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WO 2016/017693 (04.02.2016 Gazette 2016/05)(54) **METHOD FOR IMPROVED HIGH-LEVEL SECRETORY PRODUCTION OF PROTEINS**

VERFAHREN ZUR VERBESSERTEN HOCHGRADIGEN SEKRETORISCHEN PRODUKTION VON PROTEINEN

PROCÉDÉ DESTINÉ À LA PRODUCTION SÉCRÉTOIRE AMÉLIORÉE À HAUT NIVEAU DE PROTÉINES

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(56) References cited:
WO-A1-03/091431 **WO-A1-2009/057813**
JP-A- S62 104 585 **JP-A- 2000 078 978**
US-A- 5 612 198

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- **AHMAD M ET AL: "Protein expression in Pichia pastoris: recent achievements and perspectives for heterologous protein production", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 98, no. 12, 1 June 2014 (2014-06-01), pages 5301-5317, XP002745344, ISSN: 1432-0614, DOI: 10.1007/S00253-014-5732-5 [retrieved on 2014-04-18]**

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- CREGG,J.: 'The Pichia System' ARCHIVE, WAYBACK MACHINE, [Online] XP055394752 Retrieved from the Internet:
<URL:HTTP://WWW.PICHIA.COM/SCIENCE-CENTER/JAMES-M-CREGG-PH-D/URL:HTTPS://WEB.ARCHIVE.ORG/WEB/20140408110740/HTTP://WWW.PICHIA.COM/SCIENCE-CENTER/JAMES-M-CREGG-PH-D/> [retrieved on 2015-09-29]
- CHIRUVOLU,V. ET AL.: 'Recombinant protein production in an alcohol oxidase-defective strain of *Pichia pastoris* in fedbatch fermentations' ENZYME AND MICROBIAL TECHNOLOGY vol.21, no. 4, 01 September 1997, pages 277 - 283, XP055390303 DOI: 10.1016/S0141-0229(97)00042-2
- TSCHOPP,J.F. ET AL.: 'High-Level Secretion of Glycosylated Invertase in the Methylotrophic Yeast' BIOTECHNOLOGY vol. 5, no. 12, 05 December 1987, pages 1305 - 1308, XP001313021
- None

Description

Technical Field

5 [0001] The present invention relates to a method for high-level secretory production of a protein in yeast.

Background Art

10 [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.

15 [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 20 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 25 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).

30 [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 35 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 oxidation of substrate proteins is reoxidized by oxidative ERO1 localized in the vicinity of the membrane (Non- 40 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 45 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 dimer, 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).

50 [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).

55 [0006] Even if the productivity of target protein secretion into media is improved with coexpression 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 methylo trophic 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 CO₂ 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 (dasl) 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 methylo trophic 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 methylo trophic 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 methylo trophic 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]

- 45 [Patent Document 1] WO 2009/057813
- [Patent Document 2] JP S62-104585 A (1987)

[Non-Patent Document]

[0011]

- 50 [Non-Patent Document 1] Glover JR, Lindquist S, Hsp104, Hsp70, and Hsp40: A novel chaperone system that rescues previously aggregated proteins. *Cell* (1998) 94:73-82
- [Non-Patent Document 2] Benjamin P. Tu and Jonathan S. Weissman, Oxidative protein folding in eukaryotes: mechanisms and consequences. *J. Cell Biol.* (2004) 164:341-346
- [Non-Patent Document 3] Mezghrani, A., Fassio, A., Benham, A., Simmen, T., Braakman, I., and Sitia, R., Manipulation of oxidative protein folding and PD1redox state in mammalian cells. *EMBO J.* (2001) 20: 6288-6296
- [Non-Patent Document 4] Frand, A. R. and C. A. Kaiser, Eroip oxidizes protein disulfide isomerase in a pathway for disulfide bond formation in the endoplasmic reticulum. *Mol. Cell* (1999) 4:469-477

- [Non-Patent Document 5] Per Norgaard, Vibeke Westphal, Christine Tachibana, Lene Alsoe, Bjorn Holst, Jakob R. Winther, Functional Differences in Yeast Protein Disulfide Isomerases. *J. Cell Biology* (2001) 152(3): 553-562,
- [Non-Patent Document 6] Marcus Mayer, Ursula Kies, Robert Kammermeier, and Johannes Buchner, BiP and PDI Cooperate in the Oxidative Folding of Antibodies in Vitro, *J. Biol. Chem.* (2000) 275(38): 29421-29425.
- [Non-Patent Document 7] Cox JS., Shamu CE., Walter P., Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* (1993) 73:1197-1206
- [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
- [Non-Patent Document 9] Susana Silberstein, Gabriel Schlenstedt, Pam A. Silver, and Reid Gilmore, A Role for the DnaJ Homologue Scj1p in Protein Folding in the Yeast Endoplasmic Reticulum. *J. Cell Biol.* (1998) 143(4): 921-933
- [Non-Patent Document 10] Morozkina EV, Marchenko AN, Keruchenko JS, Keruchenko ID, Khotchenkov VP, Popov VO, and Benevolensky SV, Proteinase B disruption is required for high level production of human mechano-growth factor in *Saccharomyces cerevisiae*. *J. Mol. Microbiol. Biotechnol.* (2010) 18(3): 188-194
- [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
- [Non-Patent Document 13] Susanne O. SORENSEN, H. Bart VAN DEN HAZEL, Morten C. KIELLAND-BRANDT, and Jakob R. WINTHER, pH-dependent processing of yeast procarboxypeptidase Y by proteinase A in vivo and in vitro. *Eur. J. Biochem.* (1994) 220: 19-27
- [Non-Patent Document 14] Ida J. van der Kleia, Yurimoto 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
- [Non-Patent Document 17] Sakai Y, Yoshida H, Yurimoto H, Yoshida N, Fukuya H, Takabe K, Kato N, Production of fungal fructosyl amino acid oxidase useful for diabetic diagnosis in the peroxisome of *Candida boidinii*. *FEBS Lett.* (1999) 459, 233-237
- [Non-Patent Document 18] Nishikawa M, Hagishita T, Yurimoto H, Kato N, Sakai Y, Hatanaka T, Primary structure and expression of peroxisomal acetylspermidine oxidase in the methylotrophic yeast *Candida boidinii*. *FEBS Lett.* (2000) 476, 150-154

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

5 the *aox1* gene has been disrupted, and
a protease gene has been disrupted, wherein optionally the protease gene is a *prb1* gene.

[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:

- 10 (a) a gene encoding PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1, or HSP104 derived from *Ogataea minuto* (*O. minuto*);
(b) a gene encoding PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5, or EUG1 derived from *Saccharomyces cerevisiae* (*S. cerevisiae*);
15 (c) a gene encoding PDI, ERO1-L α , ERO1-L β , 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:

- 20 (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*;
25 (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:

- 30 (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*;
35 (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-L β 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*,
40 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 yeast is a methylotrophic yeast.

[6] The transformed yeast according to any of [1] to [5], which comprises a gene encoding a target protein introduced thereinto.

[7] Use of the transformed yeast according to any of [1] to [6] for the production of a target protein.

[8] A method for producing a protein comprising culturing the transformed yeast according to [6] in a medium and sampling a target protein from the culture product.

[9] The method for producing a protein according to [8], wherein culture is conducted under conditions in which protease activity is inhibited.

[10] The method for producing a protein according to [8] or [9], wherein culture is conducted in a medium with a pH of 6.0 to 7.5.

[11] The method for producing a protein according to any of [8] to [10], wherein a nitrogen source is added to the medium.

[12] The method for producing a protein according to [8] to [11], wherein (a) methanol is not added to the medium or (b) the amount of methanol added to the medium is 2% (v/v) or less.

[13] A method for producing a transformed yeast comprising step (i) in addition to both step (ii) and (iii):

- (i) a step of introducing a chaperone gene into yeast; and
 (ii) a step of disrupting the *aox1* gene in yeast; and
 (iii) a step of disrupting the *prbl* gene in yeast,
 optionally further comprising a step of introducing a gene encoding a target protein.

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[0016] Further disclosed herein are:

- (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. cerevisiae*);
 - (c) a gene encoding PDI, ERO1-L α , ERO1-L β , 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-L β 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 *prbl* 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 *prbl* gene.
- (9) The transformed yeast according to any of (1) to (8), wherein the yeast is a methylo trophic 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

[0017] 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 as claimed resulting from the introduction of a chaperone gene, the disruption of the aox1 gene, and the disruption of a 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).

[0018] 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

[0019]

[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 (onaPI 1007: 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 Δ aox1+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 Δ aox1+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 Δ aox1+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 Δ aox1+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 Δ aox1+PEK dprb1 strain) (carbon source starvation-induced culture)].

Embodiments for Carrying out the Invention

1. Transformed yeast

[0020] The transformed yeast of the present invention is obtained by introduction of a chaperone gene, disruption of the aox1 gene, and 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 disclosure.

(Host cells)

[0021] Host cells to be transformed are preferably yeast strains. Examples of yeast strains include methylo-trophic yeast strains such as *Ogataea minuta*, *Pichia lindneri*, *Pichia pastoris*, *Hansenula polymorpha* (*Pichia angusta*), and *Candida boidinii* and yeast strains such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, and *Shizosaccharomyces pombe*, with methylo-trophic yeast strains being preferable. A specific example of the *Ogataea minuta* strain is the *Ogataea minuta* YK3 strain (Δ och1 Δ pep4 Δ prb1 Δ yps1 Δ ura3 Δ ade1), and a specific example of the *Saccharomyces cerevisiae* strain is the *Saccharomyces cerevisiae* BY4741 strain (MATa Δ his3 Δ leu2 Δ met15 Δ ura3), although yeast strains are not limited thereto.

(Introduction of chaperone gene)

[0022] 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)), EROI (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*).

[0023] 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.

[0024] 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/>).

[0025] 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-L α (GenBank Accession No. AF081886; SEQ ID NO: 71 (the base sequence); SEQ ID NO: 72 (the amino acid sequence)), ERO1-L β (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)).

[0026] 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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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 pdl1 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.

[0034] 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 pdl1 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-L β 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.

[0035] 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."

[0036] 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 (Cregg 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)

[0037] The transformed yeast of the present disclosure 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.

[0038] 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.

[0039] 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 prbl gene and the pep4 gene derived from *Pichia pastoris*, the prbl 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.

[0040] Accordingly, the transformed yeast of the present disclosure 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 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.

[0041] 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.

[0042] 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.

[0043] 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. 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.

[0044] 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)

[0045] 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.

[0046] 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.

[0047] 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.

[0048] 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 methylotrophic 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.

[0049] 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 (α MF), a secretory signal sequence of *S. cerevisiae*-derived invertase (SUC2), and a secretory signal sequence of human-derived α -galactosidase.

[0050] 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, adel, ade2, ura3, and ura5 genes.

[0051] 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.

35 2. Method for producing protein

[0052] In the present invention, proteins can be produced by culturing the transformed yeast as claimed 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.

[0053] 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(II) 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.

[0054] 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.

[0055] 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).

[0056] 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.

[0057] 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.

[0058] 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 aox1 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] 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

[0064] 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.

[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

[0065] 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'-GCAAGCTTCTTCGCAAACAGCTTTG-3': SEQ ID NO: 1), the AOXp rv primer (5'-GAACCCGGAACAGAAATCTAGATTTCGTAAGTCGTAAG-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-containing 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'-CTGTTCCCAGGGTTCTGGATCCGAGACGGTGCCGACTC-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-containing 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 *Hind*III and *Kpn*I to obtain a DNA fragment comprising the *aox1* gene promoter and the terminator cassette.

[0066] 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 *Hind*III 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.

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

(1) Preparation of DNA fragment for *ura3* gene disruption

[0067] 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'-GGTACCAAGTACTGGAAA-3': SEQ ID NO: 5), the dURA rv primer (5'-CAGATAAACAGGGCGACT TTTCGGGTACGTGACT-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-containing 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 AGTCGCCTGTTATCTG-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- containing 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.

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

[0068] 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 (OD₆₀₀ = 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 MgCl₂) 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'-ATTCGAG-CATCGCCGTG-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).

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

(1) Preparation of vector for aox1 gene disruption

[0069] Fig. 2 shows forms of gene disruption using a DNA fragment comprising the aox1 ORF promoter and the 45 terminator region. pOMEA-Z1 constructed in Example 1 was subjected to double digestion with *Hind*III 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 *Hind*III and *Kpn*I to obtain the pOMEU1 plasmid.

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

[0070] 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.

[0071] Subsequently, the strain into which the *aox1* gene-disrupting plasmid had been introduced was inoculated into a 50-ml polypropylene 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 CO₂ 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'-ATGGCTATTCCCTGACGAATT-3': SEQ ID NO: 11) and the AOX ORF rv primer (5'-TTA-GAATCTAGCCAGACCCTTC-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 (Δ*aox1* strain).

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

[0072] The *onaP1 1007* 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 *NotI*, followed by ethanol precipitation, and the resultant DNA (1 µg) was used. The constructed *onaP11007* was digested with the restriction enzyme *NotI* 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 *pdi 1* 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'-TTACAACCTCGTGTGAGCC-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'-TTATAGCTCAAACGATAACAG-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).

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

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

[0073] 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 *HindIII* and *KpnI* 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 Dop*prb1*F primer (5'-CAAGCT-TCGTTGGCAGCAGTGGAG-3': SEQ ID NO: 18) and the Dop*prb1*R 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 *Hind*III-*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 *Hind*III site that had been introduced into the DoprblF primer and the restriction enzyme *Kpn*I site that had been introduced into the DoprblR 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.

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

[0074] The pdPRB 1 plasmid was introduced into the NBRC 10746 strain via electroporation. The pdPRB 1 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 pdPRB 1 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 dprbl 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 dprbl check fw primer (5'-ATGAAGTTATCCCAGTCTGCTG-3': SEQ ID NO: 23) designed within the prb1 ORF sequence and the Hyg-t primer (5'-CAAAGGAATAGATCCCCAT-3': SEQ ID NO: 24) designed in the hygromycin-resistant gene within the pdPRB 1 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, dprbl strain).

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

[0075] The pdPRB1 plasmid was introduced into the NBRC10746+PEK strain described in Example 4 via electroporation. The pdPRB 1 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 dprbl check rv primer (5'-CTAATCGAACAAATCAGCAACC-3': SEQ ID NO: 22) designed within the pdPRB 1 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 dprbl check fw primer (5'-ATGAAGTTATCCCAGTCTGCTG-3': SEQ ID NO: 23) designed within the prb1 ORF sequence and the Hyg-t primer (5'-CAAAGGAATAGATATCCCCAT-3': SEQ ID NO: 24) designed in the hygromycin-resistant gene within the pdPRB 1 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 prb 1::hyg, NBRC 10746+PEK dprb1 strains).

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

[0076] 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 $\times g$ and 4°C for 5 minutes.

[0077] 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'-TTA-CAACTCGTCGTGAGCC-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'-TTATAGCTCCAAAC-GATACAG-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 , $\text{ura3}::\text{pdi1}/\text{ero1}/\text{kar2}$, $\Delta\text{aox1+PEK}$ strains).

[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

[0078] The pdPRB1 plasmid was introduced into the $\Delta\text{aox1+PEK}$ 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 $\mu\text{g}/\text{ml}$, 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 $\mu\text{g}/\text{ml}$, 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 $\mu\text{g}/\text{ml}$ 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 $\times g$ and 4°C for 5 minutes.

[0079] The resulting supernatant as a DNA solution was inspected with the use of the M13 RV primer (5'-CAG-GAACAGCTATGAC-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 , $\text{ura3}::\text{pdi1}/\text{ero1}/\text{kar2}$, $\text{prb1}::\text{hyg}$, $\Delta\text{aox1+PEK}$ dprb1 strains).

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

(1) Preparation of kex2 expression plasmid

[0080] 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).

(2) Preparation of KEX2-producing strain

5 (2-1) Preparation of KEX2-producing strain derived from chaperone-introduced strain

[0081] 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 *Bg*/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.

[0082] 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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 CO₂ 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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).

40 (2-2) Preparation of KEX2-producing strain derived from a strain into which a chaperone had been introduced and in which the prbl gene had been interrupted

[0083] The kex2 expression plasmid was introduced into the NBRC10746+PEK dprbl strain described in Example 6 via electroporation. The kex2 expression plasmid was digested with the restriction enzyme *Bg*/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.

[0084] 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 CO₂ 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 prbl gene had been interrupted (ura3::pdi1/ero1/kar2, prb1::hyg, NBRC10746+PEK dprbl).

5 10 (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

10 15 [0085] 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.

20 20 [0086] 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 CO₂ 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

25 25 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-MF 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°C for 5 minutes was added dropwise to a nitrocellulose membrane

30 30 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).

35 35 (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 prbl gene had been interrupted

[0087] The kex2 expression plasmid was introduced into the Δaox1+PEK dprbl 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.

[0088] 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 CO₂ 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

50 50 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°C 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 ($\Delta aox1$, *ura3::pdi1/ero1/kar2*, *prb1::hyg*, $\Delta aox1+PEK$ *dprb1* strain).

(3) Comparison of secretory production amounts of KEX2

[0089] 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 μ g/ml (in the case of *dprb1*-derived strain, containing Zeocin at a final concentration of 200 μ g/ml and hygromycin B at a final concentration of 200 μ g/ml), and the strains were allowed to proliferate at 28°C for about 2 days. The 2xYP-P6-dp medium (800 μ l) 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 CO₂ 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 μ l of the 2xYP-P6-dpn medium containing 40 μ M of rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was added, and 100 μ l of the 2xYP-P6-dpn medium containing 40 μ M 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 $\Delta aox1+PEK$ - and $\Delta aox1+PEK$ *dprb1*-derived strains, 100 μ l of the 2xYP-P6-dpn medium containing 40 μ M rhodanine-3-acetic acid derivative 1c, 5%(w/v) glycerin, and 5%(v/v) methanol was added, and 100 μ l of the 2xYP-P6-dpn medium containing 40 μ M 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 $\times g$ 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.

[0090] 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 $\Delta aox1+PEK$ *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 $\Delta aox1+PEK$ *dprb1* strain exhibited improvement in secretory production that was about 1.8 times greater than that of the control sample. In particular, the $\Delta aox1+PEK$ *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.

[Example 10] Comparison of KEX2 protein enzyme activity

[0091] 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 $\times g$ 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 μ l of the diluted sample was mixed with 100 μ l of the substrate solution (400 mM Tris (pH 7.0), 2 mM CaCl₂, 0.2% lubrol, 100 μ M BOC-Leu-Arg-Arg-MCA), the mixture was subjected to reaction at 28°C for 30 minutes, and 50 μ l 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 $\Delta aox1+PEK$ *dprb1* strain exhibited improvement in enzyme activity that was about 1.8 times greater than that of the control (the NBRC 10746+PEK strain).

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

(1) Preparation of hsa expression plasmid

5 [0092] 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
10 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).

(2) Preparation of HSA-producing strain

15 (2-1) Preparation of HSA-producing strain derived from chaperone-introduced strain

20 [0093] 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 μ g) was used. After electroporation had been conducted under the conditions described
25 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 μ g/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 μ g/ml.

30 [0094] 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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 μ l) 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 CO₂ 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 μ l of the 2xYP-P6-dpn medium containing 40 μ M 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 0.355 N KOH)] was added, and 100 μ l of the 2xYP-P6-dpn medium containing 40 μ M 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 \times 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
35 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 μ l, 423437, Cosmo Bio, Co., Ltd.) was added to 5 μ l of the culture supernatant, 1 μ l of the culture supernatant resulting from a reaction conducted at 100°C 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).

40 (2-2) Preparation of HSA-producing strain derived from a strain into which a chaperone had been introduced and in
50 which the prb1 gene had been interrupted

55 [0095] The hsa expression plasmid was introduced into the NBRC10746+PEK dprbl 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 μ g) 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 μ g/ml and hygromycin B at a final concentration of 200 μ g/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 μ g/ml and hygromycin B at a final concentration of 200

μg/ml.

[0096] 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 CO₂ 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 dprbl).

(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

[0097] 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 *Bg*/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.

[0098] 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 CO₂ 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°C 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).

(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

[0099] The hsa expression plasmid was introduced into the Δaox1+PEK dprbl strain described in Example 8 via electroporation. The hsa expression plasmid was digested with the restriction enzyme *Bg*/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.

[0100] 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 CO₂ 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-dp 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-dp 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).

(3) Comparison of secretory production amounts of HSA

[0101] 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, 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 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 CO₂ 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-dp medium containing 40 µM rhodanine-3-acetic acid derivative 1c and 10%(v/v) methanol was added, and 100 µl of the 2xYP-P6-dp 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 the Δaox1+PEK- and Δaox1+PEK dprb1-derived strains, 100 µl of the 2xYP-P6-dp 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-dp 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).

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

[0102] 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 polypropylene 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 dprbl-derived strain, 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 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.

[0103] 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 dprbl-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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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.

[0104] 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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 dprbl-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.

[0105] 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 dprbl 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 dprbl 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 dprbl 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.

[0106] 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.

[0107] 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 dprbl 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 dprbl strain (Jar3) (improved from 2486 mg/l to 7104 mg/l). The amount of methanol added when culturing the Δaox1+PEK dprbl 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 Δaox1+PEK dprbl 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).

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

[0108] The amount of the HSA protein secreted and produced by the HSA-producing strain derived from the Δaox1+PEK dprbl 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 polypropylene 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.

[0109] 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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.

[0110] 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 KH₂PO₄, 0.15 M (NH₄)₂SO₄, 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.

[0111] 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.

[0112] 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.

[0113] 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

[0114] The present invention is applicable to the field of production of protein pharmaceuticals, such as antigen or antibody.

SEQUENCE LISTING

[0115]

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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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10	act gtg gct ctc atc gag ggt gag aac aac atc aat aac cca tgg Thr Val Ala Leu Ile Glu Gly Gly Glu Asn Asn Ile Asn Asn Pro Trp 35 40 45	144
15	gtc tac ctt cct ggt gtc tat cca aga aac atg aga ctc gac tcc aag Val Tyr Leu Pro Gly Val Tyr Pro Arg Asn Met Arg Leu Asp Ser Lys 50 55 60	192
	acg gct acc ttc tac aac tcg aga cca tcc aag cac ctg aac ggc aga Thr Ala Thr Phe Tyr Asn Ser Arg Pro Ser Lys His Leu Asn Gly Arg 65 70 75 80	240
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25	aac ttc ctc atg tac acc aga gcc tcg gcc tcc gac tac gac gac tgg Asn Phe Leu Met Tyr Thr Arg Ala Ser Ala Ser Asp Tyr Asp Asp Trp 100 105 110	336
30	gag caa gag gga tgg acc acc gac gag ctg ctt ccg ctc atg aag aag Glu Gln Glu Gly Trp Thr Thr Asp Glu Leu Leu Pro Leu Met Lys Lys 115 120 125	384
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35	gac ggt ccg atc aag gtc tcc ttc ggt aac tac acc tac cca act gcc Asp Gly Pro Ile Lys Val Ser Phe Gly Asn Tyr Thr Tyr Pro Thr Ala 145 150 155 160	480
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45	gat ctt gaa gac ctc aag gcc tcg cac gga gct gag tac tgg ctc aag Asp Leu Glu Asp Leu Lys Ala Ser His Gly Ala Glu Tyr Trp Leu Lys 180 185 190	576

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10	acc aag gct gac aag gtg atc att gag aac ggc gtt gct gtc ggt gtc Thr Lys Ala Asp Lys Val Ile Ile Glu Asn Gly Val Ala Val Gly Val 225	230	235	720
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15	cac cca ctc tac ccg gtt gac tct cca gcc cgt gcc aag gac ttg gat His Pro Leu Tyr Pro Val Asp Ser Pro Ala Arg Ala Lys Asp Leu Asp 485 490 495			1488
20	ctc gag aca tgc aag gca ttt gct gga cca aac cac ttc acc gcc aac Leu Glu Thr Cys Lys Ala Phe Ala Gly Pro Asn His Phe Thr Ala Asn 500 505 510			1536
25	ttg tac cac ggt tcc tgg act gtt cca att gag aag cca acg cca aag Leu Tyr His Gly Ser Trp Thr Val Pro Ile Glu Lys Pro Thr Pro Lys 515 520 525			1584
30	aac gac tcg cac gtg acc tgc aac cag gtc gag atc ttc tcc gac att Asn Asp Ser His Val Thr Cys Asn Gln Val Glu Ile Phe Ser Asp Ile 530 535 540			1632
35	gac tac tct gcc gag gac gat gag gct att gtc aag tac atc aag gag Asp Tyr Ser Ala Glu Asp Asp Glu Ala Ile Val Lys Tyr Ile Lys Glu 545 550 555 560			1680
40	cac act gag acc acc tgg cac tgt ttg gga acc tgt tcg atg gct cca His Thr Glu Thr Trp His Cys Leu Gly Thr Cys Ser Met Ala Pro 565 570 575			1728
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<213> Ogataea minuta

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15	Val Tyr Leu Pro Gly Val Tyr Pro Arg Asn Met Arg Leu Asp Ser Lys			
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20	Thr Ala Thr Phe Tyr Asn Ser Arg Pro Ser Lys His Leu Asn Gly Arg			
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25	Arg Ala Ile Val Pro Cys Ala Asn Ile Leu Gly Gly Ser Ser Ile			
	85	90	95	
30	Asn Phe Leu Met Tyr Thr Arg Ala Ser Ala Ser Asp Tyr Asp Asp Trp			
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45	Asp Gly Pro Ile Lys Val Ser Phe Gly Asn Tyr Thr Tyr Pro Thr Ala			
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50	Gln Asp Phe Leu Arg Ala Cys Glu Ser Gln Gly Ile Pro Phe Asn Asp			
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60	Trp Ile Asn Arg Asp Leu Gly Arg Arg Ser Asp Ser Ala His Ala Tyr			
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65	Ile His Pro Thr Met Arg Asn Lys Ser Asn Leu Phe Leu Ile Thr Ser			
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10 Ser Pro Leu Val Leu Gln Arg Ser Gly Ile Gly Ala Ala His Lys Leu
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50 Phe Phe Gly Asp His Thr Lys Ile Pro Asn Gly Lys Phe Phe Thr Met
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55 Phe His Phe Leu Glu Tyr Pro Phe Ser Arg Gly Phe Val Tyr Ala Val
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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

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5 Leu Tyr His Gly Ser Trp Thr Val Pro Ile Glu Lys Pro Thr Pro Lys
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15 Asp Tyr Ser Ala Glu Asp Asp Glu Ala Ile Val Lys Tyr Ile Lys Glu
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20 Gln Glu Gly Ser Lys Ile Ala Pro Lys Gly Gly Val Val Asp Ala Arg
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Pro Val Ala Arg Arg Leu Leu Asn Leu Met Glu Ser Lys Lys Thr Asn
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5	ttg gac aag ctg gga ccg ttc att tgt ctg gtc aag aca cac atc gac Leu Asp Lys Leu Gly Pro Phe Ile Cys Leu Val Lys Thr His Ile Asp 50 55 60	192
10	att gtg gaa gac ttt tcg tac gaa aac acc gtg gtg ccg ctg ctg aaa Ile Val Glu Asp Phe Ser Tyr Glu Asn Thr Val Val Pro Leu Leu Lys 65 70 75 80	240
15	ctg gcc aag aaa cac aac ttc atg atc ttc gag gac cga aaa ttt gcc Leu Ala Lys Lys His Asn Phe Met Ile Phe Glu Asp Arg Lys Phe Ala 85 90 95	288
	gat ata ggc aac acc gtc aaa ctc cag tac aag gga gga gtt tac caa Asp Ile Gly Asn Thr Val Lys Leu Gln Tyr Lys Gly Val Tyr Gln 100 105 110	336
20	atc gca aag tgg gcc gat atc acc aac gcc cac gga gtg acc ggc tcg Ile Ala Lys Trp Ala Asp Ile Thr Asn Ala His Gly Val Thr Gly Ser 115 120 125	384
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30	cca aga ggt ctg ctc atg ctg gct gag ctg tcg tct gaa ggc tcg ctc Pro Arg Gly Leu Leu Met Leu Ala Glu Leu Ser Ser Glu Gly Ser Leu 145 150 155 160	480
	gcg tac gga gag tac acc aaa aag acg gtt gaa atc gca aag tcc gac Ala Tyr Gly Tyr Thr Lys Lys Thr Val Glu Ile Ala Lys Ser Asp 165 170 175	528
35	aga gat ttt gtg atc ggt ttc att gcg caa aac gac atg ggt ggc cgc Arg Asp Phe Val Ile Gly Phe Ile Ala Gln Asn Asp Met Gly Gly Arg 180 185 190	576
40	gat gag ggc ttc gac tgg ctc atc atg acc cca ggt gtc gga ctc gac Asp Glu Gly Phe Asp Trp Leu Ile Met Thr Pro Gly Val Gly Leu Asp 195 200 205	624
45	gac acc ggt gac gct ctg ggc cag cag tac cgc acg gtc agc gcc gtt Asp Thr Gly Asp Ala Leu Gly Gln Gln Tyr Arg Thr Val Ser Ala Val 210 215 220	672
	atg aag acg gga act gac atc ata atc gtg ggc agg gga ctg ttc ggc Met Lys Thr Gly Thr Asp Ile Ile Val Gly Arg Gly Leu Phe Gly 225 230 235 240	720
50	aag gga aga gac cct gtc gtg gaa ggc gaa aga tac aga aag gct gga Lys Gly Arg Asp Pro Val Val Glu Gly Glu Arg Tyr Arg Lys Ala Gly 245 250 255	768
55	tgg gac gct tat ttg agt cgt gtc gca tga Trp Asp Ala Tyr Leu Ser Arg Val Ala 260 265	798

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<213> Ogataea minuta

5 <400> 30

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

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

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

15 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

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

25 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

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

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

40 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

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

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

55 Met Lys Thr Gly Thr Asp Ile Ile Val Gly Arg Gly Leu Phe Gly

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225

230

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5 Lys Gly Arg Asp Pro Val Val Glu Gly Glu Arg Tyr Arg Lys Ala Gly
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Trp Asp Ala Tyr Leu Ser Arg Val Ala
260 265

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<220>
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<222> (1)..(1620)

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1	5	10	15	
5	gca gtg gag gcc ttg gtc atc ccg tta ttt gac gac ttg cca gca gag Ala Val Glu Ala Leu Val Ile Pro Leu Phe Asp Asp Leu Pro Ala Glu	96		
	20	25	30	
10	ttt gcc ctt gtt cca atg gat gcg aaa gcg gaa gtc att tct gac gtt Phe Ala Leu Val Pro Met Asp Ala Lys Ala Glu Val Ile Ser Asp Val	144		
	35	40	45	
15	cct gtc gac tcg gcc att agt gat gct cct atc gcg gca cta aat gat Pro Val Asp Ser Ala Ile Ser Asp Ala Pro Ile Ala Ala Leu Asn Asp	192		
	50	55	60	
20	gct cca agc cct ctc gtc aca tcg ctg atc gca tct caa aat ttg att Ala Pro Ser Pro Leu Val Thr Ser Leu Ile Ala Ser Gln Asn Leu Ile	240		
	65	70	75	80
25	cca aac tct tat att gtc gtt ttc aag aat ggc cta gct tcc ggg gca Pro Asn Ser Tyr Ile Val Val Phe Lys Asn Gly Leu Ala Ser Gly Ala	288		
	85	90	95	
30	gtt gac ttc cac atg gag tgg ctc aag gaa acg cac tcc caa acc ctg Val Asp Phe His Met Glu Trp Leu Lys Glu Thr His Ser Gln Thr Leu	336		
	100	105	110	
35	gct gct ttg tct aag gac atg cca gca gaa gaa ttg gcc gcc gaa ggt Ala Ala Leu Ser Lys Asp Met Pro Ala Glu Glu Leu Ala Ala Glu Gly	384		
	115	120	125	
40	ttc gtt tcc gaa agc att gat ctt act gag gtg ttt agc atc tcc gat Phe Val Ser Glu Ser Ile Asp Leu Thr Glu Val Phe Ser Ile Ser Asp	432		
	130	135	140	
45	ttg ttc agt gga tat acc gga tac ttc ccg gag aag gtg gtt gac ctc Leu Phe Ser Gly Tyr Thr Gly Tyr Phe Pro Glu Lys Val Val Asp Leu	480		
	145	150	155	160
50	atc aga aga cac cct gac gtg gcg ttc gtt gag cag gac tcg aga gtt	528		

55

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	Ile Arg Arg His Pro Asp Val Ala Phe Val Glu Gln Asp Ser Arg Val	165	170	175	
5	Phe Ala Asp Lys Ser Ser Thr Gln Asn Gly Ala Pro Trp Gly Leu Ser	180	185	190	576
	aga atc tct cac aga gag cct ctc agt ctc ggc aat ttc aac gag tac				624
10	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
15	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
20	Thr Gly Ile Asn Val Lys His Glu Gln Phe Gly Gly Arg Ala Glu Trp	225	230	235	
	240				
25	ggt aag acc atc cca acc ggt 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
30	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	
	aag aac tcc aaa att gtc gca gtg aag gtt ttg aga tct aac ggt tct				864
35	Lys Asn Ser Lys Ile Val Ala Val Lys Val Leu Arg Ser Asn Gly Ser	290	295	300	
	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	305	310	315	
	320				
40	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	325	330	335	
	340				
45	gcc aac atg tct ttg gga ggt ggc aag tct cct gct ctt gac ttg gct				1008
	Ala Asn Met Ser Leu Gly Gly Lys Ser Pro Ala Leu Asp Leu Ala	345	350	355	
	360				
50	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	365			
	370				
	aac gac aat gct gac gca tgc aac tat tct cct gct gct gca gag aac				1104
55	Asn Asp Asn Ala Asp Ala Cys Asn Tyr Ser Pro Ala Ala Ala Glu Asn	375	380	385	
	390				
	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	395	400	405	
	410				
	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	415			
	420				
	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	425			
	430				

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	aca tcg atg gcc tcc cct cac gtt tgt ggt ctg ttg acc tac ttt ttg Thr Ser Met Ala Ser Pro His Val Cys Gly Ieu Leu Thr Tyr Phe Ieu 420 425 430	1296
5	agc ttg caa cca gaa tcg tcg ttg ttt tct tcg gca gct atc tcc Ser Leu Gln Pro Glu Ser Ser Ser Leu Phe Ser Ser Ala Ala Ile Ser 435 440 445	1344
10	cct gct cag ctg aag aac ctg atc aag ttt ggt acg aag aac gtt Pro Ala Gln Leu Lys Lys Asn Leu Ile Lys Phe Gly Thr Lys Asn Val 450 455 460	1392
15	ttg tct gag att cca tcg gac gga acc cca aat att ctc att tac aac Leu Ser Glu Ile Pro Ser Asp Gly Thr Pro Asn Ile Leu Ile Tyr Asn 465 470 475 480	1440
	ggt gct ggc aag aac atc agt gac ttc tgg gcg ttt gaa gac gag gac Gly Ala Gly Lys Asn Ile Ser Asp Phe Trp Ala Phe Glu Asp Glu Ala 485 490 495	1488
20	tcg gcc aag tcc gac ttg aag aag gct gtc gat att gcc aca agt gtt Ser Ala Lys Ser Asp Leu Lys Ala Val Asp Ile Ala Thr Ser Val 500 505 510	1536
25	gac tta gac ctg caa gat atc aag gag aag ttc aac cat att ttg gag Asp Leu Asp Leu Gln Asp Ile Lys Glu Lys Phe Asn His Ile Leu Glu 515 520 525	1584
30	gag gtc gcc gaa gag gtt gct gat ttg ttc gat tag Glu Val Ala Glu Glu Val Ala Asp Leu Phe Asp 530 535	1620
35	<210> 32 <211> 539 <212> PRT <213> Ogataea minuta	
40	<400> 32	
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45	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	
50	Pro Val Asp Ser Ala Ile Ser Asp Ala Pro Ile Ala Ala Leu Asn Asp 50 55 60	
55	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	

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

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

10 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

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

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

25 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

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

35 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

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

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

50 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 Lys Ser Pro Ala Leu Asp Leu Ala
325 330 335

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

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

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

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

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

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

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

30 Pro Ala Gln Leu Lys Lys Asn Leu Ile Lys Phe Gly Thr Lys Asn Val
 450 455 460

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

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

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

50 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

55 <210> 33
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50 <400> 33

Met Lys Val Arg Lys Tyr Ile Thr Leu Cys Phe Trp Trp Ala Phe Ser
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55 Thr Ser Ala Leu Val Ser Ser Gln Gln Ile Pro Leu Lys Asp His Thr
 20 25 30

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

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

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

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

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

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

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

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

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

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

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

55 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

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275

280

285

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

10 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

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

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

25 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

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

35 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

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

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

50 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

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

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

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805 810

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<211> 609

10 <212> PRT

<213> Homo sapiens

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20 Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
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25 His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

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

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

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

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

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

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

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

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

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

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

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

10 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

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

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

25 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

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

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

40 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

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

50 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

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

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

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

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

15 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

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

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

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

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

40 Leu
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50 <400> 35

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Met Lys Leu Phe Gly Leu Thr Thr Leu Thr Ser Ile Leu Ala Ala Leu
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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

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	aac cca ttg gtt ctg gcg gag ttt ttt gct cca tgg tgt ggc cac tgc Asn Pro Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys 50 55 60			192
10	aag aag ctg ggt cca gaa ttc agc gca gcc gca gac cag ctg gtg gag Lys Lys Leu Gly Pro Glu Phe Ser Ala Ala Asp Gln Leu Val Glu 65 70 75 80			240
15	aag aac atc aag ctt gca cag atc gac tgt acc gag gaa aga gat ctg Lys Asn Ile Lys Leu Ala Gln Ile Asp Cys Thr Glu Glu Arg Asp Leu 85 90 95			288
	tgt tcg tcg cac gga atc aga gga tac cca act ttg aag gtg ttc agg Cys Ser Ser His Gly Ile Arg Gly Tyr Pro Thr Leu Lys Val Phe Arg 100 105 110			336
20	ggc gct agt gag cct gct gac tac caa ggc gcc aga gaa cag gaa gct Gly Ala Ser Glu Pro Ala Asp Tyr Gln Gly Ala Arg Glu Gln Glu Ala 115 120 125			384
25	att gtc agt caa atg atc aag ctt tct tta cct gct gtt tcc gtc att Ile Val Ser Gln Met Ile Lys Leu Ser Leu Pro Ala Val Ser Val Ile 130 135 140			432
	gag gat tct gcc gac ctg ttt gat acc att gca gaa gtc tcc gac gcc Glu Asp Ser Ala Asp Leu Phe Asp Thr Ile Ala Glu Val Ser Asp Ala 145 150 155 160			480
30	ctc att gtg caa gtg ttt cct gcg gga gct gct cag tct tcc aac gag Leu Ile Val Gln Val Phe Pro Ala Gly Ala Ala Gln Ser Ser Asn Glu 165 170 175			528
35	acg ttc tac gaa gtc gcc aac gaa ctg aga aac gac ttt gtt ttt gtc Thr Phe Tyr Glu Val Ala Asn Glu Leu Arg Asn Asp Phe Val Phe Val 180 185 190			576
40	tcc acc act aac gag ggg tac gtg aaa aag tac gcg aag gac tca aag Ser Thr Asn Glu Gly Tyr Val Lys Lys Tyr Ala Lys Asp Ser Lys 195 200 205			624
	tca cct gct tat gtc atc ttc agg caa gga gaa aag gtt gaa gat gcg Ser Pro Ala Tyr Val Ile Phe Arg Gln Gly Glu Lys Val Glu Asp Ala 210 215 220			672
45	tcc aca tac acc gga aag act gtt gac gac act cac ttg aag cag ttc Ser Thr Tyr Thr Gly Lys Thr Val Asp Asp Thr His Leu Lys Gln Phe 225 230 235 240			720
	atc aat acc gaa acc aaa cct ctg ttt ggt gaa atc acc ggc aac act Ile Asn Thr Glu Thr Lys Pro Leu Phe Gly Glu Ile Thr Gly Asn Thr 245 250 255			768
50	tcc aag acc tac atg gag gcc gag ctt cct ttg gcg tac ttt ttc tgg Phe Lys Thr Tyr Met Glu Ala Glu Leu Pro Leu Ala Tyr Phe Phe Trp 260 265 270			816
55	gac gaa gag tct caa agg gcc gag gtc gct gac atc atc acc gag ctg			864

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	Asp Glu Glu Ser Gln Arg Ala Glu Val Ala Asp Ile Ile Thr Glu Leu		
	275	280	285
5	gcc aag aag ttt aga gga gag atg aac ttt gtt ggt ttg gaa gcc aag Ala Lys Lys Phe Arg Gly Glu Met Asn Phe Val Gly Leu Glu Ala Lys		912
	290	295	300
10	aga tac ggt atg cat gcc aag aac ctc aac atg gag gaa aag ttc ccc Arg Tyr Gly Met His Ala Lys Asn Leu Asn Met Glu Glu Lys Phe Pro		960
	305	310	315
	ttg ttc gcc atc cac gat ttg acc gga aac ctc aag tac ggt att tct Leu Phe Ala Ile His Asp Leu Thr Gly Asn Leu Lys Tyr Gly Ile Ser		1008
	325	330	335
15	caa gag tct gat ctt gac gtc aag gaa atc cct aag ttc gtt gag gat Gln Glu Ser Asp Leu Asp Val Lys Glu Ile Pro Lys Phe Val Glu Asp		1056
	340	345	350
20	ttc aag aag ggc aag ttg caa gca att gtc aag tct gag cca att cca Phe Lys Lys Gly Lys Leu Gln Ala Ile Val Lys Ser Glu Pro Ile Pro		1104
	355	360	365
25	gaa gtc caa gag gag tcc gtg tac cac ctg gtt gga cac gag cac gac Glu Val Gln Glu Ser Val Tyr His Leu Val Gly His Glu His Asp		1152
	370	375	380
30	gcc atc acc aag cag aag aag gac gtt ttg gtt gag tac tac gcc cca Ala Ile Thr Lys Gln Lys Asp Val Leu Val Glu Tyr Tyr Ala Pro		1200
	385	390	395
	405	410	415
35	tgg tgt gga cac tgc aag aag ctg gct cca act tac gaa att ttg gcc Trp Cys Gly His Cys Lys Leu Ala Pro Thr Tyr Glu Ile Leu Ala		1248
	420	425	430
40	agc atc tac cag aac gac act gat gcc aag gaa aag gtt gtg att gcc Ser Ile Tyr Gln Asn Asp Thr Asp Ala Lys Glu Lys Val Val Ile Ala		1296
	445	440	445
45	aag att gac cac act gcc aac gat gtt gcc ggt gtc gac atc gcc ggt Lys Ile Asp His Thr Ala Asn Asp Val Ala Gly Val Asp Ile Ala Gly		1344
	465	470	475
50	tat cca acc atc atc ttg tat cct ggt gac gaa tct gag ccg gtt gtg Tyr Pro Thr Ile Ile Leu Tyr Pro Gly Asp Glu Ser Glu Pro Val Val		1392
	450	455	460
55	tac gag ggt tct aga act cta gag gct ctc agt tca ttc atc aag gag Tyr Glu Gly Ser Arg Thr Leu Glu Ala Leu Ser Ser Phe Ile Lys Glu		1440
	485	490	495
	500	505	510
	cac gac gag ttg taa His Asp Glu Leu		1551
	515		

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	Thr Val Val Ser Ala Thr Glu Glu Pro Ala Val Ala Ser Pro Asp Ser
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15	Ala Val Val Lys Leu Thr Ala Asp Thr Phe Glu Ser Phe Ile Lys Glu
	35 40 45
20	Asn Pro Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys
	50 55 60
25	Lys Lys Leu Gly Pro Glu Phe Ser 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
30	Cys Ser Ser His Gly Ile Arg Gly Tyr Pro Thr Leu Lys Val Phe Arg
	100 105 110
35	Gly Ala Ser Glu Pro Ala Asp Tyr Gln Gly Ala Arg Glu Gln Glu Ala
	115 120 125
40	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
45	Leu Ile Val Gln Val Phe Pro Ala Gly Ala Ala Gln Ser Ser Asn Glu
	165 170 175
50	Thr Phe Tyr Glu Val Ala Asn Glu Leu Arg Asn Asp Phe Val Phe Val
	180 185 190
55	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

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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
5 245 250 255

Phe Lys Thr Tyr Met Glu Ala Glu Leu Pro Leu Ala Tyr Phe Phe Trp
10 260 265 270

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
15 290 295 300

Arg Tyr Gly Met His Ala Lys Asn Leu Asn Met Glu Glu Lys Phe Pro
20 305 310 315 320

Leu Phe Ala Ile His Asp Leu Thr Gly Asn Leu Lys Tyr Gly Ile Ser
25 325 330 335

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
30 355 360 365

Glu Val Gln Glu Glu Ser Val Tyr His Leu Val Gly His Glu His Asp
35 370 375 380

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
40 405 410 415

Ser Ile Tyr Gln Asn Asp Thr Asp Ala Lys Glu Lys Val Val Ile Ala
45 420 425 430

Lys Ile Asp His Thr Ala Asn Asp Val Ala Gly Val Asp Ile Ala Gly
50 435 440 445

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
55 465 470 475 480

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Lys Gly Ser Asn Gly Val Asp Ala Leu Ser Ile Lys Glu Ser Arg Val
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5 Glu Lys Glu Ala Asp Ala Gln Ala Asp Ala Pro Asp Ala Gly Val Ala
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10 His Asp Glu Leu
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 Met Lys Val Ala Ser Leu Ile Ala Leu Val Val Thr Pro Ile Ile Ala
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30 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

35 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

40 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

45 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

50 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

55 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

60 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

65 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

70 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

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	145	150	155	160	
5	gtg aag aga atc aag ctc aac aaa ctt gat gaa ttt ctc aaa ccg aat Val Lys Arg Ile Lys Leu Asn Lys Leu Asp Glu Phe Leu Lys Pro Asn 165 170 175				528
10	gaa aag agc aga gtc ttg ctg gtg act tca aaa agc act ctt tcg ccg Glu Lys Ser Arg Val Leu Leu Val Thr Ser Lys Ser Thr Leu Ser Pro 180 185 190				576
15	gtt ttc aag agc ctg tcg atc gat ttt ctc gac tca gtc acg ttg gca Val Phe Lys Ser Leu Ser Ile Asp Phe Leu Asp Ser Val Thr Leu Ala 195 200 205				624
20	tac ctc act ctg agc gaa aac gac tcc gaa ggt aga gac aaa ctg ctg Tyr Leu Thr Leu Ser Glu Asn Asp Ser Glu Gly Arg Asp Lys Leu Leu 210 215 220				672
25	gaa aag att cct gcc ctc aaa gcg gac ttc aaa gtc ccg act tta ctc Glu Lys Ile Pro Ala Leu Lys Ala Asp Phe Lys Val Pro Thr Leu Leu 225 230 235 240				720
30	gcc atc gac aag gga acg aaa aat gtg acg gtt tat gat tcc gaa tcg Ala Ile Asp Lys Gly Thr Lys Asn Val Thr Val Tyr Asp Ser Glu Ser 245 250 255				768
35	atg tcg aaa aaa gag ctg acg aag ttc atg tct aag ttc ggc cag cca Met Ser Lys Lys Glu Leu Thr Lys Phe Met Ser Lys Phe Gly Gln Pro 260 265 270				816
40	caa gag ggg gca atg agc gaa aga ggg ggc atc ttg aaa gga atc aag Gln Glu Gly Ala Met Ser Glu Arg Gly Gly Ile Leu Lys Gly Ile Lys 275 280 285				864
45	aag ggt gct tac aag agc ttc aaa gat tac aaa aag aag atg caa caa Lys Gly Ala Tyr Lys Ser Phe Lys Asp Tyr Lys Lys Lys Met Gln Gln 290 295 300				912
	gct ctt gaa aaa gat gag cta tga Ala Leu Glu Lys Asp Glu Leu 305 310				936
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Met Lys Val Ala Ser Leu Ile Ala Leu Val Val Thr Pro Ile Ile Ala
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5 Ala Thr Gly Val Val Ala Asp Pro Gln Gln Gln Ala Lys Arg Pro Gly
20 25 30

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

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

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

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

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

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

25 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

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

35 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

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

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

50 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
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55 Lys Gly Ala Tyr Lys Ser Phe Lys Asp Tyr Lys Lys Lys Met Gln Gln
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Ala Leu Glu Lys Asp Glu Leu
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20	aag aga tcc ata tat gac agg tac gga gag gaa ggc ttg aaa ggc ggt Lys Arg Ser Ile Tyr Asp Arg Tyr Gly Glu Gly Leu Lys Gly Gly 20 25 30	96
25	gca ggg ggc gga gga gga caa cac cac gat ccg ttc gac atg ttt Ala Gly Gly Gly Gly Gln His His Asp Pro Phe Asp Met Phe 35 40 45	144
30	gcc aac ttt ttc ggc ggc gcc ggt ggg cgt caa caa gca cg gga gtt Ala Asn Phe Phe Gly Ala Gly Arg Gln Gln Ala Arg Gly Val 50 55 60	192
35	cca aga ggg tcg gac att gcc acg gag atg gag ttt acc ttg aaa gag Pro Arg Gly Ser Asp Ile Ala Thr Glu Met Glu Phe Thr Leu Lys Glu 65 70 75 80	240
40	ttt tac aac gga gtg aat agc gac ttt tca ctc gaa ctg caa gac atc Phe Tyr Asn Gly Val Asn Ser Asp Phe Ser Leu Glu Leu Gln Asp Ile 85 90 95	288
45	tgt gac cgt tgt gac gga agc ggg tcg cag gac ggg aaa gtg cac aag Cys Asp Arg Cys Asp Gly Ser Gly Ser Gln Asp Gly Lys Val His Lys 100 105 110	336
50	tgt tct cga tgc aat ggt cgt ggc cg gtc tta gtg aag aga cag ttg Cys Ser Arg Cys Asn Gly Arg Gly Arg Val Leu Val Lys Arg Gln Leu 115 120 125	384
55	ggt cct ggc atg ttc cag cag atg gag tcg gcg tgt ccc gac tgt cgt Gly Pro Gly Met Phe Gln Gln Met Glu Ser Ala Cys Pro Asp Cys Arg 130 135 140	432
60	gga gca gga aaa cag att act cac cat tgc aag aag tgt cgg ggt ggc Gly Ala Gly Lys Gln Ile Thr His His Cys Lys Lys Cys Arg Gly Gly 145 150 155 160	480
65	ggg gtt gtc cgt gga att cgc aac ttc aac atc cac ctt gag cca gga Gly Val Val Arg Gly Ile Arg Asn Phe Asn Ile His Leu Glu Pro Gly 165 170 175	528
70	act ccg cgc gac cac gtc gaa gtg tac gag ggt cag tcc gac agg tct Thr Pro Arg Asp His Val Glu Val Tyr Glu Gly Gln Ser Asp Arg Ser 180 185 190	576

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	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	
5	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	
10	gag atc ttg acg ctg agc gag tct ctg aag 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	
15	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	
20	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	
25	gag tat gtg gtg ttg tat ccg 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	
30	ttg cac gac gag ctg tga	930
	Leu His Asp Glu Leu	
	305	
	<210> 40	
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	<213> Ogataea minuta	
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	Lys Arg Ser Ile Tyr Asp Arg Tyr Gly Glu Glu Gly Leu Lys Gly Gly	
	20 25 30	
45	Ala Gly Gly Gly Gly Gly Gln His His Asp Pro Phe Asp Met Phe	
	35 40 45	
50	Ala Asn Phe Phe Gly Ala Gly Gly Arg Gln Gln Ala Arg Gly Val	
	50 55 60	
55	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	

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

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

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

15 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

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

25 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

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

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

40 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

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

50 Leu His Asp Glu Leu
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EP 3 196 304 B1

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5	gtt atg gcg tca gcg tcc aaa gtg atg gag ctg aac gac aag aac ttt Val Met Ala Ser Ala Ser Lys Val Met Glu Leu Asn Asp Lys Asn Phe 20 25 30	96
10	gac gag gtg gtt ctc aac tcc gga aag acc tcg cta gtg gaa ttc tac Asp Glu Val Val Leu Asn Ser Gly Lys Thr Ser Leu Val Glu Phe Tyr 35 40 45	144
15	gcg tcg tgg tgc agt cac tgc aag aag ttg gag cct act tgg gaa gag Ala Ser Trp Cys Ser His Cys Lys Lys Leu Glu Pro Thr Trp Glu Glu 50 55 60	192
20	ctg gcc tcg gcg tac gga aac aag aac gat atc cag atc gtc aag atc Leu Ala Ser Ala Tyr Gly Asn Lys Asn Asp Ile Gln Ile Val Lys Ile 65 70 75 80	240
25	gac gct gac gaa aac gga aac gtg gga aga aaa ttc gga atc aag gga Asp Ala Asp Glu Asn Gly Asn Val Gly Arg Lys Phe Gly Ile Lys Gly 85 90 95	288
30	ttt ccc acg ctg aaa ctg ttc aaa aaa gat gat ctc aac aac cca gtg Phe Pro Thr Leu Lys Leu Phe Lys Lys Asp Asp Leu Asn Asn Pro Val 100 105 110	336
35	gaa ttt gaa ggc tcc agg gac ttc cat tct ttc acc aac ttc att gct Glu Phe Glu Gly Ser Arg Asp Phe His Ser Phe Thr Asn Phe Ile Ala 115 120 125	384
40	gca cac acg ggt atc aag gct gcc aac gcg gtt ccc act gag ccg tcc Ala His Thr Gly Ile Lys Ala Ala Asn Ala Val Pro Thr Glu Pro Ser 130 135 140	432
45	aaa gtg gtg gaa ctg cac gat gga aat ttg gag gag ctt gtt aag gag Lys Val Val Glu Leu His Asp Gly Asn Leu Glu Glu Leu Val Lys Glu 145 150 155 160	480
50	cag gga aaa aat gct ctt ttt gca atc acc gca gag tgg tgt ggt tac Gln Gly Lys Asn Ala Leu Phe Ala Ile Thr Ala Glu Trp Cys Gly Tyr 165 170 175	528
55	tgc aag aag ctc aag cct aca tgg gag cag ctg gct gcc gtt ttc caa Cys Lys Lys Leu Lys Pro Thr Trp Glu Gln Leu Ala Ala Val Phe Gln 180 185 190	576
	ggc gac gag gaa aac atc ttg att gga cag gtc caa acc acc ggc gat Gly Asp Glu Glu Asn Ile Leu Ile Gly Gln Val Gln Thr Thr Gly Asp 195 200 205	624
	aac cca aca gaa tgg atc cag gag aaa tac aac ctc cag tcg ttc ccc Asn Pro Thr Glu Trp Ile Gln Glu Lys Tyr Asn Leu Gln Ser Phe Pro 210 215 220	672
	aca ata gtc ttc atc gag aag ggc aac ctg gac gag cct gtg ttc tat Thr Ile Val Phe Ile Glu Lys Gly Asn Leu Asp Glu Pro Val Phe Tyr	720

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	225	230	235	240	
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	gcc gga act cac cg ^g aac gag aaa ggc gag ctg gac tcc gag gcc ggg Ala Gly Thr His Arg Asn Glu Lys Gly Glu Leu Asp Ser Glu Ala Gly 260	265		270	816
10	ctg ata cac gca gtc gac gag ctg gtt gag cag ttt gtc ggt tcc tcg Leu Ile His Ala Val Asp Glu Leu Val Glu Gln Phe Val Gly Ser Ser 275	280		285	864
15	agc agc ggc aga aaa aat ctg gtt ccg aaa ttc ttg gaa gct ttg aaa Ser Ser Gly Arg Lys Asn Leu Val Pro Lys Phe Leu Glu Ala Leu Lys 290	295	300		912
20	tcg gct gac acc gac aat gca ttg tcg aaa gaa gtg aaa tac tac aac Ser Ala Asp Thr Asp Asn Ala Leu Ser Lys Glu Val Lys Tyr Tyr Asn 305	310	315	320	960
	aag atc atc cat acg atg gtc aac ggt ccc ttt gac ttc gtc gcg aaa Lys Ile Ile His Thr Met Val Asn Gly Pro Phe Asp Phe Val Ala Lys 325		330	335	1008
25	gaa acc gct aga ctg gag tcg cta ctg aag tcg gat ctg tct tcc cga Glu Thr Ala Arg Leu Glu Ser Leu Leu Lys Ser Asp Leu Ser Ser Arg 340	345		350	1056
30	gcc aga gac tca gct tcc ttt aga ctc aac atc ctc aag ttt ttc agc Ala Arg Asp Ser Ala Ser Phe Arg Leu Asn Ile Leu Lys Phe Phe Ser 355	360	365		1104
35	gat cct gcc cct cca gcc aag gat gag ctg tga Asp Pro Ala Pro Pro Ala Lys Asp Glu Leu 370	375			1137
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5 Val Met Ala Ser Ala Ser Lys Val Met Glu Leu Asn Asp Lys Asn Phe
20 25 30

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

15 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

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	65	70	75	80
5	Asp Ala Asp Glu Asn Gly Asn Val Gly Arg Lys Phe Gly Ile Lys Gly 85 90 95			
10	Phe Pro Thr Leu Lys Leu Phe Lys Lys Asp Asp Leu Asn Asn Pro Val 100 105 110			
15	Glu Phe Glu Gly Ser Arg Asp Phe His Ser Phe Thr Asn Phe Ile Ala 115 120 125			
20	Ala His Thr Gly Ile Lys Ala Ala Asn Ala Val Pro Thr Glu Pro Ser 130 135 140			
25	Lys Val Val Glu Leu His Asp Gly Asn Leu Glu Glu Leu Val Lys Glu 145 150 155 160			
30	Gln Gly Lys Asn Ala Leu Phe Ala Ile Thr Ala Glu Trp Cys Gly Tyr 165 170 175			
35	Cys Lys Lys Leu Lys Pro Thr Trp Glu Gln Leu Ala Ala Val Phe Gln 180 185 190			
40	Gly Asp Glu Glu Asn Ile Leu Ile Gly Gln Val Gln Thr Thr Gly Asp 195 200 205			
45	Asn Pro Thr Glu Trp Ile Gln Glu Lys Tyr Asn Leu Gln Ser Phe Pro 210 215 220			
50	Thr Ile Val Phe Ile Glu Lys Gly Asn Leu Asp Glu Pro Val Phe Tyr 225 230 235 240			
55	Pro Tyr Gly Arg Glu Leu Gly Asp Leu Val Glu Phe Val Asn Thr Gln 245 250 255			
60	Ala Gly Thr His Arg Asn Glu Lys Gly Glu Leu Asp Ser Glu Ala Gly 260 265 270			
65	Leu Ile His Ala Val Asp Glu Leu Val Glu Gln Phe Val Gly Ser Ser 275 280 285			
70	Ser Ser Gly Arg Lys Asn Leu Val Pro Lys Phe Leu Glu Ala Leu Lys 290 295 300			
75	Ser Ala Asp Thr Asp Asn Ala Leu Ser Lys Glu Val Lys Tyr Tyr Asn 305 310 315 320			

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

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

10 Ala Arg Asp Ser Ala Ser Phe Arg Leu Asn Ile Leu Lys Phe Phe Ser
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Asp Pro Ala Pro Pro Ala Lys Asp Glu Leu
370 375

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5	15	
	ctg acg tgg ttg atc tgt gcg att tgc gct gtt ccc gga gct ggg ttg Leu Thr Trp Leu Ile Cys Ala Ile Cys Ala Val Pro Gly Ala Gly Leu	96
	20	25
	30	
10	cag gag att tcg tcc ttg gag agg aaa ccg gct tac ctg tcg ccg cag Gln Glu Ile Ser Ser Leu Glu Arg Lys Pro Ala Tyr Leu Ser Pro Gln	144
	35	40
	45	
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	60	
	tac gag acg ttc acg ggg tcc aag gtg agt gag agt tcg aac gtg act Tyr Glu Thr Phe Thr Gly Ser Lys Val Ser Glu Ser Ser Asn Val Thr	240
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	75	80
20	ttt ggc cag atc aac gcg ctc aac aac gaa atc aga ccg gtt ttg cac Phe Gly Gln Ile Asn Ala Leu Asn Asn Glu Ile Arg Pro Val Leu His	288
	85	90
	95	
25	gat ctg att aac gag aac ttc ttc aaa atc ttt cga ctc aac ctg tac Asp Leu Ile Asn Glu Asn Phe Phe Lys Ile Phe Arg Leu Asn Leu Tyr	336
	100	105
	110	
30	aag gag tgt ccg ttc tgg tcg agt tcg gag gga ttt tgc atg cac aag Lys Glu Cys Pro Phe Trp Ser Ser Glu Gly Phe Cys Met His Lys	384
	115	120
	125	
	agc tgt gcc gtg gac acc att gac gac tgg aaa gat ctt ccg gag ata Ser Cys Ala Val Asp Thr Ile Asp Asp Trp Lys Asp Leu Pro Glu Ile	432
	130	135
	140	
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	tgg cag ccc gag gct ctg ggt cggt atc gag tcg ttg acg cga gaa ccg Trp Gln Pro Glu Ala Leu Gly Arg Ile Glu Ser Leu Thr Arg Glu Pro 145 150 155 160	480
5	cct acg acg ata tct gac gcg gga aat ggc tcg tgt gtc gct gca ggc Pro Thr Thr Ile Ser Asp Ala Gly Asn Gly Ser Cys Val Ala Ala Gly 165 170 175	528
10	gga cgg agc acg cgg gat tac tgc gaa ctg gac gag gtc aac gag gac Gly Arg Ser Thr Arg Asp Tyr Cys Glu Leu Asp Glu Val Asn Glu Asp 180 185 190	576
15	tcg gta tac gtg aat ctg gtg gac aat ccc gag aga ttc acg ggg tac Ser Val Tyr Val Asn Leu Val Asp Asn Pro Glu Arg Phe Thr Gly Tyr 195 200 205	624
20	gga gga gat cag tcg ttc caa att tgg cgc agc att tac aac gag aac Gly Gly Asp Gln Ser Phe Gln Ile Trp Arg Ser Ile Tyr Asn Glu Asn 210 215 220	672
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30	aag ttg atc agt gga atg cac tcg atc tcg act cat ctg acc aac Lys Leu Ile Ser Gly Met His Ser Ser Ile Ser Thr His Leu Thr Asn 245 250 255	768
35	gag tac ctg aac ttc aag acc aag cag tat gga cag gat ctc aag cag Glu Tyr Leu Asn Phe Lys Thr Lys Gln Tyr Gly Gln Asp Leu Lys Gln 260 265 270	816
40	ttc atg atc cgg gtg ggg gac ttc cct gac cgg ttc gag aac ttg tat Phe Met Ile Arg Val Gly Asp Phe Pro Asp Arg Phe Glu Asn Leu Tyr 275 280 285	864
45	ctg aac tac gtt ctg gtg aag tcc ttg atc aag ctg gag cag tcg Leu Asn Tyr Val Leu Val Lys Ser Leu Ile Lys Leu Glu Gln Ser 290 295 300	912
50	ggt gtg ctg gac aac ctc cag ttc tgt gac gag gag gtg ttt cag acg Gly Val Leu Asp Asn Leu Gln Phe Cys Asp Glu Glu Val Phe Gln Thr 305 310 315 320	960
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65	cac tcg ctg ttc cag agc gag gac tcg acg tat ctg aag gac gag ttc His Ser Leu Phe Gln Ser Glu Asp Ser Thr Tyr Leu Lys Asp Glu Phe 355 360 365	1104
70	agt gag aac ttc agg aac gtg tcg cgg atc atg gat tgt gtc cac tgc Ser Glu Asn Phe Arg Asn Val Ser Arg Ile Met Asp Cys Val His Cys 370 375 380	1152
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5	ggc aag aat ttc cag atc tcc aaa atc gag ctg gtc gcc ctg atc aac Gly Lys Asn Phe Gln Ile Ser Lys Ile Glu Leu Val Ala Leu Ile Asn 420 425 430	1296
10	acg ttt gac agg ctg tcc aag agc gtg cac gcc atc gga aac ttc aaa Thr Phe Asp Arg Leu Ser Lys Ser Val His Ala Ile Gly Asn Phe Lys 435 440 445	1344
15	caa ctg tac gat ctg aga atg aaa cag gag gaa gaa ggg ggg tcc atg Gln Leu Tyr Asp Leu Arg Met Lys Gln Glu Glu Gly Gly Ser Met 450 455 460	1392
	att act gca gac acg ttt gac ttg gag caa ttg ctg ttg aca gac cag Ile Thr Ala Asp Thr Phe Asp Leu Glu Gln Leu Leu Thr Asp Gln 465 470 475 480	1440
20	acg gtg gac gta ttc ggc cag agc act tct gag cca gaa acc ccg tcg Thr Val Asp Val Phe Gly Gln Ser Thr Ser Glu Pro Glu Thr Pro Ser 485 490 495	1488
25	gac gtt aga tat ccc gac aga aca cgg ggc tcg ctc gtt ccc gag ggg Asp Val Arg Tyr Pro Asp Arg Thr Arg Gly Ser Leu Val Pro Glu Gly 500 505 510	1536
30	ctc ggc gag gcg ttc aag aca gag ctg tac agc gtt tat cag gcg ttc Leu Gly Ala Phe Lys Thr Glu Leu Tyr Ser Val Tyr Gln Ala Phe 515 520 525	1584
	tac ttt gtc gtg acc agc tac acc atg ttc ccc aag ctg atc tac aac Tyr Phe Val Val Thr Ser Tyr Thr Met Phe Pro Lys Leu Ile Tyr Asn 530 535 540	1632
35	tac ctg ctg atc cgg gtg tac tgg tgg aac atc ttt gtg ggt cat Tyr Leu Leu Ile Arg Val Val Tyr Trp Trp Asn Ile Phe Val Gly His 545 550 555 560	1680
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5 Leu Thr Trp Leu Ile Cys Ala Ile Cys Ala Val Pro Gly Ala Gly Leu
20 25 30

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

15

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

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

10 Phe Gly Gln Ile Asn Ala Leu Asn Asn Glu Ile Arg Pro Val Leu His
85 90 95

15 Asp Leu Ile Asn Glu Asn Phe Phe Lys Ile Phe Arg Leu Asn Leu Tyr
100 105 110

20 Lys Glu Cys Pro Phe Trp Ser Ser Ser Glu Gly Phe Cys Met His Lys
115 120 125

25 Ser Cys Ala Val Asp Thr Ile Asp Asp Trp Lys Asp Leu Pro Glu Ile
130 135 140

30 Trp Gln Pro Glu Ala Leu Gly Arg Ile Glu Ser Leu Thr Arg Glu Pro
145 150 155 160

35 Pro Thr Thr Ile Ser Asp Ala Gly Asn Gly Ser Cys Val Ala Ala Gly
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40 Gly Arg Ser Thr Arg Asp Tyr Cys Glu Leu Asp Glu Val Asn Glu Asp
180 185 190

45 Ser Val Tyr Val Asn Leu Val Asp Asn Pro Glu Arg Phe Thr Gly Tyr
195 200 205

50 Gly Gly Asp Gln Ser Phe Gln Ile Trp Arg Ser Ile Tyr Asn Glu Asn
210 215 220

55 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

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5 Gly Val Leu Asp Asn Leu Gln Phe Cys Asp Glu Glu Val Phe Gln Thr
 305 310 315 320

10 Lys Glu Lys Glu Leu Lys Arg Glu Leu Lys Glu Met Ile Ser Pro Phe
 325 330 335

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

20 His Ser Leu Phe Gln Ser Glu Asp Ser Thr Tyr Leu Lys Asp Glu Phe
 355 360 365

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

30 Asp Arg Cys Arg Leu Trp Gly Lys Val Gln Thr Thr Gly Tyr Gly Thr
 385 390 395 400

35 Ala Leu Lys Ile Leu Phe Glu Leu Asp Ala Ser Asp Ser His Glu Leu
 405 410 415

40 Gly Lys Asn Phe Gln Ile Ser Lys Ile Glu Leu Val Ala Leu Ile Asn
 420 425 430

45 Thr Phe Asp Arg Leu Ser Lys Ser Val His Ala Ile Gly Asn Phe Lys
 435 440 445

50 Gln Leu Tyr Asp Leu Arg Met Lys Gln Glu Glu Gly Gly Ser Met
 450 455 460

55 Ile Thr Ala Asp Thr Phe Asp Leu Glu Gln Leu Leu Leu Thr Asp Gln
 465 470 475 480

60 Thr Val Asp Val Phe Gly Gln Ser Thr Ser Glu Pro Glu Thr Pro Ser
 485 490 495

65 Asp Val Arg Tyr Pro Asp Arg Thr Arg Gly Ser Leu Val Pro Glu Gly
 500 505 510

70 Leu Gly Glu Ala Phe Lys Thr Glu Leu Tyr Ser Val Tyr Gln Ala Phe
 515 520 525

75 Tyr Phe Val Val Thr Ser Tyr Thr Met Phe Pro Lys Leu Ile Tyr Asn
 530 535 540

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Tyr Leu Leu Ile Arg Val Val Tyr Trp Trp Asn Ile Phe Val Gly His
545 550 555 560

5 Val His Glu Asp Phe Asp Val Asp Arg Leu Tyr Arg Leu Glu Leu
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5	15	
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	20	25
	30	
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	35	40
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15	tat ctc aag acg ttg att gag aga ggt cga ttt gac tgg act gct ttt Tyr Leu Lys Thr Leu Ile Glu Arg Gly Arg Phe Asp Trp Thr Ala Phe	192
	50	55
	60	
	gaa aga gcc gtt aat aaa gca gtg gtg cgg ctc cca agc gtg gcc ggt Glu Arg Ala Val Asn Lys Ala Val Val Arg Leu Pro Ser Val Ala Gly	240
	65	70
	75	80
20	tcc aac acc gag cca agc att tcc gcc tcc gcc gcc agc att atc acc Ser Asn Thr Glu Pro Ser Ile Ser Ala Ser Ala Ala Ser Ile Ile Thr	288
	85	90
	95	
25	aac gca tcc aag atc aag gca cag cag aaa gac tcg tac atc ggc caa Asn Ala Ser Lys Ile Lys Ala Gln Gln Lys Asp Ser Tyr Ile Gly Gln	336
	100	105
	110	
30	gac cac att ctc tcg gcc ctt ttg gac gat tct agt atc cag gct gtg Asp His Ile Leu Ser Ala Leu Leu Asp Asp Ser Ser Ile Gln Ala Val	384
	115	120
	125	
	ctg aag gaa gcg aac gtc aag ccc gac gca ttg aag aca cag atc gtt Leu Lys Glu Ala Asn Val Lys Pro Asp Ala Leu Lys Thr Gln Ile Val	432
	130	135
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35	gaa ctc aga ggc aat cag aga att gat tct cgt caa gct gat tcg tct Glu Leu Arg Gly Asn Gln Arg Ile Asp Ser Arg Gln Ala Asp Ser Ser	480
	145	150
	155	160
40	cag aag ttt gag ttt ctg tcc aag tac gcc ctt gat ctt acc gag cag Gln Lys Phe Glu Phe Leu Ser Lys Tyr Ala Leu Asp Leu Thr Glu Gln	528
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5	aga agg gcc att cgg gtt ctt tcg aga cgg gcc aag tcg aac ccg tgt Arg Arg Ala Ile Arg Val Leu Ser Arg Arg Ala Lys Ser Asn Pro Cys 195 200 205	624
10	ctg att gga gat cct ggt ggt aag act agt att gtt gag gga gtc Leu Ile Gly Asp Pro Gly Val Gly Lys Thr Ser Ile Val Glu Gly Val 210 215 220	672
	gca cag agg ata gtg gac aac gat gtt cct acc gtt tta cag ggg tgc Ala Gln Arg Ile Val Asp Asn Asp Val Pro Thr Val Leu Gln Gly Cys 225 230 235 240	720
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25	agc tcg aac tcg atg atc atc ttg ttc atc gac gag atc cac atg ttg Ser Ser Asn Ser Met Ile Ile Leu Phe Ile Asp Glu Ile His Met Leu 275 280 285	864
	atg ggc gat gga aag tcg gac gct gcc aac ttg ctg aag cct gct ctg Met Gly Asp Gly Lys Ser Asp Ala Ala Asn Leu Leu Lys Pro Ala Leu 290 295 300	912
30	gct aga ggt cag ttc cac tgc atc ggt gct gac act acc gtc acc gag tac Ala Arg Gly Gln Phe His Cys Ile Gly Ala Thr Thr Val Thr Glu Tyr 305 310 315 320	960
35	aga aag cac atc gag aag gac ggt gct ttc gag aga aga ttt cag aga Arg Lys His Ile Glu Lys Asp Gly Ala Phe Glu Arg Arg Phe Gln Arg 325 330 335	1008
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45	agt gcc ctg gtt acg gct gct caa ctg gcc tcc aga tac ctc acc tac Ser Ala Leu Val Thr Ala Ala Gln Leu Ala Ser Arg Tyr Leu Thr Tyr 370 375 380	1152
	aga aag ctt ccg gac tct gca gtg gat ctg att gac gag tcc gcc gct Arg Lys Leu Pro Asp Ser Ala Val Asp Leu Ile Asp Glu Ser Ala Ala 385 390 395 400	1200
50	gga gtc gct gtt gcc agg gac tcc aag ccg gag gag ctg gac tcc aag Gly Val Ala Val Ala Arg Asp Ser Lys Pro Glu Glu Leu Asp Ser Lys 405 410 415	1248
55	gag aga cag cta cag ctg atc gag gtt gag atc aat gct ctg gaa aga Glu Arg Gln Leu Gln Leu Ile Glu Val Glu Ile Asn Ala Leu Glu Arg 420 425 430	1296

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15	aag tta gac gac ctc gaa gtt aag gcg caa gat gcg gag aga aga cac Lys Leu Asp Asp Leu Glu Val Lys Ala Gln Asp Ala Glu Arg Arg His 485 490 495	1488
20	gac tct cag acc att gcg gac ctg cggt atg ttt gcc att ccg gac gtg Asp Ser Gln Thr Ile Ala Asp Leu Arg Met Phe Ala Ile Pro Asp Val 500 505 510	1536
25	aaa cgc aga att gag gag ttg gaa cag aaa gtg gtt gaa gaa gag gcc Lys Arg Arg Ile Glu Glu Leu Glu Gln Lys Val Val Glu Glu Glu Ala 515 520 525	1584
30	act tct gaa gat ttc atg gtg aag aac gtt gtt ggt tcg gag caa gtt Thr Ser Glu Asp Phe Met Val Lys Asn Val Val Gly Ser Glu Gln Val 530 535 540	1632
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	acc gcc ggt tac gtt ggc tac gag gag ggt ggt atg ttg acc aac cag Thr Ala Gly Tyr Val Gly Tyr Glu Glu Gly Gly Met Leu Thr Asn Gln 660 665 670	2016
	ctt ttg aga aga ccg tac tct gtg gtg ttg ttc gac gag gtc gag aag Leu Leu Arg Arg Pro Tyr Ser Val Val Leu Phe Asp Glu Val Glu Lys	2064

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15	gtg atc atg acg tcc aac ctc ggc gcg gag tac atc aac gca tca aag Val Ile Met Thr Ser Asn Leu Gly Ala Glu Tyr Ile Asn Ala Ser Lys 725 730 735			2208
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15	His Phe Leu Ala Ala Met Thr Pro Thr Ser Ser Glu Gly Glu Ala Ile 35 40 45
20	Tyr Leu Lys Thr Leu Ile Glu Arg Gly Arg Phe Asp Trp Thr Ala Phe 50 55 60
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65	Arg Arg Ala Ile Arg Val Leu Ser Arg Arg Ala Lys Ser Asn Pro Cys 195 200 205
70	Leu Ile Gly Asp Pro Gly Val Gly Lys Thr Ser Ile Val Glu Gly Val 210 215 220
75	Ala Gln Arg Ile Val Asp Asn Asp Val Pro Thr Val Leu Gln Gly Cys 225 230 235 240

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Lys Leu Tyr Ser Leu Asp Leu Gly Ala Leu Lys Ala Gly Ala Lys Tyr
245 250 255

5 Gln Gly Glu Phe Glu Glu Arg Leu Lys Gly Val Leu Ser Asp Ile Glu
260 265 270

10 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
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15 Ala Arg Gly Gln Phe His Cys Ile Gly Ala Thr Thr Val Thr Glu Tyr
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20 Arg Lys His Ile Glu Lys Asp Gly Ala Phe Glu Arg Arg Phe Gln Arg
325 330 335

25 Ile Asp Val Arg Glu Pro Thr Ile Arg Glu Thr Val Ala Ile Leu Arg
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Gly Leu Gln Pro Arg Tyr Glu Ile His His Gly Val Arg Ile Leu Asp
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30 Ser Ala Leu Val Thr Ala Ala Gln Leu Ala Ser Arg Tyr Leu Thr Tyr
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45 Asp Gln Asp Ala Asp Thr Ser Thr Lys Glu Arg Leu Glu Gln Ala Arg
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50 Gln Arg Arg Gln Asn Leu 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

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485 490 495

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Asp Ser Gln Thr Ile Ala Asp Leu Arg Met Phe Ala Ile Pro Asp Val
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725 730 735

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740 745 750

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Lys Gly His Phe Arg Pro Glu Phe Leu Asn Arg Ile Ser Ala Thr Val
 755 760 765

5 Val Phe Asn Arg Leu Ser Arg His Ala Ile Ala Lys Ile Val Arg Leu
 770 775 780

10 Arg Leu Lys Glu Ile Glu Glu Arg Phe Glu Ala Asn Gly Lys Ser Ile
 785 790 795 800

15 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

20 Glu Ile Leu Asn His Leu Ala Val Met Val Leu Asn Gly Gln Val Leu
 835 840 845

25 Asp Lys Glu Glu Val Gln Ile Thr Thr Gly Ser Lys Gly Leu Ser Val
 850 855 860

30 Val Pro Asn His Asp Ile Glu Asp Glu Ala Met Asp Val Asp Val Asp
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35 Glu Trp Thr Asp Ala Ala Asp Asp Asp Ser Gly Tyr Gly Ser Pro
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Asp Leu Asp

<210> 47

<211> 1998

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40 <213> Ogataea minuta

<220>

<221> CDS

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55 gca gtg ctg ttg gta gtc cta cct ttg gct tca caa caa ttc gtg gaa 96
 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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	35	40	45	
5	acc act tac tca tgt ggt gtg atg aaa gct ggt aga gag atc Thr Thr Tyr Ser Cys Val Gly Val Met Lys Ala Gly Arg Val Glu Ile 50 55 60			192
	ctt gcc aat gac cag ggt aac aga att act cca tct tat gtg gca ttt Leu Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser Tyr Val Ala Phe 65 70 75 80			240
10	act gat gaa gag aga ctt gtc gga gat gcc gca aag aac cag att gcc Thr Asp Glu Glu Arg Leu Val Gly Asp Ala Ala Lys Asn Gln Ile Ala 85 90 95			288
15	tcc aac cca agc aac aca atc ttc gat atc aag aga ctc ata gga cac Ser Asn Pro Ser Asn Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly His 100 105 110			336
	aga ttt gac gat aag gtt gtg caa aaa gag att gca cac ctc cct tac Arg Phe Asp Asp Lys Val Val Gln Lys Glu Ile Ala His Leu Pro Tyr 115 120 125			384
20	aag atc aga aac caa gag ggc aga ccg gtc gtt gag gcc act gtc aat Lys Ile Arg Asn Gln Glu Gly Arg Pro Val Val Glu Ala Thr Val Asn 130 135 140			432
25	gga gag gtg acc acg ttc acg gcc gaa gaa gtt tcg gcc atg atc ttg Gly Glu Val Thr Phe Thr Ala Glu Glu Val Ser Ala Met Ile Leu 145 150 155 160			480
	gga aag atg aag caa att gct gaa gat tat ctc gga aag aag gtt acc Gly Lys Met Lys Gln Ile Ala Glu Asp Tyr Leu Gly Lys Val Thr 165 170 175			528
30	cat gct gtt gtc acg gtt cct gca tac ttt aac gac gcc caa aga cag His Ala Val Thr Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln 180 185 190			576
35	gcc act aag gat gct ggt acc att gcc ggt ctg gaa gtt ttg aga att Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Glu Val Leu Arg Ile 195 200 205			624
40	gtt aac gag cct act gcc gct gca att gct tac ggt ctc gac aag acg Val Asn Glu Pro Thr Ala Ala Ile Ala Tyr Gly Leu Asp Lys Thr 210 215 220			672
	gac gaa gag aag cat atc att gtt tac gat ttg ggt gga gga act ttt Asp Glu Glu Lys His Ile Ile Val Tyr Asp Leu Gly Gly Thr Phe 225 230 235 240			720
45	gat gtt tct ctg ttg aca att gca ggt gga gct ttc gag gtt cgt gcc Asp Val Ser Leu Leu Thr Ile Ala Gly Gly Ala Phe Glu Val Arg Ala 245 250 255			768
50	acc gct ggt gat acc cat ctt ggt ggt gag gac ttt gat tac aga gtt Thr Ala Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Tyr Arg Val 260 265 270			816
	gtc aga cat ttc atc aag gtg ttt aag aag aag cat ggc att gat atc Val Arg His Phe Ile Lys Val Phe Lys Lys His Gly Ile Asp Ile 275 280 285			864
55	agt gat aat cca aag gct ctt gct aaa ttg aag aga gaa gtt gaa aaa			912

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	Ser Asp Asn Pro Lys Ala Leu Ala Lys Leu Lys Arg Glu Val Glu Lys			
	290	295	300	
5	gct aag aga acc ttg tct tct caa atg tcc acc aga att gag att gac Ala Lys Arg Thr Leu Ser Ser Gln Met Ser Thr Arg Ile Glu Ile Asp			960
	305	310	315	320
10	tcg ttc gct gac ggt att gac ttc tcc gag tcc tta tcc agg gcc aag Ser Phe Ala Asp Gly Ile Asp Phe Ser Glu Ser Leu Ser Arg Ala Lys			1008
	325	330	335	
	ttc gag gaa ttg aac att gag ttg ttc aaa aag acc ttg aag cct gtt Phe Glu Glu Leu Asn Ile Glu Leu Phe Lys Lys Thr Leu Lys Pro Val			1056
	340	345	350	
15	caa cgt gtt ctt gaa gac gcc aaa ttc aag gtt tca gaa att gat gac Gln Arg Val Leu Glu Asp Ala Lys Phe Lys Val Ser Glu Ile Asp Asp			1104
	355	360	365	
20	att gtc ttg gtt ggt ggt tcc acg aga att cca aag gtg caa gag ttg Ile Val Leu Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Glu Leu			1152
	370	375	380	
	ctg gaa agt tac ttc aac ggc aag caa gtg tcc aag gga att aac cca Leu Glu Ser Tyr Phe Asn Gly Lys Gln Val Ser Lys Gly Ile Asn Pro			1200
	385	390	395	400
25	gat gaa gct gtt gct tac ggt gcg gct gtt caa gct ggt gtc ctc tct Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser			1248
	405	410	415	
30	ggt gaa gaa ggc gtt gaa gac att gtt ttg att gat gtg aat cca tta Gly Glu Glu Gly Val Glu Asp Ile Val Leu Ile Asp Val Asn Pro Leu			1296
	420	425	430	
35	act ttg ggt atc gag acc tcc ggc ggt gtc atg acc act ttg att aag Thr Leu Gly Ile Glu Thr Ser Gly Gly Val Met Thr Thr Leu Ile Lys			1344
	435	440	445	
	aga aac act gca att cca acc aag aag tct caa att ttc tct act gct Arg Asn Thr Ala Ile Pro Thr Lys Lys Ser Gln Ile Phe Ser Thr Ala			1392
	450	455	460	
40	gct gac aat caa cct gtt gtc ttg atc caa gtc tat gaa ggt gag aga Ala Asp Asn Gln Pro Val Val Leu Ile Gln Val Tyr Glu Gly Glu Arg			1440
	465	470	475	480
45	gcc atg gca aag gat aac aat ttg cta gga aag ttc gag ttg aag gat Ala Met Ala Lys Asp Asn Asn Leu Ieu Gly Lys Phe Glu Leu Lys Asp			1488
	485	490	495	
50	att cct cca gcc cca aga ggt acc cca caa att gag gtg act ttc act Ile Pro Pro Ala Pro Arg Gly Thr Pro Gln Ile Glu Val Thr Phe Thr			1536
	500	505	510	
	ctg gac tcc aac gga atc ctg aag gtt gct gcc act gat aaa ggt act Leu Asp Ser Asn Gly Ile Leu Lys Val Ala Ala Thr Asp Lys Gly Thr			1584
	515	520	525	
55	ggt aag tct aac tct atc aca atc aca aac gac aag ggt aag ctt tcg Gly Lys Ser Asn Ser Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser			1632
	530	535	540	

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aag gag gag att gag aag aag gtt gag gag gcc gaa cag tat gct caa Lys Glu Glu Ile Glu Lys Lys Val Glu Ala Glu Gln Tyr Ala Gln 545 550 555 560	1680
5 caa gat aag gag gtc aga gag aag atc gag agc aga aac gga ctt gag Gln Asp Lys Glu Val Arg Glu Lys Ile Glu Ser Arg Asn Gly Leu Glu 565 570 575	1728
10 aac tac gcc cac tcg ttg aaa aac caa gtg aac gat gag acc gga ttc Asn Tyr Ala His Ser Leu Lys Asn Gln Val Asn Asp Glu Thr Gly Phe 580 585 590	1776
15 ggc tcc aag ctt gat gag gat gac aag gaa act ttg ttg gat gcc atc Gly Ser Lys Leu Asp Glu Asp Asp Lys Glu Thr Leu Leu Asp Ala Ile 595 600 605	1824
20 aac gag gca ttg gag tac ttg gac gac aac ttt gag acc gca aca aag Asn Glu Ala Leu Glu Tyr Leu Asp Asp Asn Phe Glu Thr Ala Thr Lys 610 615 620	1872
25 caa gac ttt gag gat cag aag gaa aaa ttg agt aag gtt gct tac cca Gln Asp Phe Glu Asp Gln Lys Glu Lys Leu Ser Lys Val Ala Tyr Pro 625 630 635 640	1920
30 att act tca aag ttg tat gat acg cca cct act agt gac gaa gat gat Ile Thr Ser Lys Leu Tyr Asp Thr Pro Pro Thr Ser Asp Glu Asp Asp 645 650 655	1968
35 gag gat gac tgg gat cat gat gag ctg tga Glu Asp Asp Trp Asp His Asp Glu Leu 660 665	1998
40 Met Phe Lys Phe Asn Arg Ser Val Leu Ser Ile Ala Thr Ile Leu Tyr 1 5 10 15	
45 Ala Val Leu Leu Val Val Leu Pro Leu Ala Ser Gln Gln Phe Val Glu 20 25 30	
50 Ala Glu Ala Asn Asp Asn Tyr Gly Thr Val Ile Gly Ile Asp Leu Gly 35 40 45	
55 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	
55 Thr Asp Glu Glu Arg Leu Val Gly Asp Ala Ala Lys Asn Gln Ile Ala 85 90 95	

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Ser Asn Pro Ser Asn Thr Ile Phe Asp Ile Lys Arg Leu Ile Gly His
100 105 110

5 Arg Phe Asp Asp Lys Val Val Gln Lys Glu Ile Ala His Leu Pro Tyr
115 120 125

10 Lys Ile Arg Asn Gln Glu Gly Arg Pro Val Val Glu Ala Thr Val Asn
130 135 140

15 Gly Glu Val Thr Thr Phe Thr Ala Glu Glu Val Ser Ala Met Ile Leu
145 150 155 160

20 Gly Lys Met Lys Gln Ile Ala Glu Asp Tyr Leu Gly Lys Lys Val Thr
165 170 175

25 His Ala Val Val Thr Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln
180 185 190

30 Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Glu Val Leu Arg Ile
195 200 205

35 Val Asn Glu Pro Thr Ala Ala Ile Ala Tyr Gly Leu Asp Lys Thr
210 215 220

40 Asp Glu Glu Lys His Ile Ile Val Tyr Asp Leu Gly Gly Gly Thr Phe
225 230 235 240

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

50 Thr Ala Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Tyr Arg Val
260 265 270

55 Val Arg His Phe Ile Lys Val Phe 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

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

5 Ile Val Leu Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Glu Leu
370 375 380

10 Leu Glu Ser Tyr Phe Asn Gly Lys Gln Val Ser Lys Gly Ile Asn Pro
385 390 395 400

15 Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser
405 410 415

20 Gly Glu Glu Gly Val Glu Asp Ile Val Leu Ile Asp Val Asn Pro Leu
420 425 430

25 Thr Leu Gly Ile Glu Thr Ser Gly Gly Val Met Thr Thr Leu Ile Lys
435 440 445

30 Arg Asn Thr Ala Ile Pro Thr Lys Lys Ser Gln Ile Phe Ser Thr Ala
450 455 460

35 Ala Asp Asn Gln Pro Val Val Leu Ile Gln Val Tyr Glu Gly Glu Arg
465 470 475 480

40 Ala Met Ala Lys Asp Asn Asn Leu Leu Gly Lys Phe Glu Leu Lys Asp
485 490 495

45 Ile Pro Pro Ala Pro Arg Gly Thr Pro Gln Ile Glu Val Thr Phe Thr
500 505 510

50 Leu Asp Ser Asn Gly Ile Leu Lys Val Ala Ala Thr Asp Lys Gly Thr
515 520 525

55 Gly Lys Ser Asn Ser Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser
530 535 540

60 Lys Glu Glu Ile Glu Lys Lys Val Glu Glu Ala Glu Gln Tyr Ala Gln
545 550 555 560

65 Gln Asp Lys Glu Val Arg Glu Lys Ile Glu Ser Arg Asn Gly Leu Glu
565 570 575

70 Asn Tyr Ala His Ser Leu Lys Asn Gln Val Asn Asp Glu Thr Gly Phe
580 585 590

75 Gly Ser Lys Leu Asp Glu Asp Asp Lys Glu Thr Leu Leu Asp Ala Ile

595

600

605

5 Asn Glu Ala Leu Glu Tyr Leu Asp Asp Asn Phe Glu Thr Ala Thr Lys
610 615 620

10 Gln Asp Phe Glu Asp Gln Lys Glu Lys Leu Ser Lys Val Ala Tyr Pro
625 630 635 640

15 Ile Thr Ser Lys Leu Tyr Asp Thr Pro Pro Thr Ser Asp Glu Asp Asp
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20 Glu Asp Asp Trp Asp His Asp Glu Leu
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<210> 49

<211> 1569

<212> DNA

<213> *Saccharomyces cerevisiae*

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<221> CDS

<222> (1)..(1569)

<400> 49

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5	gcc tcc tct gtt ttc gcc caa caa gag gct gtg gcc cct gaa gac tcc Ala Ser Ser Val Phe Ala Gln Glu Ala Val Ala Pro Glu Asp Ser	96		
	20	25	30	
10	gct gtc gtt aag ttg gcc acc gac tcc ttc aat gag tac att cag tcg Ala Val Val Lys Leu Ala Thr Asp Ser Phe Asn Glu Tyr Ile Gln Ser	144		
	35	40	45	
15	cac gac ttg gtg ctt gcg gag ttt ttt gct cca tgg tgt ggc cac tgt His Asp Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys	192		
	50	55	60	
	aag aac atg gct cct gaa tac gtt aaa gcc gcc gag act tta gtt gag Lys Asn Met Ala Pro Glu Tyr Val Lys Ala Ala Glu Thr Leu Val Glu	240		
	65	70	75	80
20	aaa aac att acc ttg gcc cag atc gac tgt act gaa aac cag gat ctg Lys Asn Ile Thr Leu Ala Gln Ile Asp Cys Thr Glu Asn Gln Asp Leu	288		
	85	90	95	
25	tgt atg gaa cac aac att cca ggg ttc cca agc ttg aag att ttc aaa Cys Met Glu His Asn Ile Pro Gly Phe Pro Ser Leu Lys Ile Phe Lys	336		
	100	105	110	
30	aac agc gat gtt aac aac tcg atc gat tac gag gga cct aga act gcc Asn Ser Asp Val Asn Asn Ser Ile Asp Tyr Glu Gly Pro Arg Thr Ala	384		
	115	120	125	
	gag gcc att gtc caa ttc atg atc aag caa agc caa ccg gct gtc gcc	432		

35

40

45

50

55

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	Glu Ala Ile Val Gln Phe Met Ile Lys Gln Ser Gln Pro Ala Val Ala		
	130	135	140
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	145	150	155
10	160		
	cca gtt atc gtc caa tcc ggt aag att gac gcc gac ttc aac gcc acc Pro Val Ile Val Gln Ser Gly Lys Ile Asp Ala Asp Phe Asn Ala Thr		528
	165	170	175
	ttt tac tcc atg gcc aac aaa cac ttc aac gac tac gac ttt gtc tcc Phe Tyr Ser Met Ala Asn Lys His Phe Asn Asp Tyr Asp Phe Val Ser		576
15	180	185	190
	gct gaa aac gca gac gat gat ttc aag ctt tct att tac ttg ccc tcc Ala Glu Asn Ala Asp Asp Phe Lys Leu Ser Ile Tyr Leu Pro Ser		624
	195	200	205
20	gcc atg gac gag cct gta gta tac aac ggt aag aaa gcc gat atc gct Ala Met Asp Glu Pro Val Val Tyr Asn Gly Lys Ala Asp Ile Ala		672
	210	215	220
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25	225	230	235
	240		
	ttt ggt gaa atc gac ggt tcc gtt ttc gcc caa tac gtc gaa agc ggt Phe Gly Glu Ile Asp Gly Ser Val Phe Ala Gln Tyr Val Glu Ser Gly		768
	245	250	255
30	ttg cct ttg ggt tac tta ttc tac aat gac gag gaa ttg gaa gaa Leu Pro Leu Gly Tyr Leu Phe Tyr Asn Asp Glu Glu Leu Glu Glu		816
	260	265	270
35	tac aag cct ctc ttt acc gag ttg gcc aaa aag aac aga ggt cta atg Tyr Lys Pro Leu Phe Thr Glu Leu Ala Lys Lys Asn Arg Gly Leu Met		864
	275	280	285
	aac ttt gtt agc atc gat gcc aga aaa ttc ggc aga cac gcc ggc aac Asn Phe Val Ser Ile Asp Ala Arg Lys Phe Gly Arg His Ala Gly Asn		912
	290	295	300
40	ttg aac atg aag gaa caa ttc cct cta ttt gcc atc cac gac atg act Leu Asn Met Lys Glu Gln Phe Pro Ieu Phe Ala Ile His Asp Met Thr		960
	305	310	315
	320		
45	gaa gac ttg aag tac ggt ttg cct caa ctc tct gaa gag gcg ttt gac Glu Asp Leu Lys Tyr Gly Ieu Pro Gln Leu Ser Glu Glu Ala Phe Asp		1008
	325	330	335
	gaa ttg agc gac aag atc gtg ttg gag tct aag gct att gaa tct ttg Glu Leu Ser Asp Lys Ile Val Leu Glu Ser Lys Ala Ile Glu Ser Leu		1056
50	340	345	350
	gtt aag gac ttc ttg aaa ggt gat gcc tcc cca atc gtg aag tcc caa Val Lys Asp Phe Leu Lys Gly Asp Ala Ser Pro Ile Val Lys Ser Gln		1104
	355	360	365
55	gag atc ttc gag aac caa gat tcc tct gtc ttc caa ttg gtc ggt aag Glu Ile Phe Glu Asn Gln Asp Ser Ser Val Phe Gln Leu Val Gly Lys		1152
	370	375	380

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aac cat gac gaa atc gtc aac gac cca aag aag gac gtt ctt gtt ttg Asn His Asp Glu Ile Val Asn Asp Pro Lys Lys Asp Val Leu Val Leu 385 390 395 400	1200
5 tac tat gcc cca tgg tgt ggt cac tgt aag aga ttg gcc cca act tac Tyr Tyr Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala Pro Thr Tyr 405 410 415	1248
10 caa gaa cta gct gat acc tac gcc aac gcc aca tcc gac gtt ttg att Gln Glu Leu Ala Asp Thr Tyr Ala Asn Ala Thr Ser Asp Val Leu Ile 420 425 430	1296
15 gct aaa cta gac cac act gaa aac gat gtc aga ggc gtc gta att gaa Ala Lys Leu Asp His Thr Glu Asn Asp Val Arg Gly Val Val Ile Glu 435 440 445	1344
20 ggt tac cca aca atc gtc tta tac cca ggt ggt aag aag tcc gaa tct Gly Tyr Pro Thr Ile Val Leu Tyr Pro Gly Gly Lys Lys Ser Glu Ser 450 455 460	1392
25 gtt gtg tac caa ggt tca aga tcc ttg gac tct tta ttc gac ttc atc Val Val Tyr Gln Gly Ser Arg Ser Leu Asp Ser Leu Phe Asp Phe Ile 465 470 475 480	1440
30 aag gaa aac ggt cac ttc gac gtc gac ggt aag gcc ttg tac gaa gaa Lys Glu Asn Gly His Phe Asp Val Asp Gly Lys Ala Leu Tyr Glu Glu 485 490 495	1488
35 gcc cag gaa aaa gct gct gag gaa gcc gat gct gac gct gaa ttg gct Ala Gln Glu Lys Ala Ala Glu Glu Ala Asp Ala Asp Ala Glu Leu Ala 500 505 510	1536
40 gac gaa gaa gat gcc att cac gat gaa ttg taa Asp Glu Glu Asp Ala Ile His Asp Glu Leu 515 520	1569
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60 Ala Val Val Lys Leu Ala Thr Asp Ser Phe Asn Glu Tyr Ile Gln Ser 35 40 45	45
65 His Asp Leu Val Leu Ala Glu Phe Phe Ala Pro Trp Cys Gly His Cys 50 55 60	60
70 Lys Asn Met Ala Pro Glu Tyr Val Lys Ala Ala Glu Thr Leu Val Glu 65 70 75 80	80

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

5 Cys Met Glu His Asn Ile Pro Gly Phe Pro Ser Leu Lys Ile Phe Lys
100 105 110

10 Asn Ser Asp Val Asn Asn Ser Ile Asp Tyr Glu Gly Pro Arg Thr Ala
115 120 125

15 Glu Ala Ile Val Gln Phe Met Ile Lys Gln Ser Gln Pro Ala Val Ala
130 135 140

20 Val Val Ala Asp Leu Pro Ala Tyr Leu Ala Asn Glu Thr Phe Val Thr
145 150 155 160

25 Pro Val Ile Val Gln Ser Gly Lys Ile Asp Ala Asp Phe Asn Ala Thr
165 170 175

30 Phe Tyr Ser Met Ala Asn Lys His Phe Asn Asp Tyr Asp Phe Val Ser
180 185 190

35 Ala Glu Asn Ala Asp Asp Asp Phe Lys Leu Ser Ile Tyr Leu Pro Ser
195 200 205

40 Ala Met Asp Glu Pro Val Val Tyr Asn Gly Lys Lys Ala Asp Ile Ala
210 215 220

45 Asp Ala Asp Val Phe Glu Lys Trp Leu Gln Val Glu Ala Leu Pro Tyr
225 230 235 240

50 Phe Gly Glu Ile Asp Gly Ser Val Phe Ala Gln Tyr Val Glu Ser Gly
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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

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Glu Leu Ser Asp Lys Ile Val Leu Glu Ser Lys Ala Ile Glu Ser Leu
 340 345 350

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

10 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

15 Tyr Tyr Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala Pro Thr Tyr
 405 410 415

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

25 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

30 Val Val Tyr Gln Gly Ser Arg Ser Leu Asp Ser Leu Phe Asp Phe Ile
 465 470 475 480

35 Lys Glu Asn Gly His Phe Asp Val Asp Gly Lys Ala Leu Tyr Glu Glu
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45 <210> 51
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 <213> Saccharomyces cerevisiae

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55 <400> 51

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	gag tta acg cca aaa agc ttc gat aaa gcg atc cat aac aca aat tac Glu Leu Thr Pro Lys Ser Phe Asp Lys Ala Ile His Asn Thr Asn Tyr 35 40 45				144
10	aca tca tta gtg gaa ttt tat gct ccg tgg tgc ggc cat tgt aag aag Thr Ser Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys 50 55 60				192
15	ctc tct agt acg ttc cgc aag gca gca aaa aga ttg gat ggt gta gtc Leu Ser Ser Thr Phe Arg Lys Ala Ala Lys Arg Leu Asp Gly Val Val 65 70 75 80				240
	caa gtt gct gct gta aac tgt gac ctt aac aag aat aag gct ttg tgt Gln Val Ala Ala Val Asn Cys Asp Leu Asn Lys Asn Lys Ala Leu Cys 85 90 95				288
20	gct aaa tac gac gta aac gga ttt ccc acg tta atg gta ttt agg ccc Ala Lys Tyr Asp Val Asn Gly Phe Pro Thr Leu Met Val Phe Arg Pro 100 105 110				336
25	cca aaa att gac cta tct aag cca ata gat aac gcc aaa aaa agt ttc Pro Lys Ile Asp Leu Ser Lys Pro Ile Asp Asn Ala Lys Lys Ser Phe 115 120 125				384
	agc gct cat gcc aat gaa gtg tac tca ggt gca aga act ctc gcg cct Ser Ala His Ala Asn Glu Val Tyr Ser Gly Ala Arg Thr Leu Ala Pro 130 135 140				432
30	att gtt gat ttt tct ctt tca aga ata agg tca tat gtc aaa aag ttt Ile Val Asp Phe Ser Leu Ser Arg Ile Arg Ser Tyr Val Lys Lys Phe 145 150 155 160				480
35	gtc cgt ata gat aca ctt ggc tct tta ctt aga aag tca ccc aaa ctt Val Arg Ile Asp Thr Leu Gly Ser Leu Leu Arg Lys Ser Pro Lys Leu 165 170 175				528
40	tcc gtg gtg ttg ttt tcc aaa caa gac aaa att tca ccg gtt tat aaa Ser Val Val Leu Phe Ser Lys Gln Asp Lys Ile Ser Pro Val Tyr Lys 180 185 190				576
	agc att gcc ctt gat tgg tta gga aag ttc gat ttt tat tca att tca Ser Ile Ala Leu Asp Trp Leu Gly Lys Phe Asp Phe Tyr Ser Ile Ser 195 200 205				624
45	aac aaa aaa ctc aag caa cta acc gat atg aac cca aca tat gaa aaa Asn Lys Lys Leu Lys Gln Leu Thr Asp Met Asn Pro Thr Tyr Glu Lys 210 215 220				672
50	act cct gag att ttc aaa tat ttg cag aag gtc att cct gaa cag cga Thr Pro Glu Ile Phe Lys Tyr Leu Gln Lys Val Ile Pro Glu Gln Arg 225 230 235 240				720
	cag agc gat aaa agt aag ctt gtc gtt ttt gat gca gac aaa gat aaa Gln Ser Asp Lys Ser Lys Leu Val Val Phe Asp Ala Asp Lys Asp Lys 245 250 255				768
55	ttt tgg gag tat gaa ggg aac tca atc aac aaa aat gac att tcc aaa				816

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	Phe Trp Glu Tyr Glu Gly Asn Ser Ile Asn Lys Asn Asp Ile Ser Lys			
	260	265	270	
5	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	
10	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	
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	20	25	30	
30	Glu Leu Thr Pro Lys Ser Phe Asp Lys Ala Ile His Asn Thr Asn Tyr			
	35	40	45	
35	Thr Ser Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys			
	50	55	60	
40	Leu Ser Ser Thr Phe Arg Lys Ala Ala Lys Arg Leu Asp Gly Val Val			
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	Gln Val Ala Ala Val Asn Cys Asp Leu Asn Lys Asn Lys Ala Leu Cys			
	85	90	95	
45	Ala Lys Tyr Asp Val Asn Gly Phe Pro Thr Leu Met Val Phe Arg Pro			
	100	105	110	
50	Pro Lys Ile Asp Leu Ser Lys Pro Ile Asp Asn Ala Lys Lys Ser Phe			
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55	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			
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Val Arg Ile Asp Thr Leu Gly Ser Leu Leu Arg Lys Ser Pro Lys Leu
165 170 175

5 Ser Val Val Leu Phe Ser Lys Gln Asp Lys Ile Ser Pro Val Tyr Lys
180 185 190

10 Ser Ile Ala Leu Asp Trp Leu Gly Lys Phe Asp Phe Tyr Ser Ile Ser
195 200 205

15 Asn Lys Lys Leu Lys Gln Leu Thr Asp Met Asn Pro Thr Tyr Glu Lys
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Thr Pro Glu Ile Phe Lys Tyr Leu Gln Lys Val Ile Pro Glu Gln Arg
225 230 235 240

20 Gln Ser Asp Lys Ser Lys Leu Val Val Phe Asp Ala Asp Lys Asp Lys
245 250 255

25 Phe Trp Glu Tyr Glu Gly Asn Ser Ile Asn Lys Asn Asp Ile Ser Lys
260 265 270

30 Phe Leu Arg Asp Thr Phe Ser Ile Thr Pro Asn Glu Gly Pro Phe Ser
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Arg Arg Ser Glu Tyr Ile Ala Tyr Leu Lys Thr Gly Lys Lys Pro Ile
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5 ccg cta att ttg gcg cag gat tat tat gca ata cta gag ata gac aaa Pro Leu Ile Leu Ala Gln Asp Tyr Tyr Ala Ile Leu Glu Ile Asp Lys	96
20 25 30	
10 gat gcc act gag aag gaa atc aaa tca gcg tac aga caa ttg tct aag Asp Ala Thr Glu Lys Glu Ile Lys Ser Ala Tyr Arg Gln Leu Ser Lys	144
35 40 45	

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5	ttc att gaa gtc ggc gag gca tac gat gta ttg agc gat cct gaa aag Phe Ile Glu Val Gly Glu Ala Tyr Asp Val Leu Ser Asp Pro Glu Lys 65 70 75 80	240
10	aaa aag att tat gac cag ttt ggt gca gat gct gta aag aat ggc ggt Lys Lys Ile Tyr Asp Gln Phe Gly Ala Asp Ala Val Lys Asn Gly Gly 85 90 95	288
	ggc ggt ggc ggt cca gga ggc cct ggc gca ggt gga ttc cac gat ccg Gly Gly Gly Pro Gly Gly Pro Gly Ala Gly Gly Phe His Asp Pro 100 105 110	336
15	ttt gac ata ttc gaa cg ^g atg ttt caa gga ggt cat gga ggt cct ggc Phe Asp Ile Phe Glu Arg Met Phe Gln Gly Gly His Gly Pro Gly 115 120 125	384
20	ggc gga ttt ggc cag aga cag agg cag cgt ggt cca atg atc aag gtc Gly Gly Phe Gly Gln Arg Gln Arg Gln Arg Gly Pro Met Ile Lys Val 130 135 140	432
25	cag gaa aaa cta tct tta aag cag ttt tat tcc ggg tcc tcg ata gaa Gln Glu Lys Leu Ser Leu Lys Gln Phe Tyr Ser Gly Ser Ser Ile Glu 145 150 155 160	480
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30	tct gca gat ggt aag ctg gcc caa tgt ccc gat tgt caa ggt cgt ggg Ser Ala Asp Gly Lys Leu Ala Gln Cys Pro Asp Cys Gln Gly Arg Gly 180 185 190	576
35	gtt ata ata caa gtg ctg cgc atg ggt att atg acg cag cag att caa Val Ile Ile Gln Val Leu Arg Met Gly Ile Met Thr Gln Gln Ile Gln 195 200 205	624
	cag atg tgt ggt agg tgt ggt acg gga caa att atc aaa aat gaa Gln Met Cys Gly Arg Cys Gly Thr Gly Gln Ile Ile Lys Asn Glu 210 215 220	672
40	tgc aaa aca tgt cac ggc aaa aaa gtt acc aaa aag aac aag ttc ttc Cys Lys Thr Cys His Gly Lys Lys Val Thr Lys Lys Asn Lys Phe Phe 225 230 235 240	720
45	cac gtt gac gtt cca cca ggc gca cca aga aac tac atg gac aca aga His Val Asp Val Pro Pro Gly Ala Pro Arg Asn Tyr Met Asp Thr Arg 245 250 255	768
	gtc ggc gag gct gaa aaa ggg cct gac ttt gac gcc ggt gac ttg gtc Val Gly Glu Ala Glu Lys Gly Pro Asp Phe Asp Ala Gly Asp Leu Val 260 265 270	816
50	ata gaa ttc aag gaa aag gat act gag aac atg ggt tac aga aga aga Ile Glu Phe Lys Glu Lys Asp Thr Glu Asn Met Gly Tyr Arg Arg Arg 275 280 285	864
55	ggc gac aat ctg tac aga aca gaa gtt ctt tct gct gcg gaa gcg cta Gly Asp Asn Leu Tyr Arg Thr Glu Val Leu Ser Ala Ala Glu Ala Leu 290 295 300	912

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5	tac ggc gga tgg caa aga acg ata gaa ttc ctt gat gag aac aag ccc Tyr Gly Gly Trp Gln Arg Thr Ile Glu Phe Leu Asp Glu Asn Lys Pro 305 310 315 320	960
10	gtt aag tta tct aga ccc gct cat gta gtt gtc tcc aat ggc gaa gtt Val Lys Leu Ser Arg Pro Ala His Val Val Ser Asn Gly Glu Val 325 330 335	1008
15	gaa gtc gtg aag gga ttc ggc atg ccc aag ggt agc aag ggt tac ggt Glu Val Val Lys Gly Phe Gly Met Pro Lys Gly Ser Lys Gly Tyr Gly 340 345 350	1056
20	gat ttg tac ata gac tac gtc gtt gtc atg cca aag act ttc aaa tct Asp Leu Tyr Ile Asp Tyr Val Val Met Pro Lys Thr Phe Lys Ser 355 360 365	1104
25	ggg caa aat atg ctc aaa gat gag ttg tag Gly Gln Asn Met Leu Lys Asp Glu Leu 370 375	1134
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45	Pro Leu Ile Leu Ala Gln Asp Tyr Tyr Ala Ile Leu Glu Ile Asp Lys 20 25 30	
50	Asp Ala Thr Glu Lys Glu Ile Lys Ser Ala Tyr Arg Gln Leu Ser Lys 35 40 45	
55	Lys Tyr His Pro Asp Lys Asn Ala Gly Ser Glu Glu Ala His Gln Lys 50 55 60	
60	Phe Ile Glu Val Gly Glu Ala Tyr Asp Val Leu Ser Asp Pro Glu Lys 65 70 75 80	
65	Lys Lys Ile Tyr Asp Gln Phe Gly Ala Asp Ala Val Lys Asn Gly Gly 85 90 95	
70	Gly Gly Gly Pro Gly Gly Pro Gly Ala Gly Gly Phe His Asp Pro 100 105 110	
75	Phe Asp Ile Phe Glu Arg Met Phe Gln Gly Gly His Gly Gly Pro Gly 115 120 125	
80	Gly Gly Phe Gly Gln Arg Gln Arg Gln Arg Gly Pro Met Ile Lys Val 130 135 140	

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Gln Glu Lys Leu Ser Leu Lys Gln Phe Tyr Ser Gly Ser Ser Ile Glu
 145 150 155 160

5 Phe Thr Leu Asn Leu Asn Asp Glu Cys Asp Ala Cys His Gly Ser Gly
 165 170 175

10 Ser Ala Asp Gly Lys Leu Ala Gln Cys Pro Asp Cys Gln Gly Arg Gly
 180 185 190

15 Val Ile Ile Gln Val Leu Arg Met Gly Ile Met Thr Gln Gln Ile Gln
 195 200 205

20 Gln Met Cys Gly Arg Cys Gly Gly Thr Gly Gln Ile Ile Lys Asn Glu
 210 215 220

25 Cys Lys Thr Cys His Gly Lys Lys Val Thr Lys Lys Asn Lys Phe Phe
 225 230 235 240

30 His Val Asp Val Pro Pro Gly Ala Pro Arg Asn Tyr Met Asp Thr Arg
 245 250 255

35 Val Gly Glu Ala Glu Lys Gly Pro Asp Phe Asp Ala Gly Asp Leu Val
 260 265 270

40 Ile Glu Phe Lys Glu Lys Asp Thr Glu Asn Met Gly Tyr Arg Arg Arg
 275 280 285

45 Gly Asp Asn Leu Tyr Arg Thr Glu Val Leu Ser Ala Ala Glu Ala Leu
 290 295 300

50 Tyr Gly Gly Trp Gln Arg Thr Ile Glu Phe Leu Asp Glu Asn Lys Pro
 305 310 315 320

55 Val Lys Leu Ser Arg Pro Ala His Val Val Val Ser Asn Gly Glu Val
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Glu Val Val Lys Gly Phe Gly Met Pro Lys Gly Ser Lys Gly Tyr Gly
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Gly Gln Asn Met Leu Lys Asp Glu Leu
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<210> 55
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<400> 55

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5	tct gca act tca aac aat agc tac atc gcc acc gac caa aca caa aat Ser Ala Thr Ser Asn Asn Ser Tyr Ile Ala Thr Asp Gln Thr Gln Asn 20 25 30	96
10	gcc ttt aat gac act cac ttt tgt aag gtc gac agg aat gat cac gtt Ala Phe Asn Asp Thr His Phe Cys Lys Val Asp Arg Asn Asp His Val 35 40 45	144
15	agt ccc agt tgt aac gta aca ttc aat gaa tta aat gcc ata aat gaa Ser Pro Ser Cys Asn Val Thr Phe Asn Glu Leu Asn Ala Ile Asn Glu 50 55 60	192
20	aac att aga gat gat ctt tcg gcg tta tta aaa tct gat ttc ttc aaa Asn Ile Arg Asp Asp Leu Ser Ala Leu Leu Lys Ser Asp Phe Phe Lys 65 70 75 80	240
25	tac ttt cgg ctg gat tta tac aag caa tgt tca ttt tgg gac gcc aac Tyr Phe Arg Leu Asp Leu Tyr Lys Gln Cys Ser Phe Trp Asp Ala Asn 85 90 95	288
30	gat ggt ctg tgc tta aac cgc gct tgc tct gtt gat gtc gta gag gac Asp Gly Leu Cys Leu Asn Arg Ala Cys Ser Val Asp Val Val Glu Asp 100 105 110	336
35	tgg gat aca ctg cct gag tac tgg cag cct gag atc ttg ggt agt ttc Trp Asp Thr Leu Pro Glu Tyr Trp Gln Pro Glu Ile Leu Gly Ser Phe 115 120 125	384
40	aat aat gat aca atg aag gaa gcg gat gat agc gat gac gaa tgt aag Asn Asn Asp Thr Met Lys Glu Ala Asp Asp Ser Asp Asp Glu Cys Lys 130 135 140	432
45	ttc tta gat caa cta tgt caa acc agt aaa aaa cct gta gat atc gaa Phe Leu Asp Gln Leu Cys Gln Thr Ser Lys Lys Pro Val Asp Ile Glu 145 150 155 160	480
50	gac acc atc aac tac tgt gat gta aat gac ttt aac ggt aaa aac gcc Asp Thr Ile Asn Tyr Cys Asp Val Asn Asp Phe Asn Gly Lys Asn Ala 165 170 175	528
	gtt ctg att gat tta aca gca aat ccg gaa cga ttt aca ggt tat ggt Val Leu Ile Asp Leu Thr Ala Asn Pro Glu Arg Phe Thr Gly Tyr Gly 180 185 190	576
	ggt aag caa gct ggt caa att tgg tct act atc tac caa gac aac tgt Gly Lys Gln Ala Gly Gln Ile Trp Ser Thr Ile Tyr Gln Asp Asn Cys 195 200 205	624
	ttt aca att ggc gaa act ggt gaa tca ttg gcc aaa gat gca ttt tat	672

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	Phe Thr Ile Gly Glu Thr Gly Glu Ser Leu Ala Lys Asp Ala Phe Tyr		
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225	230	235	240
10	gaa tat ttg aac acg aaa act ggt aaa tgg gag ccc aat ctg gat ttg Glu Tyr Leu Asn Thr Lys Thr Gly Lys Trp Glu Pro Asn Leu Asp Leu		768
	245	250	255
15	ttt atg gca aga atc ggg aac ttt cct gat aga gtg aca aac atg tat Phe Met Ala Arg Ile Gly Asn Phe Pro Asp Arg Val Thr Asn Met Tyr		816
	260	265	270
20	ttc aat tat gct gtt gta gct aag gct ctc tgg aaa att caa cca tat Phe Asn Tyr Ala Val Val Ala Lys Ala Leu Trp Lys Ile Gln Pro Tyr		864
	275	280	285
25	tta cca gaa ttt tca ttc tgt gat cta gtc aat aaa gaa atc aaa aac Leu Pro Glu Phe Ser Phe Cys Asp Leu Val Asn Lys Glu Ile Lys Asn		912
	290	295	300
	aaa atg gat aac gtt att tcc cag ctg gac aca aaa att ttt aac gaa Lys Met Asp Asn Val Ile Ser Gln Leu Asp Thr Lys Ile Phe Asn Glu		960
	305	310	315
30	gac tta gtt ttt gcc aac gac cta agt ttg act ttg aag gac gaa ttc Asp Leu Val Phe Ala Asn Asp Leu Ser Leu Thr Leu Lys Asp Glu Phe		1008
	325	330	335
35	aga tct cgc ttc aag aat gtc acg aag att atg gat tgt gtg caa tgt Arg Ser Arg Phe Lys Asn Val Thr Lys Ile Met Asp Cys Val Gln Cys		1056
	340	345	350
	gat aga tgt aga ttg tgg ggc aaa att caa act acc ggt tac gca act Asp Arg Cys Arg Leu Trp Gly Lys Ile Gln Thr Thr Gly Tyr Ala Thr		1104
	355	360	365
40	gcc ttg aaa att ttg ttt gaa atc aac gac gct gat gaa ttc acc aaa Ala Leu Lys Ile Leu Phe Glu Ile Asn Asp Ala Asp Glu Phe Thr Lys		1152
	370	375	380
45	caa cat att gtt ggt aag tta acc aaa tat gag ttg att gca cta tta Gln His Ile Val Gly Lys Leu Thr Lys Tyr Glu Leu Ile Ala Leu Leu		1200
	385	390	395
	395	400	
	cag act ttc ggt aga tta tct gaa tct att gaa tct gtt aac atg ttc Gln Thr Phe Gly Arg Leu Ser Glu Ser Ile Glu Ser Val Asn Met Phe		1248
	405	410	415
50	gaa aaa atg tac ggg aaa agg tta aac ggt tct gaa aac agg tta agc Glu Lys Met Tyr Gly Lys Arg Leu Asn Gly Ser Glu Asn Arg Leu Ser		1296
	420	425	430
	430	435	
55	tca ttc ttc caa aat aac ttc ttc aac att ttg aag gag gca ggc aaa Ser Phe Phe Gln Asn Asn Phe Phe Asn Ile Leu Lys Glu Ala Gly Lys		1344
	440	445	
	445	450	
	450	455	
	455	460	

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5	ccc aaa gca gaa ata gtt cca agg ccc tct aac ggt aca gta aat aaa Pro Lys Ala Glu Ile Val Pro Arg Pro Ser Asn Gly Thr Val Asn Lys 485 490 495	1488
10	tgg aag aaa gct tgg aat act gaa gtt aac aac gtt tta gaa gca ttc Trp Lys Lys Ala Trp Asn Thr Glu Val Asn Asn Val Leu Glu Ala Phe 500 505 510	1536
15	aga ttt att tat aga agc tat ttg gat tta ccc agg aac atc tgg gaa Arg Phe Ile Tyr Arg Ser Tyr Leu Asp Leu Pro Arg Asn Ile Trp Glu 515 520 525	1584
20	tta tct ttg atg aag gta tac aaa ttt tgg aat aaa ttc atc ggt gtt Leu Ser Leu Met Lys Val Tyr Lys Phe Trp Asn Lys Phe Ile Gly Val 530 535 540	1632
25	gct gat tac gtt agt gag gag aca cga gag cct att tcc tat aag cta Ala Asp Tyr Val Ser Glu Glu Thr Arg Glu Pro Ile Ser Tyr Lys Leu 545 550 555 560	1680
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40	Ser Ala Thr Ser Asn Asn Ser Tyr Ile Ala Thr Asp Gln Thr Gln Asn 20 25 30	
45	Ala Phe Asn Asp Thr His Phe Cys Lys Val Asp Arg Asn Asp His Val 35 40 45	
50	Ser Pro Ser Cys Asn Val Thr Phe Asn Glu Leu Asn Ala Ile Asn Glu 50 55 60	
55	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	

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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
5 130 135 140

Phe Leu Asp Gln Leu Cys Gln Thr Ser Lys Lys Pro Val Asp Ile Glu
10 145 150 155 160

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
20 195 200 205

Phe Thr Ile Gly Glu Thr Gly Glu Ser Leu Ala Lys Asp Ala Phe Tyr
25 210 215 220

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
30 245 250 255

Phe Met Ala Arg Ile Gly Asn Phe Pro Asp Arg Val Thr Asn Met Tyr
35 260 265 270

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
40 290 295 300

Lys Met Asp Asn Val Ile Ser Gln Leu Asp Thr Lys Ile Phe Asn Glu
45 305 310 315 320

Asp Leu Val Phe Ala Asn Asp Leu Ser Leu Thr Leu Lys Asp Glu Phe
50 325 330 335

Arg Ser Arg Phe Lys Asn Val Thr Lys Ile Met Asp Cys Val Gln Cys
55 340 345 350

Asp Arg Cys Arg Leu Trp Gly Lys Ile Gln Thr Thr Gly Tyr Ala Thr
355 360 365

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Ala Leu Lys Ile Leu Phe Glu Ile Asn Asp Ala Asp Glu Phe Thr Lys
370 375 380

5 Gln His Ile Val Gly Lys Leu Thr Lys Tyr Glu Leu Ile Ala Leu Leu
385 390 395 400

10 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

15 Ser Phe Phe Gln Asn Asn Phe Phe Asn Ile Leu Lys Glu Ala Gly Lys
435 440 445

20 Ser Ile Arg Tyr Thr Ile Glu Asn Ile Asn Ser Thr Lys Glu Gly Lys
450 455 460

25 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

30 Trp Lys Lys Ala Trp Asn Thr Glu Val Asn Asn Val Leu Glu Ala Phe
500 505 510

35 Arg Phe Ile Tyr Arg Ser Tyr Leu Asp Leu Pro Arg Asn Ile Trp Glu
515 520 525

40 Leu Ser Leu Met Lys Val Tyr Lys Phe Trp Asn Lys Phe Ile Gly Val
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10	gta gaa gat tgc tta att aag gca atg cca ggt gat aaa gtt aag gtt Val Glu Asp Cys Leu Ile Lys Ala Met Pro Gly Asp Lys Val Lys Val 35 40 45	144
15	cat tat aca gga tct tta gaa tcg gga act gta ttt gac tca agt His Tyr Thr Gly Ser Leu Leu Glu Ser Gly Thr Val Phe Asp Ser Ser 50 55 60	192
	tat tca aga ggc tct cct atc gct ttt gaa ctt ggc gtt ggc aga gta Tyr Ser Arg Gly Ser Pro Ile Ala Phe Glu Leu Gly Val Gly Arg Val 65 70 75 80	240
20	att aaa ggt tgg gat caa ggt gtt gcc ggc atg tgc gtt ggc gaa aaa Ile Lys Gly Trp Asp Gln Gly Val Ala Gly Met Cys Val Gly Glu Lys 85 90 95	288
25	aga aag ctg caa att cca agt tct ttg gcc tac gga gaa aga ggt gtc Arg Lys Leu Gln Ile Pro Ser Ser Leu Ala Tyr Gly Glu Arg Gly Val 100 105 110	336
30	cca ggc gtc att cct cca agt gct gat ttg gtg ttt gat gtc gaa ttg Pro Gly Val Ile Pro Pro Ser Ala Asp Leu Val Phe Asp Val Glu Leu 115 120 125	384
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50	Val Glu Asp Cys Leu Ile Lys Ala Met Pro Gly Asp Lys Val Lys Val 35 40 45	
55	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	

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65

70

75

80

5 Ile Lys Gly Trp Asp Gln Gly Val Ala Gly Met Cys Val Gly Glu Lys
85 90 95

10 Arg Lys Leu Gln Ile Pro Ser Ser Leu Ala Tyr Gly Glu Arg Gly Val
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15 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
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	20	25
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10	cga ttg aac aag aat tta aaa gta gac act gaa tcc ttg cca aaa tac Arg Leu Asn Lys Asn Leu Lys Val Asp Thr Glu Ser Leu Pro Lys Tyr	144
	35	40
	45	
15	caa tgg atc gct ggg cag ttg gaa caa aac tgc atg act gcg gat cca Gln Trp Ile Ala Gly Gln Leu Glu Gln Asn Cys Met Thr Ala Asp Pro	192
	50	55
	60	
	gca agt gaa aat atg tca gac gta att caa cta gcc aat caa ata tac Ala Ser Glu Asn Met Ser Asp Val Ile Gln Leu Ala Asn Gln Ile Tyr	240
	65	70
	75	80
20	tac aaa att ggg ctg atc caa tta tcc aac gat caa cat cta aga gct Tyr Lys Ile Gly Leu Ile Gln Leu Ser Asn Asp Gln His Leu Arg Ala	288
	85	90
	95	
25	att aac aca ttt gaa aaa atc gtt ttt aat gaa act tac aaa ggt tct Ile Asn Thr Phe Glu Lys Ile Val Phe Asn Glu Thr Tyr Lys Gly Ser	336
	100	105
	110	
30	ttt ggg aag ctg gcg gaa aag agg cta caa gag ctg tat gtc gat ttt Phe Gly Lys Leu Ala Glu Lys Arg Leu Gln Glu Leu Tyr Val Asp Phe	384
	115	120
	125	
	ggg atg tgg gac aag gtg cat cag aag gat gat cag tat gcg aaa tat	432
35		
40		
45		
50		
55		

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	Gly Met Trp Asp Lys Val His Gln Lys Asp Asp Gln Tyr Ala Lys Tyr			
	130	135	140	
5	ctg tcc ttg aat gaa acc atc aga aac aaa ata tca tcc aaa gac gtt Leu Ser Ile Asn Glu Thr Ile Arg Asn Lys Ile Ser Ser Lys Asp Val			480
	145	150	155	160
10	tct gtg gag gaa gat att tct gag ctg cta cgc ata acg ccg tac gat Ser Val Glu Glu Asp Ile Ser Glu Leu Leu Arg Ile Thr Pro Tyr Asp			528
	165	170	175	
15	gtt aac gtc ctc tcc acg cac atc gat gtt ctt ttt cac aaa cta gct Val Asn Val Leu Ser Thr His Ile Asp Val Leu Phe His Lys Leu Ala			576
	180	185	190	
20	gaa gaa att gac gtt tcg tta gct gct gct atc att ttg gat tac gaa Glu Glu Ile Asp Val Ser Leu Ala Ala Ile Ile Leu Asp Tyr Glu			624
	195	200	205	
25	aca atc ctc gac aag cat ttg gct agc tta agc ata gat aca aga ctt Thr Ile Leu Asp Lys His Leu Ala Ser Leu Ser Ile Asp Thr Arg Leu			672
	210	215	220	
30	tcg att cat tat gtc ata tct gtt tta cag acc ttt gta ctt aac tca Ser Ile His Tyr Val Ile Ser Val Leu Gln Thr Phe Val Leu Asn Ser			720
	225	230	235	240
35	gat gcg tcg ttc aat ata aga aaa tgc ctt tcc att gat atg gac tat Asp Ala Ser Phe Asn Ile Arg Lys Cys Leu Ser Ile Asp Met Asp Tyr			768
	245	250	255	
40	gat aaa tgt aaa aaa cta agc ctg act att tcc aaa ttg aac aag gtg Asp Lys Cys Lys Lys Leu Ser Leu Thr Ile Ser Lys Leu Asn Lys Val			816
	260	265	270	
45	aat cca tca aaa aga cag atc ctg gat cca gca aca tat gca ttt gag Asn Pro Ser Lys Arg Gln Ile Leu Asp Pro Ala Thr Tyr Ala Phe Glu			864
	275	280	285	
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	290	295	300	
55	gat aag aag cca ttt att aca cca atg aaa att ctt aac aaa gat aca Asp Lys Lys Pro Phe Ile Thr Pro Met Lys Ile Leu Asn Lys Asp Thr			960
	305	310	315	320
60	aac ttt aaa aac aac tac ttc ttt tta gag gaa att atc aaa caa ttg Asn Phe Lys Asn Asn Tyr Phe Phe Ile Glu Glu Ile Ile Lys Gln Leu			1008
	325	330	335	
65	ata gaa gac gtt caa ctg tcg aga cct ttg gca aaa aat tta ttc gaa Ile Glu Asp Val Gln Leu Ser Arg Pro Leu Ala Lys Asn Leu Phe Glu			1056
	340	345	350	
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	355	360	365	
75	acc gat tat cta gta tac att gat tcc att ctt tgt cag gct tct agc Thr Asp Tyr Leu Val Tyr Ile Asp Ser Ile Leu Cys Gln Ala Ser Ser			1152
	370	375	380	

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	atg agt ccg gac gtc aag aga gct aaa ctg gct gcg ccg ttc tgt aaa Met Ser Pro Asp Val Lys Arg Ala Lys Leu Ala Ala Pro Phe Cys Lys 385 390 395 400	1200
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	gta tgg aat tcc aat cct cat ttg ctg atg tat atg gta aac tca ata Val Trp Asn Ser Asn Pro His Leu Leu Met Tyr Met Val Asn Ser Ile 435 440 445	1344
15	ctt aat aaa agt agg tct aaa cct cat tca cag ttc aaa aag caa tta Leu Asn Lys Ser Arg Ser Lys Pro His Ser Gln Phe Lys Lys Gln Leu 450 455 460	1392
20	tat gac cag ata aac aaa ttt ttc caa gat aac ggc ctc tca gag tcg Tyr Asp Gln Ile Asn Lys Phe Phe Gln Asp Asn Gly Leu Ser Glu Ser 465 470 475 480	1440
25	acc aat cca tac gtg atg aag aac ttc cga tta tta cag aaa caa tta Thr Asn Pro Tyr Val Met Lys Asn Phe Arg Leu Leu Gln Lys Gln Leu 485 490 495	1488
	caa acc tat aaa gag cat aaa cat cgg aat ttc aac cag caa tat ttc Gln Thr Tyr Lys Glu His Lys His Arg Asn Phe Asn Gln Gln Tyr Phe 500 505 510	1536
30	caa caa caa cag cag caa caa cac caa cga cat caa gca ccc cca Gln Gln Gln Gln Gln Gln His Gln Arg His Gln Ala Pro Pro 515 520 525	1584
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20 Arg Leu Asn Lys Asn Leu Lys Val Asp Thr Glu Ser Leu Pro Lys Tyr
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25 Gln Trp Ile Ala Gly Gln Leu Glu Gln Asn Cys Met Thr Ala Asp Pro
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Ala Ser Glu Asn Met Ser Asp Val Ile Gln Leu Ala Asn Gln Ile Tyr
 65 70 75 80

30 Tyr Lys Ile Gly Leu Ile Gln Leu Ser Asn Asp Gln His Leu Arg Ala
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35 Ile Asn Thr Phe Glu Lys Ile Val Phe Asn Glu Thr Tyr Lys Gly Ser
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40 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

45 Leu Ser Leu Asn Glu Thr Ile Arg Asn Lys Ile Ser Ser Lys Asp Val
 145 150 155 160

50 Ser Val Glu Glu Asp Ile Ser Glu Leu Leu Arg Ile Thr Pro Tyr Asp
 165 170 175

55 Val Asn Val Leu Ser Thr His Ile Asp Val Leu Phe His Lys Leu Ala
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Glu Glu Ile Asp Val Ser Leu Ala Ala Ala Ile Ile Leu Asp Tyr Glu
 195 200 205

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Thr Ile Leu Asp Lys His Leu Ala Ser Leu Ser Ile Asp Thr Arg Leu
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5 Ser Ile His Tyr Val Ile Ser Val Leu Gln Thr Phe Val Leu Asn Ser
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10 Asp Ala Ser Phe Asn Ile Arg Lys Cys Leu Ser Ile Asp Met Asp Tyr
245 250 255

15 Asp Lys Cys Lys Lys Leu Ser Leu Thr Ile Ser Lys Leu Asn Lys Val
260 265 270

20 Asn Pro Ser Lys Arg Gln Ile Leu Asp Pro Ala Thr Tyr Ala Phe Glu
275 280 285

25 Asn Lys Lys Phe Arg Ser Trp Asp Arg Ile Ile Glu Phe Tyr Leu Lys
290 295 300

30 Asp Lys Lys Pro Phe Ile Thr Pro Met Lys Ile Leu Asn Lys Asp Thr
305 310 315 320

35 Asn Phe Lys Asn Asn Tyr Phe Phe Leu Glu Glu Ile Ile Lys Gln Leu
325 330 335

40 Ile Glu Asp Val Gln Leu Ser Arg Pro Leu Ala Lys Asn Leu Phe Glu
340 345 350

45 Asp Pro Pro Ile Thr Asp Gly Phe Val Lys Pro Lys Ser Tyr Tyr His
355 360 365

50 Thr Asp Tyr Leu Val Tyr Ile Asp Ser Ile Leu Cys Gln Ala Ser Ser
370 375 380

55 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

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450

455

460

5 Tyr Asp Gln Ile Asn Lys Phe Phe Gln Asp Asn Gly Leu Ser Glu Ser
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10 Thr Asn Pro Tyr Val Met Lys Asn Phe Arg Leu Leu Gln Lys Gln Leu
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15 Gln Thr Tyr Lys Glu His Lys His Arg Asn Phe Asn Gln Gln Tyr Phe
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20 Gln Gln Gln Gln Gln Gln His Gln Arg His Gln Ala Pro Pro
 515 520 525

25 Ala Ala Pro Asn Tyr Asp Pro Lys Lys Asp Tyr Tyr Lys Ile Leu Gly
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30 Val Ser Pro Ser Ala Ser Ser Lys Glu Ile Arg Lys Ala Tyr Leu Asn
 545 550 555 560

35 Leu Thr Lys Lys Tyr His Pro Asp Lys Ile Lys Ala Asn His Asn Asp
 565 570 575

40 Lys Gln Glu Ser Ile His Glu Thr Met Ser Gln Ile Asn Glu Ala Tyr
 580 585 590

45 Glu Thr Leu Ser Asp Asp Asp Lys Arg Lys Glu Tyr Asp Leu Ser Arg
 595 600 605

50 Ser Asn Pro Arg Arg Asn Thr Phe Pro Gln Gly Pro Arg Gln Asn Asn
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	20	25	30	
10	aag gcc att gtg gtt tct ccg caa gcc cca tta gaa ctt gtg ctc aca Lys Ala Ile Val Val Ser Pro Gln Ala Pro Leu Glu Leu Val Leu Thr		144	
	35	40	45	
15	cca gag gca aaa cgg aag gag ata tct ggt ctt tcg ata aaa aga tta Pro Glu Ala Lys Arg Lys Glu Ile Ser Gly Ile Ser Ile Lys Arg Leu		192	
	50	55	60	
20	cca ggt tat gga aag gat gat ccg aat ggg att gaa aga atc tac ggt Pro Gly Tyr Gly Lys Asp Asp Pro Asn Gly Ile Glu Arg Ile Tyr Gly		240	
	65	70	75	80
25	tcc gct gtt ggc agt tta gca aca agg ttt ccc caa aac aca ttg ttg Ser Ala Val Gly Ser Leu Ala Thr Arg Phe Pro Gln Asn Thr Leu Leu		288	
	85	90	95	
30	cat ttg aaa ccg cta ctt ggg aaa tca cta gaa gat gaa acc act gta His Leu Lys Pro Leu Leu Gly Lys Ser Leu Glu Asp Glu Thr Thr Val		336	
	100	105	110	
35	act ttg tat tca aaa caa cac ccc ggt tta gaa atg gta tca aca aat Thr Leu Tyr Ser Lys Gln His Pro Gly Leu Glu Met Val Ser Thr Asn		384	
	115	120	125	
40	aga agt acc ata gcc ttt tta gtt gat aat gtg gaa tat cca ttg gaa Arg Ser Thr Ile Ala Phe Leu Val Asp Asn Val Glu Tyr Pro Leu Glu		432	
	130	135	140	
45	gag tta gtg gca atg aat gtc caa gag att gcc aat aga gcc aat tca Glu Leu Val Ala Met Asn Val Gln Glu Ile Ala Asn Arg Ala Asn Ser		480	
	145	150	155	160
50	ctg ttg aag gat aga gat gca aga act gag gac ttt gta aac aag atg Leu Leu Lys Asp Arg Asp Ala Arg Thr Glu Asp Phe Val Asn Lys Met		528	
	165	170	175	
55	agt ttt aca att cct gac ttt ttt gac caa cat caa agg aaa gca ctt Ser Phe Thr Ile Pro Asp Phe Asp Gln His Gln Arg Lys Ala Leu		576	
	180	185	190	
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	195	200	205	
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	210	215	220	
70	ttt cca cca ggt gaa cag cag cat tat atc gta tat gac atg ggg agc Phe Pro Pro Gly Glu Gln Gln His Tyr Ile Val Tyr Asp Met Gly Ser		720	
	225	230	240	
75	ggt tct att aag gcc tca atg ttc tct ata ttg cag ccg gag gac act Gly Ser Ile Lys Ala Ser Met Phe Ser Ile Leu Gln Pro Glu Asp Thr		768	
	245	250	255	

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	act cag ccc gtt aca ata gaa ttt gaa gga tat ggg tat aat cca cat Thr Gln Pro Val Thr Ile Glu Phe Glu Gly Tyr Gly Tyr Asn Pro His 260 265 270	816
5	cta ggt ggt gca aag ttt aca atg gat att ggc agt ttg ata gag aat Leu Gly Gly Ala Lys Phe Thr Met Asp Ile Gly Ser Leu Ile Glu Asn 275 280 285	864
10	aag ttt ttg gaa aca cac cca gcc ata aga act gat gaa ttg cac gct Lys Phe Leu Glu Thr His Pro Ala Ile Arg Thr Asp Glu Leu His Ala 290 295 300	912
15	aat ccc aag gcc tta gca aaa atc aac caa gca gca gag aag gca aag Asn Pro Lys Ala Leu Ala Lys Ile Asn Gln Ala Ala Glu Lys Ala Lys 305 310 315 320	960
20	tta att tta agc gcc aat tct gag gca agt att aac ata gaa tca ctg Leu Ile Leu Ser Ala Asn Ser Glu Ala Ser Ile Asn Ile Glu Ser Leu 325 330 335	1008
25	atc aac gat att gat ttc cgt act tct ata act aga cag gaa ttc gaa Ile Asn Asp Ile Asp Phe Arg Thr Ser Ile Thr Arg Gln Glu Phe Glu 340 345 350	1056
30	gaa ttt att gca gac tcg tta ttg gac att gtc aaa ccc ata aat gac Glu Phe Ile Ala Asp Ser Leu Leu Asp Ile Val Lys Pro Ile Asn Asp 355 360 365	1104
35	gct gtt aca aaa caa ttc ggt ggc tat gga aca aat tta cct gag ata Ala Val Thr Lys Gln Phe Gly Gly Tyr Gly Thr Asn Leu Pro Glu Ile 370 375 380	1152
40	aat ggg gtc att ttg gcg gga ggc tct tcc cga att ccc att gtg cag Asn Gly Val Ile Leu Ala Gly Gly Ser Ser Arg Ile Pro Ile Val Gln 385 390 395 400	1200
45	gat caa tta atc aaa ctc gta tcc gaa gaa aaa gtg ttg aga aat gtc Asp Gln Leu Ile Lys Leu Val Ser Glu Glu Lys Val Leu Arg Asn Val 405 410 415	1248
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60	gta aat act tat tca ttc aaa tta tca aac gaa tct gaa ctg tat gat Val Asn Thr Tyr Ser Phe Lys Leu Ser Asn Glu Ser Glu Leu Tyr Asp 450 455 460	1392
65	gtg ttc acg cgc gga agt gct tat cca aac aaa aca tct att ttg aca Val Phe Thr Arg Gly Ser Ala Tyr Pro Asn Lys Thr Ser Ile Leu Thr 465 470 475 480	1440
70	aac acg act gat tcg att cct aat aat ttt acc att gac tta ttt gag Asn Thr Thr Asp Ser Ile Pro Asn Asn Phe Thr Ile Asp Leu Phe Glu 485 490 495	1488
75	aat ggt aaa ttg ttc gaa act atc aca gtt aat tca gga gct ata aag Asn Gly Lys Leu Phe Glu Thr Ile Thr Val Asn Ser Gly Ala Ile Lys 500 505 510	1536

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	aat tca tat tcc tct gat aag tgc tcg tca gga gtt gcg tat aac att Asn Ser Tyr Ser Ser Asp Lys Cys Ser Ser Gly Val Ala Tyr Asn Ile 515 520 525	1584
5	act ttc gac ttg tcc agt gat aga tta ttc tct att caa gag gtt aac Thr Phe Asp Leu Ser Ser Asp Arg Ile Phe Ser Ile Gln Glu Val Asn 530 535 540	1632
10	tgc att tgt cag agc gaa aat gac ata ggt aac tcc aag caa att aag Cys Ile Cys Gln Ser Glu Asn Asp Ile Gly Asn Ser Lys Gln Ile Lys 545 550 555 560	1680
15	aac aaa ggc agc cgt ttg gct ttt act tct gag gat gtt gag atc aaa Asn Lys Gly Ser Arg Leu Ala Phe Thr Ser Glu Asp Val Glu Ile Lys 565 570 575	1728
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25	ctc gat aaa cag gat aag gaa aga ttt caa ttc caa gaa aat tta aac Leu Asp Lys Gln Asp Lys Glu Arg Phe Gln Phe Gln Glu Asn Leu Asn 595 600 605	1824
30	gtt ctt gaa agt aac ttg tat gat gct aga aac ctg cta atg gat gat Val Leu Glu Ser Asn Leu Tyr Asp Ala Arg Asn Leu Leu Met Asp Asp 610 615 620	1872
35	gaa gtt atg caa aat gga cca aaa tcc caa gta gaa gag tta tcg gag Glu Val Met Gln Asn Gly Pro Lys Ser Gln Val Glu Glu Leu Ser Glu 625 630 635 640	1920
40	atg gtt aaa gta tat ttg gat tgg ctc gaa gat gca tcc ttt gat act Met Val Lys Val Tyr Leu Asp Trp Leu Glu Asp Ala Ser Phe Asp Thr 645 650 655	1968
45	gac cct gag gat ata gtt agc aga att aga gaa att gga ata tta aaa Asp Pro Glu Asp Ile Val Ser Arg Ile Arg Glu Ile Gly Ile Leu Lys 660 665 670	2016
50	aag aaa ata gaa ctt tac atg gat tct gca aag gaa cct ttg aac tct Lys Lys Ile Glu Leu Tyr Met Asp Ser Ala Lys Glu Pro Leu Asn Ser 675 680 685	2064
55	caa caa ttt aaa gga atg ctt gaa gaa ggc cat aag tta ctt cag gct Gln Gln Phe Lys Gly Met Ile Glu Glu Gly His Lys Leu Leu Gln Ala 690 695 700	2112
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15	cat tta aga aac ttc cgc cta caa aag aga aag gag gaa aag ttg aaa His Leu Arg Asn Phe Arg Leu Gln Lys Arg Lys Glu Glu Lys Leu Lys 820 825 830			2496
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25	acc acc gag tcg aat cca agt tct gag gaa gac att ttg cat gat gaa Thr Thr Glu Ser Asn Pro Ser Ser Glu Glu Asp Ile Leu His Asp Glu 865 870 875 880			2640
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10 Lys Ala Ile Val Val Ser Pro Gln Ala Pro Leu Glu Leu Val Leu Thr
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Pro Gly Tyr Gly Lys Asp Asp Pro Asn Gly Ile Glu Arg Ile Tyr Gly
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85 90 95

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His Leu Lys Pro Leu Leu Gly Lys Ser Leu Glu Asp Glu Thr Thr Val
100 105 110

5 Thr Leu Tyr Ser Lys Gln His Pro Gly Leu Glu Met Val Ser Thr Asn
115 120 125

10 Arg Ser Thr Ile Ala Phe Leu Val Asp Asn Val Glu Tyr Pro Leu Glu
130 135 140

15 Glu Leu Val Ala Met Asn Val Gln Glu Ile Ala Asn Arg Ala Asn Ser
145 150 155 160

20 Leu Leu Lys Asp Arg Asp Ala Arg Thr Glu Asp Phe Val Asn Lys Met
165 170 175

25 Ser Phe Thr Ile Pro Asp Phe Asp Gln His Gln Arg Lys Ala Leu
180 185 190

30 Leu Asp Ala Ser Ser Ile Thr Thr Gly Ile Glu Glu Thr Tyr Leu Val
195 200 205

35 Ser Glu Gly Met Ser Val Ala Val Asn Phe Val Leu Lys Gln Arg Gln
210 215 220

40 Phe Pro Pro Gly Glu Gln Gln His Tyr Ile Val Tyr Asp Met Gly Ser
225 230 235 240

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

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260 265 270

55 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

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	Ala Val Thr Lys Gln Phe Gly Gly Tyr Gly Thr Asn Leu Pro Glu Ile 370	375	380
10	Asn Gly Val Ile Leu Ala Gly Gly Ser Ser Arg Ile Pro Ile Val Gln 385	390	395
	Asp Gln Leu Ile Lys Leu Val Ser Glu Glu Lys Val Leu Arg Asn Val 405	410	415
15	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
20	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
25	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
30	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
35	Cys Ile Cys Gln Ser Glu Asn Asp Ile Gly Asn Ser Lys Gln Ile Lys 545	550	555
	Asn Lys Gly Ser Arg Leu Ala Phe Thr Ser Glu Asp Val Glu Ile Lys 565	570	575
40	Arg Leu Ser Pro Ser Glu Arg Ser Arg Leu His Glu His Ile Lys Leu 580	585	590
45			
50			
55			

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Leu Asp Lys Gln Asp Lys Glu Arg Phe Gln Phe Gln Glu Asn Leu Asn
595 600 605

5 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
10 625 630 635 640

Met Val Lys Val Tyr Leu Asp Trp Leu Glu Asp Ala Ser Phe Asp Thr
645 650 655

15 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
20 675 680 685

Gln Gln Phe Lys Gly Met Leu Glu Glu Gly His Lys Leu Leu Gln Ala
25 690 695 700

Ile Glu Thr His Lys Asn Thr Val Glu Glu Phe Leu Ser Gln Phe Glu
705 710 715 720

30 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
35 740 745 750

Thr Leu Thr Ser Phe Lys Asn Ser Ile Ser Glu Ile Glu Lys Phe Leu
755 760 765

40 Ala Lys Asn Leu Phe Gly Glu Asp Leu Arg Glu His Leu Phe Glu Ile
770 775 780

45 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
50 805 810 815

His Leu Arg Asn Phe Arg Leu Gln Lys Arg Lys Glu Glu Lys Leu Lys
820 825 830

55 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

5 Thr Thr Glu Ser Asn Pro Ser Ser Glu Glu Asp Ile Leu His Asp Glu
 865 870 875 880

Leu

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<210> 63
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<213> *Saccharomyces cerevisiae*

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Met Lys Leu His Gly Phe Leu Phe Ser Val Leu Ser Thr Cys Val Val
1 5 10 15

30

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

35

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

40

ata aaa tac tac act tct tgg tgc caa cat tgt aaa act ctg gcc cca 192
Ile Lys Tyr Tyr Ser Trp Cys Gln His Cys Lys Thr Leu Ala Pro
50 55 60

45

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

50

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

55

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

60

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

65

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

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	agg aac cta aag agt tta agc aac ttt atc gat act gta aga agt aaa Arg Asn Leu Lys Ser Leu Ser Asn Phe Ile Asp Thr Val Arg Ser Lys 165 170 175	528
5	gat aca gaa gaa aga ttc ata gaa cat att ttc gat gat tct agg aat Asp Thr Glu Glu Arg Phe Ile Glu His Ile Phe Asp Asp Ser Arg Asn 180 185 190	576
10	tgc aat gaa gaa tta cgt tct caa cag ctt ctg tgt aaa gct ggt aaa Cys Asn Glu Glu Leu Arg Ser Gln Gln Leu Leu Cys Lys Ala Gly Lys 195 200 205	624
15	gaa tac tac tct gat act tta tct aaa tta tac ggt gac gtg aat ggg Glu Tyr Tyr Ser Asp Thr Leu Ser Lys Leu Tyr Gly Asp Val Asn Gly 210 215 220	672
20	ctg gaa aag gaa agg cga aga cta gaa gct tta att aag caa aat gga Leu Glu Lys Glu Arg Arg Leu Glu Ala Leu Ile Lys Gln Asn Gly 225 230 235 240	720
25	gat gac ttg agt aaa gaa gtt aaa gaa aaa ctg aaa atc att cgt cta Asp Asp Leu Ser Lys Glu Val Lys Glu Lys Leu Lys Ile Ile Arg Leu 245 250 255	768
30	caa ttg agc cta tta tca cac ata gaa gac cag tta gaa gat acc agt Gln Leu Ser Leu Leu Ser His Ile Glu Asp Gln Leu Glu Asp Thr Ser 260 265 270	816
35	agt cat gac gag ctt tga Ser His Asp Glu Leu 275	834
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55	Ile Glu Gln Tyr Phe Asp Ile Cys Asn Arg Asn Asp Ser Tyr Thr Met 35 40 45	
60	Ile Lys Tyr Tyr Thr Ser Trp Cys Gln His Cys Lys Thr Leu Ala Pro 50 55 60	
65	Val Tyr Glu Glu Leu Gly Glu Leu Tyr Ala Lys Lys Ala Asn Lys Asp 65 70 75 80	
70	Asp Thr Pro Ile Asn Phe Leu Glu Val Asn Cys Glu Phe Phe Gly Pro 85 90 95	

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Thr Leu Cys Thr Asp Leu Pro Gly Phe Pro Ile Ile Glu Leu Val Lys
 100 105 110

5 Pro Arg Thr Lys Pro Leu Val Leu Pro Lys Leu Asp Trp Ser Ser Met
 115 120 125

10 Lys Phe His Glu Arg Leu Trp Gln Arg Ile Lys Thr Trp Phe Asn Asn
 130 135 140

15 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

20 Asp Thr Glu Glu Arg Phe Ile Glu His Ile Phe Asp Asp Ser Arg Asn
 180 185 190

25 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

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

35 Asp Asp Leu Ser Lys Glu Val Lys Glu Lys Leu Lys Ile Ile Arg Leu
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40 Gln Leu Ser Leu Leu Ser His Ile Glu Asp Gln Leu Glu Asp Thr Ser
 260 265 270

Ser His Asp Glu Leu
 275

45 <210> 65
 <211> 888
 <212> DNA
 <213> Saccharomyces cerevisiae

50 <220>
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	1	5	10	15	
5	ct a tca tat gct ttt acc acc att gaa aca gaa att ttc caa tta caa Leu Ser Tyr Ala Phe Thr Thr Ile Glu Thr Glu Ile Phe Gln Leu Gln 20 25 30				96
	aat gaa ata agt acg aaa tat ggc cca gat atg aac ttc tac aag ttc Asn Glu Ile Ser Thr Lys Tyr Gly Pro Asp Met Asn Phe Tyr Lys Phe 35 40 45				144
10	ttg aag tta cct aaa ctg cag aat tct agt aca aag gag att aca aaa Leu Lys Leu Pro Lys Leu Gln Asn Ser Ser Thr Lys Glu Ile Thr Lys 50 55 60				192
15	aac tta aga aag cta tcc aag aag tac cat ccg gat aag aac cct aaa Asn Leu Arg Lys Leu Ser Lys Lys Tyr His Pro Asp Lys Asn Pro Lys 65 70 75 80				240
	tac cgt aaa ttg tat gaa agg tta aac ctc gct act caa att ctt tca Tyr Arg Lys Leu Tyr Glu Arg Leu Asn Leu Ala Thr Gln Ile Leu Ser 85 90 95				288
20	aac agc tct aat cgt aag att tat gat tat tat cta cag aat ggc ttt Asn Ser Ser Asn Arg Lys Ile Tyr Asp Tyr Tyr Leu Gln Asn Gly Phe 100 105 110				336
25	cct aac tat gat ttc cat aag ggt ggt ttt tat ttt tcc aga atg aag Pro Asn Tyr Asp Phe His Lys Gly Gly Phe Tyr Phe Ser Arg Met Lys 115 120 125				384
	cct aag act tgg ttc ctg ctg gcc ttt att tgg ata gtc gtt aat att Pro Lys Thr Trp Phe Leu Leu Ala Phe Ile Trp Ile Val Val Asn Ile 130 135 140				432
30	ggg cag tat atc att tct att att caa tat cgt tct caa aga tca aga Gly Gln Tyr Ile Ile Ser Ile Ile Gln Tyr Arg Ser Gln Arg Ser Arg 145 150 155 160				480
35	att gaa aac ttc atc agt cag tgt aaa caa cag gat gat acc aat gga Ile Glu Asn Phe Ile Ser Gln Cys Lys Gln Gln Asp Asp Thr Asn Gly 165 170 175				528
40	cta ggc gta aaa caa cta acg ttt aaa caa cat gaa aag gat gag ggt Leu Gly Val Lys Gln Leu Thr Phe Lys Gln His Glu Lys Asp Glu Gly 180 185 190				576
	aaa agt ttg gtt gta agg ttt agc gat gtc tat gtt gta gag cct gat Lys Ser Leu Val Val Arg Phe Ser Asp Val Tyr Val Val Glu Pro Asp 195 200 205				624
45	gga agt gaa aca cta att tcg cca gat acc ttg gat aaa cct tca gta Gly Ser Glu Thr Leu Ile Ser Pro Asp Thr Leu Asp Lys Pro Ser Val 210 215 220				672
50	aag aac tgt ttg ttt tgg aga ata cct gct tcg gtt tgg aac atg acg Lys Asn Cys Leu Phe Trp Arg Ile Pro Ala Ser Val Trp Asn Met Thr 225 230 235 240				720
	ttt ggc aaa tct gtt ggt agc gca gga aaa gaa gaa ata ata acg gat Phe Gly Lys Ser Val Gly Ser Ala Gly Lys Glu Glu Ile Ile Thr Asp 245 250 255				768
55	agt aaa aag tat gat ggt aac caa aca aaa aag ggg aac aaa gta aaa				816

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Ser Lys Lys Tyr Asp Gly Asn Gln Thr Lys Lys Gly Asn Lys Val Lys
 260 265 270

5 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

10 aaa gtg atc tat tca cgt aaa tga 888
 Lys Val Ile Tyr Ser Arg Lys
 290 295

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20 <400> 66

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 1 5 10 15

30 Leu Ser Tyr Ala Phe Thr Thr Ile Glu Thr Glu Ile Phe Gln Leu Gln
 20 25 30

35 Asn Glu Ile Ser Thr Lys Tyr Gly Pro Asp Met Asn Phe Tyr Lys Phe
 35 40 45

40 45 Leu Lys Leu Pro Lys Leu Gln Asn Ser Ser Thr Lys Glu Ile Thr Lys
 50 55 60

45 50 Asn Leu Arg Lys Leu Ser Lys Lys Tyr His Pro Asp Lys Asn Pro Lys
 65 70 75 80

55 60 Tyr Arg Lys Leu Tyr Glu Arg Leu Asn Leu Ala Thr Gln Ile Leu Ser
 85 90 95

65 70 75 80 Asn Ser Ser Asn Arg Lys Ile Tyr Asp Tyr Tyr Leu Gln Asn Gly Phe
 100 105 110

75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

115 120 125 130 135 140 145 150 155 160 165 170 175

125 130 135 140 145 150 155 160 165 170 175

135 140 145 150 155 160 165 170 175

145 150 155 160 165 170 175

155 160 165 170 175

165 170 175

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Leu Gly Val Lys Gln Leu Thr Phe Lys Gln His Glu Lys Asp Glu Gly
180 185 190

5 Lys Ser Leu Val Val Arg Phe Ser Asp Val Tyr Val Val Glu Pro Asp
195 200 205

10 Gly Ser Glu Thr Leu Ile Ser Pro Asp Thr Leu Asp Lys Pro Ser Val
210 215 220

15 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

20 Ser Lys Lys Tyr Asp Gly Asn Gln Thr Lys Lys Gly Asn Lys Val Lys
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25 Lys Gly Ser Ala Lys Lys Gly Gln Lys Lys Met Glu Leu Pro Asn Gly
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Lys Val Ile Tyr Ser Arg Lys
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30 <210> 67

<211> 1554

<212> DNA

<213> *Saccharomyces cerevisiae*

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<221> CDS
<222> (1)..(1554)

40 <400> 67

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EP 3 196 304 B1

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5 ttt gct tct ttc acg ttg gct gaa aac agc gca aga gct acg ccg gga Phe Ala Ser Phe Thr Leu Ala Glu Asn Ser Ala Arg Ala Thr Pro Gly 20 25 30	96
10 tca gat tta ctc gtt cta aca gag aag aaa ttt aaa tca ttc atc gaa Ser Asp Leu Leu Val Leu Thr Glu Lys Lys Phe Lys Ser Phe Ile Glu 35 40 45	144
15 tct cat ccg tta gtc ctc gtc gag ttt ttt gct cca tgg tgt ttg cat Ser His Pro Leu Val Leu Val Glu Phe Phe Ala Pro Trp Cys Leu His 50 55 60	192
20 tct cag atc tta cgc cct cac tta gaa gag gcc gcc tct att tta aag Ser Gln Ile Leu Arg Pro His Leu Glu Glu Ala Ala Ser Ile Leu Lys 65 70 75 80	240
25	
30	
35	
40	
45	
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	gag cat aac gtc cca gtt caa att gat tgt gag gct aac agt atg Glu His Asn Val Pro Val Val Gln Ile Asp Cys Glu Ala Asn Ser Met 85 90 95	288
5	gtt tgc ctg caa caa act ata aat acc tac cca acc ttg aaa atc ttt Val Cys Leu Gln Gln Thr Ile Asn Thr Tyr Pro Thr Leu Lys Ile Phe 100 105 110	336
10	aaa aat ggt cgt att ttt gat ggt caa gtc tat cgc ggt gtc aag atc Lys Asn Gly Arg Ile Phe Asp Gly Gln Val Tyr Arg Gly Val Lys Ile 115 120 125	384
15	acc gat gaa atc act cag tac atg att cag cta tac gag gct tct gtc Thr Asp Glu Ile Thr Gln Tyr Met Ile Gln Leu Tyr Glu Ala Ser Val 130 135 140	432
20	att tat tta aat tcc gaa gat gaa atc caa cca tac ttg gaa aat gca Ile Tyr Leu Asn Ser Glu Asp Glu Ile Gln Pro Tyr Leu Glu Asn Ala 145 150 155 160	480
25	act tta cca gta gta ata aac aga ggc ttg aca ggc ttg aat gaa acg Thr Leu Pro Val Val Ile Asn Arg Gly Leu Thr Gly Leu Asn Glu Thr 165 170 175	528
30	tat caa gaa gtc gca ctg gac ctt gct gag gat tac gtc ttt tta tcc Tyr Gln Glu Val Ala Leu Asp Leu Ala Glu Asp Tyr Val Phe Leu Ser 180 185 190	576
35	ctt cta gat tca gaa gat aag tca tta tca atc cac ttg cca aac act Leu Leu Asp Ser Glu Asp Lys Ser Leu Ser Ile His Leu Pro Asn Thr 195 200 205	624
40	aca gaa cca att ctg ttt gat gga aat gta gac tct ttg gtc gga aat Thr Glu Pro Ile Leu Phe Asp Gly Asn Val Asp Ser Leu Val Gly Asn 210 215 220	672
45	tcc gtt gct cta act cag tgg tta aaa gtg gta att tta cct tac ttt Ser Val Ala Leu Thr Gln Trp Leu Lys Val Val Ile Leu Pro Tyr Phe 225 230 235 240	720
50	acc gac atc gaa cct gat ctc ttc ccc aag tac att tct agc aat ttg Thr Asp Ile Glu Pro Asp Leu Phe Pro Lys Tyr Ile Ser Ser Asn Leu 245 250 255	768
55	cgg ttg gct tac ttc ttt tat act tct gag gaa gaa ttg gaa gat tac Pro Leu Ala Tyr Phe Phe Tyr Thr Ser Glu Glu Glu Leu Glu Asp Tyr 260 265 270	816
60	act gat ctt ttc acg cag tta ggt aag gaa aat cgt ggc caa ata aat Thr Asp Leu Phe Thr Gln Leu Gly Lys Glu Asn Arg Gly Gln Ile Asn 275 280 285	864
65	ttc att gca tta aac tct aca atg ttc cca cac cac gtt aga ttc cta Phe Ile Ala Leu Asn Ser Thr Met Phe Pro His His Val Arg Phe Leu 290 295 300	912
70	aat atg aga gaa cag ttc cca tta ttt gct atc cat aat atg atc aat Asn Met Arg Glu Gln Phe Pro Leu Phe Ala Ile His Asn Met Ile Asn 305 310 315 320	960
75	aat ctg aaa tat ggt tta cca caa cta cca gaa gaa gag tac gcg aaa Asn Leu Lys Tyr Gly Leu Pro Gln Leu Pro Glu Glu Glu Tyr Ala Lys 325 330 335	1008

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	tta gaa aaa cca caa cca cta gac aga gat atg atc gtt cag ttg gta Leu Glu Lys Pro Gln Pro Leu Asp Arg Asp Met Ile Val Gln Leu Val 340 345 350	1056
5	aaa gat tac cgt gaa ggt act gcc aag cca att gtt aag tca gaa gag Lys Asp Tyr Arg Glu Gly Thr Ala Lys Pro Ile Val Lys Ser Glu Glu 355 360 365	1104
10	att cca aaa gaa caa aag tcc aat gtt tat aaa ata gtt ggg aag aca Ile Pro Lys Glu Gln Lys Ser Asn Val Tyr Lys Ile Val Gly Lys Thr 370 375 380	1152
15	cat gac gac att gtt cat gat gat gac aag gat gtc ctt gtc aaa tat His Asp Asp Ile Val His Asp Asp Asp Lys Asp Val Leu Val Lys Tyr 385 390 395 400	1200
	tac gcg aca tgg tgt att cat agt aaa agg ttt gcg cct att tac gaa Tyr Ala Thr Trp Cys Ile His Ser Lys Arg Phe Ala Pro Ile Tyr Glu 405 410 415	1248
20	gaa att gca aat gtc tta gca tct gat gaa tct gtt cgc gat aaa atc Glu Ile Ala Asn Val Leu Ala Ser Asp Glu Ser Val Arg Asp Lys Ile 420 425 430	1296
25	ttg atc gcc gaa gta gat tca ggg gca aat gat atc tta agt ttt cct Leu Ile Ala Glu Val Asp Ser Gly Ala Asn Asp Ile Leu Ser Phe Pro 435 440 445	1344
30	gtg aca gga tat cca acc att gct ttg tat cct gcc gga aat aac tct Val Thr Gly Tyr Pro Thr Ile Ala Leu Tyr Pro Ala Gly Asn Asn Ser 450 455 460	1392
	aag cct att atc ttc aat aaa att aga aat ttg gaa gat gtt ttc gaa Lys Pro Ile Ile Phe Asn Lys Ile Arg Asn Leu Glu Asp Val Phe Glu 465 470 475 480	1440
35	ttt atc aag gaa tca ggt aca cat cac att gac ggc cag gca att tat Phe Ile Lys Glu Ser Gly Thr His His Ile Asp Gly Gln Ala Ile Tyr 485 490 495	1488
40	gat aaa ttg cac cag gcc aag gat tct gaa gtg tct act gaa gat acc Asp Lys Leu His Gln Ala Lys Asp Ser Glu Val Ser Thr Glu Asp Thr 500 505 510	1536
45	gta cat gat gaa tta taa Val His Asp Glu Leu 515	1554
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Ser Asp Leu Leu Val Leu Thr Glu Lys Lys Phe Lys Ser Phe Ile Glu
 35 40 45

5 Ser His Pro Leu Val Leu Val Glu Phe Phe Ala Pro Trp Cys Leu His
 50 55 60

10 Ser Gln Ile Leu Arg Pro His Leu Glu Glu Ala Ala Ser Ile Leu Lys
 65 70 75 80

15 Glu His Asn Val Pro Val Val Gln Ile Asp Cys Glu Ala Asn Ser Met
 85 90 95

20 Val Cys Leu Gln Gln Thr Ile Asn Thr Tyr Pro Thr Leu Lys Ile Phe
 100 105 110

25 Lys Asn Gly Arg Ile Phe Asp Gly Gln Val Tyr Arg Gly Val Lys Ile
 115 120 125

30 Thr Asp Glu Ile Thr Gln Tyr Met Ile Gln Leu Tyr Glu Ala Ser Val
 130 135 140

35 Ile Tyr Leu Asn Ser Glu Asp Glu Ile Gln Pro Tyr Leu Glu Asn Ala
 145 150 155 160

40 Thr Leu Pro Val Val Ile Asn Arg Gly Leu Thr Gly Leu Asn Glu Thr
 165 170 175

45 Tyr Gln Glu Val Ala Leu Asp Leu Ala Glu Asp Tyr Val Phe Leu Ser
 180 185 190

50 Leu Leu Asp Ser Glu Asp Lys Ser Leu Ser Ile His Leu Pro Asn Thr
 195 200 205

55 Thr Glu Pro Ile Leu Phe Asp Gly Asn Val Asp Ser Leu Val Gly Asn
 210 215 220

60 Ser Val Ala Leu Thr Gln Trp Leu Lys Val Val Ile Leu Pro Tyr Phe
 225 230 235 240

65 Thr Asp Ile Glu Pro Asp Leu Phe Pro Lys Tyr Ile Ser Ser Asn Leu
 245 250 255

70 Pro Leu Ala Tyr Phe Phe Tyr Thr Ser Glu Glu Glu Leu Glu Asp Tyr
 260 265 270

75 Thr Asp Leu Phe Thr Gln Leu Gly Lys Glu Asn Arg Gly Gln Ile Asn

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275

280

285

5 Phe Ile Ala Leu Asn Ser Thr Met Phe Pro His His Val Arg Phe Leu
 290 295 300

10 Asn Met Arg Glu Gln Phe Pro Leu Phe Ala Ile His Asn Met Ile Asn
 305 310 315 320

15 Asn Leu Lys Tyr Gly Leu Pro Gln Leu Pro Glu Glu Glu Tyr Ala Lys
 325 330 335

20 Leu Glu Lys Pro Gln Pro Leu Asp Arg Asp Met Ile Val Gln Leu Val
 340 345 350

25 Lys Asp Tyr Arg Glu Gly Thr Ala Lys Pro Ile Val Lys Ser Glu Glu
 355 360 365

30 Ile Pro Lys Glu Gln Lys Ser Asn Val Tyr Lys Ile Val Gly Lys Thr
 370 375 380

35 His Asp Asp Ile Val His Asp Asp Asp Lys Asp Val Leu Val Lys Tyr
 385 390 395 400

40 Tyr Ala Thr Trp Cys Ile His Ser Lys Arg Phe Ala Pro Ile Tyr Glu
 405 410 415

45 Glu Ile Ala Asn Val Leu Ala Ser Asp Glu Ser Val Arg Asp Lys Ile
 420 425 430

50 Leu Ile Ala Glu Val Asp Ser Gly Ala Asn Asp Ile Leu Ser Phe Pro
 435 440 445

55 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
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Phe Ile Lys Glu Ser Gly Thr His His Ile Asp Gly Gln Ala Ile Tyr
 485 490 495

50 Asp Lys Leu His Gln Ala Lys Asp Ser Glu Val Ser Thr Glu Asp Thr
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55 Val His Asp Glu Leu
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<210> 69
<211> 1527
<212> DNA
<213> Homo sapiens

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<220>
<221> CDS
<222> (1)..(1527)

10 <400> 69

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5	gcc gac gcc ccc gag gag gag gac cac gtc ctg gtg ctg cgaa agc Ala Asp Ala Pro Glu Glu Asp His Val Leu Val Leu Arg Lys Ser 20 25 30	96
10	aac ttc gcg gag gcg ctg gcg gcc cac aag tac ctg ctg gtg gag ttc Asn Phe Ala Glu Ala Leu Ala His Lys Tyr Leu Leu Val Glu Phe 35 40 45	144
15	tat gcc cct tgg tgt ggc cac tgc aag gct ctg gcc cct gag tat gcc Tyr Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu Tyr Ala 50 55 60	192
	aaa gcc gct ggg aag ctg aag gca gaa ggt tcc gag atc agg ttg gcc Lys Ala Ala Gly Lys Leu Lys Ala Glu Gly Ser Glu Ile Arg Leu Ala 65 70 75 80	240
20	aag gtg gac gcc acg gag gag tct gac ctg gcc cag cag tac ggc gtg Lys Val Asp Ala Thr Glu Glu Ser Asp Leu Ala Gln Gln Tyr Gly Val 85 90 95	288
25	cgc ggc tat ccc acc atc aag ttc ttc agg aat gga gac acg gct tcc Arg Gly Tyr Pro Thr Ile Lys Phe Phe Arg Asn Gly Asp Thr Ala Ser 100 105 110	336
30	ccc aag gaa tat aca gct ggc aga gag gct gat gac atc gtg aac tgg Pro Lys Glu Tyr Thr Ala Gly Arg Glu Ala Asp Asp Ile Val Asn Trp 115 120 125	384
35	ctg aag aag cgc acg ggc ccg gct gcc acc acc ctg cct gac ggc gca Leu Lys Arg Thr Gly Pro Ala Ala Thr Thr Leu Pro Asp Gly Ala 130 135 140	432
40	gct gca gag tcc ttg gtg gag tcc agc gag gtg gct gtc atc ggc ttc Ala Ala Glu Ser Leu Val Glu Ser Ser Glu Val Ala Val Ile Gly Phe 145 150 155 160	480
	ttc aag gac gtg gag tcg gac tct gcc aag cag ttt ttg cag gca gca Phe Lys Asp Val Glu Ser Asp Ser Ala Lys Gln Phe Leu Gln Ala Ala 165 170 175	528
45	gag gcc atc gat gac ata cca ttt ggg atc act tcc aac agt gac gtg Glu Ala Ile Asp Asp Ile Pro Phe Gly Ile Thr Ser Asn Ser Asp Val 180 185 190	576
	ttc tcc aaa tac cag ctc gac aaa gat ggg gtt gtc ctc ttt aag aag Phe Ser Lys Tyr Gln Leu Asp Lys Asp Gly Val Val Leu Phe Lys Lys 195 200 205	624

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	ttt gat gaa ggc cg ^g aac aac ttt gaa ggg gag gtc acc aag gag aac Phe Asp Glu Gly Arg Asn Asn Phe Glu Gly Glu Val Thr Lys Glu Asn 210 215 220	672
5	ctg ctg gac ttt atc aaa cac aac cag ctg ccc ctt gtc atc gag ttc Leu Leu Asp Phe Ile Lys His Asn Gln Leu Pro Leu Val Ile Glu Phe 225 230 235 240	720
10	acc gag cag aca gcc cc ^g aag att ttt gga ggt gaa atc aag act cac Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly Glu Ile Lys Thr His 245 250 255	768
15	atc ctg ctg ttc ttg ccc aag agt gtg tct gac tat gac ggc aaa ctg Ile Leu Leu Phe Leu Pro Lys Ser Val Ser Asp Tyr Asp Gly Lys Leu 260 265 270	816
20	agc aac ttc aaa aca gca gcc gag agc ttc aag ggc aag atc ctg ttc Ser Asn Phe Lys Thr Ala Ala Glu Ser Phe Lys Gly Lys Ile Leu Phe 275 280 285	864
25	atc ttc atc gac agc gac cac acc gac aac cag cgc atc ctc gag ttc Ile Phe Ile Asp Ser Asp His Thr Asp Asn Gln Arg Ile Leu Glu Phe 290 295 300	912
30	ttt ggc ctg aag aag gaa gag tgc cc ^g gcc gtg cgc ctc atc acc ctg Phe Gly Leu Lys Lys Glu Glu Cys Pro Ala Val Arg Leu Ile Thr Leu 305 310 315 320	960
35	gag gag gag atg acc aag tac aag ccc gaa tcg gag gag ctg acg gca Glu Glu Glu Met Thr Lys Tyr Lys Pro Glu Ser Glu Glu Leu Thr Ala 325 330 335	1008
40	gag agg atc aca gag ttc tgc cac cgc ttc ctg gag ggc aaa atc aag Glu Arg Ile Thr Glu Phe Cys His Arg Phe Leu Glu Gly Lys Ile Lys 340 345 350	1056
45	ccc cac ctg atg agc cag gag ctg cc ^g gag gac tgg gac aag cag cct Pro His Leu Met Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys Gln Pro 355 360 365	1104
50	gtc aag gtg ctt gtt ggg aag aac ttt gaa gac gtg gct ttt gat gag Val Lys Val Leu Val Gly Lys Asn Phe Glu Asp Val Ala Phe Asp Glu 370 375 380	1152
55	aaa aaa aac gtc ttt gtg gag ttc tat gcc cca tgg tgt ggt cac tgc Lys Lys Asn Val Phe Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys 385 390 395 400	1200
60	aaa cag ttg gct ccc att tgg gat aaa ctg gga gag acg tac aag gac Lys Gln Leu Ala Pro Ile Trp Asp Lys Leu Gly Glu Thr Tyr Lys Asp 405 410 415	1248
65	cat gag aac atc gtc atc gcc aag atg gac tcg act gcc aac gag gtg His Glu Asn Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn Glu Val 420 425 430	1296
70	gag gcc gtc aaa gtg cac agc ttc ccc aca ctc aag ttc ttt cct gcc Glu Ala Val Lys Val His Ser Phe Pro Thr Leu Lys Phe Phe Pro Ala 435 440 445	1344
75	agt gcc gac agg acg gtc att gat tac aac ggg gaa cgc acg ctg gat Ser Ala Asp Arg Thr Val Ile Asp Tyr Asn Gly Glu Arg Thr Leu Asp 450 455 460	1392

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ggt ttt aag aaa ttc ctg gag agc ggt ggc cag gat ggg gca ggg gat
Gly Phe Lys Lys Phe Leu Glu Ser Gly Gly Gln Asp Gly Ala Gly Asp
465 470 475 480

1440

5 gat gac gat ctc gag gac ctg gaa gaa gca gag gag cca gac atg gag
Asp Asp Asp Leu Glu Asp Leu Glu Ala Glu Glu Pro Asp Met Glu
485 490 495

1488

10 gaa gac gat gat cag aaa gct gtg aaa gat gaa ctg taa
Glu Asp Asp Asp Gln Lys Ala Val Lys Asp Glu Leu
500 505

1527

<210> 70

<211> 508

<212> PRT

<213> Homo sapiens

<400> 70

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Met Leu Arg Arg Ala Leu Leu Cys Leu Ala Val Ala Ala Leu Val Arg
1 5 10 15

5 Ala Asp Ala Pro Glu Glu Glu Asp His Val Val Leu Arg Lys Ser
20 25 30

10 Asn Phe Ala Glu Ala Leu Ala His Lys Tyr Leu Leu Val Glu Phe
35 40 45

15 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

20 Lys Val Asp Ala Thr Glu Glu Ser Asp Leu Ala Gln Gln Tyr Gly Val
85 90 95

25 Arg Gly Tyr Pro Thr Ile Lys Phe Phe Arg Asn Gly Asp Thr Ala Ser
100 105 110

30 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

35 Ala Ala Glu Ser Leu Val Glu Ser Ser Glu Val Ala Val Ile Gly Phe
145 150 155 160

40 Phe Lys Asp Val Glu Ser Asp Ser Ala Lys Gln Phe Leu Gln Ala Ala
165 170 175

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Glu Ala Ile Asp Asp Ile Pro Phe Gly Ile Thr Ser Asn Ser Asp Val
180 185 190

5 Phe Ser Lys Tyr Gln Leu Asp Lys Asp Gly Val Val Leu Phe Lys Lys
195 200 205

10 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

15 Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly Glu Ile Lys Thr His
245 250 255

20 Ile Leu Leu Phe Leu Pro Lys Ser Val Ser Asp Tyr Asp Gly Lys Leu
260 265 270

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

30 Ile Phe Ile Asp Ser Asp His Thr Asp Asn Gln Arg Ile Leu Glu Phe
290 295 300

35 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

40 Glu Arg Ile Thr Glu Phe Cys His Arg Phe Leu Glu Gly Lys Ile Lys
340 345 350

45 Pro His Leu Met Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys Gln Pro
355 360 365

50 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

55 His Glu Asn Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn Glu Val

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420

425

430

5 Glu Ala Val Lys Val His Ser Phe Pro Thr Leu Lys Phe Phe Pro Ala
435 440 445

10 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

15 Asp Asp Asp Leu Glu Asp Leu Glu Glu Glu Pro Asp Met Glu
485 490 495

20 Glu Asp Asp Asp Gln Lys Ala Val Lys Asp Glu Leu
500 505

<210> 71

<211> 1407

<212> DNA

25 <213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1407)

30 <400> 71

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5	ctg ctc agc tcg ggc cac gga gag gag cag ccc ccg gag aca gca gca Leu Leu Ser Ser Gly His Glu Glu Gln Pro Pro Glu Thr Ala Ala 20 25 30	96
10	cag agg tgc ttc tgc cag gtt agt ggt tac ttg gat gat tgt acc tgt Gln Arg Cys Phe Cys Gln Val Ser Gly Tyr Leu Asp Asp Cys Thr Cys 35 40 45	144
15	gat gtt gaa acc att gat aga ttt aat aac tac agg ctt ttc cca aga Asp Val Glu Thr Ile Asp Arg Phe Asn Asn Tyr Arg Leu Phe Pro Arg 50 55 60	192
20	cta caa aaa ctt ctt gaa agt gac tac ttt agg tat tac aag gta aac Leu Gln Lys Leu Leu Glu Ser Asp Tyr Phe Arg Tyr Tyr Lys Val Asn 65 70 75 80	240
25	ctg aag agg ccg tgt cct ttc tgg aat gac atc agc cag tgt gga aga Leu Lys Arg Pro Cys Pro Phe Trp Asn Asp Ile Ser Gln Cys Gly Arg 85 90 95	288
30	agg gac tgt gct gtc aaa cca tgt caa tct gat gaa gtt cct gat gga Arg Asp Cys Ala Val Lys Pro Cys Gln Ser Asp Glu Val Pro Asp Gly 100 105 110	336
35	att aaa tct gcg agc tac aag tat tct gaa gaa gcc aat aat ctc att Ile Lys Ser Ala Ser Tyr Lys Ser Glu Glu Ala Asn Asn Leu Ile	384
40		
45		
50		
55		

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	115	120	125	
5	gaa gaa tgt gaa caa gct gaa cga ctt gga gca gtg gat gaa tct ctg Glu Glu Cys Glu Gln Ala Glu Arg Leu Gly Ala Val Asp Glu Ser Leu 130 135 140			432
10	agt gag gaa aca cag aag gct gtt ctt cag tgg acc aag cat gat gat Ser Glu Glu Thr Gln Lys Ala Val Leu Gln Trp Thr Lys His Asp Asp 145 150 155 160			480
15	tct tca gat aac ttc tgt gaa gct gat gac att cag tcc cct gaa gct Ser Ser Asp Asn Phe Cys Glu Ala Asp Asp Ile Gln Ser Pro Glu Ala 165 170 175			528
20	gaa tat gta gat ttg ctt ctt aat cct gag cgc tac act ggt tac aag Glu Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys 180 185 190			576
25	gga cca gat gct tgg aaa ata tgg aat gtc atc tac gaa gaa aac tgt Gly Pro Asp Ala Trp Lys Ile Trp Asn Val Ile Tyr Glu Glu Asn Cys 195 200 205			624
30	ttt aag cca cag aca att aaa aga cct tta aat cct ttg gct tct ggt Phe Lys Pro Gln Thr Ile Lys Arg Pro Leu Asn Pro Leu Ala Ser Gly 210 215 220			672
35	caa ggg aca agt gaa gag aac act ttt tac agt tgg cta gaa ggt ctc Gln Gly Thr Ser Glu Glu Asn Thr Phe Tyr Ser Trp Leu Glu Gly Leu 225 230 235 240			720
40	tgt gta gaa aaa aga gca ttc tac aga ctt ata tct ggc cta cat gca Cys Val Glu Lys Arg Ala Phe Tyr Arg Leu Ile Ser Gly Leu His Ala 245 250 255			768
45	agc att aat gtg cat ttg agt gca aga tat ctt tta caa gag acc ttg Ser Ile Asn Val His Leu Ser Ala Arg Tyr Leu Leu Gln Glu Thr Trp 260 265 270			816
50	tta gaa aag aaa tgg gga cac aac att aca gaa ttt caa cag cga ttt Leu Glu Lys Lys Trp Gly His Asn Ile Thr Glu Phe Gln Gln Arg Phe 275 280 285			864
55	gat gga att ttg act gaa gga gaa ggt cca aga agg ctt aag aac ttg Asp Gly Ile Leu Thr Glu Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu 290 295 300			912
	tat ttt ctc tac tta ata gaa cta agg gct tta tcc aaa gtg tta cca Tyr Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Leu Pro 305 310 315 320			960
	ttc ttc gag cgc cca gat ttt caa ctc ttt act gga aat aaa att cag Phe Phe Glu Arg Pro Asp Phe Gln Leu Phe Thr Gly Asn Lys Ile Gln 325 330 335			1008
	gat gag gaa aac aaa atg tta ctt ctg gaa ata ctt cat gaa atc aag Asp Glu Glu Asn Lys Met Leu Leu Leu Glu Ile Leu His Glu Ile Lys 340 345 350			1056
	tca ttt cct ttg cat ttt gat gag aat tca ttt ttt gct ggg gat aaa Ser Phe Pro Leu His Phe Asp Glu Asn Ser Phe Phe Ala Gly Asp Lys 355 360 365			1104
	aaa gaa gca cac aaa cta aag gag gac ttt cga ctg cat ttt aga aat			1152

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	Lys Glu Ala His Lys Leu Lys Glu Asp Phe Arg Leu His Phe Arg Asn			
	370	375	380	
5	att tca aga att atg gat tgt gtt ggt tgt ttt aaa tgt cgt ctg tgg Ile Ser Arg Ile Met Asp Cys Val Gly Cys Phe Lys Cys Arg Leu Trp			1200
	385	390	395	400
10	gga aag ctt cag act cag ggt ttg ggc act gct ctg aag atc tta ttt Gly Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe			1248
	405	410	415	
15	tct gag aaa ttg ata gca aat atg cca gaa agt gga cct agt tat gaa Ser Glu Lys Leu Ile Ala Asn Met Pro Glu Ser Gly Pro Ser Tyr Glu			1296
	420	425	430	
20	ttc cat cta acc aga caa gaa ata gta tca tta ttc aac gca ttt gga Phe His Leu Thr Arg Gln Glu Ile Val Ser Leu Phe Asn Ala Phe Gly			1344
	435	440	445	
25	aga att tct aca agt gtg aaa gaa tta gaa aac ttc agg aac ttg tta Arg Ile Ser Thr Ser Val Lys Glu Leu Glu Asn Phe Arg Asn Leu Leu			1392
	450	455	460	
	cag aat att cat taa Gln Asn Ile His			1407
	465			
30	<210> 72 <211> 468 <212> PRT <213> Homo sapiens			
	<400> 72			
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40	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			
45	Asp Val Glu Thr Ile Asp Arg Phe Asn Asn Tyr Arg Leu Phe Pro Arg 50 55 60			
50	Leu Gln Lys Leu Leu Glu Ser Asp Tyr Phe Arg Tyr Tyr Lys Val Asn 65 70 75 80			
55	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			

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Ile Lys Ser Ala Ser Tyr Lys Tyr Ser Glu Glu Ala Asn Asn Leu Ile
115 120 125

5 Glu Glu Cys Glu Gln Ala Glu Arg Leu Gly Ala Val Asp Glu Ser Leu
130 135 140

10 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

15 Glu Tyr Val Asp Leu Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys
180 185 190

20 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

25 Gln Gly Thr Ser Glu Glu Asn Thr Phe Tyr Ser Trp Leu Glu Gly Leu
225 230 235 240

30 Cys Val Glu Lys Arg Ala Phe Tyr Arg Leu Ile Ser Gly Leu His Ala
245 250 255

35 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

40 Asp Gly Ile Leu Thr Glu Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu
290 295 300

45 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

50 Asp Glu Glu Asn Lys Met Leu Leu Leu Glu Ile Leu His Glu Ile Lys
340 345 350

55 Ser Phe Pro Leu His Phe Asp Glu Asn Ser Phe Phe Ala Gly Asp Lys
355 360 365

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Lys Glu Ala His Lys Leu Lys Glu Asp Phe Arg Leu His Phe Arg Asn
370 375 380

5 Ile Ser Arg Ile Met Asp Cys Val Gly Cys Phe Lys Cys Arg Leu Trp
385 390 395 400

10 Gly Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe
405 410 415

15 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

20 Arg Ile Ser Thr Ser Val Lys Glu Leu Glu Asn Phe Arg Asn Leu Leu
450 455 460

25 Gln Asn Ile His
465

<210> 73

<211> 1404

<212> DNA

30 <213> Homo sapiens

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<221> CDS

<222> (1)..(1404)

35 <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

5 gcg gtg cag ctg ctg gtc acc ctg agc ttc ctg cgg agc gtc gtc gag
 Ala Val Gln Leu Leu Val Thr Leu Ser Phe Leu Arg Ser Val Val Glu
 20 25 30 96

10 gcg cag gtc act gga gtt ctg gat gat tgc ttg tgt gat att gac agc
 Ala Gln Val Thr Gly Val Leu Asp Asp Cys Leu Cys Asp Ile Asp Ser
 35 40 45 144

15 atc gat aac ttc aat acc tac aaa atc ttc ccc aaa ata aaa aaa ttg
 Ile Asp Asn Phe Asn Thr Tyr Lys Ile Phe Pro Lys Ile Lys Lys Leu
 50 55 60 192

caa gag aga gac tat ttt cgt tat tac aag gtt aat ctg aag cga cct
 Gln Glu Arg Asp Tyr Phe Arg Tyr Tyr Lys Val Asn Leu Lys Arg Pro
 65 70 75 80 240

20 tgt cct ttc tgg gca gaa gat ggc cac tgt tca ata aaa gac tgt cat
 Cys Pro Phe Trp Ala Glu Asp Gly His Cys Ser Ile Lys Asp Cys His
 85 90 95 288

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	gtg gag ccc tgt cca gag agt aaa att ccg gtt gga ata aaa gct ggg Val Glu Pro Cys Pro Glu Ser Lys Ile Pro Val Gly Ile Lys Ala Gly 100 105 110	336
5	cat tct aat aag tac ttg aaa atg gca aac aat acc aaa gaa tta gaa His Ser Asn Lys Tyr Leu Lys Met Ala Asn Asn Thr Lys Glu Leu Glu 115 120 125	384
10	gtt tgt gag caa gct aat aaa ctg gga gca att aac agc aca tta agt Val Cys Glu Gln Ala Asn Lys Leu Gly Ala Ile Asn Ser Thr Leu Ser 130 135 140	432
15	aat caa agc aaa gaa gct ttc att gac tgg gca aga tat gat gat tca Asn Gln Ser Lys Glu Ala Phe Ile Asp Trp Ala Arg Tyr Asp Asp Ser 145 150 155 160	480
20	cgg gat cac ttt tgt gaa ctt gat gat gag aga tct cca gct gct cag Arg Asp His Phe Cys Glu Leu Asp Asp Glu Arg Ser Pro Ala Ala Gln 165 170 175	528
25	tat gta gac cta ttg ctg aac cca gag cgt tac act ggc tat aaa ggg Tyr Val Asp Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys Gly 180 185 190	576
30	acc tct gca tgg aga gtg tgg aac agc atc tat gaa gag aac tgt ttc Thr Ser Ala Trp Arg Val Trp Asn Ser Ile Tyr Glu Glu Asn Cys Phe 195 200 205	624
35	aag cct cga tct gtt tat cgt cct tta aat cct ctg gcg cct agc cga Lys Pro Arg Ser Val Tyr Arg Pro Leu Asn Pro Leu Ala Pro Ser Arg 210 215 220	672
40	ggc gaa gat gat gga gaa tca ttc tac aca tgg cta gaa ggt ttg tgt Gly Glu Asp Asp Gly Glu Ser Phe Tyr Thr Trp Leu Glu Gly Leu Cys 225 230 235 240	720
45	ctg gag aaa aga gtc ttc tat aag ctt ata tgg gga ctt cat gct agc Leu Glu Lys Arg Val Phe Tyr Lys Leu Ile Ser Gly Leu His Ala Ser 245 250 255	768
50	atc aat tta cat cta tgc gca aat tat ctt ttg gaa gaa acc tgg ggt Ile Asn Leu His Leu Cys Ala Asn Tyr Leu Leu Glu Glu Thr Trp Gly 260 265 270	816
55	aag ccc agt tgg gga cct aat att aaa gaa ttc aaa cac cgc ttt gac Lys Pro Ser Trp Gly Pro Asn Ile Lys Glu Phe Lys His Arg Phe Asp 275 280 285	864
	cct gtg gaa acc aag gga gaa ggt cca aga agg ctc aag aat ctt tac Pro Val Glu Thr Lys Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu Tyr 290 295 300	912
	ttt tta tac ttg att gag ctt cga gct ttg tca aag gtg gct cca tat Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Ala Pro Tyr 305 310 315 320	960
	ttt gag cgc tca att gtc gat ctt tac act gga aat gca gaa gaa gat Phe Glu Arg Ser Ile Val Asp Leu Tyr Thr Gly Asn Ala Glu Glu Asp 325 330 335	1008
	gct gac aca aaa act ctt cta ctg aat atc ttt caa gat aca aag tcc Ala Asp Thr Lys Thr Leu Leu Asn Ile Phe Gln Asp Thr Lys Ser	1056

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	340	345	350	
5	ttt ccc atg cac ttt gat gag aaa tcc atg ttt gca ggt gac aaa aaa Phe Pro Met His Phe Asp Glu Lys Ser Met Phe Ala Gly Asp Lys Lys 355 360 365			1104
	ggg gcc aag tca cta aag gag gaa ttc cga tta cat ttc aag aat atc Gly Ala Lys Ser Leu Lys Glu Glu Phe Arg Leu His Phe Lys Asn Ile 370 375 380			1152
10	tcc cgt ata atg gac tgt gtt gga tgt gac aaa tgc aga tta tgg gga Ser Arg Ile Met Asp Cys Val Gly Cys Asp Lys Cys Arg Leu Trp Gly 385 390 395 400			1200
15	aaa tta cag act cag ggt tta gga act gcc ctg aag ata tta ttc tct Lys Leu Gln Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe Ser 405 410 415			1248
20	gaa aaa gaa atc caa aag ctt cca gag aat agt cca tct aaa ggc ttc Glu Lys Glu Ile Gln Lys Leu Pro Glu Asn Ser Pro Ser Lys Gly Phe 420 425 430			1296
	caa ctc acc cga cag gaa ata gtt gct ctt tta aat gct ttt gga agg Gln Leu Thr Arg Gln Glu Ile Val Ala Leu Leu Asn Ala Phe Gly Arg 435 440 445			1344
25	ctt tct aca agt ata aga gac tta cag aat ttt aaa gtc tta tta caa Leu Ser Thr Ser Ile Arg Asp Leu Gln Asn Phe Lys Val Leu Leu Gln 450 455 460			1392
30	cac agt agg taa His Ser Arg 465			1404
	<210> 74			
35	<211> 467			
	<212> PRT			
	<213> Homo sapiens			
	<400> 74			
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Met Ser Gln Gly Val Arg Arg Ala Gly Ala Gly Gln Gly Val Ala Ala
1 5 10 15

5 Ala Val Gln Leu Leu Val Thr Leu Ser Phe Leu Arg Ser Val Val Glu
20 25 30

10 Ala Gln Val Thr Gly Val Leu Asp Asp Cys Leu Cys Asp Ile Asp Ser
35 40 45

15 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

20 Cys Pro Phe Trp Ala Glu Asp Gly His Cys Ser Ile Lys Asp Cys His

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	85	90	95
5	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		
10	120		
	125		
15	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		
20	150	155	160
	Arg Asp His Phe Cys Glu Leu Asp Asp Glu Arg Ser Pro Ala Ala Gln 165		
25	170	175	
	Tyr Val Asp Leu Leu Asn Pro Glu Arg Tyr Thr Gly Tyr Lys Gly 180		
	185	190	
30	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		
35	215	220	
	Gly Glu Asp Asp Gly Glu Ser Phe Tyr Thr Trp Leu Glu Gly Leu Cys 225		
	230	235	240
40	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	
45	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		
50	295	300	
	Phe Leu Tyr Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Ala Pro Tyr 305		
	310	315	320
55	Phe Glu Arg Ser Ile Val Asp Leu Tyr Thr Gly Asn Ala Glu Glu Asp 325	330	335

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Ala Asp Thr Lys Thr Leu Leu Leu Asn Ile Phe Gln Asp Thr Lys Ser
340 345 350

5 Phe Pro Met His Phe Asp Glu Lys Ser Met Phe Ala Gly Asp Lys Lys
355 360 365

10 Gly Ala Lys Ser Leu Lys Glu Glu Phe Arg Leu His Phe Lys Asn Ile
370 375 380

15 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

20 Glu Lys Glu Ile Gln Lys Leu Pro Glu Asn Ser Pro Ser Lys Gly Phe
420 425 430

25 Gln Leu Thr Arg Gln Glu Ile Val Ala Leu Leu Asn Ala Phe Gly Arg
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1 5 10 15	
5	
cgg gcc gag gag gag gac aag aag gag gac gtg ggc acg gtg gtc ggc Arg Ala Glu Glu Asp Lys Lys Glu Asp Val Gly Thr Val Val Gly	96
20 25 30	
10	
atc gac ctg ggg acc acc tac tcc tgc gtc ggc gtg ttc aag aac ggc Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Gly Val Phe Lys Asn Gly	144
35 40 45	
15	
cgc gtg gag atc atc gcc aac gat cag ggc aac cgc atc acg ccg tcc Arg Val Glu Ile Ile Ala Asn Asp Gln Gly Asn Arg Ile Thr Pro Ser	192
50 55 60	

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	tat gtc gcc ttc act cct gaa ggg gaa cgt ctg att ggc gat gcc gcc Tyr Val Ala Phe Thr Pro Glu Gly Glu Arg Leu Ile Gly Asp Ala Ala 65 70 75 80	240
5	aag aac cag ctc acc tcc aac ccc gag aac acg gtc ttt gac gcc aag Lys Asn Gln Leu Thr Ser Asn Pro Glu Asn Thr Val Phe Asp Ala Lys 85 90 95	288
10	cgg ctc atc ggc cgc acg tgg aat gac ccg tct gtg cag cag gac atc Arg Leu Ile Gly Arg Thr Trp Asn Asp Pro Ser Val Gln Gln Asp Ile 100 105 110	336
	aag ttc ttg ccg ttc aag gtg gtt gaa aag aaa act aaa cca tac att Lys Phe Leu Pro Phe Lys Val Val Glu Lys Lys Thr Lys Pro Tyr Ile 115 120 125	384
15	caa gtt gat att gga ggt ggg caa aca aag aca ttt gct cct gaa gaa Gln Val Asp Ile Gly Gly Gln Thr Lys Thr Phe Ala Pro Glu Glu 130 135 140	432
20	att tct gcc atg gtt ctc act aaa atg aaa gaa acc gct gag gct tat Ile Ser Ala Met Val Leu Thr Lys Met Lys Glu Thr Ala Glu Ala Tyr 145 150 155 160	480
	ttg gga aag aag gtt acc cat gca gtt gtt act gta cca gcc tat ttt Leu Gly Lys Lys Val Thr His Ala Val Val Thr Val Pro Ala Tyr Phe 165 170 175	528
25	aat gat gcc caa cgc caa gca acc aaa gac gct gga act att gct ggc Asn Asp Ala Gln Arg Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly 180 185 190	576
30	cta aat gtt atg agg atc atc aac gag cct acg gca gct gct att gct Leu Asn Val Met Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala 195 200 205	624
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40	gtc ttc gaa gtt gtg gcc act aat gga gat act cat ctg ggt gga gaa Val Phe Glu Val Val Ala Thr Asn Gly Asp Thr His Leu Gly Gly Glu 245 250 255	768
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40	cag atc ttt tct aca gct tct gat aat caa cca act gtt aca atc aag Gln Ile Phe Ser Thr Ala Ser Asp Asn Gln Pro Thr Val Thr Ile Lys 450 455 460	1392
45	gtc tat gaa ggt gaa aga ccc ctg aca aaa gac aat cat ctt ctg ggt Val Tyr Glu Gly Glu Arg Pro Leu Thr Lys Asp Asn His Leu Leu Gly 465 470 475 480	1440
50	aca ttt gat ctg act gga att cct cct gct cct cgt ggg gtc cca cag Thr Phe Asp Leu Thr Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln 485 490 495	1488
55	att gaa gtc acc ttt gag ata gat gtg aat ggt att ctt cga gtg aca Ile Glu Val Thr Phe Glu Ile Asp Val Asn Gly Ile Leu Arg Val Thr 500 505 510	1536
	gct gaa gac aag ggt aca ggg aac aaa aat aag atc aca atc acc aat Ala Glu Asp Lys Gly Thr Gly Asn Lys Asn Lys Ile Thr Ile Thr Asn 515 520 525	1584
	gac cag aat cgc ctg aca cct gaa gaa atc gaa agg atg gtt aat gat Asp Gln Asn Arg Leu Thr Pro Glu Glu Ile Glu Arg Met Val Asn Asp 530 535 540	1632
	gct gag aag ttt gct gag gaa gac aaa aag ctc aag gag cgc att gat Ala Glu Lys Phe Ala Glu Glu Asp Lys Lys Leu Lys Glu Arg Ile Asp 545 550 555 560	1680
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1 5 10 15

5 Arg Ala Glu Glu Glu Asp Lys Lys Glu Asp Val Gly Thr Val Val Gly
20 25 30

10 Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Gly Val Phe Lys Asn Gly
35 40 45

15 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

20 Lys Asn Gln Leu Thr Ser Asn Pro Glu Asn Thr Val Phe Asp Ala Lys
85 90 95

25 Arg Leu Ile Gly Arg Thr Trp Asn Asp Pro Ser Val Gln Gln Asp Ile
100 105 110

30 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 Gln Thr Lys Thr Phe Ala Pro Glu Glu

35

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130 135 140

5 Ile Ser Ala Met Val Leu Thr Lys Met Lys Glu Thr Ala Glu Ala Tyr
145 150 155 160

10 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
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15 Leu Asn Val Met Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala
195 200 205

20 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

25 Val Phe Glu Val Val Ala Thr Asn Gly Asp Thr His Leu Gly Gly Glu
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30 Asp Phe Asp Gln Arg Val Met Glu His Phe Ile Lys Leu Tyr Lys Lys
260 265 270

35 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
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40 Ala Arg Ile Glu Ile Glu Ser Phe Tyr Glu Gly Glu Asp Phe Ser Glu
305 310 315 320

45 Thr Leu Thr Arg Ala Lys Phe Glu Glu Leu Asn Met Asp Leu Phe Arg
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50 Ser Thr Met Lys Pro Val Gln Lys Val Leu Glu Asp Ser Asp Leu Lys
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Lys Ser Asp Ile Asp Glu Ile Val Leu Val Gly Gly Ser Thr Arg Ile
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55 Pro Lys Ile Gln Gln Leu Val Lys Glu Phe Phe Asn Gly Lys Glu Pro
370 375 380

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Ser Arg Gly Ile Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val
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5 Gln Ala Gly Val Leu Ser Gly Asp Gln Asp Thr Gly Asp Leu Val Leu
 405 410 415

10 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

15 Gln Ile Phe Ser Thr Ala Ser Asp Asn Gln Pro Thr Val Thr Ile Lys
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20 Val Tyr Glu Gly Glu Arg Pro Leu Thr Lys Asp Asn His Leu Leu Gly
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Ile Glu Val Thr Phe Glu Ile Asp Val Asn Gly Ile Leu Arg Val Thr
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30 Ala Glu Asp Lys Gly Thr Gly Asn Lys Asn Lys Ile Thr Ile Thr Asn
 515 520 525

35 Asp Gln Asn Arg Leu Thr Pro Glu Glu Ile Glu Arg Met Val Asn Asp
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Ala Glu Lys Phe Ala Glu Glu Asp Lys Lys Leu Lys Glu Arg Ile Asp
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40 Thr Arg Asn Glu Leu Glu Ser Tyr Ala Tyr Ser Leu Lys Asn Gln Ile
 565 570 575

45 Gly Asp Lys Glu Lys Leu Gly Gly Lys Leu Ser Ser Glu Asp Lys Glu
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50 Thr Met Glu Lys Ala Val Glu Glu Lys Ile Glu Trp Leu Glu Ser His
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Gln Asp Ala Asp Ile Glu Asp Phe Lys Ala Lys Lys Lys Glu Leu Glu
 610 615 620

55 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

5 **Claims**

1. A transformed yeast into which a chaperone gene has been introduced and in which
the aox1 gene has been disrupted, and
10 a protease gene has been disrupted, wherein optionally the protease gene is a prb1 gene.
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:
 - 15 (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. cerevisiae*);
(c) a gene encoding PDI, ERO1-L α , ERO1-L β , or GRP78 derived from a human; and
20 (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:
 - 25 (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;
30 (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:
 - 35 (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*;
40 (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-L β 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*,
45 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 yeast is a methylotrophic yeast.
 - 50 6. The transformed yeast according to any of claims 1 to 5, which comprises a gene encoding a target protein introduced thereinto.
 7. Use of the transformed yeast according to any of claims 1 to 6 for the production of a target protein.
 - 55 8. A method for producing a protein comprising culturing the transformed yeast according to claim 6 in a medium and sampling a target protein from the culture product.
 9. The method for producing a protein according to claim 8, wherein culture is conducted under conditions in which

protease activity is inhibited.

10. The method for producing a protein according to claim 8 or 9, wherein culture is conducted in a medium with a pH of 6.0 to 7.5.
- 5 11. The method for producing a protein according to any of claims 8 to 10, wherein a nitrogen source is added to the medium.
- 10 12. The method for producing a protein according to claims 8 to 11, wherein (a) methanol is not added to the medium or (b) the amount of methanol added to the medium is 2% (v/v) or less.
13. A method for producing a transformed yeast comprising step (i) in addition to both step (ii) and (iii):
 - 15 (i) a step of introducing a chaperone gene into yeast; and
 - (ii) a step of disrupting the aox1 gene in yeast; and
 - (iii) a step of disrupting the prb1 gene in yeast,
 optionally further comprising a step of introducing a gene encoding a target protein.

20 Patentansprüche

1. Transformierte Hefe, in die ein Chaperon-Gen eingebracht wurde und in der das aox1-Gen unterbrochen wurde und ein Protease-Gen unterbrochen wurde, wobei das Protease-Gen optional ein prb1-Gen ist.
- 25 2. Transformierte Hefe nach Anspruch 1, wobei das Chaperon-Gen wenigstens ein Gen ist, ausgewählt aus der Gruppe, bestehend aus den Genen (a) bis (d) unten:
 - (a) ein Gen, welches PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1 oder HSP104, abgeleitet von *Ogataea minuta* (*O. minuta*), codiert;
 - 30 (b) ein Gen, welches PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5 oder EUG1, abgeleitet von *Saccharomyces cerevisiae* (*S. cerevisiae*), codiert;
 - (c) ein Gen, welches PDI, ERO1-L α , ERO1-L β oder GRP78, abgeleitet von einem Menschen, codiert; und
 - (d) ein Gen, welches 95% oder mehr Sequenzhomologie zu einer Basensequenz irgendeines der Gene (a) bis (c) zeigt.
- 35 3. Transformierte Hefe gemäß Anspruch 1, wobei das Chaperon-Gen wenigstens ein Gen ist, ausgewählt aus der Gruppe, bestehend aus den Genen (a) bis (g) unten:
 - (a) ein Gen, welches PDI1, abgeleitet von *O. minuta*, codiert;
 - 40 (b) ein Gen, welches ERO1, abgeleitet von *O. minuta*, codiert;
 - (c) ein Gen, welches Kar2, abgeleitet von *O. minuta*, codiert;
 - (d) ein Gen, welches PDI1, abgeleitet von *S. cerevisiae*, codiert;
 - (e) ein Gen, welches PDI, abgeleitet von einem Menschen, codiert;
 - (f) ein Gen, welches ERO1, abgeleitet von einem Menschen, codiert; und
 - 45 (g) ein Gen, welches 95% oder mehr Sequenzhomologie zu einer Basensequenz irgendeines der Gene (a) bis (f) zeigt.
- 50 4. Transformierte Hefe gemäß Anspruch 1, wobei das Chaperon-Gen irgendeines der Chaperon-Gene (a) bis (g) unten ist:
 - (a) eine Kombination aus einem Gen, welches PDI1 codiert, einem Gen, welches ERO1 codiert, und einem Gen, welches Kar2 codiert, abgeleitet von *O. minuta*;
 - (b) eine Kombination aus einem Gen, welches PDI1 codiert, und einem Gen, welches Kar2 codiert, abgeleitet von *O. minuta*;
 - 55 (c) eine Kombination aus einem Gen, welches PDI, abgeleitet von einem Menschen, codiert, und einem Gen, welches ERO1, abgeleitet von *O. minuta*, codiert;
 - (d) eine Kombination aus einem Gen, welches PDI1 codiert, und einem Gen, welches ERO1 codiert, abgeleitet von *O. minuta*;

(e) eine Kombination aus einem Gen, welches PDI, abgeleitet von einem Menschen, codiert, einem Gen, welches ERO1-L β , abgeleitet von einem Menschen, codiert, und einem Gen, welches GRP78, abgeleitet von einem Menschen, codiert;
 5 (f) eine Kombination aus einem Gen, welches PDI, abgeleitet von einem Menschen, codiert, einem Gen, welches ERO1, abgeleitet von *O. minuta*, codiert, und einem Gen, welches GRP78, abgeleitet von einem Menschen, codiert; und
 (g) ein Gen, welches 95% oder mehr Sequenzhomologie zu einer Basensequenz irgendeines der Gene (a) bis
 (f) zeigt.

- 10 5. Transformierte Hefe gemäß irgendeinem der Ansprüche 1 bis 4, wobei die Hefe eine methylotrophe Hefe ist.
 6. Transformierte Hefe gemäß irgendeinem der Ansprüche 1 bis 5, welche ein Gen umfasst, das ein dort eingebrachtes Zielprotein codiert.
 15 7. Verwendung der transformierten Hefe gemäß irgendeinem der Ansprüche 1 bis 6 zur Produktion eines Zielproteins.
 8. Verfahren zum Produzieren eines Proteins, umfassend das Kultivieren der transformierten Hefe gemäß Anspruch 6 in einem Medium und Entnahme eines Zielproteins aus dem Kulturprodukt.
 20 9. Verfahren zum Produzieren eines Proteins gemäß Anspruch 8, wobei die Kultur unter Bedingungen durchgeführt wird, in denen Proteaseaktivität inhibiert ist.
 10. Verfahren zum Produzieren eines Proteins gemäß Anspruch 8 oder 9, wobei die Kultur in einem Medium mit einem pH-Wert von 6,0 bis 7,5 durchgeführt wird.
 25 11. Verfahren zum Produzieren eines Proteins gemäß irgendeinem der Ansprüche 8 bis 10, wobei eine Stickstoffquelle zu dem Medium hinzugefügt wird.
 12. Verfahren zum Produzieren eines Proteins gemäß den Ansprüchen 8 bis 11, wobei (a) Methanol nicht zu dem Medium hinzugefügt wird oder (b) die Menge an zu dem Medium hinzugefügtem Methanol 2% (v/v) oder weniger ist.
 30 13. Verfahren zum Produzieren einer transformierten Hefe, umfassend Schritt (i) zusätzlich zu sowohl Schritt (ii) als auch Schritt (iii):
 35 (i) einen Schritt des Einbringens eines Chaperon-Gens in Hefe; und
 (ii) einen Schritt des Unterbrechens des aox1-Gens in Hefe; und
 (iii) einen Schritt des Unterbrechens des prb1-Gens in Hefe,
 optional weiterhin umfassend einen Schritt des Einbringens eines ein Zielprotein codierenden Gens.

40 Revendications

1. Levure transformée dans laquelle un gène chaperon a été introduit et dans laquelle le gène aox1 a été interrompu, et un gène de protéase a été interrompu, dans laquelle facultativement le gène de protéase est un gène prb1.
 45 2. Levure transformée selon la revendication 1, dans laquelle le gène chaperon est au moins un gène sélectionné dans le groupe consistant en les gènes (a) à (d) ci-dessous :
 (a) un gène codant pour PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1, ou HSP104 dérivé de *Ogataea minuta* (*O. minuta*) ;
 50 (b) un gène codant pour PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5, ou EUG1 dérivé de *Saccharomyces cerevisiae* (*S. cerevisiae*) ;
 (c) un gène codant pour PDI, ERO1-L α , ERO1-L β , ou GRP78 dérivé d'un humain ; et
 55 (d) un gène présentant 95 % ou plus d'homologie de séquence avec une séquence de base de l'un quelconque des gènes (a) à (c).
 3. Levure transformée selon la revendication 1, dans laquelle le gène chaperon est au moins un gène sélectionné dans le groupe consistant en les gènes (a) à (g) ci-dessous :

- (a) un gène codant pour PDI1 dérivé de *O. minuta* ;
 (b) un gène codant pour ERO1 dérivé de *O. minuta* ;
 (c) un gène codant pour Kar2 dérivé de *O. minuta* ;
 (d) un gène codant pour PDI1 dérivé de *S. cerevisiae* ;
 5 (e) un gène codant pour la PDI dérivé d'un humain ;
 (f) un gène codant pour ERO1 dérivé d'un humain ; et
 (g) un gène présentant 95 % ou plus d'homologie de séquence avec une séquence de bases de l'un quelconque des gènes (a) à (f).

10 4. Levure transformée selon la revendication 1, dans laquelle le gène chaperon est l'un quelconque des gènes chaperon (a) à (g) ci-dessous :

- (a) une combinaison d'un gène codant pour PDI1, d'un gène codant pour ERO1, et d'un gène codant pour Kar2 dérivés de *O. minuta* ;
 15 (b) une combinaison d'un gène codant pour PDI1 et d'un gène codant pour Kar2 dérivés de *O. minuta* ;
 (c) une combinaison d'un gène codant pour PDI dérivé d'un humain et d'un gène codant pour ERO1 dérivé de *O. minuta* ;
 (d) une combinaison d'un gène codant pour PDI1 et d'un gène codant pour ERO1 dérivés de *O. minuta* ;
 20 (e) une combinaison d'un gène codant pour PDI dérivé d'un humain, d'un gène codant pour ERO1-L β dérivé d'un humain, et d'un gène codant pour GRP78 dérivé d'un humain ;
 (f) une combinaison d'un gène codant pour PDI dérivé d'un humain, d'un gène codant pour ERO1 dérivé de *O. minuta*, et d'un gène codant pour GRP78 dérivé d'un humain ; et
 (g) un gène présentant 95 % ou plus d'homologie de séquence avec une séquence de bases de l'un quelconque des gènes (a) à (f).

25 5. Levure transformée selon l'une quelconque des revendications 1 à 4, dans laquelle la levure est une levure méthylotrophe.

30 6. Levure transformée selon l'une quelconque des revendications 1 à 5, qui comprend un gène codant pour une protéine cible introduite dans celui-ci.

7. Utilisation de la levure transformée selon l'une quelconque des revendications 1 à 6 pour la production d'une protéine cible.

35 8. Procédé de production d'une protéine comprenant la mise en culture de la levure transformée selon la revendication 6 dans un milieu et l'échantillonnage d'une protéine cible à partir du produit de culture.

9. Procédé de production d'une protéine selon la revendication 8, dans lequel la culture est menée dans des conditions dans lesquelles l'activité des protéases est inhibée.

40 10. Procédé de production d'une protéine selon la revendication 8 ou 9, dans lequel la culture est menée dans un milieu ayant un pH de 6,0 à 7,5.

11. Procédé de production d'une protéine selon l'une quelconque des revendications 8 à 10, dans lequel une source 45 d'azote est ajoutée au milieu.

12. Procédé de production d'une protéine selon les revendications 8 à 11, dans lequel (a) du méthanol n'est pas ajouté au milieu ou (b) la quantité de méthanol ajoutée au milieu est de 2 % (v/v) ou moins.

50 13. Procédé de production d'une levure transformée comprenant l'étape (i) en plus des deux étapes (ii) et (iii) :

- (i) une étape d'introduction d'un gène chaperon dans la levure ; et
 (ii) une étape d'interruption du gène aox1 dans la levure ; et
 55 (iii) une étape d'interruption du gène prb1 dans la levure,
 facultativement comprenant en outre une étape d'introduction d'un gène codant pour une protéine cible.

Fig. 1

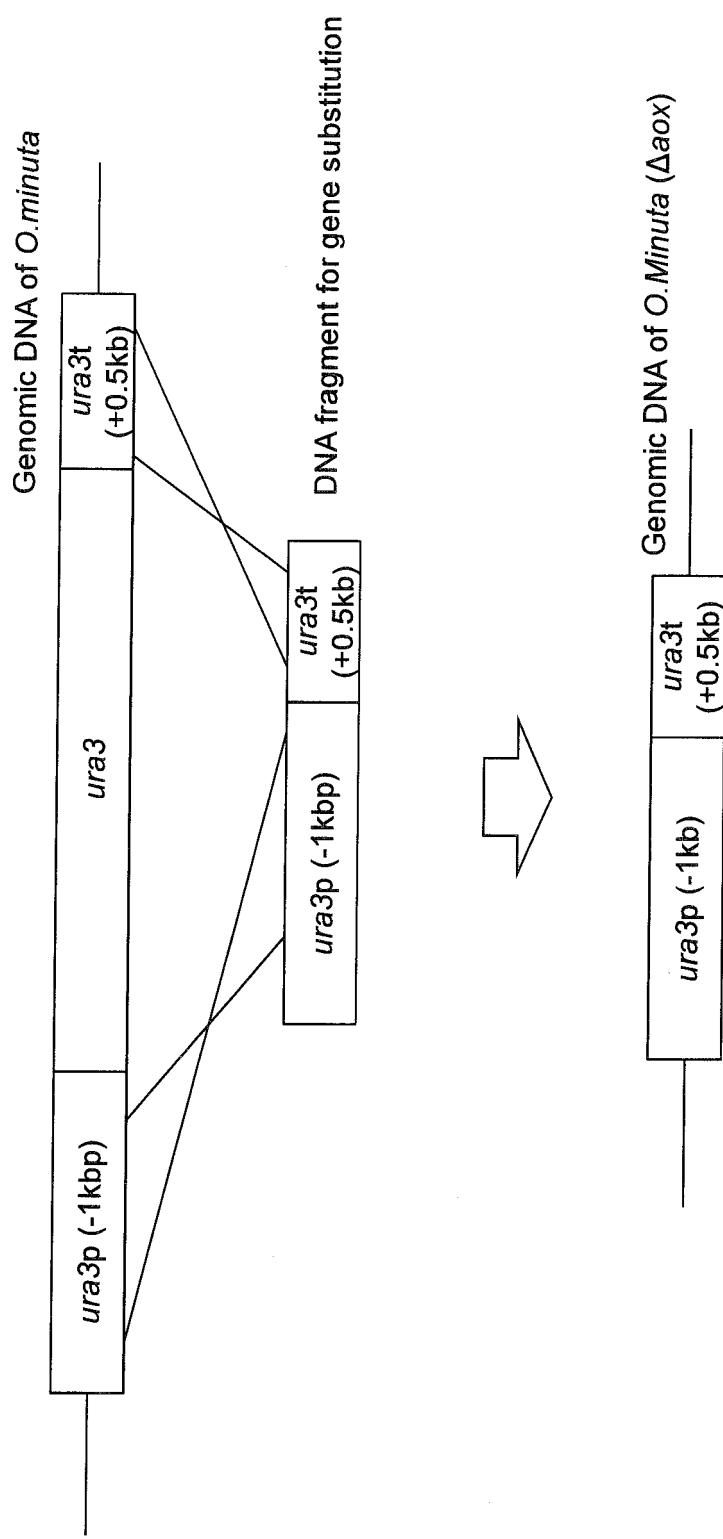


Fig. 2

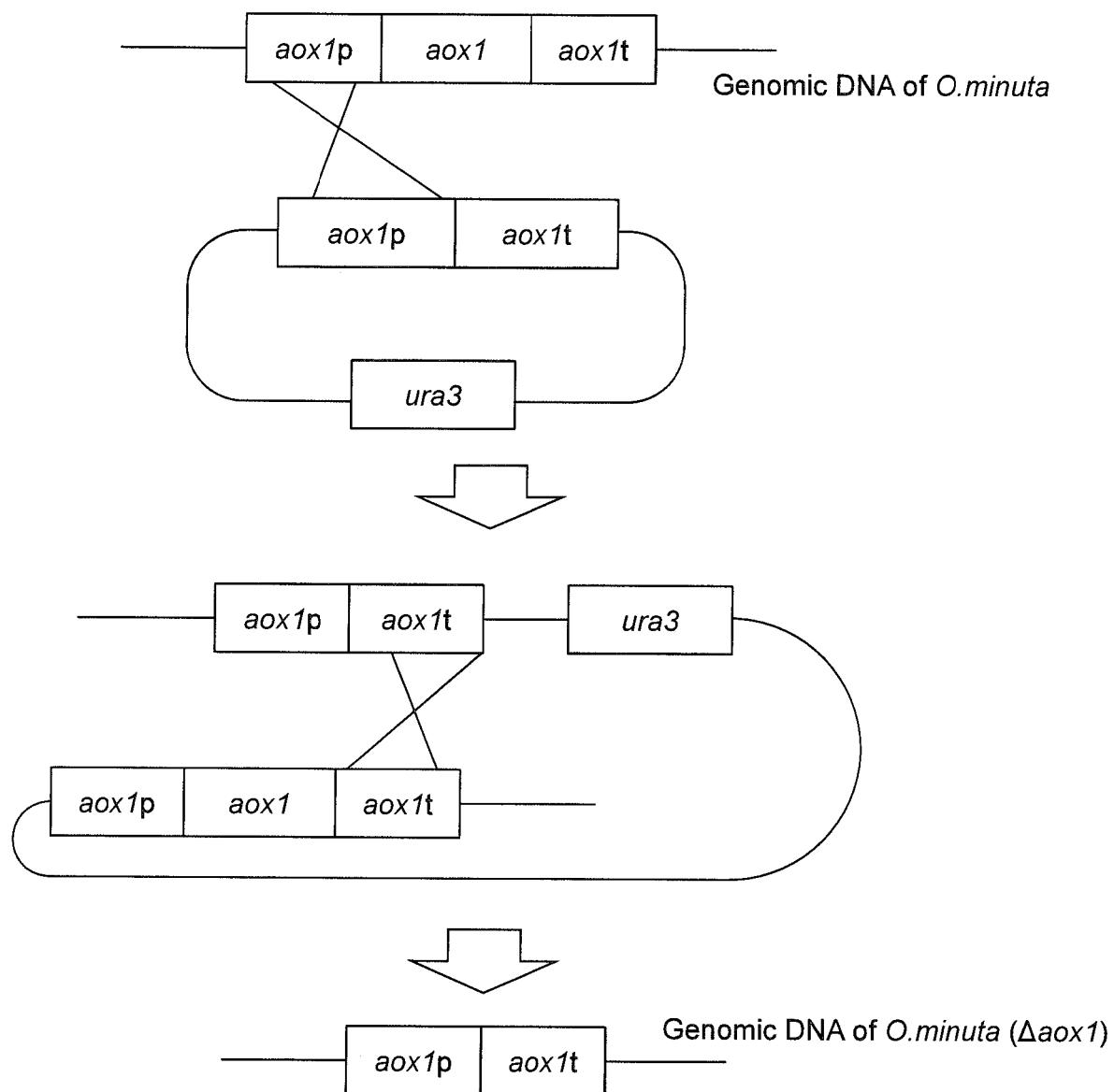


Fig. 3

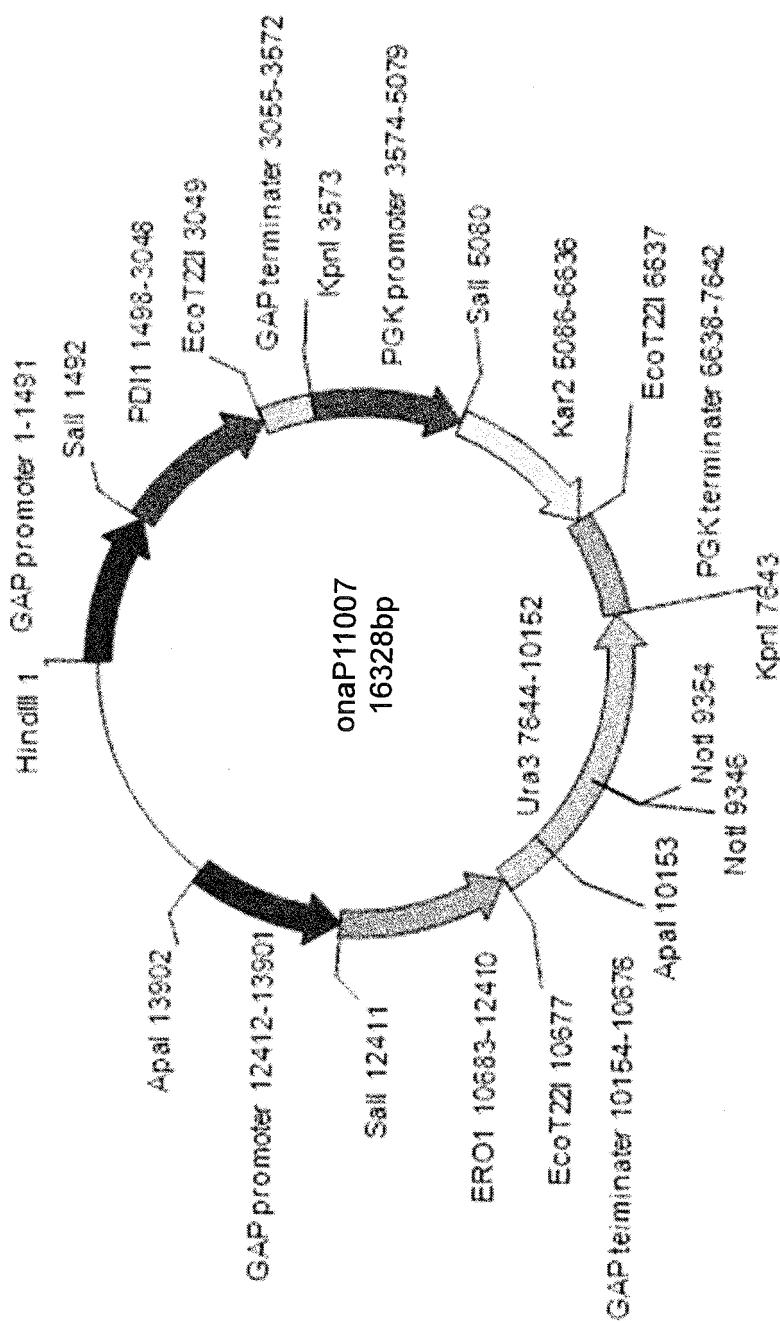


Fig. 4

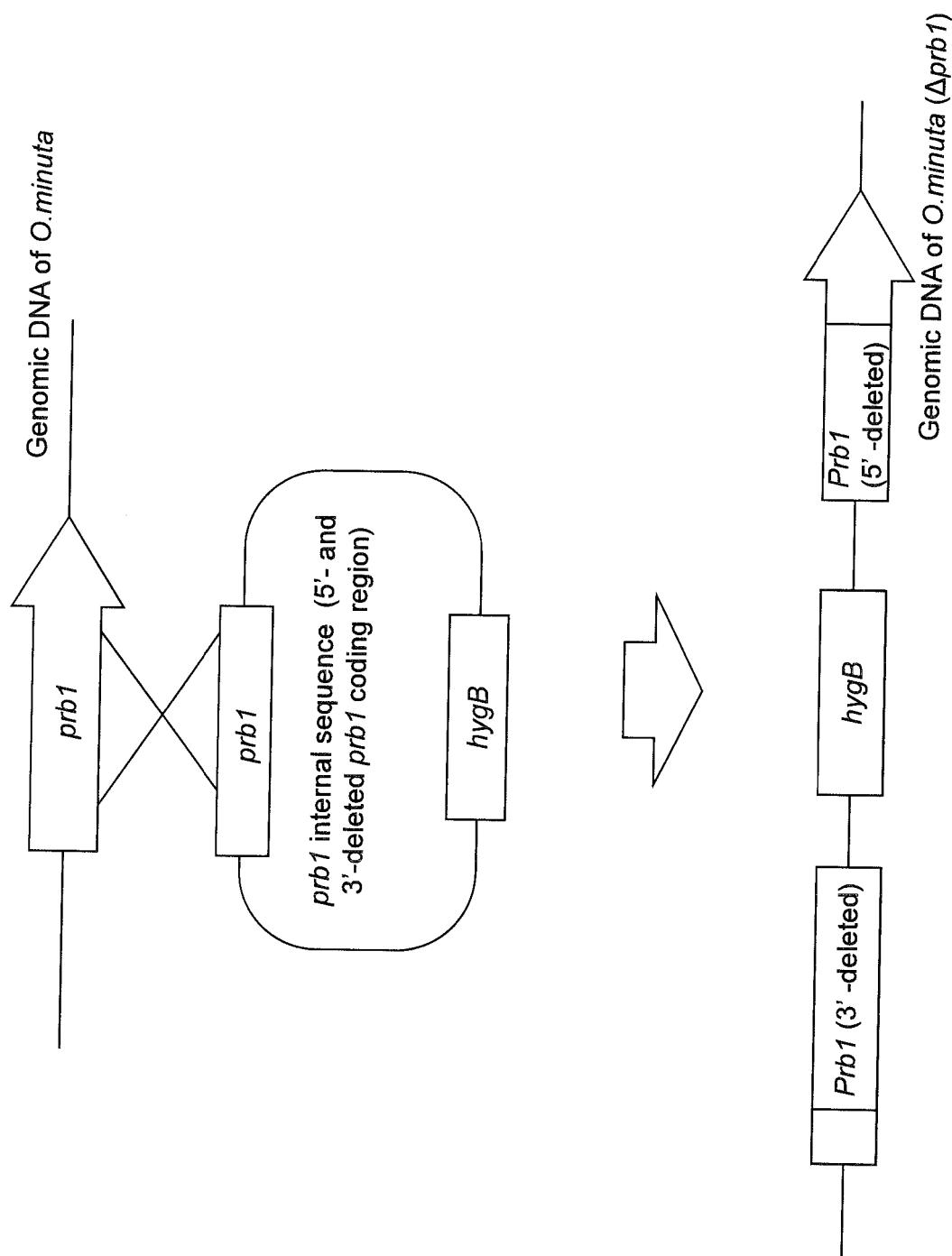


Fig. 5

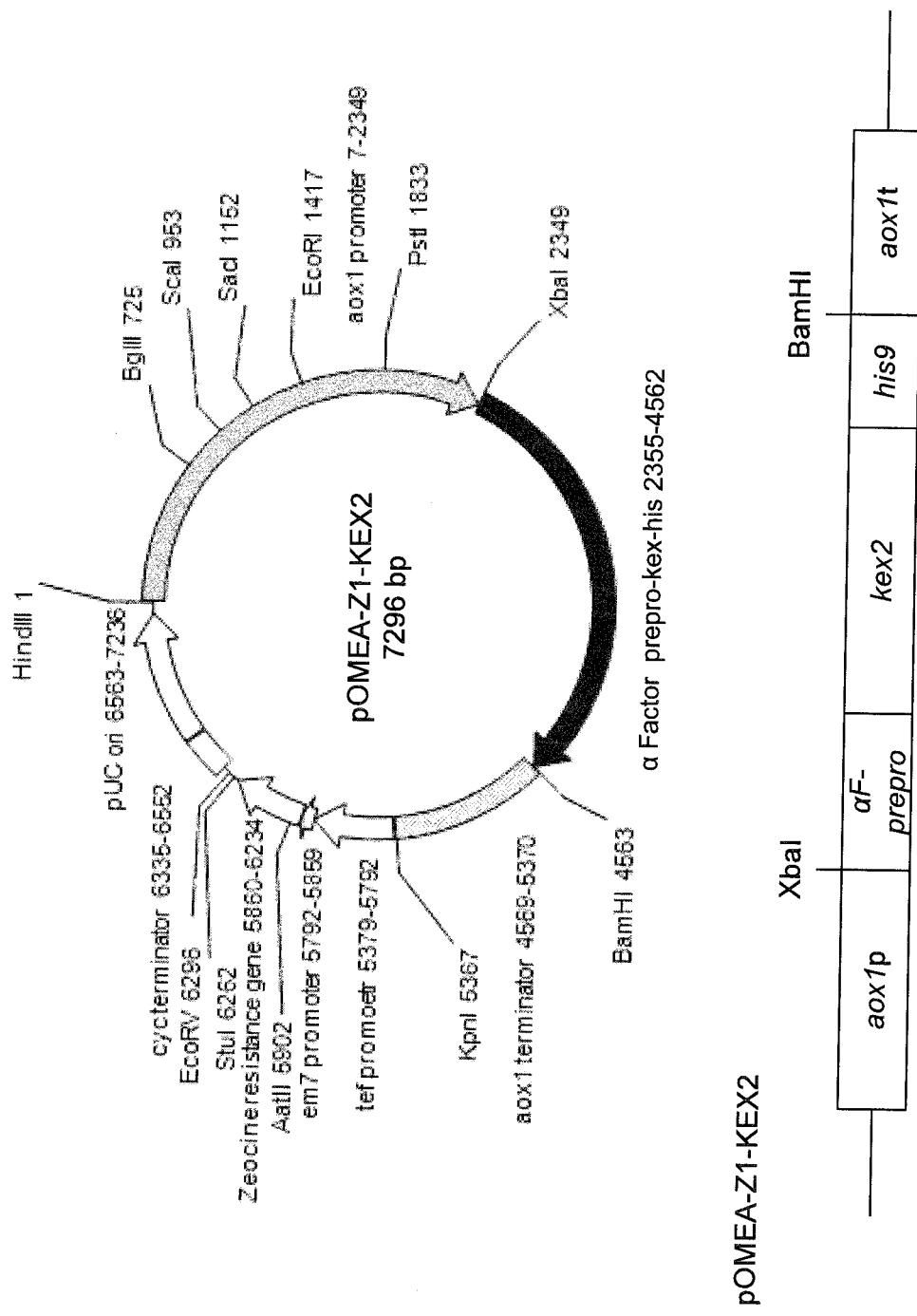


Fig. 6-1

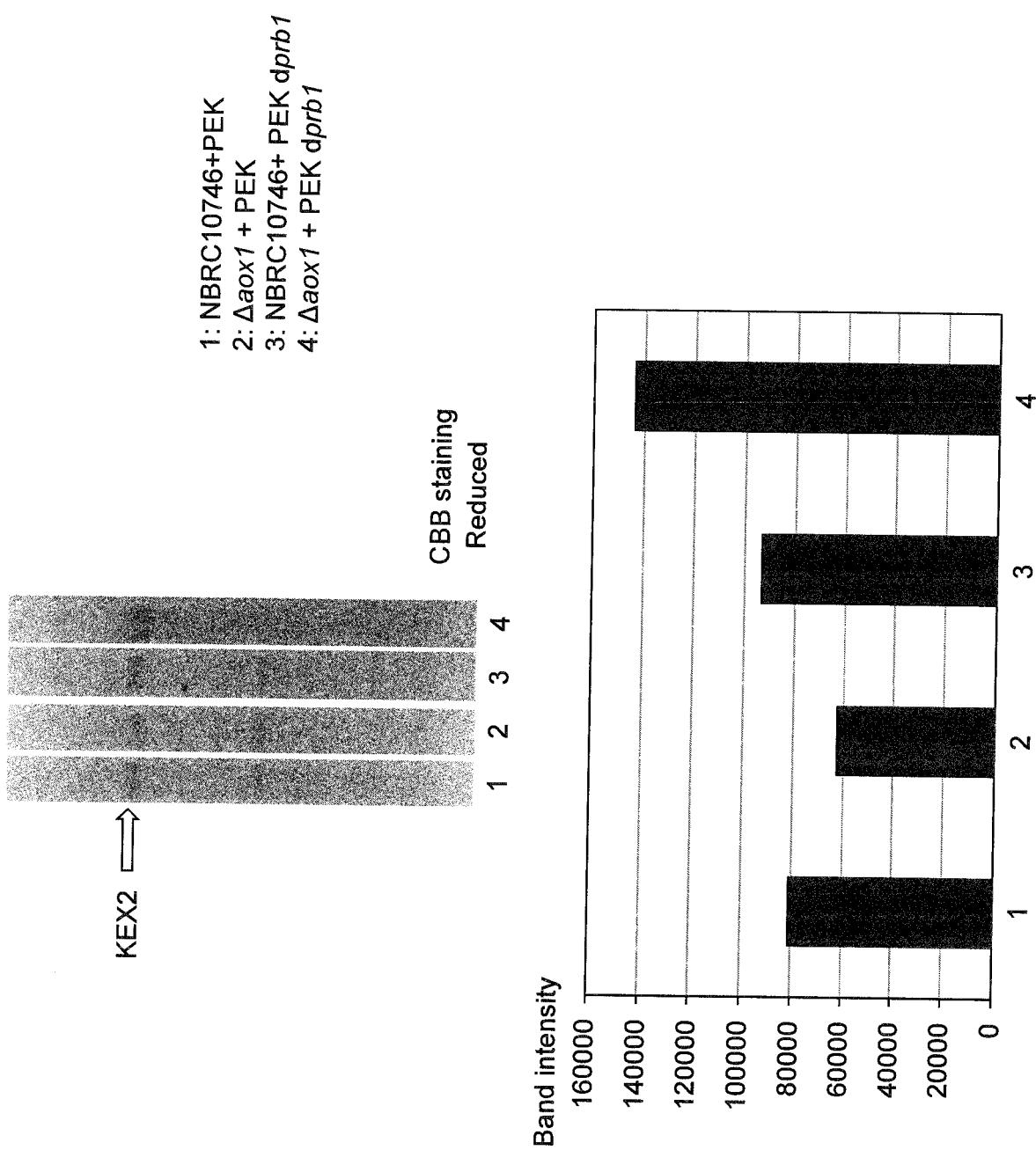


Fig. 6-2

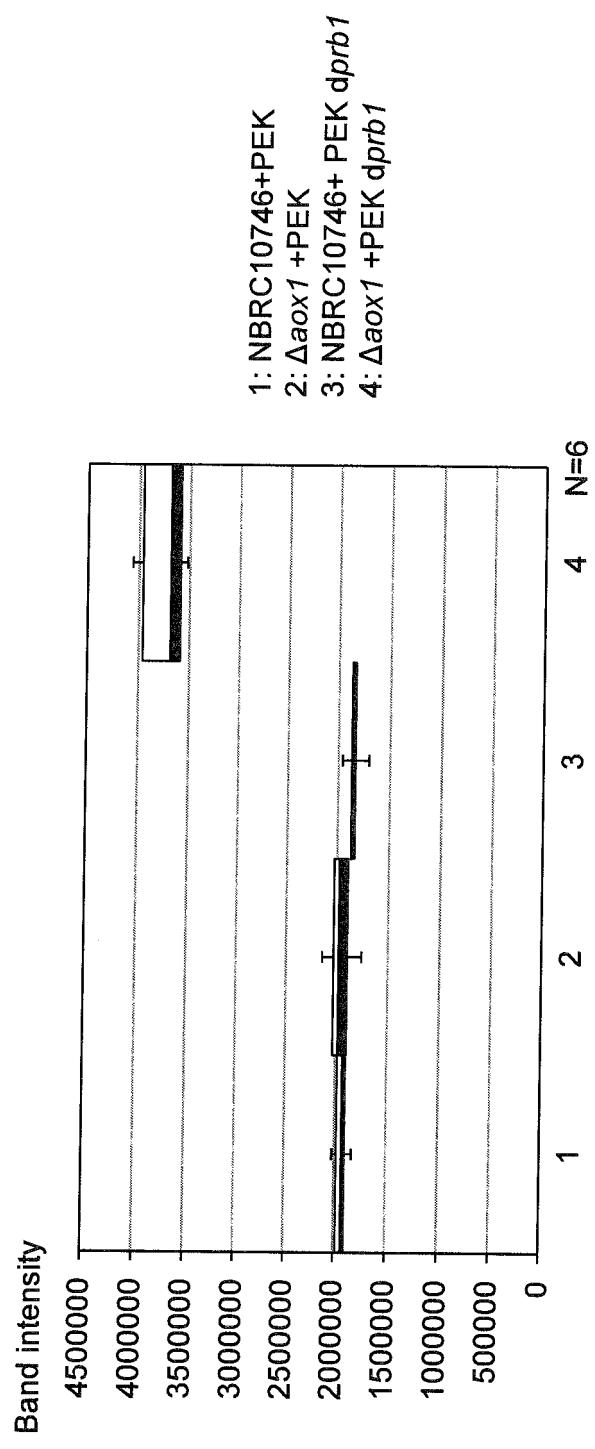
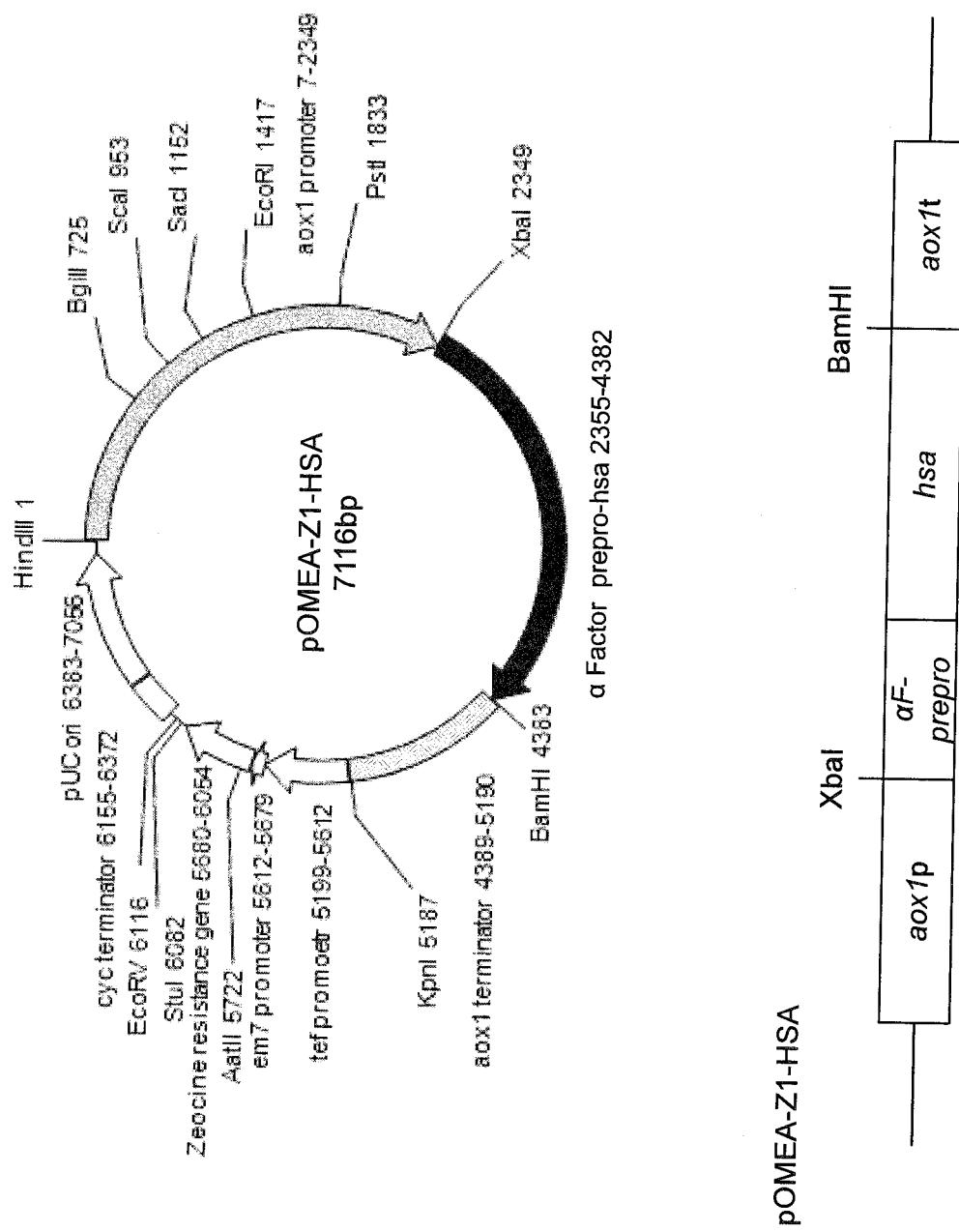


Fig. 7



pOMEA-Z1-HSA

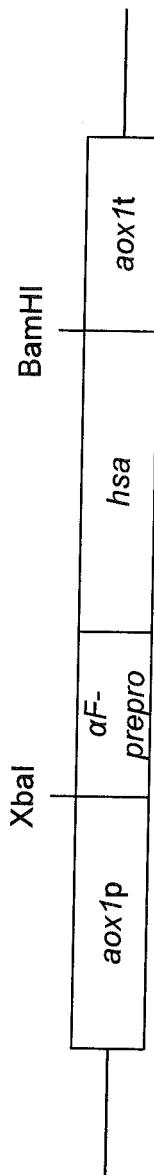
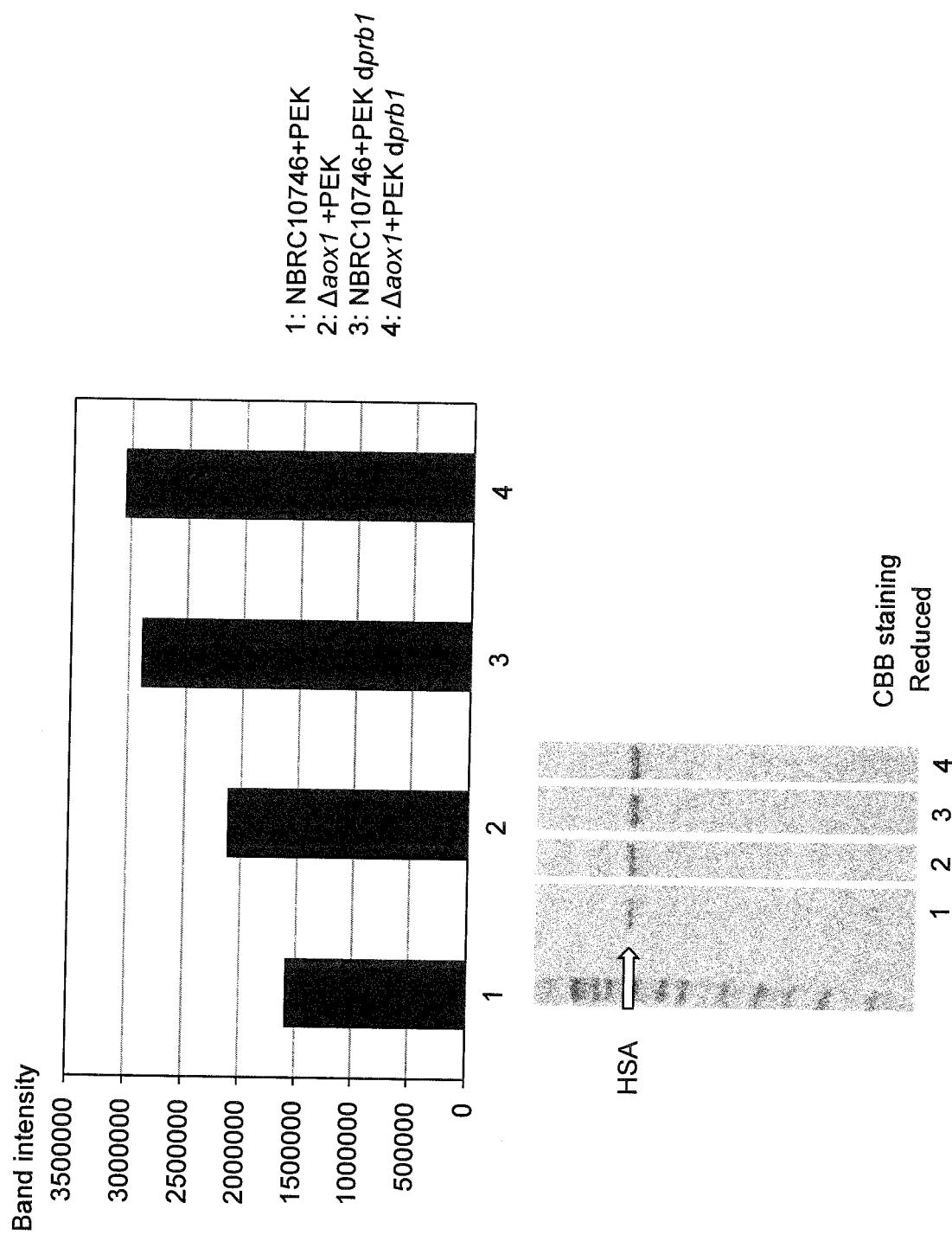


Fig. 8



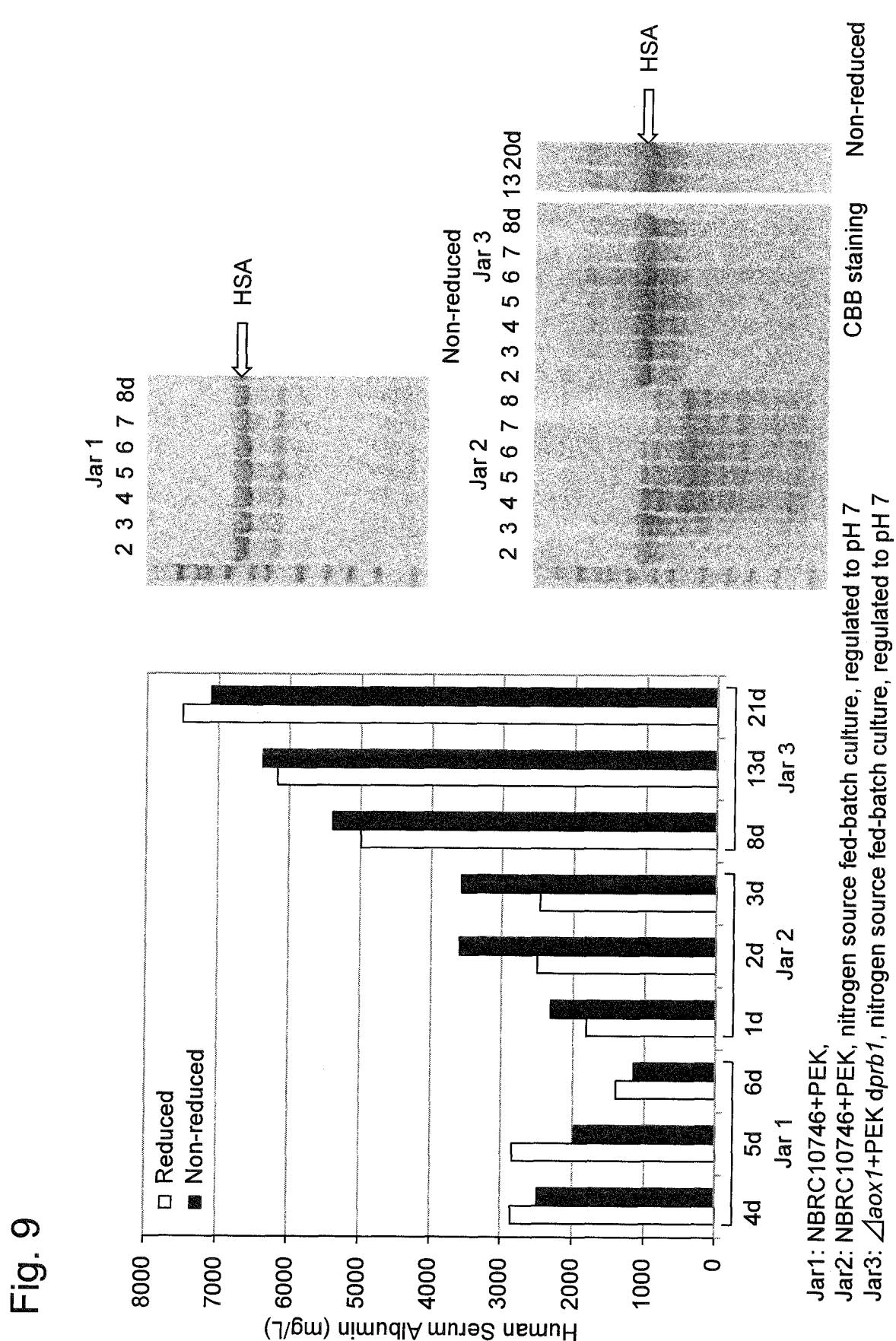
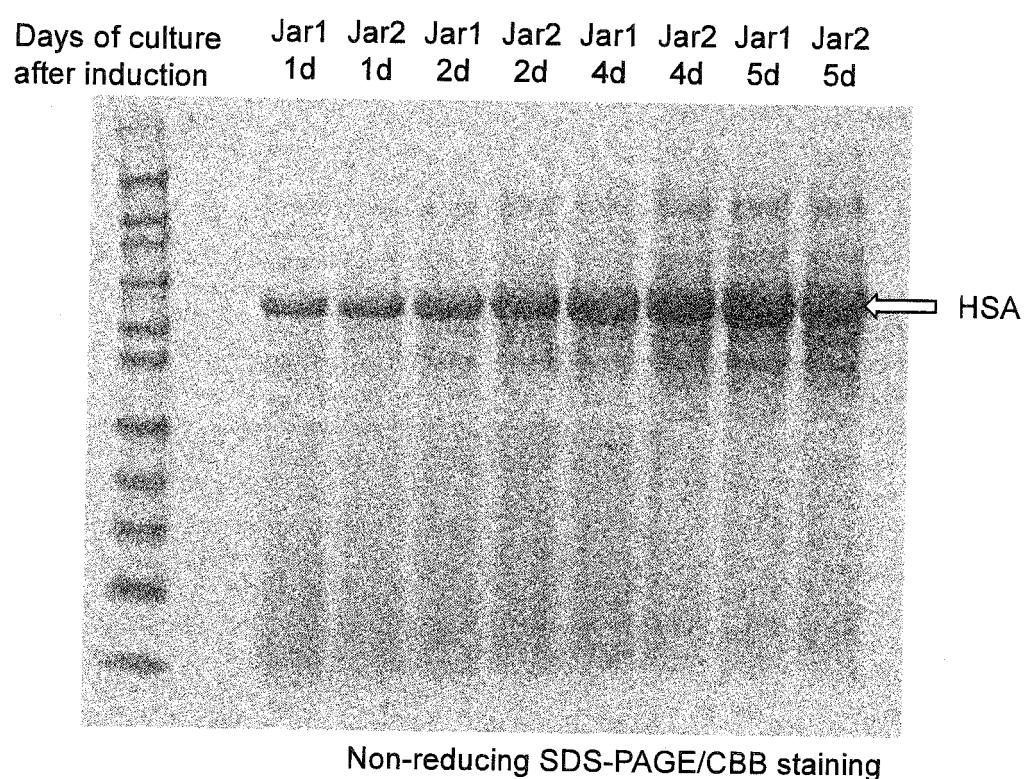


Fig. 10



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2009057813 A [0010] [0066] [0069] [0072] [0073]
- JP S62104585 A [0010]
- JP 2014155272 A [0018] [0115]
- JP 2000078978 A [0039]

Non-patent literature cited in the description

- **GLOVER JR ; LINDQUIST S.** Hsp104, Hsp70, and Hsp40: A novel chaperone system that rescues previously aggregated proteins. *Cell*, 1998, vol. 94, 73-82 [0011]
- **BENJAMIN P. TU ; JONATHAN S. WEISSMAN.** Oxidative protein folding in eukaryotes: mechanisms and consequences. *J. Cell Biol.*, 2004, vol. 164, 341-346 [0011]
- **MEZGHRAINI, A. ; FASSIO, A. ; BENHAM, A. ; SIMMEN, T. ; BRAAKMAN, I. ; SITIA, R.** Manipulation of oxidative protein folding and PDIredox state in mammalian cells. *EMBO J.*, 2001, vol. 20, 6288-6296 [0011]
- **FRAND, A. R. ; C. A. KAISER.** Ero1p oxidizes protein disulfide isomerase in a pathway for disulfide bond formation in the endoplasmic reticulum. *Mol. Cell*, 1999, vol. 4, 469-477 [0011]
- **PER NORGAAARD ; VIBEKE WESTPHAL ; CHRISTINE TACHIBANA ; LENE ALSOE ; BJORN HOLST ; JAKOB R. WINTHER.** Functional Differences in Yeast Protein Disulfide Isomerases. *J. Cell Biology*, 2001, vol. 152 (3), 553-562 [0011]
- **MARCUS MAYER ; URSULA KIES ; ROBERT KAMMERMEIER ; JOHANNES BUCHNER.** BiP and PDI Cooperate in the Oxidative Folding of Antibodies in Vitro. *J. Biol. Chem.*, 2000, vol. 275 (38), 29421-29425 [0011]
- **COX JS. ; SHAMU CE. ; WALTER P.** Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell*, 1993, vol. 73, 1197-1206 [0011]
- **SIDRAUSKI C. ; WALTER P.** The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell*, 1997, vol. 90 (6), 1031-1039 [0011]
- **SUSANA SILBERSTEIN ; GABRIEL SCHLENSTEDT ; PAM A. SILVER ; REID GILMORE.** A Role for the DnaJ Homologue Scj1p in Protein Folding in the Yeast Endoplasmic Reticulum. *J. Cell Biol.*, 1998, vol. 143 (4), 921-933 [0011]
- **MOROZKINA EV ; MARCHENKO AN ; KERUCHENKO JS ; KERUCHENKO ID ; KHOTCHENKOV VP ; POPOV VO ; BENEVOLEN-SKY SV.** Proteinase B disruption is required for high level production of human mechano-growth factor in *Saccharomyces cerevisiae*. *J. Mol. Microbiol. Biotechnol.*, 2010, vol. 18 (3), 188-194 [0011]
- **H. BART VAN DEN HAZEL ; MORTEN C. KIELLAND-BRANDT ; JAKOB R. WINTHER.** Autocatalysis of proteinase A initiates activation of yeast vacuolar zymogens. *Eur. J. Biochem.*, 1992, vol. 207, 277-283 [0011]
- **VICKI L. NEBES ; 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, vol. 266 (34), 22851-22857 [0011]
- **SUSANNE O. SORENSEN ; H. BART VAN DEN HAZEL ; MORTEN C. KIELLAND-BRANDT ; JAKOB R. WINTHER.** pH-dependent processing of yeast procarboxypeptidase Y by proteinase A in vivo and in vitro. *Eur. J. Biochem.*, 1994, vol. 220, 19-27 [0011]
- **IDA J. VAN DER KLEIA ; YURIMOTO H ; SAKAI Y ; VENHUISA M.** The significance of peroxisomes in methanol metabolism in methylotrophic yeast. *Biochim. Biophys. Acta*, 2006, vol. 1763, 1453-1462 [0011]
- **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, vol. 1493 (1-2), 56-63 [0011]
- **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, vol. 65 (3), 627-633 [0011]
- **SAKAI Y ; YOSHIDA H ; YURIMOTO H ; YOSHIDA N ; FUKUYA H ; TAKABE K ; KATO N.** Production of fungal fructosyl amino acid oxidase useful for diabetic diagnosis in the peroxisome of *Candida boidinii*. *FEBS Lett.*, 1999, vol. 459, 233-237 [0011]

- NISHIKAWA M ; HAGISHITA T ; YURIMOTO H ; KATO N ; SAKAI Y ; HATANAKA T. Primary structure and expression of peroxisomal acetylspermidine oxidase in the methylotrophic yeast *Candida boidinii*. *FEBS Lett.*, 2000, vol. 476, 150-154 [0011]
- SAMBROOK, J. et al. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 [0029]
- ITO et al. *Agric. Biol. Chem.*, 1984, vol. 48, 341 [0036]
- BECKER, D. M. et al. *Methods. Enzymol.*, 1990, vol. 194, 182-187 [0036]
- CREGGH et al. *Mol. Cell. Biol.*, 1985, vol. 5, 3376 [0036]
- ITOH, H. *J. Bacteriol.*, 1983, vol. 153, 163-168 [0036]
- BOEKER et al. *Mol. Gen. Genet.*, 1984, vol. 197, 345-346 [0043]
- BOEKER et al. *Methods Enzymol.*, 1987, vol. 154, 165-174 [0043]
- *Bioorganic & Medicinal Chemistry Letters*, 2004, vol. 14, 3975 [0055]