile Ala Leu Asp Trp Leu Gly Lys Phe Asp Phe Tyr Ser Ile Ser
195 200 205

aac aaa aaa ctc aag caa cta acc gat atg aac cca aca tat gaa aaa 672
Asn Lys Lys Leu Lys Gln Leu Thr Asp Met Asn Pro Thr Tyr Glu Lys
210 215 220

act cct gag att ttc aaa tat ttg cag aag gtc att cct gaa cag cga 720
Thr Pro Glu Ile Phe Lys Tyr Leu Gln Lys Val Ile Pro Glu Gln Arg
225 230 235 240

cag agc gat aaa agt aag ctt gtc gtt ttt gat gca gac aaa gat aaa 768
Gln Ser Asp Lys Ser Lys Leu Val Val Phe Asp Ala Asp Lys Asp Lys
245 250 255

ttt tgg gag tat gaa ggg aac tca atc aac aaa aat gac att tcc aaa 816
Phe Trp Glu Tyr Glu Gly Asn Ser Ile Asn Lys Asn Asp Ile Ser Lys
260 265 270

ttt ctg cgg gac act ttt agt att acc ccc aat gag ggt cct ttt agt 864
Phe Leu Arg Asp Thr Phe Ser Ile Thr Pro Asn Glu Gly Pro Phe Ser
275 280 285

aga cgt tct gaa tat att gct tac tta aaa act ggc aag aag cca att 912
Arg Arg Ser Glu Tyr Ile Ala Tyr Leu Lys Thr Gly Lys Lys Pro Ile
290 295 300

aaa aag aac cat tcc tcc tca gga aac aag cac gac gaa ttg tag 957
Lys Lys Asn His Ser Ser Ser Gly Asn Lys His Asp Glu Leu
305 310 315

<210> 52
<211> 318
<212> PRT
<213> Saccharomyces cerevisiae

<400> 52

Met Leu Phe Leu Asn Ile Ile Lys Leu Leu Leu Gly Leu Phe Ile Met
1 5 10 15

Asn Glu Val Lys Ala Gln Asn Phe Tyr Asp Ser Asp Pro His Ile Ser
20 25 30

Glu Leu Thr Pro Lys Ser Phe Asp Lys Ala Ile His Asn Thr Asn Tyr
35 40 45

Thr Ser Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys
50 55 60

Leu Ser Ser Thr Phe Arg Lys Ala Ala Lys Arg Leu Asp Gly Val Val
65 70 75 80

Gln Val Ala Ala Val Asn Cys Asp Leu Asn Lys Asn Lys Ala Leu Cys
85 90 95

Ala Lys Tyr Asp Val Asn Gly Phe Pro Thr Leu Met Val Phe Arg Pro
100 105 110

Pro Lys Ile Asp Leu Ser Lys Pro Ile Asp Asn Ala Lys Lys Ser Phe
115 120 125

Ser Ala His Ala Asn Glu Val Tyr Ser Gly Ala Arg Thr Leu Ala Pro
130 135 140

Ile Val Asp Phe Ser Leu Ser Arg Ile Arg Ser Tyr Val Lys Lys Phe

145 150 155 160

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

Ser Val Val Leu Phe Ser Lys Gln Asp Lys Ile Ser Pro Val Tyr Lys
180 185 190

Ser Ile Ala Leu Asp Trp Leu Gly Lys Phe Asp Phe Tyr Ser Ile Ser
195 200 205

Asn Lys Lys Leu Lys Gln Leu Thr Asp Met Asn Pro Thr Tyr Glu Lys
210 215 220

Thr Pro Glu Ile Phe Lys Tyr Leu Gln Lys Val Ile Pro Glu Gln Arg
225 230 235 240

Gln Ser Asp Lys Ser Lys Leu Val Val Phe Asp Ala Asp Lys Asp Lys
245 250 255

Phe Trp Glu Tyr Glu Gly Asn Ser Ile Asn Lys Asn Asp Ile Ser Lys
260 265 270

Phe Leu Arg Asp Thr Phe Ser Ile Thr Pro Asn Glu Gly Pro Phe Ser
275 280 285

Arg Arg Ser Glu Tyr Ile Ala Tyr Leu Lys Thr Gly Lys Lys Pro Ile
290 295 300

Lys Lys Asn His Ser Ser Ser Gly Asn Lys His Asp Glu Leu
305 310 315

<210> 53
<211> 1134
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS
<222> (1)..(1134)

<400> 53
atg att cca aaa tta tat ata cat ttg ata cta tct tta ttg ttg ttg 48
Met Ile Pro Lys Leu Tyr Ile His Leu Ile Leu Ser Leu Leu Leu Leu
1 5 10 15

ccg cta att ttg gcg cag gat tat tat gca ata cta gag ata gac aaa 96
Pro Leu Ile Leu Ala Gln Asp Tyr Tyr Ala Ile Leu Glu Ile Asp Lys
20 25 30

gat gcc act gag aag gaa atc aaa tca gcg tac aga caa ttg tct aag 144
Asp Ala Thr Glu Lys Glu Ile Lys Ser Ala Tyr Arg Gln Leu Ser Lys
35 40 45

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aag tac cat ccg gat aaa aat gct ggg agc gaa gaa gcc cat caa aaa      192
Lys Tyr His Pro Asp Lys Asn Ala Gly Ser Glu Glu Ala His Gln Lys
    50                  55                  60

ttc att gaa gtc ggc gag gca tac gat gta ttg agc gat cct gaa aag      240
Phe Ile Glu Val Gly Glu Ala Tyr Asp Val Leu Ser Asp Pro Glu Lys
65                  70                  75                  80

aaa aag att tat gac cag ttt ggt gca gat gct gta aag aat ggc ggt      288
Lys Lys Ile Tyr Asp Gln Phe Gly Ala Asp Ala Val Lys Asn Gly Gly
                85                  90                  95

ggc ggt ggc ggt cca gga ggc cct ggc gca ggt gga ttc cac gat ccg      336
Gly Gly Gly Gly Pro Gly Gly Pro Gly Ala Gly Gly Phe His Asp Pro
            100                 105                 110

ttt gac ata ttc gaa cgg atg ttt caa gga ggt cat gga ggt cct ggc      384
Phe Asp Ile Phe Glu Arg Met Phe Gln Gly Gly His Gly Gly Pro Gly
        115                 120                 125

ggc gga ttt ggc cag aga cag agg cag cgt ggt cca atg atc aag gtc      432
Gly Gly Phe Gly Gln Arg Gln Arg Gln Arg Gly Pro Met Ile Lys Val
    130                 135                 140

cag gaa aaa cta tct tta aag cag ttt tat tcc ggg tcc tcg ata gaa      480
Gln Glu Lys Leu Ser Leu Lys Gln Phe Tyr Ser Gly Ser Ser Ile Glu
145                 150                 155                 160

ttt act tta aac cta aac gat gaa tgt gat gca tgc cat ggt agt ggc      528
Phe Thr Leu Asn Leu Asn Asp Glu Cys Asp Ala Cys His Gly Ser Gly
                165                 170                 175

tct gca gat ggt aag ctg gcc caa tgt ccc gat tgt caa ggt cgt ggg      576
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Ser Ala Asp Gly Lys Leu Ala Gln Cys Pro Asp Cys Gln Gly Arg Gly
180 185 190

gtt ata ata caa gtg ctg cgc atg ggt att atg acg cag cag att caa 624
Val Ile Ile Gln Val Leu Arg Met Gly Ile Met Thr Gln Gln Ile Gln
195 200 205

cag atg tgt ggt agg tgt ggt ggt acg gga caa att atc aaa aat gaa 672
Gln Met Cys Gly Arg Cys Gly Gly Thr Gly Gln Ile Ile Lys Asn Glu
210 215 220

tgc aaa aca tgt cac ggc aaa aaa gtt acc aaa aag aac aag ttc ttc 720
Cys Lys Thr Cys His Gly Lys Lys Val Thr Lys Lys Asn Lys Phe Phe
225 230 235 240

cac gtt gac gtt cca cca ggc gca cca aga aac tac atg gac aca aga 768
His Val Asp Val Pro Pro Gly Ala Pro Arg Asn Tyr Met Asp Thr Arg
245 250 255

gtc ggc gag gct gaa aaa ggg cct gac ttt gac gcc ggt gac ttg gtc 816
Val Gly Glu Ala Glu Lys Gly Pro Asp Phe Asp Ala Gly Asp Leu Val
260 265 270

ata gaa ttc aag gaa aag gat act gag aac atg ggt tac aga aga aga 864
Ile Glu Phe Lys Glu Lys Asp Thr Glu Asn Met Gly Tyr Arg Arg Arg
275 280 285

ggc gac aat ctg tac aga aca gaa gtt ctt tct gct gcg gaa gcg cta 912
Gly Asp Asn Leu Tyr Arg Thr Glu Val Leu Ser Ala Ala Glu Ala Leu
290 295 300

tac ggc gga tgg caa aga acg ata gaa ttc ctt gat gag aac aag ccc 960
Tyr Gly Gly Trp Gln Arg Thr Ile Glu Phe Leu Asp Glu Asn Lys Pro

305 310 315 320

gtt aag tta tct aga ccc gct cat gta gtt gtc tcc aat ggc gaa gtt 1008
Val Lys Leu Ser Arg Pro Ala His Val Val Val Ser Asn Gly Glu Val
325 330 335

gaa gtc gtg aag gga ttc ggc atg ccc aag ggt agc aag ggt tac ggt 1056
Glu Val Val Lys Gly Phe Gly Met Pro Lys Gly Ser Lys Gly Tyr Gly
340 345 350

gat ttg tac ata gac tac gtc gtt gtc atg cca aag act ttc aaa tct 1104
Asp Leu Tyr Ile Asp Tyr Val Val Val Met Pro Lys Thr Phe Lys Ser
355 360 365

ggg caa aat atg ctc aaa gat gag ttg tag 1134
Gly Gln Asn Met Leu Lys Asp Glu Leu
370 375

<210> 54
<211> 377
<212> PRT
<213> Saccharomyces cerevisiae

<400> 54

Met Ile Pro Lys Leu Tyr Ile His Leu Ile Leu Ser Leu Leu Leu Leu
1 5 10 15

Pro Leu Ile Leu Ala Gln Asp Tyr Tyr Ala Ile Leu Glu Ile Asp Lys
20 25 30

Asp Ala Thr Glu Lys Glu Ile Lys Ser Ala Tyr Arg Gln Leu Ser Lys
35 40 45

Lys Tyr His Pro Asp Lys Asn Ala Gly Ser Glu Glu Ala His Gln Lys
50 55 60

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

Lys Lys Ile Tyr Asp Gln Phe Gly Ala Asp Ala Val Lys Asn Gly Gly
85 90 95

Gly Gly Gly Gly Pro Gly Gly Pro Gly Ala Gly Gly Phe His Asp Pro
100 105 110

Phe Asp Ile Phe Glu Arg Met Phe Gln Gly Gly His Gly Gly Pro Gly
115 120 125

Gly Gly Phe Gly Gln Arg Gln Arg Gln Arg Gly Pro Met Ile Lys Val
130 135 140

Gln Glu Lys Leu Ser Leu Lys Gln Phe Tyr Ser Gly Ser Ser Ile Glu
145 150 155 160

Phe Thr Leu Asn Leu Asn Asp Glu Cys Asp Ala Cys His Gly Ser Gly
165 170 175

Ser Ala Asp Gly Lys Leu Ala Gln Cys Pro Asp Cys Gln Gly Arg Gly
180 185 190

Val Ile Ile Gln Val Leu Arg Met Gly Ile Met Thr Gln Gln Ile Gln
195 200 205

Gln Met Cys Gly Arg Cys Gly Gly Thr Gly Gln Ile Ile Lys Asn Glu
210 215 220

Cys Lys Thr Cys His Gly Lys Lys Val Thr Lys Lys Asn Lys Phe Phe
225 230 235 240

His Val Asp Val Pro Pro Gly Ala Pro Arg Asn Tyr Met Asp Thr Arg
245 250 255

Val Gly Glu Ala Glu Lys Gly Pro Asp Phe Asp Ala Gly Asp Leu Val
260 265 270

Ile Glu Phe Lys Glu Lys Asp Thr Glu Asn Met Gly Tyr Arg Arg Arg
275 280 285

Gly Asp Asn Leu Tyr Arg Thr Glu Val Leu Ser Ala Ala Glu Ala Leu

290 295 300

Tyr Gly Gly Trp Gln Arg Thr Ile Glu Phe Leu Asp Glu Asn Lys Pro
305 310 315 320

Val Lys Leu Ser Arg Pro Ala His Val Val Val Ser Asn Gly Glu Val
325 330 335

Glu Val Val Lys Gly Phe Gly Met Pro Lys Gly Ser Lys Gly Tyr Gly
340 345 350

Asp Leu Tyr Ile Asp Tyr Val Val Val Met Pro Lys Thr Phe Lys Ser
355 360 365

Gly Gln Asn Met Leu Lys Asp Glu Leu
370 375

<210> 55
<211> 1692
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS
<222> (1)..(1692)

〈400〉 55

atg aga tta aga acc gcc att gcc aca ctg tgc ctc acg gct ttt aca 48
Met Arg Leu Arg Thr Ala Ile Ala Thr Leu Cys Leu Thr Ala Phe Thr
1 5 10 15

tct gca act tca aac aat agc tac atc gcc acc gac caa aca caa aat 96
Ser Ala Thr Ser Asn Asn Ser Tyr Ile Ala Thr Asp Gln Thr Gln Asn
20 25 30

gcc ttt aat gac act cac ttt tgt aag gtc gac agg aat gat cac gtt 144
Ala Phe Asn Asp Thr His Phe Cys Lys Val Asp Arg Asn Asp His Val
35 40 45

agt ccc agt tgt aac gta aca ttc aat gaa tta aat gcc ata aat gaa 192
Ser Pro Ser Cys Asn Val Thr Phe Asn Glu Leu Asn Ala Ile Asn Glu
50 55 60

aac att aga gat gat ctt tcg gcg tta tta aaa tct gat ttc ttc aaa 240
Asn Ile Arg Asp Asp Leu Ser Ala Leu Leu Lys Ser Asp Phe Phe Lys
65 70 75 80

tac ttt cgg ctg gat tta tac aag caa tgt tca ttt tgg gac gcc aac 288
Tyr Phe Arg Leu Asp Leu Tyr Lys Gln Cys Ser Phe Trp Asp Ala Asn
85 90 95

gat ggt ctg tgc tta aac cgc gct tgc tct gtt gat gtc gta gag gac 336
Asp Gly Leu Cys Leu Asn Arg Ala Cys Ser Val Asp Val Val Glu Asp
100 105 110

tgg gat aca ctg cct gag tac tgg cag cct gag atc ttg ggt agt ttc 384
Trp Asp Thr Leu Pro Glu Tyr Trp Gln Pro Glu Ile Leu Gly Ser Phe
115 120 125

aat aat gat aca atg aag gaa gcg gat gat agc gat gac gaa tgt aag 432
Asn Asn Asp Thr Met Lys Glu Ala Asp Asp Ser Asp Asp Glu Cys Lys
130 135 140

ttc tta gat caa cta tgt caa acc agt aaa aaa cct gta gat atc gaa 480
Phe Leu Asp Gln Leu Cys Gln Thr Ser Lys Lys Pro Val Asp Ile Glu
145 150 155 160

gac acc atc aac tac tgt gat gta aat gac ttt aac ggt aaa aac gcc 528
Asp Thr Ile Asn Tyr Cys Asp Val Asn Asp Phe Asn Gly Lys Asn Ala
165 170 175

gtt ctg att gat tta aca gca aat ccg gaa cga ttt aca ggt tat ggt 576
Val Leu Ile Asp Leu Thr Ala Asn Pro Glu Arg Phe Thr Gly Tyr Gly
180 185 190

ggt aag caa gct ggt caa att tgg tct act atc tac caa gac aac tgt 624
Gly Lys Gln Ala Gly Gln Ile Trp Ser Thr Ile Tyr Gln Asp Asn Cys
195 200 205

ttt aca att ggc gaa act ggt gaa tca ttg gcc aaa gat gca ttt tat 672
Phe Thr Ile Gly Glu Thr Gly Glu Ser Leu Ala Lys Asp Ala Phe Tyr
210 215 220

aga ctt gta tcc ggt ttc cat gcc tct atc ggt act cac tta tca aag 720
Arg Leu Val Ser Gly Phe His Ala Ser Ile Gly Thr His Leu Ser Lys
225 230 235 240

gaa tat ttg aac acg aaa act ggt aaa tgg gag ccc aat ctg gat ttg 768
Glu Tyr Leu Asn Thr Lys Thr Gly Lys Trp Glu Pro Asn Leu Asp Leu
245 250 255

ttt atg gca aga atc ggg aac ttt cct gat aga gtg aca aac atg tat 816

Phe Met Ala Arg Ile Gly Asn Phe Pro Asp Arg Val Thr Asn Met Tyr
260 265 270

ttc aat tat gct gtt gta gct aag gct ctc tgg aaa att caa cca tat 864
Phe Asn Tyr Ala Val Val Ala Lys Ala Leu Trp Lys Ile Gln Pro Tyr
275 280 285

tta cca gaa ttt tca ttc tgt gat cta gtc aat aaa gaa atc aaa aac 912
Leu Pro Glu Phe Ser Phe Cys Asp Leu Val Asn Lys Glu Ile Lys Asn
290 295 300

aaa atg gat aac gtt att tcc cag ctg gac aca aaa att ttt aac gaa 960
Lys Met Asp Asn Val Ile Ser Gln Leu Asp Thr Lys Ile Phe Asn Glu
305 310 315 320

gac tta gtt ttt gcc aac gac cta agt ttg act ttg aag gac gaa ttc 1008
Asp Leu Val Phe Ala Asn Asp Leu Ser Leu Thr Leu Lys Asp Glu Phe
325 330 335

aga tct cgc ttc aag aat gtc acg aag att atg gat tgt gtg caa tgt 1056
Arg Ser Arg Phe Lys Asn Val Thr Lys Ile Met Asp Cys Val Gln Cys
340 345 350

gat aga tgt aga ttg tgg ggc aaa att caa act acc ggt tac gca act 1104
Asp Arg Cys Arg Leu Trp Gly Lys Ile Gln Thr Thr Gly Tyr Ala Thr
355 360 365

gcc ttg aaa att ttg ttt gaa atc aac gac gct gat gaa ttc acc aaa 1152
Ala Leu Lys Ile Leu Phe Glu Ile Asn Asp Ala Asp Glu Phe Thr Lys
370 375 380

caa cat att gtt ggt aag tta acc aaa tat gag ttg att gca cta tta 1200
Gln His Ile Val Gly Lys Leu Thr Lys Tyr Glu Leu Ile Ala Leu Leu

385 390 395 400

cag act ttc ggt aga tta tct gaa tct att gaa tct gtt aac atg ttc 1248
Gln Thr Phe Gly Arg Leu Ser Glu Ser Ile Glu Ser Val Asn Met Phe
405 410 415

gaa aaa atg tac ggg aaa agg tta aac ggt tct gaa aac agg tta agc 1296
Glu Lys Met Tyr Gly Lys Arg Leu Asn Gly Ser Glu Asn Arg Leu Ser
420 425 430

tca ttc ttc caa aat aac ttc ttc aac att ttg aag gag gca ggc aaa 1344
Ser Phe Phe Gln Asn Asn Phe Phe Asn Ile Leu Lys Glu Ala Gly Lys
435 440 445

tcg att cgt tac acc ata gag aac atc aat tcc act aaa gaa gga aag 1392
Ser Ile Arg Tyr Thr Ile Glu Asn Ile Asn Ser Thr Lys Glu Gly Lys
450 455 460

aaa aag act aac aat tct caa tca cat gta ttt gat gat tta aaa atg 1440
Lys Lys Thr Asn Asn Ser Gln Ser His Val Phe Asp Asp Leu Lys Met
465 470 475 480

ccc aaa gca gaa ata gtt cca agg ccc tct aac ggt aca gta aat aaa 1488
Pro Lys Ala Glu Ile Val Pro Arg Pro Ser Asn Gly Thr Val Asn Lys
485 490 495

tgg aag aaa gct tgg aat act gaa gtt aac aac gtt tta gaa gca ttc 1536
Trp Lys Lys Ala Trp Asn Thr Glu Val Asn Asn Val Leu Glu Ala Phe
500 505 510

aga ttt att tat aga agc tat ttg gat tta ccc agg aac atc tgg gaa 1584
Arg Phe Ile Tyr Arg Ser Tyr Leu Asp Leu Pro Arg Asn Ile Trp Glu
515 520 525

tta tct ttg atg aag gta tac aaa ttt tgg aat aaa ttc atc ggt gtt 1632
Leu Ser Leu Met Lys Val Tyr Lys Phe Trp Asn Lys Phe Ile Gly Val
530 535 540

gct gat tac gtt agt gag gag aca cga gag cct att tcc tat aag cta 1680
Ala Asp Tyr Val Ser Glu Glu Thr Arg Glu Pro Ile Ser Tyr Lys Leu
545 550 555 560

gat ata caa taa 1692
Asp Ile Gln

<210> 56
<211> 563
<212> PRT
<213> Saccharomyces cerevisiae

<400> 56

Met Arg Leu Arg Thr Ala Ile Ala Thr Leu Cys Leu Thr Ala Phe Thr
1 5 10 15

Ser Ala Thr Ser Asn Asn Ser Tyr Ile Ala Thr Asp Gln Thr Gln Asn
20 25 30

Ala Phe Asn Asp Thr His Phe Cys Lys Val Asp Arg Asn Asp His Val
35 40 45

Ser Pro Ser Cys Asn Val Thr Phe Asn Glu Leu Asn Ala Ile Asn Glu
50 55 60

Asn Ile Arg Asp Asp Leu Ser Ala Leu Leu Lys Ser Asp Phe Phe Lys
65 70 75 80

Tyr Phe Arg Leu Asp Leu Tyr Lys Gln Cys Ser Phe Trp Asp Ala Asn
85 90 95

Asp Gly Leu Cys Leu Asn Arg Ala Cys Ser Val Asp Val Val Glu Asp
100 105 110

Trp Asp Thr Leu Pro Glu Tyr Trp Gln Pro Glu Ile Leu Gly Ser Phe
115 120 125

Asn Asn Asp Thr Met Lys Glu Ala Asp Asp Ser Asp Asp Glu Cys Lys
130 135 140

Phe Leu Asp Gln Leu Cys Gln Thr Ser Lys Lys Pro Val Asp Ile Glu
145 150 155 160

Asp Thr Ile Asn Tyr Cys Asp Val Asn Asp Phe Asn Gly Lys Asn Ala
165 170 175

Val Leu Ile Asp Leu Thr Ala Asn Pro Glu Arg Phe Thr Gly Tyr Gly

180 185 190

Gly Lys Gln Ala Gly Gln Ile Trp Ser Thr Ile Tyr Gln Asp Asn Cys
195 200 205

Phe Thr Ile Gly Glu Thr Gly Glu Ser Leu Ala Lys Asp Ala Phe Tyr
210 215 220

Arg Leu Val Ser Gly Phe His Ala Ser Ile Gly Thr His Leu Ser Lys
225 230 235 240

Glu Tyr Leu Asn Thr Lys Thr Gly Lys Trp Glu Pro Asn Leu Asp Leu
245 250 255

Phe Met Ala Arg Ile Gly Asn Phe Pro Asp Arg Val Thr Asn Met Tyr
260 265 270

Phe Asn Tyr Ala Val Val Ala Lys Ala Leu Trp Lys Ile Gln Pro Tyr
275 280 285

Leu Pro Glu Phe Ser Phe Cys Asp Leu Val Asn Lys Glu Ile Lys Asn
290 295 300

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

Asp Leu Val Phe Ala Asn Asp Leu Ser Leu Thr Leu Lys Asp Glu Phe
325 330 335

Arg Ser Arg Phe Lys Asn Val Thr Lys Ile Met Asp Cys Val Gln Cys
340 345 350

Asp Arg Cys Arg Leu Trp Gly Lys Ile Gln Thr Thr Gly Tyr Ala Thr
355 360 365

Ala Leu Lys Ile Leu Phe Glu Ile Asn Asp Ala Asp Glu Phe Thr Lys
370 375 380

Gln His Ile Val Gly Lys Leu Thr Lys Tyr Glu Leu Ile Ala Leu Leu
385 390 395 400

Gln Thr Phe Gly Arg Leu Ser Glu Ser Ile Glu Ser Val Asn Met Phe
405 410 415

Glu Lys Met Tyr Gly Lys Arg Leu Asn Gly Ser Glu Asn Arg Leu Ser
420 425 430

Ser Phe Phe Gln Asn Asn Phe Phe Asn Ile Leu Lys Glu Ala Gly Lys
435 440 445

Ser Ile Arg Tyr Thr Ile Glu Asn Ile Asn Ser Thr Lys Glu Gly Lys
450 455 460

Lys Lys Thr Asn Asn Ser Gln Ser His Val Phe Asp Asp Leu Lys Met
465 470 475 480

Pro Lys Ala Glu Ile Val Pro Arg Pro Ser Asn Gly Thr Val Asn Lys
485 490 495

Trp Lys Lys Ala Trp Asn Thr Glu Val Asn Asn Val Leu Glu Ala Phe
500 505 510

Arg Phe Ile Tyr Arg Ser Tyr Leu Asp Leu Pro Arg Asn Ile Trp Glu
515 520 525

Leu Ser Leu Met Lys Val Tyr Lys Phe Trp Asn Lys Phe Ile Gly Val
530 535 540

Ala Asp Tyr Val Ser Glu Glu Thr Arg Glu Pro Ile Ser Tyr Lys Leu
545 550 555 560

Asp Ile Gln

<210> 57

<211> 408

<212> DNA

<213> Saccharomyces cerevisiae

<220>

<221> CDS

<222> (1)..(408)

<400> 57

atg atg ttt aat att tac ctt ttc gtc act ttt ttt tcc acc att ctt 48
Met Met Phe Asn Ile Tyr Leu Phe Val Thr Phe Phe Ser Thr Ile Leu
1 5 10 15

gca ggt tcc ctg tca gat ttg gaa atc ggt att atc aag aga ata ccg 96
Ala Gly Ser Leu Ser Asp Leu Glu Ile Gly Ile Ile Lys Arg Ile Pro
20 25 30

gta gaa gat tgc tta att aag gca atg cca ggt gat aaa gtt aag gtt 144
Val Glu Asp Cys Leu Ile Lys Ala Met Pro Gly Asp Lys Val Lys Val
35 40 45

cat tat aca gga tct tta tta gaa tcg gga act gta ttt gac tca agt 192
His Tyr Thr Gly Ser Leu Leu Glu Ser Gly Thr Val Phe Asp Ser Ser
50 55 60

tat tca aga ggc tct cct atc gct ttt gaa ctt ggc gtt ggc aga gta 240
Tyr Ser Arg Gly Ser Pro Ile Ala Phe Glu Leu Gly Val Gly Arg Val
65 70 75 80

att aaa ggt tgg gat caa ggt gtt gcc ggc atg tgc gtt ggc gaa aaa 288
Ile Lys Gly Trp Asp Gln Gly Val Ala Gly Met Cys Val Gly Glu Lys

85 90 95

aga aag ctg caa att cca agt tct ttg gcc tac gga gaa aga ggt gtc 336
Arg Lys Leu Gln Ile Pro Ser Ser Leu Ala Tyr Gly Glu Arg Gly Val
100 105 110

cca ggc gtc att cct cca agt gct gat ttg gtg ttt gat gtc gaa ttg 384
Pro Gly Val Ile Pro Pro Ser Ala Asp Leu Val Phe Asp Val Glu Leu
115 120 125

gta gac gtg aaa tca gcc gcc tag 408
Val Asp Val Lys Ser Ala Ala
130 135

<210> 58
<211> 135
<212> PRT
<213> Saccharomyces cerevisiae

<400> 58

Met Met Phe Asn Ile Tyr Leu Phe Val Thr Phe Phe Ser Thr Ile Leu
1 5 10 15

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

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

His Tyr Thr Gly Ser Leu Leu Glu Ser Gly Thr Val Phe Asp Ser Ser
50 55 60

Tyr Ser Arg Gly Ser Pro Ile Ala Phe Glu Leu Gly Val Gly Arg Val
65 70 75 80

Ile Lys Gly Trp Asp Gln Gly Val Ala Gly Met Cys Val Gly Glu Lys
85 90 95

Arg Lys Leu Gln Ile Pro Ser Ser Leu Ala Tyr Gly Glu Arg Gly Val
100 105 110

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

Val Asp Val Lys Ser Ala Ala
130 135

<210> 59
<211> 1938
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS

<222> (1)..(1938)

<400> 59

atg ata ctg atc tcg gga tac tgt ctt tta gtg tat agc gtt att ttg 48
Met Ile Leu Ile Ser Gly Tyr Cys Leu Leu Val Tyr Ser Val Ile Leu
1 5 10 15

cca gta ctg ata tcg gct tct aag tta tgt gat ttg gct gag tta caa 96
Pro Val Leu Ile Ser Ala Ser Lys Leu Cys Asp Leu Ala Glu Leu Gln
20 25 30

cga ttg aac aag aat tta aaa gta gac act gaa tcc ttg cca aaa tac 144
Arg Leu Asn Lys Asn Leu Lys Val Asp Thr Glu Ser Leu Pro Lys Tyr
35 40 45

caa tgg atc gct ggg cag ttg gaa caa aac tgc atg act gcg gat cca 192
Gln Trp Ile Ala Gly Gln Leu Glu Gln Asn Cys Met Thr Ala Asp Pro
50 55 60

gca agt gaa aat atg tca gac gta att caa cta gcc aat caa ata tac 240
Ala Ser Glu Asn Met Ser Asp Val Ile Gln Leu Ala Asn Gln Ile Tyr
65 70 75 80

tac aaa att ggg ctg atc caa tta tcc aac gat caa cat cta aga gct 288
Tyr Lys Ile Gly Leu Ile Gln Leu Ser Asn Asp Gln His Leu Arg Ala
85 90 95

att aac aca ttt gaa aaa atc gtt ttt aat gaa act tac aaa ggt tct 336
Ile Asn Thr Phe Glu Lys Ile Val Phe Asn Glu Thr Tyr Lys Gly Ser
100 105 110

ttt ggg aag ctg gcg gaa aag agg cta caa gag ctg tat gtc gat ttt 384
Phe Gly Lys Leu Ala Glu Lys Arg Leu Gln Glu Leu Tyr Val Asp Phe

115 120 125

ggg atg tgg gac aag gtg cat cag aag gat gat cag tat gcg aaa tat 432
Gly Met Trp Asp Lys Val His Gln Lys Asp Asp Gln Tyr Ala Lys Tyr
130 135 140

ctg tcc ttg aat gaa acc atc aga aac aaa ata tca tcc aaa gac gtt 480
Leu Ser Leu Asn Glu Thr Ile Arg Asn Lys Ile Ser Ser Lys Asp Val
145 150 155 160

tct gtg gag gaa gat att tct gag ctg cta cgc ata acg ccg tac gat 528
Ser Val Glu Glu Asp Ile Ser Glu Leu Leu Arg Ile Thr Pro Tyr Asp
165 170 175

gtt aac gtc ctc tcc acg cac atc gat gtt ctt ttt cac aaa cta gct 576
Val Asn Val Leu Ser Thr His Ile Asp Val Leu Phe His Lys Leu Ala
180 185 190

gaa gaa att gac gtt tcg tta gct gct gct atc att ttg gat tac gaa 624
Glu Glu Ile Asp Val Ser Leu Ala Ala Ala Ile Ile Leu Asp Tyr Glu
195 200 205

aca atc ctc gac aag cat ttg gct agc tta agc ata gat aca aga ctt 672
Thr Ile Leu Asp Lys His Leu Ala Ser Leu Ser Ile Asp Thr Arg Leu
210 215 220

tcg att cat tat gtc ata tct gtt tta cag acc ttt gta ctt aac tca 720
Ser Ile His Tyr Val Ile Ser Val Leu Gln Thr Phe Val Leu Asn Ser
225 230 235 240

gat gcg tcg ttc aat ata aga aaa tgc ctt tcc att gat atg gac tat 768
Asp Ala Ser Phe Asn Ile Arg Lys Cys Leu Ser Ile Asp Met Asp Tyr
245 250 255

gat aaa tgt aaa aaa cta agc ctg act att tcc aaa ttg aac aag gtg 816
Asp Lys Cys Lys Lys Leu Ser Leu Thr Ile Ser Lys Leu Asn Lys Val
260 265 270

aat cca tca aaa aga cag atc ctg gat cca gca aca tat gca ttt gag 864
Asn Pro Ser Lys Arg Gln Ile Leu Asp Pro Ala Thr Tyr Ala Phe Glu
275 280 285

aac aaa aag ttt aga agt tgg gat aga att att gaa ttt tat ttg aag 912
Asn Lys Lys Phe Arg Ser Trp Asp Arg Ile Ile Glu Phe Tyr Leu Lys
290 295 300

gat aag aag cca ttt att aca cca atg aaa att ctt aac aaa gat aca 960
Asp Lys Lys Pro Phe Ile Thr Pro Met Lys Ile Leu Asn Lys Asp Thr
305 310 315 320

aac ttt aaa aac aac tac ttc ttt tta gag gaa att atc aaa caa ttg 1008
Asn Phe Lys Asn Asn Tyr Phe Phe Leu Glu Glu Ile Ile Lys Gln Leu
325 330 335

ata gaa gac gtt caa ctg tcg aga cct ttg gca aaa aat tta ttc gaa 1056
Ile Glu Asp Val Gln Leu Ser Arg Pro Leu Ala Lys Asn Leu Phe Glu
340 345 350

gat ccc cca ata acc gat ggt ttt gtc aaa cca aaa tca tac tat cat 1104
Asp Pro Pro Ile Thr Asp Gly Phe Val Lys Pro Lys Ser Tyr Tyr His
355 360 365

acc gat tat cta gta tac att gat tcc att ctt tgt cag gct tct agc 1152
Thr Asp Tyr Leu Val Tyr Ile Asp Ser Ile Leu Cys Gln Ala Ser Ser
370 375 380

atg agt ccg gac gtc aag aga gct aaa ctg gct gcg ccg ttc tgt aaa 1200
Met Ser Pro Asp Val Lys Arg Ala Lys Leu Ala Ala Pro Phe Cys Lys
385 390 395 400

aag agt ttg agg cat tca cta aca cta gaa aca tgg aaa cac tat cag 1248
Lys Ser Leu Arg His Ser Leu Thr Leu Glu Thr Trp Lys His Tyr Gln
405 410 415

gat gct aag tcc gag caa aaa cct tta cct gag acg gta ttg agt gat 1296
Asp Ala Lys Ser Glu Gln Lys Pro Leu Pro Glu Thr Val Leu Ser Asp
420 425 430

gta tgg aat tcc aat cct cat ttg ctg atg tat atg gta aac tca ata 1344
Val Trp Asn Ser Asn Pro His Leu Leu Met Tyr Met Val Asn Ser Ile
435 440 445

ctt aat aaa agt agg tct aaa cct cat tca cag ttc aaa aag caa tta 1392
Leu Asn Lys Ser Arg Ser Lys Pro His Ser Gln Phe Lys Lys Gln Leu
450 455 460

tat gac cag ata aac aaa ttt ttc caa gat aac ggc ctc tca gag tcg 1440
Tyr Asp Gln Ile Asn Lys Phe Phe Gln Asp Asn Gly Leu Ser Glu Ser
465 470 475 480

acc aat cca tac gtg atg aag aac ttc cga tta tta cag aaa caa tta 1488
Thr Asn Pro Tyr Val Met Lys Asn Phe Arg Leu Leu Gln Lys Gln Leu
485 490 495

caa acc tat aaa gag cat aaa cat cgg aat ttc aac cag caa tat ttc 1536
Gln Thr Tyr Lys Glu His Lys His Arg Asn Phe Asn Gln Gln Tyr Phe
500 505 510

caa caa caa caa cag cag caa caa cac caa cga cat caa gca ccc cca 1584

Gln Gln Gln Gln Gln Gln Gln Gln His Gln Arg His Gln Ala Pro Pro
515 520 525

gca gcg cct aac tac gac cca aaa aag gac tat tat aaa att ctt gga 1632
Ala Ala Pro Asn Tyr Asp Pro Lys Lys Asp Tyr Tyr Lys Ile Leu Gly
530 535 540

gta tcg cct agt gct agt tcg aaa gaa ata agg aaa gca tat tta aat 1680
Val Ser Pro Ser Ala Ser Ser Lys Glu Ile Arg Lys Ala Tyr Leu Asn
545 550 555 560

tta acc aaa aaa tac cac cca gac aaa ata aag gcc aac cat aac gac 1728
Leu Thr Lys Lys Tyr His Pro Asp Lys Ile Lys Ala Asn His Asn Asp
565 570 575

aaa caa gaa tca att cac gaa act atg tca caa atc aat gaa gcg tac 1776
Lys Gln Glu Ser Ile His Glu Thr Met Ser Gln Ile Asn Glu Ala Tyr
580 585 590

gaa aca tta agt gat gac gat aaa agg aag gaa tac gat ctt tcc aga 1824
Glu Thr Leu Ser Asp Asp Asp Lys Arg Lys Glu Tyr Asp Leu Ser Arg
595 600 605

tca aac ccc cgc cgc aac act ttt cct cag ggg cct agg caa aat aac 1872
Ser Asn Pro Arg Arg Asn Thr Phe Pro Gln Gly Pro Arg Gln Asn Asn
610 615 620

atg ttc aaa aat cca gga agt ggc ttc cca ttc gga aat ggc ttt aaa 1920
Met Phe Lys Asn Pro Gly Ser Gly Phe Pro Phe Gly Asn Gly Phe Lys
625 630 635 640

atg aat ttt ggg ctt tga 1938
Met Asn Phe Gly Leu

645

<210> 60

<211> 645

<212> PRT

<213> Saccharomyces cerevisiae

<400> 60

Met Ile Leu Ile Ser Gly Tyr Cys Leu Leu Val Tyr Ser Val Ile Leu
1 5 10 15

Pro Val Leu Ile Ser Ala Ser Lys Leu Cys Asp Leu Ala Glu Leu Gln
20 25 30

Arg Leu Asn Lys Asn Leu Lys Val Asp Thr Glu Ser Leu Pro Lys Tyr
35 40 45

Gln Trp Ile Ala Gly Gln Leu Glu Gln Asn Cys Met Thr Ala Asp Pro
50 55 60

Ala Ser Glu Asn Met Ser Asp Val Ile Gln Leu Ala Asn Gln Ile Tyr
65 70 75 80

Tyr Lys Ile Gly Leu Ile Gln Leu Ser Asn Asp Gln His Leu Arg Ala
85 90 95

Ile Asn Thr Phe Glu Lys Ile Val Phe Asn Glu Thr Tyr Lys Gly Ser
100 105 110

Phe Gly Lys Leu Ala Glu Lys Arg Leu Gln Glu Leu Tyr Val Asp Phe
115 120 125

Gly Met Trp Asp Lys Val His Gln Lys Asp Asp Gln Tyr Ala Lys Tyr
130 135 140

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

Ser Val Glu Glu Asp Ile Ser Glu Leu Leu Arg Ile Thr Pro Tyr Asp
165 170 175

Val Asn Val Leu Ser Thr His Ile Asp Val Leu Phe His Lys Leu Ala
180 185 190

Glu Glu Ile Asp Val Ser Leu Ala Ala Ala Ile Ile Leu Asp Tyr Glu
195 200 205

Thr Ile Leu Asp Lys His Leu Ala Ser Leu Ser Ile Asp Thr Arg Leu
210 215 220

Ser Ile His Tyr Val Ile Ser Val Leu Gln Thr Phe Val Leu Asn Ser
225 230 235 240

Asp Ala Ser Phe Asn Ile Arg Lys Cys Leu Ser Ile Asp Met Asp Tyr
245 250 255

Asp Lys Cys Lys Lys Leu Ser Leu Thr Ile Ser Lys Leu Asn Lys Val
260 265 270

Asn Pro Ser Lys Arg Gln Ile Leu Asp Pro Ala Thr Tyr Ala Phe Glu
275 280 285

Asn Lys Lys Phe Arg Ser Trp Asp Arg Ile Ile Glu Phe Tyr Leu Lys
290 295 300

Asp Lys Lys Pro Phe Ile Thr Pro Met Lys Ile Leu Asn Lys Asp Thr
305 310 315 320

Asn Phe Lys Asn Asn Tyr Phe Phe Leu Glu Glu Ile Ile Lys Gln Leu
325 330 335

Ile Glu Asp Val Gln Leu Ser Arg Pro Leu Ala Lys Asn Leu Phe Glu
340 345 350

Asp Pro Pro Ile Thr Asp Gly Phe Val Lys Pro Lys Ser Tyr Tyr His

355 360 365

Thr Asp Tyr Leu Val Tyr Ile Asp Ser Ile Leu Cys Gln Ala Ser Ser
370 375 380

Met Ser Pro Asp Val Lys Arg Ala Lys Leu Ala Ala Pro Phe Cys Lys
385 390 395 400

Lys Ser Leu Arg His Ser Leu Thr Leu Glu Thr Trp Lys His Tyr Gln
405 410 415

Asp Ala Lys Ser Glu Gln Lys Pro Leu Pro Glu Thr Val Leu Ser Asp
420 425 430

Val Trp Asn Ser Asn Pro His Leu Leu Met Tyr Met Val Asn Ser Ile
435 440 445

Leu Asn Lys Ser Arg Ser Lys Pro His Ser Gln Phe Lys Lys Gln Leu
450 455 460

Tyr Asp Gln Ile Asn Lys Phe Phe Gln Asp Asn Gly Leu Ser Glu Ser
465 470 475 480

Thr Asn Pro Tyr Val Met Lys Asn Phe Arg Leu Leu Gln Lys Gln Leu
485 490 495

Gln Thr Tyr Lys Glu His Lys His Arg Asn Phe Asn Gln Gln Tyr Phe
500 505 510

Gln Gln Gln Gln Gln Gln Gln Gln His Gln Arg His Gln Ala Pro Pro
515 520 525

Ala Ala Pro Asn Tyr Asp Pro Lys Lys Asp Tyr Tyr Lys Ile Leu Gly
530 535 540

Val Ser Pro Ser Ala Ser Ser Lys Glu Ile Arg Lys Ala Tyr Leu Asn
545 550 555 560

Leu Thr Lys Lys Tyr His Pro Asp Lys Ile Lys Ala Asn His Asn Asp
565 570 575

Lys Gln Glu Ser Ile His Glu Thr Met Ser Gln Ile Asn Glu Ala Tyr
580 585 590

Glu Thr Leu Ser Asp Asp Asp Lys Arg Lys Glu Tyr Asp Leu Ser Arg
595 600 605

Ser Asn Pro Arg Arg Asn Thr Phe Pro Gln Gly Pro Arg Gln Asn Asn
610 615 620

Met Phe Lys Asn Pro Gly Ser Gly Phe Pro Phe Gly Asn Gly Phe Lys
625 630 635 640

Met Asn Phe Gly Leu
645

<210> 61
<211> 2646
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS
<222> (1)..(2646)

<400> 61
atg cga aac gtt tta agg ctt tta ttt tta aca gct ttt gtt gct ata 48
Met Arg Asn Val Leu Arg Leu Leu Phe Leu Thr Ala Phe Val Ala Ile
1 5 10 15

ggg tct tta gca gcc gtt tta ggt gtt gat tac ggt cag caa aat atc 96
Gly Ser Leu Ala Ala Val Leu Gly Val Asp Tyr Gly Gln Gln Asn Ile
20 25 30

aag gcc att gtg gtt tct ccg caa gcc cca tta gaa ctt gtg ctc aca 144
Lys Ala Ile Val Val Ser Pro Gln Ala Pro Leu Glu Leu Val Leu Thr
35 40 45

cca gag gca aaa cgg aag gag ata tct ggt ctt tcg ata aaa aga tta 192

Pro Glu Ala Lys Arg Lys Glu Ile Ser Gly Leu Ser Ile Lys Arg Leu
50 55 60

cca ggt tat gga aag gat gat ccg aat ggg att gaa aga atc tac ggt 240
Pro Gly Tyr Gly Lys Asp Asp Pro Asn Gly Ile Glu Arg Ile Tyr Gly
65 70 75 80

tcc gct gtt ggc agt tta gca aca agg ttt ccc caa aac aca ttg ttg 288
Ser Ala Val Gly Ser Leu Ala Thr Arg Phe Pro Gln Asn Thr Leu Leu
85 90 95

cat ttg aaa ccg cta ctt ggg aaa tca cta gaa gat gaa acc act gta 336
His Leu Lys Pro Leu Leu Gly Lys Ser Leu Glu Asp Glu Thr Thr Val
100 105 110

act ttg tat tca aaa caa cac ccc ggt tta gaa atg gta tca aca aat 384
Thr Leu Tyr Ser Lys Gln His Pro Gly Leu Glu Met Val Ser Thr Asn
115 120 125

aga agt acc ata gcc ttt tta gtt gat aat gtg gaa tat cca ttg gaa 432
Arg Ser Thr Ile Ala Phe Leu Val Asp Asn Val Glu Tyr Pro Leu Glu
130 135 140

gag tta gtg gca atg aat gtc caa gag att gcc aat aga gcc aat tca 480
Glu Leu Val Ala Met Asn Val Gln Glu Ile Ala Asn Arg Ala Asn Ser
145 150 155 160

ctg ttg aag gat aga gat gca aga act gag gac ttt gta aac aag atg 528
Leu Leu Lys Asp Arg Asp Ala Arg Thr Glu Asp Phe Val Asn Lys Met
165 170 175

agt ttt aca att cct gac ttt ttt gac caa cat caa agg aaa gca ctt 576
Ser Phe Thr Ile Pro Asp Phe Phe Asp Gln His Gln Arg Lys Ala Leu

180 185 190

tta gat gcc agt tca ata acc aca gga atc gaa gag aca tat ctg gtt 624
Leu Asp Ala Ser Ser Ile Thr Thr Gly Ile Glu Glu Thr Tyr Leu Val
195 200 205

agt gaa ggg atg tct gtt gca gtt aac ttt gta tta aag cag cgc caa 672
Ser Glu Gly Met Ser Val Ala Val Asn Phe Val Leu Lys Gln Arg Gln
210 215 220

ttt cca cca ggt gaa cag cag cat tat atc gta tat gac atg ggg agc 720
Phe Pro Pro Gly Glu Gln Gln His Tyr Ile Val Tyr Asp Met Gly Ser
225 230 235 240

ggt tct att aag gcc tca atg ttc tct ata ttg cag ccg gag gac act 768
Gly Ser Ile Lys Ala Ser Met Phe Ser Ile Leu Gln Pro Glu Asp Thr
245 250 255

act cag ccc gtt aca ata gaa ttt gaa gga tat ggg tat aat cca cat 816
Thr Gln Pro Val Thr Ile Glu Phe Glu Gly Tyr Gly Tyr Asn Pro His
260 265 270

cta ggt ggt gca aag ttt aca atg gat att ggc agt ttg ata gag aat 864
Leu Gly Gly Ala Lys Phe Thr Met Asp Ile Gly Ser Leu Ile Glu Asn
275 280 285

aag ttt ttg gaa aca cac cca gcc ata aga act gat gaa ttg cac gct 912
Lys Phe Leu Glu Thr His Pro Ala Ile Arg Thr Asp Glu Leu His Ala
290 295 300

aat ccc aag gcc tta gca aaa atc aac caa gca gca gag aag gca aag 960
Asn Pro Lys Ala Leu Ala Lys Ile Asn Gln Ala Ala Glu Lys Ala Lys
305 310 315 320

tta att tta agc gcc aat tct gag gca agt att aac ata gaa tca ctg 1008
Leu Ile Leu Ser Ala Asn Ser Glu Ala Ser Ile Asn Ile Glu Ser Leu
325 330 335

atc aac gat att gat ttc cgt act tct ata act aga cag gaa ttc gaa 1056
Ile Asn Asp Ile Asp Phe Arg Thr Ser Ile Thr Arg Gln Glu Phe Glu
340 345 350

gaa ttt att gca gac tcg tta ttg gac att gtc aaa ccc ata aat gac 1104
Glu Phe Ile Ala Asp Ser Leu Leu Asp Ile Val Lys Pro Ile Asn Asp
355 360 365

gct gtt aca aaa caa ttc ggt ggc tat gga aca aat tta cct gag ata 1152
Ala Val Thr Lys Gln Phe Gly Gly Tyr Gly Thr Asn Leu Pro Glu Ile
370 375 380

aat ggg gtc att ttg gcg gga ggc tct tcc cga att ccc att gtg cag 1200
Asn Gly Val Ile Leu Ala Gly Gly Ser Ser Arg Ile Pro Ile Val Gln
385 390 395 400

gat caa tta atc aaa ctc gta tcc gaa gaa aaa gtg ttg aga aat gtc 1248
Asp Gln Leu Ile Lys Leu Val Ser Glu Glu Lys Val Leu Arg Asn Val
405 410 415

aat gct gat gaa tca gct gtg aat ggt gtt gtt atg aga ggg atc aag 1296
Asn Ala Asp Glu Ser Ala Val Asn Gly Val Val Met Arg Gly Ile Lys
420 425 430

tta tct aat tcg ttt aag acc aag ccg tta aat gtt gtt gac cgt tct 1344
Leu Ser Asn Ser Phe Lys Thr Lys Pro Leu Asn Val Val Asp Arg Ser
435 440 445

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gta aat act tat tca ttc aaa tta tca aac gaa tct gaa ctg tat gat     1392
Val Asn Thr Tyr Ser Phe Lys Leu Ser Asn Glu Ser Glu Leu Tyr Asp
    450                 455                 460

gtg ttc acg cgc gga agt gct tat cca aac aaa aca tct att ttg aca     1440
Val Phe Thr Arg Gly Ser Ala Tyr Pro Asn Lys Thr Ser Ile Leu Thr
465                 470                 475                 480

aac acg act gat tcg att cct aat aat ttt acc att gac tta ttt gag     1488
Asn Thr Thr Asp Ser Ile Pro Asn Asn Phe Thr Ile Asp Leu Phe Glu
                485                 490                 495

aat ggt aaa ttg ttc gaa act atc aca gtt aat tca gga gct ata aag     1536
Asn Gly Lys Leu Phe Glu Thr Ile Thr Val Asn Ser Gly Ala Ile Lys
            500                 505                 510

aat tca tat tcc tct gat aag tgc tcg tca gga gtt gcg tat aac att     1584
Asn Ser Tyr Ser Ser Asp Lys Cys Ser Ser Gly Val Ala Tyr Asn Ile
        515                 520                 525

act ttc gac ttg tcc agt gat aga tta ttc tct att caa gag gtt aac     1632
Thr Phe Asp Leu Ser Ser Asp Arg Leu Phe Ser Ile Gln Glu Val Asn
    530                 535                 540

tgc att tgt cag agc gaa aat gac ata ggt aac tcc aag caa att aag     1680
Cys Ile Cys Gln Ser Glu Asn Asp Ile Gly Asn Ser Lys Gln Ile Lys
545                 550                 555                 560

aac aaa ggc agc cgt ttg gct ttt act tct gag gat gtt gag atc aaa     1728
Asn Lys Gly Ser Arg Leu Ala Phe Thr Ser Glu Asp Val Glu Ile Lys
                565                 570                 575

agg ctt tct cct tca gaa cgt tcg cgt ttg cat gag cat atc aag ttg     1776
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Arg Leu Ser Pro Ser Glu Arg Ser Arg Leu His Glu His Ile Lys Leu
580 585 590

ctc gat aaa cag gat aag gaa aga ttt caa ttc caa gaa aat tta aac 1824
Leu Asp Lys Gln Asp Lys Glu Arg Phe Gln Phe Gln Glu Asn Leu Asn
595 600 605

gtt ctt gaa agt aac ttg tat gat gct aga aac ctg cta atg gat gat 1872
Val Leu Glu Ser Asn Leu Tyr Asp Ala Arg Asn Leu Leu Met Asp Asp
610 615 620

gaa gtt atg caa aat gga cca aaa tcc caa gta gaa gag tta tcg gag 1920
Glu Val Met Gln Asn Gly Pro Lys Ser Gln Val Glu Glu Leu Ser Glu
625 630 635 640

atg gtt aaa gta tat ttg gat tgg ctc gaa gat gca tcc ttt gat act 1968
Met Val Lys Val Tyr Leu Asp Trp Leu Glu Asp Ala Ser Phe Asp Thr
645 650 655

gac cct gag gat ata gtt agc aga att aga gaa att gga ata tta aaa 2016
Asp Pro Glu Asp Ile Val Ser Arg Ile Arg Glu Ile Gly Ile Leu Lys
660 665 670

aag aaa ata gaa ctt tac atg gat tct gca aag gaa cct ttg aac tct 2064
Lys Lys Ile Glu Leu Tyr Met Asp Ser Ala Lys Glu Pro Leu Asn Ser
675 680 685

caa caa ttt aaa gga atg ctt gaa gaa ggc cat aag tta ctt cag gct 2112
Gln Gln Phe Lys Gly Met Leu Glu Glu Gly His Lys Leu Leu Gln Ala
690 695 700

ata gaa acc cat aag aat acc gtt gaa gaa ttt ttg agt caa ttt gaa 2160
Ile Glu Thr His Lys Asn Thr Val Glu Glu Phe Leu Ser Gln Phe Glu

705 710 715 720

acc gag ttt gcg gat acc ata gat aat gtt aga gaa gaa ttt aaa aag 2208
Thr Glu Phe Ala Asp Thr Ile Asp Asn Val Arg Glu Glu Phe Lys Lys
725 730 735

att aag caa cca gcg tat gtg tcg aag gcg tta tct aca tgg gag gaa 2256
Ile Lys Gln Pro Ala Tyr Val Ser Lys Ala Leu Ser Thr Trp Glu Glu
740 745 750

acc tta acc tct ttt aaa aat tcc att agc gaa ata gag aag ttc ctg 2304
Thr Leu Thr Ser Phe Lys Asn Ser Ile Ser Glu Ile Glu Lys Phe Leu
755 760 765

gca aaa aac cta ttt ggc gaa gac ctt cgt gaa cat tta ttt gaa atc 2352
Ala Lys Asn Leu Phe Gly Glu Asp Leu Arg Glu His Leu Phe Glu Ile
770 775 780

aaa tta caa ttt gat atg tat cgt acg aaa cta gag gaa aaa ctg cgt 2400
Lys Leu Gln Phe Asp Met Tyr Arg Thr Lys Leu Glu Glu Lys Leu Arg
785 790 795 800

tta ata aaa agc ggt gat gaa agt cgc tta aat gaa ata aag aag tta 2448
Leu Ile Lys Ser Gly Asp Glu Ser Arg Leu Asn Glu Ile Lys Lys Leu
805 810 815

cat tta aga aac ttc cgc cta caa aag aga aag gag gaa aag ttg aaa 2496
His Leu Arg Asn Phe Arg Leu Gln Lys Arg Lys Glu Glu Lys Leu Lys
820 825 830

aga aag ctt gaa cag gaa aaa agc aga aac aac aat gaa aca gaa tcg 2544
Arg Lys Leu Glu Gln Glu Lys Ser Arg Asn Asn Asn Glu Thr Glu Ser
835 840 845

aca gta atc aac tcg gct gac gat aaa act act att gtc aat gac aag 2592
Thr Val Ile Asn Ser Ala Asp Asp Lys Thr Thr Ile Val Asn Asp Lys
850 855 860

acc acc gag tcg aat cca agt tct gag gaa gac att ttg cat gat gaa 2640
Thr Thr Glu Ser Asn Pro Ser Ser Glu Glu Asp Ile Leu His Asp Glu
865 870 875 880

tta tag 2646
Leu

<210> 62
<211> 881
<212> PRT
<213> Saccharomyces cerevisiae

<400> 62

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Lys Ala Ile Val Val Ser Pro Gln Ala Pro Leu Glu Leu Val Leu Thr
35 40 45

Pro Glu Ala Lys Arg Lys Glu Ile Ser Gly Leu Ser Ile Lys Arg Leu

50 55 60

Pro Gly Tyr Gly Lys Asp Asp Pro Asn Gly Ile Glu Arg Ile Tyr Gly
65 70 75 80

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

His Leu Lys Pro Leu Leu Gly Lys Ser Leu Glu Asp Glu Thr Thr Val
100 105 110

Thr Leu Tyr Ser Lys Gln His Pro Gly Leu Glu Met Val Ser Thr Asn
115 120 125

Arg Ser Thr Ile Ala Phe Leu Val Asp Asn Val Glu Tyr Pro Leu Glu
130 135 140

Glu Leu Val Ala Met Asn Val Gln Glu Ile Ala Asn Arg Ala Asn Ser
145 150 155 160

Leu Leu Lys Asp Arg Asp Ala Arg Thr Glu Asp Phe Val Asn Lys Met
165 170 175

Ser Phe Thr Ile Pro Asp Phe Phe Asp Gln His Gln Arg Lys Ala Leu
180 185 190

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

Ser Glu Gly Met Ser Val Ala Val Asn Phe Val Leu Lys Gln Arg Gln
210 215 220

Phe Pro Pro Gly Glu Gln Gln His Tyr Ile Val Tyr Asp Met Gly Ser
225 230 235 240

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

Thr Gln Pro Val Thr Ile Glu Phe Glu Gly Tyr Gly Tyr Asn Pro His
260 265 270

Leu Gly Gly Ala Lys Phe Thr Met Asp Ile Gly Ser Leu Ile Glu Asn
275 280 285

Lys Phe Leu Glu Thr His Pro Ala Ile Arg Thr Asp Glu Leu His Ala
290 295 300

Asn Pro Lys Ala Leu Ala Lys Ile Asn Gln Ala Ala Glu Lys Ala Lys
305 310 315 320

Leu Ile Leu Ser Ala Asn Ser Glu Ala Ser Ile Asn Ile Glu Ser Leu
325 330 335

Ile Asn Asp Ile Asp Phe Arg Thr Ser Ile Thr Arg Gln Glu Phe Glu
340 345 350

Glu Phe Ile Ala Asp Ser Leu Leu Asp Ile Val Lys Pro Ile Asn Asp
355 360 365

Ala Val Thr Lys Gln Phe Gly Gly Tyr Gly Thr Asn Leu Pro Glu Ile
370 375 380

Asn Gly Val Ile Leu Ala Gly Gly Ser Ser Arg Ile Pro Ile Val Gln
385 390 395 400

Asp Gln Leu Ile Lys Leu Val Ser Glu Glu Lys Val Leu Arg Asn Val
405 410 415

Asn Ala Asp Glu Ser Ala Val Asn Gly Val Val Met Arg Gly Ile Lys
420 425 430

Leu Ser Asn Ser Phe Lys Thr Lys Pro Leu Asn Val Val Asp Arg Ser
435 440 445

Val Asn Thr Tyr Ser Phe Lys Leu Ser Asn Glu Ser Glu Leu Tyr Asp
450 455 460

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

Asn Thr Thr Asp Ser Ile Pro Asn Asn Phe Thr Ile Asp Leu Phe Glu
485 490 495

Asn Gly Lys Leu Phe Glu Thr Ile Thr Val Asn Ser Gly Ala Ile Lys
500 505 510

Asn Ser Tyr Ser Ser Asp Lys Cys Ser Ser Gly Val Ala Tyr Asn Ile
515 520 525

Thr Phe Asp Leu Ser Ser Asp Arg Leu Phe Ser Ile Gln Glu Val Asn
530 535 540

Cys Ile Cys Gln Ser Glu Asn Asp Ile Gly Asn Ser Lys Gln Ile Lys
545 550 555 560

Asn Lys Gly Ser Arg Leu Ala Phe Thr Ser Glu Asp Val Glu Ile Lys
565 570 575

Arg Leu Ser Pro Ser Glu Arg Ser Arg Leu His Glu His Ile Lys Leu

580 585 590

Leu Asp Lys Gln Asp Lys Glu Arg Phe Gln Phe Gln Glu Asn Leu Asn
595 600 605

Val Leu Glu Ser Asn Leu Tyr Asp Ala Arg Asn Leu Leu Met Asp Asp
610 615 620

Glu Val Met Gln Asn Gly Pro Lys Ser Gln Val Glu Glu Leu Ser Glu
625 630 635 640

Met Val Lys Val Tyr Leu Asp Trp Leu Glu Asp Ala Ser Phe Asp Thr
645 650 655

Asp Pro Glu Asp Ile Val Ser Arg Ile Arg Glu Ile Gly Ile Leu Lys
660 665 670

Lys Lys Ile Glu Leu Tyr Met Asp Ser Ala Lys Glu Pro Leu Asn Ser
675 680 685

Gln Gln Phe Lys Gly Met Leu Glu Glu Gly His Lys Leu Leu Gln Ala
690 695 700

Ile Glu Thr His Lys Asn Thr Val Glu Glu Phe Leu Ser Gln Phe Glu
705 710 715 720

Thr Glu Phe Ala Asp Thr Ile Asp Asn Val Arg Glu Glu Phe Lys Lys
725 730 735

Ile Lys Gln Pro Ala Tyr Val Ser Lys Ala Leu Ser Thr Trp Glu Glu
740 745 750

Thr Leu Thr Ser Phe Lys Asn Ser Ile Ser Glu Ile Glu Lys Phe Leu
755 760 765

Ala Lys Asn Leu Phe Gly Glu Asp Leu Arg Glu His Leu Phe Glu Ile
770 775 780

Lys Leu Gln Phe Asp Met Tyr Arg Thr Lys Leu Glu Glu Lys Leu Arg
785 790 795 800

Leu Ile Lys Ser Gly Asp Glu Ser Arg Leu Asn Glu Ile Lys Lys Leu
805 810 815

His Leu Arg Asn Phe Arg Leu Gln Lys Arg Lys Glu Glu Lys Leu Lys
820 825 830

Arg Lys Leu Glu Gln Glu Lys Ser Arg Asn Asn Asn Glu Thr Glu Ser
835 840 845

Thr Val Ile Asn Ser Ala Asp Asp Lys Thr Thr Ile Val Asn Asp Lys
850 855 860

Thr Thr Glu Ser Asn Pro Ser Ser Glu Glu Asp Ile Leu His Asp Glu
865 870 875 880

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att tta cca gcg ttg gcc tac agt gaa gct gtc acg atg gtc aag tcg 96
Ile Leu Pro Ala Leu Ala Tyr Ser Glu Ala Val Thr Met Val Lys Ser
20 25 30

att gag cag tac ttc gat atc tgc aat agg aat gat tct tac aca atg 144

Ile Glu Gln Tyr Phe Asp Ile Cys Asn Arg Asn Asp Ser Tyr Thr Met
35 40 45

ata aaa tac tac act tct tgg tgc caa cat tgt aaa act ctg gcc cca 192
Ile Lys Tyr Tyr Thr Ser Trp Cys Gln His Cys Lys Thr Leu Ala Pro
50 55 60

gta tac gaa gag ctt ggt gag cta tac gcc aaa aaa gct aat aaa gat 240
Val Tyr Glu Glu Leu Gly Glu Leu Tyr Ala Lys Lys Ala Asn Lys Asp
65 70 75 80

gat acc cca att aac ttc ctt gaa gtt aac tgt gaa ttc ttc ggg cca 288
Asp Thr Pro Ile Asn Phe Leu Glu Val Asn Cys Glu Phe Phe Gly Pro
85 90 95

act tta tgt acc gac ttg cct gga ttt cca ata att gaa ctg gtc aaa 336
Thr Leu Cys Thr Asp Leu Pro Gly Phe Pro Ile Ile Glu Leu Val Lys
100 105 110

cct cgt act aag ccc tta gtt ctt ccg aag ctc gat tgg tcg tct atg 384
Pro Arg Thr Lys Pro Leu Val Leu Pro Lys Leu Asp Trp Ser Ser Met
115 120 125

aaa ttt cat gaa aga cta tgg caa aga atc aag acg tgg ttc aac aat 432
Lys Phe His Glu Arg Leu Trp Gln Arg Ile Lys Thr Trp Phe Asn Asn
130 135 140

cct aag tac caa ctg gat acg tct agg gtt gtt cgt ttt gaa ggg agt 480
Pro Lys Tyr Gln Leu Asp Thr Ser Arg Val Val Arg Phe Glu Gly Ser
145 150 155 160

agg aac cta aag agt tta agc aac ttt atc gat act gta aga agt aaa 528
Arg Asn Leu Lys Ser Leu Ser Asn Phe Ile Asp Thr Val Arg Ser Lys

165 170 175

gat aca gaa gaa aga ttc ata gaa cat att ttc gat gat tct agg aat 576
Asp Thr Glu Glu Arg Phe Ile Glu His Ile Phe Asp Asp Ser Arg Asn
180 185 190

tgc aat gaa gaa tta cgt tct caa cag ctt ctg tgt aaa gct ggt aaa 624
Cys Asn Glu Glu Leu Arg Ser Gln Gln Leu Leu Cys Lys Ala Gly Lys
195 200 205

gaa tac tac tct gat act tta tct aaa tta tac ggt gac gtg aat ggg 672
Glu Tyr Tyr Ser Asp Thr Leu Ser Lys Leu Tyr Gly Asp Val Asn Gly
210 215 220

ctg gaa aag gaa agg cga aga cta gaa gct tta att aag caa aat gga 720
Leu Glu Lys Glu Arg Arg Arg Leu Glu Ala Leu Ile Lys Gln Asn Gly
225 230 235 240

gat gac ttg agt aaa gaa gtt aaa gaa aaa ctg aaa atc att cgt cta 768
Asp Asp Leu Ser Lys Glu Val Lys Glu Lys Leu Lys Ile Ile Arg Leu
245 250 255

caa ttg agc cta tta tca cac ata gaa gac cag tta gaa gat acc agt 816
Gln Leu Ser Leu Leu Ser His Ile Glu Asp Gln Leu Glu Asp Thr Ser
260 265 270

agt cat gac gag ctt tga 834
Ser His Asp Glu Leu
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<212> PRT

<213> Saccharomyces cerevisiae

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Ile Leu Pro Ala Leu Ala Tyr Ser Glu Ala Val Thr Met Val Lys Ser
20 25 30

Ile Glu Gln Tyr Phe Asp Ile Cys Asn Arg Asn Asp Ser Tyr Thr Met
35 40 45

Ile Lys Tyr Tyr Thr Ser Trp Cys Gln His Cys Lys Thr Leu Ala Pro
50 55 60

Val Tyr Glu Glu Leu Gly Glu Leu Tyr Ala Lys Lys Ala Asn Lys Asp
65 70 75 80

Asp Thr Pro Ile Asn Phe Leu Glu Val Asn Cys Glu Phe Phe Gly Pro
85 90 95

Thr Leu Cys Thr Asp Leu Pro Gly Phe Pro Ile Ile Glu Leu Val Lys
100 105 110

Pro Arg Thr Lys Pro Leu Val Leu Pro Lys Leu Asp Trp Ser Ser Met
115 120 125

Lys Phe His Glu Arg Leu Trp Gln Arg Ile Lys Thr Trp Phe Asn Asn
130 135 140

Pro Lys Tyr Gln Leu Asp Thr Ser Arg Val Val Arg Phe Glu Gly Ser
145 150 155 160

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

Asp Thr Glu Glu Arg Phe Ile Glu His Ile Phe Asp Asp Ser Arg Asn
180 185 190

Cys Asn Glu Glu Leu Arg Ser Gln Gln Leu Leu Cys Lys Ala Gly Lys
195 200 205

Glu Tyr Tyr Ser Asp Thr Leu Ser Lys Leu Tyr Gly Asp Val Asn Gly
210 215 220

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

Asp Asp Leu Ser Lys Glu Val Lys Glu Lys Leu Lys Ile Ile Arg Leu

245 250 255

Gln Leu Ser Leu Leu Ser His Ile Glu Asp Gln Leu Glu Asp Thr Ser
260 265 270

Ser His Asp Glu Leu
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cta tca tat gct ttt acc acc att gaa aca gaa att ttc caa tta caa 96
Leu Ser Tyr Ala Phe Thr Thr Ile Glu Thr Glu Ile Phe Gln Leu Gln
20 25 30

aat gaa ata agt acg aaa tat ggc cca gat atg aac ttc tac aag ttc 144
Asn Glu Ile Ser Thr Lys Tyr Gly Pro Asp Met Asn Phe Tyr Lys Phe
35 40 45

ttg aag tta cct aaa ctg cag aat tct agt aca aag gag att aca aaa 192
Leu Lys Leu Pro Lys Leu Gln Asn Ser Ser Thr Lys Glu Ile Thr Lys
50 55 60

aac tta aga aag cta tcc aag aag tac cat ccg gat aag aac cct aaa 240
Asn Leu Arg Lys Leu Ser Lys Lys Tyr His Pro Asp Lys Asn Pro Lys
65 70 75 80

tac cgt aaa ttg tat gaa agg tta aac ctc gct act caa att ctt tca 288
Tyr Arg Lys Leu Tyr Glu Arg Leu Asn Leu Ala Thr Gln Ile Leu Ser
85 90 95

aac agc tct aat cgt aag att tat gat tat tat cta cag aat ggc ttt 336
Asn Ser Ser Asn Arg Lys Ile Tyr Asp Tyr Tyr Leu Gln Asn Gly Phe
100 105 110

cca aac tat gat ttc cat aag ggt ggt ttt tat ttt tcc aga atg aag 384
Pro Asn Tyr Asp Phe His Lys Gly Gly Phe Tyr Phe Ser Arg Met Lys
115 120 125

cct aag act tgg ttc ctg ctg gcc ttt att tgg ata gtc gtt aat att 432
Pro Lys Thr Trp Phe Leu Leu Ala Phe Ile Trp Ile Val Val Asn Ile
130 135 140

ggg cag tat atc att tct att att caa tat cgt tct caa aga tca aga 480
Gly Gln Tyr Ile Ile Ser Ile Ile Gln Tyr Arg Ser Gln Arg Ser Arg
145 150 155 160

att gaa aac ttc atc agt cag tgt aaa caa cag gat gat acc aat gga 528
Ile Glu Asn Phe Ile Ser Gln Cys Lys Gln Gln Asp Asp Thr Asn Gly
165 170 175

cta ggc gta aaa caa cta acg ttt aaa caa cat gaa aag gat gag ggt 576
Leu Gly Val Lys Gln Leu Thr Phe Lys Gln His Glu Lys Asp Glu Gly
180 185 190

aaa agt ttg gtt gta agg ttt agc gat gtc tat gtt gta gag cct gat 624
Lys Ser Leu Val Val Arg Phe Ser Asp Val Tyr Val Val Glu Pro Asp
195 200 205

gga agt gaa aca cta att tcg cca gat acc ttg gat aaa cct tca gta 672
Gly Ser Glu Thr Leu Ile Ser Pro Asp Thr Leu Asp Lys Pro Ser Val
210 215 220

aag aac tgt ttg ttt tgg aga ata cct gct tcg gtt tgg aac atg acg 720
Lys Asn Cys Leu Phe Trp Arg Ile Pro Ala Ser Val Trp Asn Met Thr
225 230 235 240

ttt ggc aaa tct gtt ggt agc gca gga aaa gaa gaa ata ata acg gat 768
Phe Gly Lys Ser Val Gly Ser Ala Gly Lys Glu Glu Ile Ile Thr Asp
245 250 255

agt aaa aag tat gat ggt aac caa aca aaa aag ggg aac aaa gta aaa 816
Ser Lys Lys Tyr Asp Gly Asn Gln Thr Lys Lys Gly Asn Lys Val Lys
260 265 270

aag ggt tct gca aag aaa ggc caa aag aaa atg gaa ttg cct aac ggt 864
Lys Gly Ser Ala Lys Lys Gly Gln Lys Lys Met Glu Leu Pro Asn Gly
275 280 285

aaa gtg atc tat tca cgt aaa tga 888
Lys Val Ile Tyr Ser Arg Lys
290 295

<210> 66

<211> 295

<212> PRT

<213> Saccharomyces cerevisiae

<400> 66

Met Asn Gly Tyr Trp Lys Pro Ala Leu Val Val Leu Gly Leu Val Ser
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Asn Glu Ile Ser Thr Lys Tyr Gly Pro Asp Met Asn Phe Tyr Lys Phe
35 40 45

Leu Lys Leu Pro Lys Leu Gln Asn Ser Ser Thr Lys Glu Ile Thr Lys
50 55 60

Asn Leu Arg Lys Leu Ser Lys Lys Tyr His Pro Asp Lys Asn Pro Lys
65 70 75 80

Tyr Arg Lys Leu Tyr Glu Arg Leu Asn Leu Ala Thr Gln Ile Leu Ser
85 90 95

Asn Ser Ser Asn Arg Lys Ile Tyr Asp Tyr Tyr Leu Gln Asn Gly Phe
100 105 110

Pro Asn Tyr Asp Phe His Lys Gly Gly Phe Tyr Phe Ser Arg Met Lys
115 120 125

Pro Lys Thr Trp Phe Leu Leu Ala Phe Ile Trp Ile Val Val Asn Ile
130 135 140

Gly Gln Tyr Ile Ile Ser Ile Ile Gln Tyr Arg Ser Gln Arg Ser Arg
145 150 155 160

Ile Glu Asn Phe Ile Ser Gln Cys Lys Gln Gln Asp Asp Thr Asn Gly
165 170 175

Leu Gly Val Lys Gln Leu Thr Phe Lys Gln His Glu Lys Asp Glu Gly
180 185 190

Lys Ser Leu Val Val Arg Phe Ser Asp Val Tyr Val Val Glu Pro Asp
195 200 205

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

Lys Asn Cys Leu Phe Trp Arg Ile Pro Ala Ser Val Trp Asn Met Thr
225 230 235 240

Phe Gly Lys Ser Val Gly Ser Ala Gly Lys Glu Glu Ile Ile Thr Asp
245 250 255

Ser Lys Lys Tyr Asp Gly Asn Gln Thr Lys Lys Gly Asn Lys Val Lys
260 265 270

Lys Gly Ser Ala Lys Lys Gly Gln Lys Lys Met Glu Leu Pro Asn Gly
275 280 285

Lys Val Ile Tyr Ser Arg Lys
290 295

<210> 67
<211> 1554
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS
<222> (1)..(1554)

<400> 67
atg caa gtg acc aca aga ttt ata tct gcg ata gtc tcg ttt tgc ctg 48
Met Gln Val Thr Thr Arg Phe Ile Ser Ala Ile Val Ser Phe Cys Leu
1 5 10 15

ttt gct tct ttc acg ttg gct gaa aac agc gca aga gct acg ccg gga 96

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Phe Ala Ser Phe Thr Leu Ala Glu Asn Ser Ala Arg Ala Thr Pro Gly
            20                  25                  30

tca gat tta ctc gtt cta aca gag aag aaa ttt aaa tca ttc atc gaa      144
Ser Asp Leu Leu Val Leu Thr Glu Lys Lys Phe Lys Ser Phe Ile Glu
        35                  40                  45

tct cat ccg tta gtc ctc gtc gag ttt ttt gct cca tgg tgt ttg cat      192
Ser His Pro Leu Val Leu Val Glu Phe Phe Ala Pro Trp Cys Leu His
    50                  55                  60

tct cag atc tta cgc cct cac tta gaa gag gcc gcc tct att tta aag      240
Ser Gln Ile Leu Arg Pro His Leu Glu Glu Ala Ala Ser Ile Leu Lys
65                  70                  75                  80

gag cat aac gtc cca gtt gtt caa att gat tgt gag gct aac agt atg      288
Glu His Asn Val Pro Val Val Gln Ile Asp Cys Glu Ala Asn Ser Met
                85                  90                  95

gtt tgc ctg caa caa act ata aat acc tac cca acc ttg aaa atc ttt      336
Val Cys Leu Gln Gln Thr Ile Asn Thr Tyr Pro Thr Leu Lys Ile Phe
            100                 105                 110

aaa aat ggt cgt att ttt gat ggt caa gtc tat cgc ggt gtc aag atc      384
Lys Asn Gly Arg Ile Phe Asp Gly Gln Val Tyr Arg Gly Val Lys Ile
        115                 120                 125

acc gat gaa atc act cag tac atg att cag cta tac gag gct tct gtc      432
Thr Asp Glu Ile Thr Gln Tyr Met Ile Gln Leu Tyr Glu Ala Ser Val
    130                 135                 140

att tat tta aat tcc gaa gat gaa atc caa cca tac ttg gaa aat gca      480
Ile Tyr Leu Asn Ser Glu Asp Glu Ile Gln Pro Tyr Leu Glu Asn Ala
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145 150 155 160

act tta cca gta gta ata aac aga ggc ttg aca ggc ttg aat gaa acg 528
Thr Leu Pro Val Val Ile Asn Arg Gly Leu Thr Gly Leu Asn Glu Thr
165 170 175

tat caa gaa gtc gca ctg gac ctt gct gag gat tac gtc ttt tta tcc 576
Tyr Gln Glu Val Ala Leu Asp Leu Ala Glu Asp Tyr Val Phe Leu Ser
180 185 190

ctt cta gat tca gaa gat aag tca tta tca atc cac ttg cca aac act 624
Leu Leu Asp Ser Glu Asp Lys Ser Leu Ser Ile His Leu Pro Asn Thr
195 200 205

aca gaa cca att ctg ttt gat gga aat gta gac tct ttg gtc gga aat 672
Thr Glu Pro Ile Leu Phe Asp Gly Asn Val Asp Ser Leu Val Gly Asn
210 215 220

tcc gtt gct cta act cag tgg tta aaa gtg gta att tta cct tac ttt 720
Ser Val Ala Leu Thr Gln Trp Leu Lys Val Val Ile Leu Pro Tyr Phe
225 230 235 240

acc gac atc gaa cct gat ctc ttc ccc aag tac att tct agc aat ttg 768
Thr Asp Ile Glu Pro Asp Leu Phe Pro Lys Tyr Ile Ser Ser Asn Leu
245 250 255

ccg ttg gct tac ttc ttt tat act tct gag gaa gaa ttg gaa gat tac 816
Pro Leu Ala Tyr Phe Phe Tyr Thr Ser Glu Glu Glu Leu Glu Asp Tyr
260 265 270

act gat ctt ttc acg cag tta ggt aag gaa aat cgt ggc caa ata aat 864
Thr Asp Leu Phe Thr Gln Leu Gly Lys Glu Asn Arg Gly Gln Ile Asn
275 280 285

ttc att gca tta aac tct aca atg ttc cca cac cac gtt aga ttc cta 912
Phe Ile Ala Leu Asn Ser Thr Met Phe Pro His His Val Arg Phe Leu
290 295 300

aat atg aga gaa cag ttc cca tta ttt gct atc cat aat atg atc aat 960
Asn Met Arg Glu Gln Phe Pro Leu Phe Ala Ile His Asn Met Ile Asn
305 310 315 320

aat ctg aaa tat ggt tta cca caa cta cca gaa gaa gag tac gcg aaa 1008
Asn Leu Lys Tyr Gly Leu Pro Gln Leu Pro Glu Glu Glu Tyr Ala Lys
325 330 335

tta gaa aaa cca caa cca cta gac aga gat atg atc gtt cag ttg gta 1056
Leu Glu Lys Pro Gln Pro Leu Asp Arg Asp Met Ile Val Gln Leu Val
340 345 350

aaa gat tac cgt gaa ggt act gcc aag cca att gtt aag tca gaa gag 1104
Lys Asp Tyr Arg Glu Gly Thr Ala Lys Pro Ile Val Lys Ser Glu Glu
355 360 365

att cca aaa gaa caa aag tcc aat gtt tat aaa ata gtt ggg aag aca 1152
Ile Pro Lys Glu Gln Lys Ser Asn Val Tyr Lys Ile Val Gly Lys Thr
370 375 380

cat gac gac att gtt cat gat gat gac aag gat gtc ctt gtc aaa tat 1200
His Asp Asp Ile Val His Asp Asp Asp Lys Asp Val Leu Val Lys Tyr
385 390 395 400

tac gcg aca tgg tgt att cat agt aaa agg ttt gcg cct att tac gaa 1248
Tyr Ala Thr Trp Cys Ile His Ser Lys Arg Phe Ala Pro Ile Tyr Glu
405 410 415

gaa att gca aat gtc tta gca tct gat gaa tct gtt cgc gat aaa atc 1296
Glu Ile Ala Asn Val Leu Ala Ser Asp Glu Ser Val Arg Asp Lys Ile
420 425 430

ttg atc gcc gaa gta gat tca ggg gca aat gat atc tta agt ttt cct 1344
Leu Ile Ala Glu Val Asp Ser Gly Ala Asn Asp Ile Leu Ser Phe Pro
435 440 445

gtg aca gga tat cca acc att gct ttg tat cct gcc gga aat aac tct 1392
Val Thr Gly Tyr Pro Thr Ile Ala Leu Tyr Pro Ala Gly Asn Asn Ser
450 455 460

aag cct att atc ttc aat aaa att aga aat ttg gaa gat gtt ttc gaa 1440
Lys Pro Ile Ile Phe Asn Lys Ile Arg Asn Leu Glu Asp Val Phe Glu
465 470 475 480

ttt atc aag gaa tca ggt aca cat cac att gac ggc cag gca att tat 1488
Phe Ile Lys Glu Ser Gly Thr His His Ile Asp Gly Gln Ala Ile Tyr
485 490 495

gat aaa ttg cac cag gcc aag gat tct gaa gtg tct act gaa gat acc 1536
Asp Lys Leu His Gln Ala Lys Asp Ser Glu Val Ser Thr Glu Asp Thr
500 505 510

gta cat gat gaa tta taa 1554
Val His Asp Glu Leu
515

<210> 68
<211> 517
<212> PRT
<213> Saccharomyces cerevisiae

〈400〉 68

Met Gln Val Thr Thr Arg Phe Ile Ser Ala Ile Val Ser Phe Cys Leu
1 5 10 15

Phe Ala Ser Phe Thr Leu Ala Glu Asn Ser Ala Arg Ala Thr Pro Gly
20 25 30

Ser Asp Leu Leu Val Leu Thr Glu Lys Lys Phe Lys Ser Phe Ile Glu
35 40 45

Ser His Pro Leu Val Leu Val Glu Phe Phe Ala Pro Trp Cys Leu His
50 55 60

Ser Gln Ile Leu Arg Pro His Leu Glu Glu Ala Ala Ser Ile Leu Lys
65 70 75 80

Glu His Asn Val Pro Val Val Gln Ile Asp Cys Glu Ala Asn Ser Met
85 90 95

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

Lys Asn Gly Arg Ile Phe Asp Gly Gln Val Tyr Arg Gly Val Lys Ile
115 120 125

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

Ile Tyr Leu Asn Ser Glu Asp Glu Ile Gln Pro Tyr Leu Glu Asn Ala
145 150 155 160

Thr Leu Pro Val Val Ile Asn Arg Gly Leu Thr Gly Leu Asn Glu Thr
165 170 175

Tyr Gln Glu Val Ala Leu Asp Leu Ala Glu Asp Tyr Val Phe Leu Ser
180 185 190

Leu Leu Asp Ser Glu Asp Lys Ser Leu Ser Ile His Leu Pro Asn Thr
195 200 205

Thr Glu Pro Ile Leu Phe Asp Gly Asn Val Asp Ser Leu Val Gly Asn
210 215 220

Ser Val Ala Leu Thr Gln Trp Leu Lys Val Val Ile Leu Pro Tyr Phe
225 230 235 240

Thr Asp Ile Glu Pro Asp Leu Phe Pro Lys Tyr Ile Ser Ser Asn Leu
245 250 255

Pro Leu Ala Tyr Phe Phe Tyr Thr Ser Glu Glu Glu Leu Glu Asp Tyr
260 265 270

Thr Asp Leu Phe Thr Gln Leu Gly Lys Glu Asn Arg Gly Gln Ile Asn
275 280 285

Phe Ile Ala Leu Asn Ser Thr Met Phe Pro His His Val Arg Phe Leu
290 295 300

Asn Met Arg Glu Gln Phe Pro Leu Phe Ala Ile His Asn Met Ile Asn
305 310 315 320

Asn Leu Lys Tyr Gly Leu Pro Gln Leu Pro Glu Glu Glu Tyr Ala Lys
325 330 335

Leu Glu Lys Pro Gln Pro Leu Asp Arg Asp Met Ile Val Gln Leu Val
340 345 350

Lys Asp Tyr Arg Glu Gly Thr Ala Lys Pro Ile Val Lys Ser Glu Glu
355 360 365

Ile Pro Lys Glu Gln Lys Ser Asn Val Tyr Lys Ile Val Gly Lys Thr
370 375 380

His Asp Asp Ile Val His Asp Asp Asp Lys Asp Val Leu Val Lys Tyr
385 390 395 400

Tyr Ala Thr Trp Cys Ile His Ser Lys Arg Phe Ala Pro Ile Tyr Glu
405 410 415

Glu Ile Ala Asn Val Leu Ala Ser Asp Glu Ser Val Arg Asp Lys Ile
420 425 430

Leu Ile Ala Glu Val Asp Ser Gly Ala Asn Asp Ile Leu Ser Phe Pro
435 440 445

Val Thr Gly Tyr Pro Thr Ile Ala Leu Tyr Pro Ala Gly Asn Asn Ser
450 455 460

Lys Pro Ile Ile Phe Asn Lys Ile Arg Asn Leu Glu Asp Val Phe Glu
465 470 475 480

Phe Ile Lys Glu Ser Gly Thr His His Ile Asp Gly Gln Ala Ile Tyr
485 490 495

Asp Lys Leu His Gln Ala Lys Asp Ser Glu Val Ser Thr Glu Asp Thr
500 505 510

Val His Asp Glu Leu

515

<210> 69
<211> 1527
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1527)

<400> 69
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Met Leu Arg Arg Ala Leu Leu Cys Leu Ala Val Ala Ala Leu Val Arg
1 5 10 15

gcc gac gcc ccc gag gag gag gac cac gtc ctg gtg ctg cgg aaa agc 96
Ala Asp Ala Pro Glu Glu Glu Asp His Val Leu Val Leu Arg Lys Ser
20 25 30

aac ttc gcg gag gcg ctg gcg gcc cac aag tac ctg ctg gtg gag ttc 144
Asn Phe Ala Glu Ala Leu Ala Ala His Lys Tyr Leu Leu Val Glu Phe
35 40 45

tat gcc cct tgg tgt ggc cac tgc aag gct ctg gcc cct gag tat gcc 192
Tyr Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu Tyr Ala
50 55 60

aaa gcc gct ggg aag ctg aag gca gaa ggt tcc gag atc agg ttg gcc 240
Lys Ala Ala Gly Lys Leu Lys Ala Glu Gly Ser Glu Ile Arg Leu Ala
65 70 75 80

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aag gtg gac gcc acg gag gag tct gac ctg gcc cag cag tac ggc gtg      288
Lys Val Asp Ala Thr Glu Glu Ser Asp Leu Ala Gln Gln Tyr Gly Val
                85                  90                  95

cgc ggc tat ccc acc atc aag ttc ttc agg aat gga gac acg gct tcc      336
Arg Gly Tyr Pro Thr Ile Lys Phe Phe Arg Asn Gly Asp Thr Ala Ser
            100                 105                 110

ccc aag gaa tat aca gct ggc aga gag gct gat gac atc gtg aac tgg      384
Pro Lys Glu Tyr Thr Ala Gly Arg Glu Ala Asp Asp Ile Val Asn Trp
        115                 120                 125

ctg aag aag cgc acg ggc ccg gct gcc acc acc ctg cct gac ggc gca      432
Leu Lys Lys Arg Thr Gly Pro Ala Ala Thr Thr Leu Pro Asp Gly Ala
    130                 135                 140

gct gca gag tcc ttg gtg gag tcc agc gag gtg gct gtc atc ggc ttc      480
Ala Ala Glu Ser Leu Val Glu Ser Ser Glu Val Ala Val Ile Gly Phe
145                 150                 155                 160

ttc aag gac gtg gag tcg gac tct gcc aag cag ttt ttg cag gca gca      528
Phe Lys Asp Val Glu Ser Asp Ser Ala Lys Gln Phe Leu Gln Ala Ala
                165                 170                 175

gag gcc atc gat gac ata cca ttt ggg atc act tcc aac agt gac gtg      576
Glu Ala Ile Asp Asp Ile Pro Phe Gly Ile Thr Ser Asn Ser Asp Val
            180                 185                 190

ttc tcc aaa tac cag ctc gac aaa gat ggg gtt gtc ctc ttt aag aag      624
Phe Ser Lys Tyr Gln Leu Asp Lys Asp Gly Val Val Leu Phe Lys Lys
        195                 200                 205
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ttt gat gaa ggc cgg aac aac ttt gaa ggg gag gtc acc aag gag aac      672
Phe Asp Glu Gly Arg Asn Asn Phe Glu Gly Glu Val Thr Lys Glu Asn
    210                 215                 220

ctg ctg gac ttt atc aaa cac aac cag ctg ccc ctt gtc atc gag ttc      720
Leu Leu Asp Phe Ile Lys His Asn Gln Leu Pro Leu Val Ile Glu Phe
225                 230                 235                 240

acc gag cag aca gcc ccg aag att ttt gga ggt gaa atc aag act cac      768
Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly Glu Ile Lys Thr His
                245                 250                 255

atc ctg ctg ttc ttg ccc aag agt gtg tct gac tat gac ggc aaa ctg      816
Ile Leu Leu Phe Leu Pro Lys Ser Val Ser Asp Tyr Asp Gly Lys Leu
            260                 265                 270

agc aac ttc aaa aca gca gcc gag agc ttc aag ggc aag atc ctg ttc      864
Ser Asn Phe Lys Thr Ala Ala Glu Ser Phe Lys Gly Lys Ile Leu Phe
        275                 280                 285

atc ttc atc gac agc gac cac acc gac aac cag cgc atc ctc gag ttc      912
Ile Phe Ile Asp Ser Asp His Thr Asp Asn Gln Arg Ile Leu Glu Phe
    290                 295                 300

ttt ggc ctg aag aag gaa gag tgc ccg gcc gtg cgc ctc atc acc ctg      960
Phe Gly Leu Lys Lys Glu Glu Cys Pro Ala Val Arg Leu Ile Thr Leu
305                 310                 315                 320

gag gag gag atg acc aag tac aag ccc gaa tcg gag gag ctg acg gca     1008
Glu Glu Glu Met Thr Lys Tyr Lys Pro Glu Ser Glu Glu Leu Thr Ala
                325                 330                 335

gag agg atc aca gag ttc tgc cac cgc ttc ctg gag ggc aaa atc aag     1056
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Glu Arg Ile Thr Glu Phe Cys His Arg Phe Leu Glu Gly Lys Ile Lys
340 345 350

ccc cac ctg atg agc cag gag ctg ccg gag gac tgg gac aag cag cct 1104
Pro His Leu Met Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys Gln Pro
355 360 365

gtc aag gtg ctt gtt ggg aag aac ttt gaa gac gtg gct ttt gat gag 1152
Val Lys Val Leu Val Gly Lys Asn Phe Glu Asp Val Ala Phe Asp Glu
370 375 380

aaa aaa aac gtc ttt gtg gag ttc tat gcc cca tgg tgt ggt cac tgc 1200
Lys Lys Asn Val Phe Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys
385 390 395 400

aaa cag ttg gct ccc att tgg gat aaa ctg gga gag acg tac aag gac 1248
Lys Gln Leu Ala Pro Ile Trp Asp Lys Leu Gly Glu Thr Tyr Lys Asp
405 410 415

cat gag aac atc gtc atc gcc aag atg gac tcg act gcc aac gag gtg 1296
His Glu Asn Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn Glu Val
420 425 430

gag gcc gtc aaa gtg cac agc ttc ccc aca ctc aag ttc ttt cct gcc 1344
Glu Ala Val Lys Val His Ser Phe Pro Thr Leu Lys Phe Phe Pro Ala
435 440 445

agt gcc gac agg acg gtc att gat tac aac ggg gaa cgc acg ctg gat 1392
Ser Ala Asp Arg Thr Val Ile Asp Tyr Asn Gly Glu Arg Thr Leu Asp
450 455 460

ggt ttt aag aaa ttc ctg gag agc ggt ggc cag gat ggg gca ggg gat 1440
Gly Phe Lys Lys Phe Leu Glu Ser Gly Gly Gln Asp Gly Ala Gly Asp

465 470 475 480

gat gac gat ctc gag gac ctg gaa gaa gca gag gag cca gac atg gag 1488
Asp Asp Asp Leu Glu Asp Leu Glu Glu Ala Glu Glu Pro Asp Met Glu
485 490 495

gaa gac gat gat cag aaa gct gtg aaa gat gaa ctg taa 1527
Glu Asp Asp Asp Gln Lys Ala Val Lys Asp Glu Leu
500 505

<210> 70
<211> 508
<212> PRT
<213> Homo sapiens

<400> 70

Met Leu Arg Arg Ala Leu Leu Cys Leu Ala Val Ala Ala Leu Val Arg
1 5 10 15

Ala Asp Ala Pro Glu Glu Glu Asp His Val Leu Val Leu Arg Lys Ser
20 25 30

Asn Phe Ala Glu Ala Leu Ala Ala His Lys Tyr Leu Leu Val Glu Phe
35 40 45

Tyr Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu Tyr Ala
50 55 60

Lys Ala Ala Gly Lys Leu Lys Ala Glu Gly Ser Glu Ile Arg Leu Ala
65 70 75 80

Lys Val Asp Ala Thr Glu Glu Ser Asp Leu Ala Gln Gln Tyr Gly Val
85 90 95

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

Pro Lys Glu Tyr Thr Ala Gly Arg Glu Ala Asp Asp Ile Val Asn Trp
115 120 125

Leu Lys Lys Arg Thr Gly Pro Ala Ala Thr Thr Leu Pro Asp Gly Ala
130 135 140

Ala Ala Glu Ser Leu Val Glu Ser Ser Glu Val Ala Val Ile Gly Phe
145 150 155 160

Phe Lys Asp Val Glu Ser Asp Ser Ala Lys Gln Phe Leu Gln Ala Ala
165 170 175

Glu Ala Ile Asp Asp Ile Pro Phe Gly Ile Thr Ser Asn Ser Asp Val
180 185 190

Phe Ser Lys Tyr Gln Leu Asp Lys Asp Gly Val Val Leu Phe Lys Lys
195 200 205

Phe Asp Glu Gly Arg Asn Asn Phe Glu Gly Glu Val Thr Lys Glu Asn
210 215 220

Leu Leu Asp Phe Ile Lys His Asn Gln Leu Pro Leu Val Ile Glu Phe
225 230 235 240

Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly Glu Ile Lys Thr His
245 250 255

Ile Leu Leu Phe Leu Pro Lys Ser Val Ser Asp Tyr Asp Gly Lys Leu
260 265 270

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

Ile Phe Ile Asp Ser Asp His Thr Asp Asn Gln Arg Ile Leu Glu Phe
290 295 300

Phe Gly Leu Lys Lys Glu Glu Cys Pro Ala Val Arg Leu Ile Thr Leu
305 310 315 320

Glu Glu Glu Met Thr Lys Tyr Lys Pro Glu Ser Glu Glu Leu Thr Ala

325 330 335

Glu Arg Ile Thr Glu Phe Cys His Arg Phe Leu Glu Gly Lys Ile Lys
340 345 350

Pro His Leu Met Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys Gln Pro
355 360 365

Val Lys Val Leu Val Gly Lys Asn Phe Glu Asp Val Ala Phe Asp Glu
370 375 380

Lys Lys Asn Val Phe Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys
385 390 395 400

Lys Gln Leu Ala Pro Ile Trp Asp Lys Leu Gly Glu Thr Tyr Lys Asp
405 410 415

His Glu Asn Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn Glu Val
420 425 430

Glu Ala Val Lys Val His Ser Phe Pro Thr Leu Lys Phe Phe Pro Ala
435 440 445

Ser Ala Asp Arg Thr Val Ile Asp Tyr Asn Gly Glu Arg Thr Leu Asp
450 455 460

Gly Phe Lys Lys Phe Leu Glu Ser Gly Gly Gln Asp Gly Ala Gly Asp
465 470 475 480

Asp Asp Asp Leu Glu Asp Leu Glu Glu Ala Glu Glu Pro Asp Met Glu
485 490 495

Glu Asp Asp Asp Gln Lys Ala Val Lys Asp Glu Leu
500 505

<210> 71
<211> 1407
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1407)

<400> 71
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Met Gly Arg Gly Trp Gly Phe Leu Phe Gly Leu Leu Gly Ala Val Trp
1 5 10 15

ctg ctc agc tcg ggc cac gga gag gag cag ccc ccg gag aca gcg gca 96
Leu Leu Ser Ser Gly His Gly Glu Glu Gln Pro Pro Glu Thr Ala Ala
20 25 30

cag agg tgc ttc tgc cag gtt agt ggt tac ttg gat gat tgt acc tgt 144

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Gln Arg Cys Phe Cys Gln Val Ser Gly Tyr Leu Asp Asp Cys Thr Cys
        35                  40                  45

gat gtt gaa acc att gat aga ttt aat aac tac agg ctt ttc cca aga      192
Asp Val Glu Thr Ile Asp Arg Phe Asn Asn Tyr Arg Leu Phe Pro Arg
    50                  55                  60

cta caa aaa ctt ctt gaa agt gac tac ttt agg tat tac aag gta aac      240
Leu Gln Lys Leu Leu Glu Ser Asp Tyr Phe Arg Tyr Tyr Lys Val Asn
65                  70                  75                  80

ctg aag agg ccg tgt cct ttc tgg aat gac atc agc cag tgt gga aga      288
Leu Lys Arg Pro Cys Pro Phe Trp Asn Asp Ile Ser Gln Cys Gly Arg
                85                  90                  95

agg gac tgt gct gtc aaa cca tgt caa tct gat gaa gtt cct gat gga      336
Arg Asp Cys Ala Val Lys Pro Cys Gln Ser Asp Glu Val Pro Asp Gly
            100                 105                 110

att aaa tct gcg agc tac aag tat tct gaa gaa gcc aat aat ctc att      384
Ile Lys Ser Ala Ser Tyr Lys Tyr Ser Glu Glu Ala Asn Asn Leu Ile
        115                 120                 125

gaa gaa tgt gaa caa gct gaa cga ctt gga gca gtg gat gaa tct ctg      432
Glu Glu Cys Glu Gln Ala Glu Arg Leu Gly Ala Val Asp Glu Ser Leu
    130                 135                 140

agt gag gaa aca cag aag gct gtt ctt cag tgg acc aag cat gat gat      480
Ser Glu Glu Thr Gln Lys Ala Val Leu Gln Trp Thr Lys His Asp Asp
145                 150                 155                 160

tct tca gat aac ttc tgt gaa gct gat gac att cag tcc cct gaa gct      528
Ser Ser Asp Asn Phe Cys Glu Ala Asp Asp Ile Gln Ser Pro Glu Ala
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165 170 175

gaa tat gta gat ttg ctt ctt aat cct gag cgc tac act ggt tac aag 576
Glu Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys
180 185 190

gga cca gat gct tgg aaa ata tgg aat gtc atc tac gaa gaa aac tgt 624
Gly Pro Asp Ala Trp Lys Ile Trp Asn Val Ile Tyr Glu Glu Asn Cys
195 200 205

ttt aag cca cag aca att aaa aga cct tta aat cct ttg gct tct ggt 672
Phe Lys Pro Gln Thr Ile Lys Arg Pro Leu Asn Pro Leu Ala Ser Gly
210 215 220

caa ggg aca agt gaa gag aac act ttt tac agt tgg cta gaa ggt ctc 720
Gln Gly Thr Ser Glu Glu Asn Thr Phe Tyr Ser Trp Leu Glu Gly Leu
225 230 235 240

tgt gta gaa aaa aga gca ttc tac aga ctt ata tct ggc cta cat gca 768
Cys Val Glu Lys Arg Ala Phe Tyr Arg Leu Ile Ser Gly Leu His Ala
245 250 255

agc att aat gtg cat ttg agt gca aga tat ctt tta caa gag acc tgg 816
Ser Ile Asn Val His Leu Ser Ala Arg Tyr Leu Leu Gln Glu Thr Trp
260 265 270

tta gaa aag aaa tgg gga cac aac att aca gaa ttt caa cag cga ttt 864
Leu Glu Lys Lys Trp Gly His Asn Ile Thr Glu Phe Gln Gln Arg Phe
275 280 285

gat gga att ttg act gaa gga gaa ggt cca aga agg ctt aag aac ttg 912
Asp Gly Ile Leu Thr Glu Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu
290 295 300

tat ttt ctc tac tta ata gaa cta agg gct tta tcc aaa gtg tta cca 960
Tyr Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Leu Pro
305 310 315 320

ttc ttc gag cgc cca gat ttt caa ctc ttt act gga aat aaa att cag 1008
Phe Phe Glu Arg Pro Asp Phe Gln Leu Phe Thr Gly Asn Lys Ile Gln
325 330 335

gat gag gaa aac aaa atg tta ctt ctg gaa ata ctt cat gaa atc aag 1056
Asp Glu Glu Asn Lys Met Leu Leu Leu Glu Ile Leu His Glu Ile Lys
340 345 350

tca ttt cct ttg cat ttt gat gag aat tca ttt ttt gct ggg gat aaa 1104
Ser Phe Pro Leu His Phe Asp Glu Asn Ser Phe Phe Ala Gly Asp Lys
355 360 365

aaa gaa gca cac aaa cta aag gag gac ttt cga ctg cat ttt aga aat 1152
Lys Glu Ala His Lys Leu Lys Glu Asp Phe Arg Leu His Phe Arg Asn
370 375 380

att tca aga att atg gat tgt gtt ggt tgt ttt aaa tgt cgt ctg tgg 1200
Ile Ser Arg Ile Met Asp Cys Val Gly Cys Phe Lys Cys Arg Leu Trp
385 390 395 400

gga aag ctt cag act cag ggt ttg ggc act gct ctg aag atc tta ttt 1248
Gly Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe
405 410 415

tct gag aaa ttg ata gca aat atg cca gaa agt gga cct agt tat gaa 1296
Ser Glu Lys Leu Ile Ala Asn Met Pro Glu Ser Gly Pro Ser Tyr Glu
420 425 430

ttc cat cta acc aga caa gaa ata gta tca tta ttc aac gca ttt gga 1344
Phe His Leu Thr Arg Gln Glu Ile Val Ser Leu Phe Asn Ala Phe Gly
435 440 445

aga att tct aca agt gtg aaa gaa tta gaa aac ttc agg aac ttg tta 1392
Arg Ile Ser Thr Ser Val Lys Glu Leu Glu Asn Phe Arg Asn Leu Leu
450 455 460

cag aat att cat taa 1407
Gln Asn Ile His
465

<210> 72
<211> 468
<212> PRT
<213> Homo sapiens

<400> 72

Met Gly Arg Gly Trp Gly Phe Leu Phe Gly Leu Leu Gly Ala Val Trp
1 5 10 15

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

Gln Arg Cys Phe Cys Gln Val Ser Gly Tyr Leu Asp Asp Cys Thr Cys
35 40 45

Asp Val Glu Thr Ile Asp Arg Phe Asn Asn Tyr Arg Leu Phe Pro Arg

50 55 60

Leu Gln Lys Leu Leu Glu Ser Asp Tyr Phe Arg Tyr Tyr Lys Val Asn
65 70 75 80

Leu Lys Arg Pro Cys Pro Phe Trp Asn Asp Ile Ser Gln Cys Gly Arg
85 90 95

Arg Asp Cys Ala Val Lys Pro Cys Gln Ser Asp Glu Val Pro Asp Gly
100 105 110

Ile Lys Ser Ala Ser Tyr Lys Tyr Ser Glu Glu Ala Asn Asn Leu Ile
115 120 125

Glu Glu Cys Glu Gln Ala Glu Arg Leu Gly Ala Val Asp Glu Ser Leu
130 135 140

Ser Glu Glu Thr Gln Lys Ala Val Leu Gln Trp Thr Lys His Asp Asp
145 150 155 160

Ser Ser Asp Asn Phe Cys Glu Ala Asp Asp Ile Gln Ser Pro Glu Ala
165 170 175

Glu Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys
180 185 190

Gly Pro Asp Ala Trp Lys Ile Trp Asn Val Ile Tyr Glu Glu Asn Cys
195 200 205

Phe Lys Pro Gln Thr Ile Lys Arg Pro Leu Asn Pro Leu Ala Ser Gly
210 215 220

Gln Gly Thr Ser Glu Glu Asn Thr Phe Tyr Ser Trp Leu Glu Gly Leu
225 230 235 240

Cys Val Glu Lys Arg Ala Phe Tyr Arg Leu Ile Ser Gly Leu His Ala
245 250 255

Ser Ile Asn Val His Leu Ser Ala Arg Tyr Leu Leu Gln Glu Thr Trp
260 265 270

Leu Glu Lys Lys Trp Gly His Asn Ile Thr Glu Phe Gln Gln Arg Phe
275 280 285

Asp Gly Ile Leu Thr Glu Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu
290 295 300

Tyr Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Leu Pro
305 310 315 320

Phe Phe Glu Arg Pro Asp Phe Gln Leu Phe Thr Gly Asn Lys Ile Gln
325 330 335

Asp Glu Glu Asn Lys Met Leu Leu Leu Glu Ile Leu His Glu Ile Lys
340 345 350

Ser Phe Pro Leu His Phe Asp Glu Asn Ser Phe Phe Ala Gly Asp Lys
355 360 365

Lys Glu Ala His Lys Leu Lys Glu Asp Phe Arg Leu His Phe Arg Asn
370 375 380

Ile Ser Arg Ile Met Asp Cys Val Gly Cys Phe Lys Cys Arg Leu Trp
385 390 395 400

Gly Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe
405 410 415

Ser Glu Lys Leu Ile Ala Asn Met Pro Glu Ser Gly Pro Ser Tyr Glu
420 425 430

Phe His Leu Thr Arg Gln Glu Ile Val Ser Leu Phe Asn Ala Phe Gly
435 440 445

Arg Ile Ser Thr Ser Val Lys Glu Leu Glu Asn Phe Arg Asn Leu Leu
450 455 460

Gln Asn Ile His
465

<210> 73
<211> 1404
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1404)

<400> 73
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Met Ser Gln Gly Val Arg Arg Ala Gly Ala Gly Gln Gly Val Ala Ala
1 5 10 15

gcg gtg cag ctg ctg gtc acc ctg agc ttc ctg cgg agc gtc gtc gag 96
Ala Val Gln Leu Leu Val Thr Leu Ser Phe Leu Arg Ser Val Val Glu
20 25 30

gcg cag gtc act gga gtt ctg gat gat tgc ttg tgt gat att gac agc 144
Ala Gln Val Thr Gly Val Leu Asp Asp Cys Leu Cys Asp Ile Asp Ser
35 40 45

atc gat aac ttc aat acc tac aaa atc ttc ccc aaa ata aaa aaa ttg 192
Ile Asp Asn Phe Asn Thr Tyr Lys Ile Phe Pro Lys Ile Lys Lys Leu

50 55 60

caa gag aga gac tat ttt cgt tat tac aag gtt aat ctg aag cga cct 240
Gln Glu Arg Asp Tyr Phe Arg Tyr Tyr Lys Val Asn Leu Lys Arg Pro
65 70 75 80

tgt cct ttc tgg gca gaa gat ggc cac tgt tca ata aaa gac tgt cat 288
Cys Pro Phe Trp Ala Glu Asp Gly His Cys Ser Ile Lys Asp Cys His
85 90 95

gtg gag ccc tgt cca gag agt aaa att ccg gtt gga ata aaa gct ggg 336
Val Glu Pro Cys Pro Glu Ser Lys Ile Pro Val Gly Ile Lys Ala Gly
100 105 110

cat tct aat aag tac ttg aaa atg gca aac aat acc aaa gaa tta gaa 384
His Ser Asn Lys Tyr Leu Lys Met Ala Asn Asn Thr Lys Glu Leu Glu
115 120 125

gtt tgt gag caa gct aat aaa ctg gga gca att aac agc aca tta agt 432
Val Cys Glu Gln Ala Asn Lys Leu Gly Ala Ile Asn Ser Thr Leu Ser
130 135 140

aat caa agc aaa gaa gct ttc att gac tgg gca aga tat gat gat tca 480
Asn Gln Ser Lys Glu Ala Phe Ile Asp Trp Ala Arg Tyr Asp Asp Ser
145 150 155 160

cgg gat cac ttt tgt gaa ctt gat gat gag aga tct cca gct gct cag 528
Arg Asp His Phe Cys Glu Leu Asp Asp Glu Arg Ser Pro Ala Ala Gln
165 170 175

tat gta gac cta ttg ctg aac cca gag cgt tac act ggc tat aaa ggg 576
Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys Gly
180 185 190

acc tct gca tgg aga gtg tgg aac agc atc tat gaa gag aac tgt ttc 624
Thr Ser Ala Trp Arg Val Trp Asn Ser Ile Tyr Glu Glu Asn Cys Phe
195 200 205

aag cct cga tct gtt tat cgt cct tta aat cct ctg gcg cct agc cga 672
Lys Pro Arg Ser Val Tyr Arg Pro Leu Asn Pro Leu Ala Pro Ser Arg
210 215 220

ggc gaa gat gat gga gaa tca ttc tac aca tgg cta gaa ggt ttg tgt 720
Gly Glu Asp Asp Gly Glu Ser Phe Tyr Thr Trp Leu Glu Gly Leu Cys
225 230 235 240

ctg gag aaa aga gtc ttc tat aag ctt ata tcg gga ctt cat gct agc 768
Leu Glu Lys Arg Val Phe Tyr Lys Leu Ile Ser Gly Leu His Ala Ser
245 250 255

atc aat tta cat cta tgc gca aat tat ctt ttg gaa gaa acc tgg ggt 816
Ile Asn Leu His Leu Cys Ala Asn Tyr Leu Leu Glu Glu Thr Trp Gly
260 265 270

aag ccc agt tgg gga cct aat att aaa gaa ttc aaa cac cgc ttt gac 864
Lys Pro Ser Trp Gly Pro Asn Ile Lys Glu Phe Lys His Arg Phe Asp
275 280 285

cct gtg gaa acc aag gga gaa ggt cca aga agg ctc aag aat ctt tac 912
Pro Val Glu Thr Lys Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu Tyr
290 295 300

ttt tta tac ttg att gag ctt cga gct ttg tca aag gtg gct cca tat 960
Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Ala Pro Tyr
305 310 315 320

ttt gag cgc tca att gtc gat ctt tac act gga aat gca gaa gaa gat 1008
Phe Glu Arg Ser Ile Val Asp Leu Tyr Thr Gly Asn Ala Glu Glu Asp
325 330 335

gct gac aca aaa act ctt cta ctg aat atc ttt caa gat aca aag tcc 1056
Ala Asp Thr Lys Thr Leu Leu Leu Asn Ile Phe Gln Asp Thr Lys Ser
340 345 350

ttt ccc atg cac ttt gat gag aaa tcc atg ttt gca ggt gac aaa aaa 1104
Phe Pro Met His Phe Asp Glu Lys Ser Met Phe Ala Gly Asp Lys Lys
355 360 365

ggg gcc aag tca cta aag gag gaa ttc cga tta cat ttc aag aat atc 1152
Gly Ala Lys Ser Leu Lys Glu Glu Phe Arg Leu His Phe Lys Asn Ile
370 375 380

tcc cgt ata atg gac tgt gtt gga tgt gac aaa tgc aga tta tgg gga 1200
Ser Arg Ile Met Asp Cys Val Gly Cys Asp Lys Cys Arg Leu Trp Gly
385 390 395 400

aaa tta cag act cag ggt tta gga act gcc ctg aag ata tta ttc tct 1248
Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe Ser
405 410 415

gaa aaa gaa atc caa aag ctt cca gag aat agt cca tct aaa ggc ttc 1296
Glu Lys Glu Ile Gln Lys Leu Pro Glu Asn Ser Pro Ser Lys Gly Phe
420 425 430

caa ctc acc cga cag gaa ata gtt gct ctt tta aat gct ttt gga agg 1344
Gln Leu Thr Arg Gln Glu Ile Val Ala Leu Leu Asn Ala Phe Gly Arg
435 440 445

ctt tct aca agt ata aga gac tta cag aat ttt aaa gtc tta tta caa 1392

Leu Ser Thr Ser Ile Arg Asp Leu Gln Asn Phe Lys Val Leu Leu Gln
450 455 460

cac agt agg taa 1404
His Ser Arg
465

<210> 74
<211> 467
<212> PRT
<213> Homo sapiens

<400> 74

Met Ser Gln Gly Val Arg Arg Ala Gly Ala Gly Gln Gly Val Ala Ala
1 5 10 15

Ala Val Gln Leu Leu Val Thr Leu Ser Phe Leu Arg Ser Val Val Glu
20 25 30

Ala Gln Val Thr Gly Val Leu Asp Asp Cys Leu Cys Asp Ile Asp Ser
35 40 45

Ile Asp Asn Phe Asn Thr Tyr Lys Ile Phe Pro Lys Ile Lys Lys Leu
50 55 60

Gln Glu Arg Asp Tyr Phe Arg Tyr Tyr Lys Val Asn Leu Lys Arg Pro
65 70 75 80

Cys Pro Phe Trp Ala Glu Asp Gly His Cys Ser Ile Lys Asp Cys His
85 90 95

Val Glu Pro Cys Pro Glu Ser Lys Ile Pro Val Gly Ile Lys Ala Gly
100 105 110

His Ser Asn Lys Tyr Leu Lys Met Ala Asn Asn Thr Lys Glu Leu Glu
115 120 125

Val Cys Glu Gln Ala Asn Lys Leu Gly Ala Ile Asn Ser Thr Leu Ser
130 135 140

Asn Gln Ser Lys Glu Ala Phe Ile Asp Trp Ala Arg Tyr Asp Asp Ser
145 150 155 160

Arg Asp His Phe Cys Glu Leu Asp Asp Glu Arg Ser Pro Ala Ala Gln
165 170 175

Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys Gly
180 185 190

Thr Ser Ala Trp Arg Val Trp Asn Ser Ile Tyr Glu Glu Asn Cys Phe
195 200 205

Lys Pro Arg Ser Val Tyr Arg Pro Leu Asn Pro Leu Ala Pro Ser Arg
210 215 220

Gly Glu Asp Asp Gly Glu Ser Phe Tyr Thr Trp Leu Glu Gly Leu Cys
225 230 235 240

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

Ile Asn Leu His Leu Cys Ala Asn Tyr Leu Leu Glu Glu Thr Trp Gly
260 265 270

Lys Pro Ser Trp Gly Pro Asn Ile Lys Glu Phe Lys His Arg Phe Asp
275 280 285

Pro Val Glu Thr Lys Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu Tyr
290 295 300

Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Ala Pro Tyr
305 310 315 320

Phe Glu Arg Ser Ile Val Asp Leu Tyr Thr Gly Asn Ala Glu Glu Asp
325 330 335

Ala Asp Thr Lys Thr Leu Leu Leu Asn Ile Phe Gln Asp Thr Lys Ser
340 345 350

Phe Pro Met His Phe Asp Glu Lys Ser Met Phe Ala Gly Asp Lys Lys
355 360 365

Gly Ala Lys Ser Leu Lys Glu Glu Phe Arg Leu His Phe Lys Asn Ile
370 375 380

Ser Arg Ile Met Asp Cys Val Gly Cys Asp Lys Cys Arg Leu Trp Gly
385 390 395 400

Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe Ser
405 410 415

Glu Lys Glu Ile Gln Lys Leu Pro Glu Asn Ser Pro Ser Lys Gly Phe
420 425 430

Gln Leu Thr Arg Gln Glu Ile Val Ala Leu Leu Asn Ala Phe Gly Arg
435 440 445

Leu Ser Thr Ser Ile Arg Asp Leu Gln Asn Phe Lys Val Leu Leu Gln
450 455 460

His Ser Arg

465

<210> 75
<211> 1965
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1965)

<400> 75
atg aag ctc tcc ctg gtg gcc gcg atg ctg ctg ctg ctc agc gcg gcg 48
Met Lys Leu Ser Leu Val Ala Ala Met Leu Leu Leu Leu Ser Ala Ala
1 5 10 15

cgg gcc gag gag gag gac aag aag gag gac gtg ggc acg gtg gtc ggc 96
Arg Ala Glu Glu Glu Asp Lys Lys Glu Asp Val Gly Thr Val Val Gly
20 25 30

atc gac ctg ggg acc acc tac tcc tgc gtc ggc gtg ttc aag aac ggc 144
Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Gly Val Phe Lys Asn Gly
35 40 45

cgc gtg gag atc atc gcc aac gat cag ggc aac cgc atc acg ccg tcc 192
Arg Val Glu Ile Ile Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser
50 55 60

tat gtc gcc ttc act cct gaa ggg gaa cgt ctg att ggc gat gcc gcc 240
Tyr Val Ala Phe Thr Pro Glu Gly Glu Arg Leu Ile Gly Asp Ala Ala
65 70 75 80

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aag aac cag ctc acc tcc aac ccc gag aac acg gtc ttt gac gcc aag      288
Lys Asn Gln Leu Thr Ser Asn Pro Glu Asn Thr Val Phe Asp Ala Lys
                85                  90                  95

cgg ctc atc ggc cgc acg tgg aat gac ccg tct gtg cag cag gac atc      336
Arg Leu Ile Gly Arg Thr Trp Asn Asp Pro Ser Val Gln Gln Asp Ile
            100                 105                 110

aag ttc ttg ccg ttc aag gtg gtt gaa aag aaa act aaa cca tac att      384
Lys Phe Leu Pro Phe Lys Val Val Glu Lys Lys Thr Lys Pro Tyr Ile
        115                 120                 125

caa gtt gat att gga ggt ggg caa aca aag aca ttt gct cct gaa gaa      432
Gln Val Asp Ile Gly Gly Gly Gln Thr Lys Thr Phe Ala Pro Glu Glu
    130                 135                 140

att tct gcc atg gtt ctc act aaa atg aaa gaa acc gct gag gct tat      480
Ile Ser Ala Met Val Leu Thr Lys Met Lys Glu Thr Ala Glu Ala Tyr
145                 150                 155                 160

ttg gga aag aag gtt acc cat gca gtt gtt act gta cca gcc tat ttt      528
Leu Gly Lys Lys Val Thr His Ala Val Val Thr Val Pro Ala Tyr Phe
                165                 170                 175

aat gat gcc caa cgc caa gca acc aaa gac gct gga act att gct ggc      576
Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly
            180                 185                 190

cta aat gtt atg agg atc atc aac gag cct acg gca gct gct att gct      624
Leu Asn Val Met Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala
        195                 200                 205
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tat ggc ctg gat aag agg gag ggg gag aag aac atc ctg gtg ttt gac      672
Tyr Gly Leu Asp Lys Arg Glu Gly Glu Lys Asn Ile Leu Val Phe Asp
    210                 215                 220

ctg ggt ggc gga acc ttc gat gtg tct ctt ctc acc att gac aat ggt      720
Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu Thr Ile Asp Asn Gly
225                 230                 235                 240

gtc ttc gaa gtt gtg gcc act aat gga gat act cat ctg ggt gga gaa      768
Val Phe Glu Val Val Ala Thr Asn Gly Asp Thr His Leu Gly Gly Glu
                245                 250                 255

gac ttt gac cag cgt gtc atg gaa cac ttc atc aaa ctg tac aaa aag      816
Asp Phe Asp Gln Arg Val Met Glu His Phe Ile Lys Leu Tyr Lys Lys
            260                 265                 270

aag acg ggc aaa gat gtc agg aaa gac aat aga gct gtg cag aaa ctc      864
Lys Thr Gly Lys Asp Val Arg Lys Asp Asn Arg Ala Val Gln Lys Leu
        275                 280                 285

cgg cgc gag gta gaa aag gcc aaa cgg gcc ctg tct tct cag cat caa      912
Arg Arg Glu Val Glu Lys Ala Lys Arg Ala Leu Ser Ser Gln His Gln
    290                 295                 300

gca aga att gaa att gag tcc ttc tat gaa gga gaa gac ttt tct gag      960
Ala Arg Ile Glu Ile Glu Ser Phe Tyr Glu Gly Glu Asp Phe Ser Glu
305                 310                 315                 320

acc ctg act cgg gcc aaa ttt gaa gag ctc aac atg gat ctg ttc cgg     1008
Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn Met Asp Leu Phe Arg
                325                 330                 335

tct act atg aag ccc gtc cag aaa gtg ttg gaa gat tct gat ttg aag     1056
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Ser Thr Met Lys Pro Val Gln Lys Val Leu Glu Asp Ser Asp Leu Lys
340 345 350

aag tct gat att gat gaa att gtt ctt gtt ggt ggc tcg act cga att 1104
Lys Ser Asp Ile Asp Glu Ile Val Leu Val Gly Gly Ser Thr Arg Ile
355 360 365

cca aag att cag caa ctg gtt aaa gag ttc ttc aat ggc aag gaa cca 1152
Pro Lys Ile Gln Gln Leu Val Lys Glu Phe Phe Asn Gly Lys Glu Pro
370 375 380

tcc cgt ggc ata aac cca gat gaa gct gta gcg tat ggt gct gct gtc 1200
Ser Arg Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val
385 390 395 400

cag gct ggt gtg ctc tct ggt gat caa gat aca ggt gac ctg gta ctg 1248
Gln Ala Gly Val Leu Ser Gly Asp Gln Asp Thr Gly Asp Leu Val Leu
405 410 415

ctt gat gta tgt ccc ctt aca ctt ggt att gaa act gtg gga ggt gtc 1296
Leu Asp Val Cys Pro Leu Thr Leu Gly Ile Glu Thr Val Gly Gly Val
420 425 430

atg acc aaa ctg att cca agg aac aca gtg gtg cct acc aag aag tct 1344
Met Thr Lys Leu Ile Pro Arg Asn Thr Val Val Pro Thr Lys Lys Ser
435 440 445

cag atc ttt tct aca gct tct gat aat caa cca act gtt aca atc aag 1392
Gln Ile Phe Ser Thr Ala Ser Asp Asn Gln Pro Thr Val Thr Ile Lys
450 455 460

gtc tat gaa ggt gaa aga ccc ctg aca aaa gac aat cat ctt ctg ggt 1440
Val Tyr Glu Gly Glu Arg Pro Leu Thr Lys Asp Asn His Leu Leu Gly

465 470 475 480

aca ttt gat ctg act gga att cct cct gct cct cgt ggg gtc cca cag 1488
Thr Phe Asp Leu Thr Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln
485 490 495

att gaa gtc acc ttt gag ata gat gtg aat ggt att ctt cga gtg aca 1536
Ile Glu Val Thr Phe Glu Ile Asp Val Asn Gly Ile Leu Arg Val Thr
500 505 510

gct gaa gac aag ggt aca ggg aac aaa aat aag atc aca atc acc aat 1584
Ala Glu Asp Lys Gly Thr Gly Asn Lys Asn Lys Ile Thr Ile Thr Asn
515 520 525

gac cag aat cgc ctg aca cct gaa gaa atc gaa agg atg gtt aat gat 1632
Asp Gln Asn Arg Leu Thr Pro Glu Glu Ile Glu Arg Met Val Asn Asp
530 535 540

gct gag aag ttt gct gag gaa gac aaa aag ctc aag gag cgc att gat 1680
Ala Glu Lys Phe Ala Glu Glu Asp Lys Lys Leu Lys Glu Arg Ile Asp
545 550 555 560

act aga aat gag ttg gaa agc tat gcc tat tct cta aag aat cag att 1728
Thr Arg Asn Glu Leu Glu Ser Tyr Ala Tyr Ser Leu Lys Asn Gln Ile
565 570 575

gga gat aaa gaa aag ctg gga ggt aaa ctt tcc tct gaa gat aag gag 1776
Gly Asp Lys Glu Lys Leu Gly Gly Lys Leu Ser Ser Glu Asp Lys Glu
580 585 590

acc atg gaa aaa gct gta gaa gaa aag att gaa tgg ctg gaa agc cac 1824
Thr Met Glu Lys Ala Val Glu Glu Lys Ile Glu Trp Leu Glu Ser His
595 600 605

caa gat gct gac att gaa gac ttc aaa gct aag aag aag gaa ctg gaa 1872
Gln Asp Ala Asp Ile Glu Asp Phe Lys Ala Lys Lys Lys Glu Leu Glu
610 615 620

gaa att gtt caa cca att atc agc aaa ctc tat gga agt gca ggc cct 1920
Glu Ile Val Gln Pro Ile Ile Ser Lys Leu Tyr Gly Ser Ala Gly Pro
625 630 635 640

ccc cca act ggt gaa gag gat aca gca gaa aaa gat gag ttg tag 1965
Pro Pro Thr Gly Glu Glu Asp Thr Ala Glu Lys Asp Glu Leu
645 650

<210> 76
<211> 654
<212> PRT
<213> Homo sapiens

<400> 76

Met Lys Leu Ser Leu Val Ala Ala Met Leu Leu Leu Leu Ser Ala Ala
1 5 10 15

Arg Ala Glu Glu Glu Asp Lys Lys Glu Asp Val Gly Thr Val Val Gly
20 25 30

Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Gly Val Phe Lys Asn Gly
35 40 45

Arg Val Glu Ile Ile Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser
50 55 60

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

Lys Asn Gln Leu Thr Ser Asn Pro Glu Asn Thr Val Phe Asp Ala Lys
85 90 95

Arg Leu Ile Gly Arg Thr Trp Asn Asp Pro Ser Val Gln Gln Asp Ile
100 105 110

Lys Phe Leu Pro Phe Lys Val Val Glu Lys Lys Thr Lys Pro Tyr Ile
115 120 125

Gln Val Asp Ile Gly Gly Gly Gln Thr Lys Thr Phe Ala Pro Glu Glu
130 135 140

Ile Ser Ala Met Val Leu Thr Lys Met Lys Glu Thr Ala Glu Ala Tyr
145 150 155 160

Leu Gly Lys Lys Val Thr His Ala Val Val Thr Val Pro Ala Tyr Phe
165 170 175

Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly

180 185 190

Leu Asn Val Met Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala
195 200 205

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

Leu Gly Gly Gly Thr Phe Asp Val Ser Leu Leu Thr Ile Asp Asn Gly
225 230 235 240

Val Phe Glu Val Val Ala Thr Asn Gly Asp Thr His Leu Gly Gly Glu
245 250 255

Asp Phe Asp Gln Arg Val Met Glu His Phe Ile Lys Leu Tyr Lys Lys
260 265 270

Lys Thr Gly Lys Asp Val Arg Lys Asp Asn Arg Ala Val Gln Lys Leu
275 280 285

Arg Arg Glu Val Glu Lys Ala Lys Arg Ala Leu Ser Ser Gln His Gln
290 295 300

Ala Arg Ile Glu Ile Glu Ser Phe Tyr Glu Gly Glu Asp Phe Ser Glu
305 310 315 320

Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn Met Asp Leu Phe Arg
325 330 335

Ser Thr Met Lys Pro Val Gln Lys Val Leu Glu Asp Ser Asp Leu Lys
340 345 350

Lys Ser Asp Ile Asp Glu Ile Val Leu Val Gly Gly Ser Thr Arg Ile
355 360 365

Pro Lys Ile Gln Gln Leu Val Lys Glu Phe Phe Asn Gly Lys Glu Pro
370 375 380

Ser Arg Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val
385 390 395 400

Gln Ala Gly Val Leu Ser Gly Asp Gln Asp Thr Gly Asp Leu Val Leu
405 410 415

Leu Asp Val Cys Pro Leu Thr Leu Gly Ile Glu Thr Val Gly Gly Val
420 425 430

Met Thr Lys Leu Ile Pro Arg Asn Thr Val Val Pro Thr Lys Lys Ser
435 440 445

Gln Ile Phe Ser Thr Ala Ser Asp Asn Gln Pro Thr Val Thr Ile Lys
450 455 460

Val Tyr Glu Gly Glu Arg Pro Leu Thr Lys Asp Asn His Leu Leu Gly
465 470 475 480

Thr Phe Asp Leu Thr Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln
485 490 495

Ile Glu Val Thr Phe Glu Ile Asp Val Asn Gly Ile Leu Arg Val Thr
500 505 510

Ala Glu Asp Lys Gly Thr Gly Asn Lys Asn Lys Ile Thr Ile Thr Asn
515 520 525

Asp Gln Asn Arg Leu Thr Pro Glu Glu Ile Glu Arg Met Val Asn Asp
530 535 540

Ala Glu Lys Phe Ala Glu Glu Asp Lys Lys Leu Lys Glu Arg Ile Asp
545 550 555 560

Thr Arg Asn Glu Leu Glu Ser Tyr Ala Tyr Ser Leu Lys Asn Gln Ile
565 570 575

Gly Asp Lys Glu Lys Leu Gly Gly Lys Leu Ser Ser Glu Asp Lys Glu
580 585 590

Thr Met Glu Lys Ala Val Glu Glu Lys Ile Glu Trp Leu Glu Ser His
595 600 605

Gln Asp Ala Asp Ile Glu Asp Phe Lys Ala Lys Lys Lys Glu Leu Glu
610 615 620

Glu Ile Val Gln Pro Ile Ile Ser Lys Leu Tyr Gly Ser Ala Gly Pro
625 630 635 640

Pro Pro Thr Gly Glu Glu Asp Thr Ala Glu Lys Asp Glu Leu
645 650