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(57) Abstract: The present invention relates to novel engineered Pichia strains with improved fermentation yields for expressing heterologous proteins with improved N-glycosylation quality, as well as to methods of generating such strains.

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ENGINEERED *PICHIA* STRAINS WITH IMPROVED FERMENTATION YIELD AND N-GLYCOSYLATION QUALITY

This application claims the benefit of U.S. provisional patent application no. 61/553,801; filed October 31, 2011; which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to novel engineered *Pichia* strains with improved fermentation yields for expressing heterologous proteins with improved N-glycosylation quality, as well as to methods of generating such strains.

BACKGROUND OF THE INVENTION

The methylotrophic yeast *Pichia pastoris* is one of the most widely used expression hosts for genetic engineering. This ascomycetous single-celled budding yeast has been used for the heterologous expression of hundreds of proteins (Lin-Cereghino, Curr Opin Biotech, 2002; Macauley-Patrick, Yeast, 2005). Importantly, *P. pastoris* is a lower eukaryote which provides the further advantage of having basic machinery for protein folding and post-translational modifications.

As a protein expression system, *P. pastoris* provides the advantages of a microbial system with facile genetics, shorter cycle times and the capability of achieving high cell densities. Secreted protein productivities have routinely been reported in the multi-gram per liter ranges. Several promoter systems are available for expression of proteins, for example, the methanol-inducible AOX1 promoter. The AOX1 promoter is a desirable feature of the *P. pastoris* system because it is tightly regulated and highly induced upon exposure to methanol (Cregg, Biotechnology, 1993, 11:905-910). The native Aox1p can be expressed up to 30% of total cellular protein when cells are grown on methanol. A drawback to this system is that cultivation on methanol during large scale fermentation can be complicated.

Constitutive promoter systems have been developed using the GAPDH promoter and more recently the TEF promoter (Waterham, Gene 1997, 186: 37-44; Ahn, Appl Microb Biotech, 2007, 74:601-608). These promoters are not as strong as AOX1, but, in some instances have lead to yield higher levels of secreted product than expression by AOX1, probably due to cultivation on a more energetically rich carbon source such as glycerol or glucose. However, such alternative promoter systems can be unpredictable for heterologous protein production.

Engineered *Pichia* strains have been utilized as an alternative host system for producing recombinant glycoproteins with human-like glycosylation. However, the extensive genetic modifications have also caused fundamental changes in cell wall structures in many glycoengineered yeast strains, predisposing some glyco-engineered strains to cell lysis and reduced cell robustness during fermentation.

Certain glyco-engineered strains have substantial reductions in cell viability as well as a marked increase in intracellular protease leakage into the fermentation broth, resulting in a reduction in both recombinant product yield and quality. Current strategies for identifying robust glyco-engineered *Pichia* production strains rely heavily on screening a large number of clones using various platforms such as 96-deep-well plates, 5ml mini-scale fermenters (Micro24), and 0.5L-scale bioreactors (DasGip) to empirically identify clones that are compatible for large-scale (40L and above) fermentation processes (Barnard et al. 2010). Despite the fact that high-throughput screening has been successfully used to identify several *Pichia* hosts capable of producing recombinant mAb with yields in excess of 1 g/L (anti-RSV and anti-Her2) (Potgieter et al. 2009; Zhang et al. 2011), these large-scale screening approach is very resource-intensive and time-consuming, and often only identify clones with incremental increases in cell-robustness.

Therefore, host strains that have improved robustness and the ability to produce high quality human-like proteins would be of value and interest to the field. Here, we present engineered *Pichia* host strains having a deletion, nonsense mutation, or other modification resulting in a truncation of a *P. pastoris* gene *XRN1*, which under bioprocess conditions produce both higher titer protein products that also exhibit improved N-glycosylation compared to protein produced produced in *XRN1* naïve parental strains under similar production conditions. These strains are especially useful for heterologous gene expression and production of therapeutic proteins.

SUMMARY OF THE INVENTION

The present invention relates to a modified *Pichia* sp. host cell wherein the host cell has been modified to reduce or eliminate expression of a functional gene product of a nucleic acid sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:76. In additional embodiments, the modified host cell comprises a disruption or deletion in the nucleic acid sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:76. In further embodiments, the host cell further comprises disruption or deletion of one or more of a functional gene product encoding an alpha-1,6-mannosyltransferase activity, mannosylphosphate transferase activity, and β-mannosyltransferase activity.

In yet additional embodiments, the invention further comprises one or more nucleic acid sequences of interest. In certain embodiments, the nucleic acid sequences of interest encode one or more glycosylation enzymes or oligosaccharyltransferases. In certain embodiments, the glycosylation enzymes or oligosaccharyltransferases are selected from the group consisting of glycosidases, mannosidases, phosphomannosidases, phosphatases, nucleotide sugar transporters, mannosyltransferases, the N-acetylglucosaminyltransferases, the UDP-N-acetylglucosamine transporters, the galactosyltransferases, the sialyltransferases, the protein mannosyltransferases, and the oligosaccharyltransferases STT3A, STT3B, STT3C and STT3D.

In yet additional embodiments, the nucleic acid sequences of interest encode one or more therapeutic proteins. In certain embodiments, the therapeutic proteins are selected from the group consisting of an immunoglobulin heavy chain variable domain (optionally wherein the variable domain is linked to an immunoglobulin heavy chain constant domain), an immunoglobulin light chain variable domain (optionally wherein the variable domain is linked to an immunoglobulin light chain constant domain), kringle domains of the human plasminogen, erythropoietin, cytokines, coagulation factors, soluble IgE receptor α-chain, IgG, IgG fragments, IgM, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α-1 antitrypsin, DNase II , insulin, Fc-fusions, and HSA-fusions.

The present invention further provides a *Pichia sp.* host cell comprising a disruption or deletion of the *XRN1* gene in the genomic DNA of the host cell that encodes a protein having of a nucleic acid sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:76. In yet additional embodiments, the host cell further comprises a nucleic acid sequence of interest.

In yet additional embodiments, the modified host cell of the present invention produces proteins with improved N-glycosylation compared with the *XRN1* naïve parental host cell under similar culture conditions.

In yet additional embodiments, the invention relates to a method for producing glycoprotein compositions in *Pichia sp.* host cells, said method comprising growing the modified host cells described herein under inducing conditions.

In further embodiments, the host cell further comprises disruption or deletion of one or more of a functional gene product encoding an alpha-1,6-mannosyltransferase activity, mannosylphosphate transferase activity, β-mannosyltransferase activity, or a dolichol-P-Man dependent alpha(1-3) mannosyltransferase activity.

In yet additional embodiments, the invention further comprises one or more nucleic acid sequences of interest. In certain embodiments, the nucleic acid sequences of interest encode one or more glycosylation enzymes or oligosaccharyltransferases. In certain embodiments, the glycosylation enzymes or oligosaccharyltransferases are selected from the group consisting of glycosidases, mannosidases, phosphomannosidases, phosphatases, nucleotide sugar transporters, mannosyltransferases, the N-acetylglucosaminyltransferases, the UDP-N-acetylglucosamine transporters, the galactosyltransferases, the sialyltransferases, the protein mannosyltransferases, and the oligosaccharyltransferases STT3A, STT3B, STT3C and STT3D.

In yet additional embodiments, the nucleic acid sequences of interest encode one or more therapeutic proteins. In certain embodiments, the therapeutic proteins are selected from the group consisting of kringle domains of the human plasminogen, erythropoietin, cytokines, coagulation factors, soluble IgE receptor α-chain, IgG, IgG fragments, IgM, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein,

angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α-1 antitrypsin, DNase II,α-feto proteins, insulin, Fc-fusions, and HSA-fusions.

In certain embodiments, the invention also provides host cells comprising a disruption, deletion or mutation of a nucleic acid sequence selected from the group consisting of the coding sequence of the *P. pastoris XRN1* gene, a nucleic acid sequence that is a degenerate variant of the coding sequence of the *P. pastoris XRN1* gene and related nucleic acid sequences and fragments, in which the host cells have a reduced activity of the polypeptide encoded by the nucleic acid sequence compared to a host cell without the disruption, deletion or mutation.

In addition, the invention provides methods for the genetic integration of a heterologous nucleic acid sequence into a host cell comprising a disruption or deletion of the *P. pastoris XRN1* gene in the genomic DNA of the host cell. These methods comprise the step of introducing a sequence of interest into the host cell comprising a disrupted, deleted or mutated nucleic acid sequence derived from a sequence selected from the group consisting of the coding sequence of the *P. pastoris XRN1* gene, a nucleic acid sequence that is a degenerate variant of the coding sequence of the *P. pastoris XRN1* gene and related nucleic acid sequences and fragments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 A-H shows the genealogy of *P. pastoris* strain YGLY12501 (Figure 1F), YGLY13992 (Figure 1G), and strain YGLY14836 (Figure 1H) beginning from wild-type strain NRRL-Y11430 (Figure 1A).

Figures 2 A-C shows the genealogy of *P. pastoris* glycoinsulin producing strain **YGLY21058 (Figure 2A)** beginning from glycoengineering strain **YGLY7961.**

Figure 3 shows as map of plasmid pGLY7392. Plasmid pGLY7392 is an integration vector that targets the *XRN1/KEM1* loci contains a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by nucleic acid molecules comprising *lacZ* repeats (lacZ repeat) which in turn is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *XRN1* gene (PpXRN1-5') and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *XRN1* gene (Pp XRN1-3').

Figure 4 shows a map of plasmid pGLY6. Plasmid pGLY6 is an integration vector that targets the *URA5* locus and contains a nucleic acid molecule comprising the *S. cerevisiae* invertase gene or transcription unit (ScSUC2) flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *P. pastoris URA5* gene (PpURA5-5') and on the other side by a nucleic acid molecule comprising the a nucleotide sequence from the 3' region of the *P. pastoris URA5* gene (PpURA5-3').

Figure 5 shows a map of plasmid pGLY40. Plasmid pGLY40 is an integration vector that targets the *OCH1* locus and contains a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by nucleic acid molecules

comprising *lacZ* repeats (lacZ repeat) which in turn is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *OCH1* gene (PpOCH1-5') and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *OCH1* gene (PpOCH1-3').

Figure 6 shows a map of plasmid pGLY43a. Plasmid pGLY43a is an integration vector that targets the *BMT2* locus and contains a nucleic acid molecule comprising the *K. lactis* UDP-*N*-acetylglucosamine (UDP-GlcNAc) transporter gene or transcription unit (KlGlcNAc Transp.) adjacent to a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by nucleic acid molecules comprising *lacZ* repeats (lacZ repeat). The adjacent genes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *BMT2* gene (PpPBS2-5') and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *BMT2* gene (PpPBS2-3').

Figure 7 shows a map of plasmid pGLY48. Plasmid pGLY48 is an integration vector that targets the *MNN4L1* locus and contains an expression cassette comprising a nucleic acid molecule encoding the mouse homologue of the UDP-GlcNAc transporter (MmGlcNAc Transp.) open reading frame (ORF) operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GAPDH* promoter (PpGAPDH Prom) and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* termination sequence (ScCYC TT) adjacent to a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by *lacZ* repeats (lacZ repeat) and in which the expression cassettes together are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *P. pastoris MNN4L1* gene (PpMNN4L1-5') and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *MNN4L1* gene (PpMNN4L1-3').

Figure 8 shows as map of plasmid pGLY45. Plasmid pGLY45 is an integration vector that targets the *PNO1/MNN4* loci contains a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by nucleic acid molecules comprising *lacZ* repeats (lacZ repeat) which in turn is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *PNO1* gene (PpPNO1-5') and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *MNN4* gene (PpMNN4-3').

Figure 9 shows a map of plasmid pGLY1430. Plasmid pGLY1430 is a KINKO integration vector that targets the *ADE1* locus without disrupting expression of the locus and contains in tandem four expression cassettes encoding (1) the human GlcNAc transferase I catalytic domain (codon optimized) fused at the *N*-terminus to *P. pastoris SEC12* leader peptide (CO-NA10), (2) mouse homologue of the UDP-GlcNAc transporter (MmTr), (3) the mouse mannosidase IA catalytic domain (FB) fused at the *N*-terminus to *S. cerevisiae SEC12* leader peptide (FB8), and (4) the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by

lacZ repeats (lacZ). All flanked by the 5' region of the *ADE1* gene and ORF (ADE1 5' and ORF) and the 3' region of the *ADE1* gene (PpADE1-3'). PpPMA1 prom is the *P. pastoris PMA1* promoter; PpPMA1 TT is the *P. pastoris PMA1* termination sequence; SEC4 is the *P. pastoris SEC4* promoter; OCH1 TT is the *P. pastoris OCH1* termination sequence; ScCYC TT is the *S. cerevisiae CYC* termination sequence; PpOCH1 Prom is the *P. pastoris OCH1* promoter; PpALG3 TT is the *P. pastoris ALG3* termination sequence; and PpGAPDH is the *P. pastoris GADPH* promoter.

Figure 10 shows a map of plasmid pGLY582. Plasmid pGLY582 is an integration vector that targets the *HIS1* locus and contains in tandem four expression cassettes encoding (1) the *S. cerevisiae* UDP-glucose epimerase (ScGAL10), (2) the human galactosyltransferase I (hGalT) catalytic domain fused at the N-terminus to the *S. cerevisiae KRE2-s* leader peptide (33), (3) the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by lacZ repeats (lacZ repeat), and (4) the *D. melanogaster* UDP-galactose transporter (DmUGT). All flanked by the 5' region of the *HIS1* gene (PpHIS1-5') and the 3' region of the *HIS1* gene (PpHIS1-3'). PMA1 is the *P. pastoris PMA1* promoter; PpPMA1 TT is the *P. pastoris PMA1* termination sequence; GAPDH is the *P. pastoris GADPH* promoter and ScCYC TT is the *S. cerevisiae CYC* termination sequence; PpOCH1 Prom is the *P. pastoris OCH1* promoter and PpALG12 TT is the *P. pastoris ALG12* termination sequence.

Figure 11 shows a map of plasmid pGLY167b. Plasmid pGLY167b is an integration vector that targets the *ARG1* locus and contains in tandem three expression cassettes encoding (1) the *D. melanogaster* mannosidase II catalytic domain (codon optimized) fused at the *N*-terminus to *S. cerevisiae MNN2* leader peptide (CO-KD53), (2) the *P. pastoris HIS1* gene or transcription unit, and (3) the rat *N*-acetylglucosamine (GlcNAc) transferase II catalytic domain (codon optimized) fused at the *N*-terminus to *S. cerevisiae MNN2* leader peptide (CO-TC54). All flanked by the 5' region of the *ARG1* gene (PpARG1-5') and the 3' region of the *ARG1* gene (PpARG1-3'). PpPMA1 prom is the *P. pastoris PMA1* promoter; PpPMA1 TT is the *P. pastoris PMA1* termination sequence; PpGAPDH is the *P. pastoris GADPH* promoter; ScCYC TT is the *S. cerevisiae CYC* termination sequence; PpOCH1 Prom is the *P. pastoris OCH1* promoter; and PpALG12 TT is the *P. pastoris ALG12* termination sequence.

Figure 12 shows a map of plasmid pGLY3411 (pSH1092). Plasmid pGLY3411 (pSH1092) is an integration vector that contains the expression cassette comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by *lacZ* repeats (lacZ repeat) flanked on one side with the 5' nucleotide sequence of the *P. pastoris BMT4* gene (PpPBS4 5') and on the other side with the 3' nucleotide sequence of the *P. pastoris BMT4* gene (PpPBS4 3').

Figure 13 shows a map of plasmid pGLY3419 (pSH1110). Plasmid pGLY3430 (pSH1115) is an integration vector that contains an expression cassette comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by *lacZ* repeats (lacZ repeat) flanked

on one side with the 5' nucleotide sequence of the *P. pastoris BMT1* gene (PBS1 5') and on the other side with the 3' nucleotide sequence of the *P. pastoris BMT1* gene (PBS1 3')

Figure 14 shows a map of plasmid pGLY3421 (pSH1106). Plasmid pGLY4472 (pSH1186) contains an expression cassette comprising the *P. pastoris URA5* gene or transcription unit (PpURA5) flanked by *lacZ* repeats (lacZ repeat) flanked on one side with the 5' nucleotide sequence of the *P. pastoris BMT3* gene (PpPBS3 5') and on the other side with the 3' nucleotide sequence of the *P. pastoris BMT3* gene (PpPBS3 3').

Figure 15 shows a map of plasmid pGLY3673. Plasmid pGLY3673 is a KINKO integration vector that targets the *PRO1* locus without disrupting expression of the locus and contains expression cassettes encoding the *T. reesei* α-1,2-mannosidase catalytic domain fused at the *N*-terminus to *S. cerevisiae* αMATpre signal peptide (aMATTrMan) to target the chimeric protein to the secretory pathway and secretion from the cell.

Figure 16 shows a map of pGLY5883 encoding the light and heavy chains of an anti-Her2 antibody. The plasmid is a roll-in vector that targets the *TRP2* locus. The ORFs encoding the light and heavy chains are under the control of a *P. pastoris* AOX1 promoter and the *S. cerevisiae CYC* 3UTR transcription termination sequence. Selection of transformants uses zeocin resistance encoded by the zeocin resistance protein ($Zeocin^R$) ORF under the control of the *P. pastoris TEF1* promoter and *S. cerevisiae CYC* termination sequence.

Figure 17 shows a map of pGLY6833 encoding the light and heavy chains of an anti-Her2 antibody. The plasmid is a roll-in vector that targets the *TRP2* locus. The ORFs encoding the light and heavy chains are under the control of a *P. pastoris* AOX1 promoter and the *P. pastoris CIT1* 3UTR transcription termination sequence. Selection of transformants uses zeocin resistance encoded by the zeocin resistance protein ($Zeocin^R$) ORF under the control of the *P. pastoris TEF1* promoter and *S. cerevisiae CYC* termination sequence.

Figure 18 shows a map of plasmid pGLY3714. Plasmid pGLY3714 is a KINKO integration vector that targets the *TRP1* locus without disrupting expression of the locus and contains expression cassettes encoding the mouse mannosidase IB catalytic domain (GD) fused at the *N*-terminus to *S. cerevisiae SEC12* leader peptide (GD9) to target the chimeric enzyme to the ER or Golgi. For selecting transformants, the plasmid comprises an expression cassette encoding the Nourseothricin resistance (NAT^R) ORF (originally from pAG25 from EROSCARF, Scientific Research and Development GmbH, Daimlerstrasse 13a, D-61352 Bad Homburg, Germany, *See* Goldstein *et al.*, Yeast 15: 1541 (1999)); wherein the nucleic acid molecule encoding the ORF (SEQ ID NO:64) is operably linked to at the 5' end to a nucleic acid molecule having the *Ashbya gossypii TEF1* promoter sequence (SEQ ID NO:65) and at the 3' end to a nucleic acid molecule that has the *Ashbya gossypii TEF1* termination sequence (SEQ ID NO:66).

Figure 19 shows a map of plasmid pGLY2456. Plasmid pGLY2456 is a KINKO integration vector that targets the *TRP2* locus without disrupting expression of the locus and contains six expression cassettes encoding (1) the mouse CMP-sialic acid transporter codon

optimized (CO mCMP-Sia Transp), (2) the human UDP-GlcNAc 2-epimerase/*N*-acetylmannosamine kinase codon optimized (CO hGNE), (3) the *Pichia pastoris ARG1* gene or transcription unit, (4) the human CMP-sialic acid synthase codon optimized (CO hCMP-NANA S), (5) the human N-acetylneuraminate-9-phosphate synthase codon optimized (CO hSIAP S), and, (6) the mouse a-2,6-sialyltransferase catalytic domain codon optimized fused at the *N*-terminus to *S. cerevisiae KRE2* leader peptide (comST6-33). All flanked by the 5' region of the *TRP2* gene and ORF (PpTRP2 5') and the 3' region of the *TRP2* gene (PpTRP2-3'). PpPMA1 prom is the *P. pastoris PMA1* promoter; PpPMA1 TT is the *P. pastoris PMA1* termination sequence; CYC TT is the *S. cerevisiae CYC* termination sequence; PpTEF Prom is the *P. pastoris TEF1* promoter; PpTEF TT is the *P. pastoris TEF1* termination sequence; PpALG3 TT is the *P. pastoris ALG3* termination sequence; and pGAP is the *P. pastoris GAPDH* promoter.

Figure 20 shows a map of plasmid pGLY5048 (pSH1275). Plasmid pGLY5048 (pSH1275) is an integration vector that targets the *STE13* locus and contains expression cassettes encoding (1) the *T. reesei* α-1,2-mannosidase catalytic domain fused at the *N*-terminus to *S. cerevisiae* αMATpre signal peptide (aMATTrMan) to target the chimeric protein to the secretory pathway and secretion from the cell and (2) the *P. pastoris URA5* gene or transcription unit.

Figure 21 shows a map of plasmid pGLY5019 (pSH1246). Plasmid pGLY5019 (pSH1246) is an integration vector that targets the *DAP2* locus and contains an expression cassette comprising a nucleic acid molecule encoding the Nourseothricin resistance (NAT^R) ORF operably linked to the *Ashbya gossypii TEF1* promoter and *A. gossypii TEF1* termination sequences flanked one side with the 5' nucleotide sequence of the *P. pastoris DAP2* gene and on the other side with the 3' nucleotide sequence of the *P. pastoris DAP2* gene.

Figure 22 shows a map of plasmid pGLY5085 (pSH1312). Plasmid pGLY5085 (pSH1312) is a KINKO plasmid for introducing a second set of the genes involved in producing sialylated *N*-glycans into *P. pastoris*. The plasmid is similar to plasmid YGLY2456 except that the *P. pastoris ARG1* gene has been replaced with an expression cassette encoding hygromycin resistance (HygR) and the plasmid targets the *P. pastoris TRP5* locus. The six tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region and ORF of the *TRP5* gene ending at the stop codon followed by a *P. pastoris ALG3* termination sequence and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *TRP5* gene.

Figure 23 shows map of plasmid **pGLY4362,** which is a roll-in integration plasmid that targets the *TRP2* or *AOX1p* loci, includes an expression cassette encoding an insulin

precursor fusion protein comprising a Yps1ss peptide fused to a TA57 propeptide fused to an *N*-terminal spacer fused to the human insulin B-chain with a P28N substitution fused to a C-peptide consisting of the amino acid sequence AAK fused to the human insulin A-chain.

DETAILED DESCRIPTION OF THE INVENTION

Molecular Biology

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See*, e.g., James M. Cregg (Editor), *Pichia* Protocols (Methods in Molecular Biology), Humana Press (2010), Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Taylor and Drickamer, Introduction to Glycobiology, Oxford Univ. Press (2003); Worthington Enzyme Manual, Worthington Biochemical Corp., Freehold, N.J.; Handbook of Biochemistry: Section A Proteins, Vol I, CRC Press (1976); Handbook of Biochemistry: Section A Proteins, Vol II, CRC Press (1976); Essentials of Glycobiology, Cold Spring Harbor Laboratory Press (1999), Animal Cell Culture (R.I. Freshney, ed. (1986)); Immobilized Cells And Enzymes (IRL Press, (1986)); B. Perbal, A Practical Guide To Molecular Cloning (1984);

A "polynucleotide", "nucleic acid "includes DNA and RNA in single stranded form, double-stranded form or otherwise.

A "polynucleotide sequence" or "nucleotide sequence" is a series of nucleotide bases (also called "nucleotides") in a nucleic acid, such as DNA or RNA, and means a series of two or more nucleotides. Any polynucleotide comprising a nucleotide sequence set forth herein (*e.g.*, promoters of the present invention) forms part of the present invention.

A "coding sequence" or a sequence "encoding" an expression product, such as an RNA or polypeptide is a nucleotide sequence (*e.g.*, heterologous polynucleotide) that, when expressed, results in

production of the product (*e.g.*, a heterologous polypeptide such as an immunoglobulin heavy chain and/or light chain).

As used herein, the term "oligonucleotide" refers to a nucleic acid, generally of no more than about 100 nucleotides (*e.g.*, 30, 40, 50, 60, 70, 80, or 90), that may be hybridizable to a polynucleotide molecule. Oligonucleotides can be labeled, *e.g.*, by incorporation of ^{32}P-nucleotides, ^{3}H-nucleotides, ^{14}C-nucleotides, ^{35}S-nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated.

A "protein", "peptide" or "polypeptide" (*e.g.*, a heterologous polypeptide such as an immunoglobulin heavy chain and/or light chain) includes a contiguous string of two or more amino acids.

A "protein sequence", "peptide sequence" or "polypeptide sequence" or "amino acid sequence" refers to a series of two or more amino acids in a protein, peptide or polypeptide.

The term "isolated polynucleotide" or "isolated polypeptide" includes a polynucleotide or polypeptide, respectively, which is partially or fully separated from other components that are normally found in cells or in recombinant DNA expression systems or any other contaminant. These components include, but are not limited to, cell membranes, cell walls, ribosomes, polymerases, serum components and extraneous genomic sequences. The scope of the present invention includes the isolated polynucleotides set forth herein, *e.g.*, the promoters set forth herein; and methods related thereto, *e.g.*, as discussed herein.

An isolated polynucleotide or polypeptide will, preferably, be an essentially homogeneous composition of molecules but may contain some heterogeneity.

"Amplification" of DNA as used includes the use of polymerase chain reaction (PCR) to increase the concentration of a particular DNA sequence within a mixture of DNA sequences. For a description of PCR see Saiki, *et al.*, Science (1988) 239:487.

In general, a "promoter" or "promoter sequence" is a DNA regulatory region capable of binding an RNA polymerase in a cell (*e.g.*, directly or through other promoter-bound proteins or substances) and initiating transcription of a coding sequence to which it operably links.

A coding sequence (*e.g.*, of a heterologous polynucleotide, *e.g.*, reporter gene or immunoglobulin heavy and/or light chain) is "operably linked to", "under the control of", "functionally associated with" or "operably associated with" a transcriptional and translational control sequence (*e.g.*, a promoter of the present invention) when the sequence directs RNA polymerase mediated transcription of the coding sequence into RNA, preferably mRNA, which then may be RNA spliced (if it contains introns) and, optionally, translated into a protein encoded by the coding sequence.

The present invention includes vectors or cassettes which comprise modified XRN1 including nonsense mutations, truncations, deletions, knock-outs, or overexpression cassettes, including promoters optionally operably linked to a heterologous polynucleotide. The term "vector" includes a vehicle (*e.g.*, a plasmid) by which a DNA or RNA sequence can be introduced into a host cell, so as to transform the host and, optionally, promote expression and/or replication of the introduced sequence. Suitable vectors

for use herein include plasmids, integratable DNA fragments, and other vehicles that may facilitate introduction of the nucleic acids into the genome of a host cell (*e.g.*, *Pichia pastoris*). Plasmids are the most commonly used form of vector but all other forms of vectors which serve a similar function and which are, or become, known in the art are suitable for use herein. See, *e.g.*, Pouwels, *et al.*, Cloning Vectors: A Laboratory Manual, 1985 and Supplements, Elsevier, N.Y., and Rodriguez *et al.* (eds.), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, 1988, Buttersworth, Boston, MA.

A polynucleotide (*e.g.*, a heterologous polynucleotide, *e.g.*, encoding an immunoglobulin heavy chain and/or light chain), operably linked to a promoter, may be expressed in an expression system. The term "expression system" means a host cell and compatible vector which, under suitable conditions, can express a protein or nucleic acid which is carried by the vector and introduced to the host cell. Common expression systems include fungal host cells (*e.g.*, *Pichia pastoris*) and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors.

The term methanol-induction refers to increasing expression of a polynucleotide (*e.g.*, a heterologous polynucleotide) operably linked to a methanol-inducible promoter in a host cell of the present invention by exposing the host cells to methanol.

The term methanol-repression refers to decreasing expression of a polynucleotide (*e.g.*, a heterologous polynucleotide) operably linked to a methanol-repressible promoter in a host cell of the present invention by exposing the host cells to methanol.

The following references regarding the BLAST algorithm are herein incorporated by reference: **BLAST ALGORITHMS:** Altschul, S.F., *et al.*, J. Mol. Biol. (1990) 215:403-410; Gish, W., *et al.*, Nature Genet. (1993) 3:266-272; Madden, T.L., *et al.*, Meth. Enzymol. (1996) 266:131-141; Altschul, S.F., *et al.*, Nucleic Acids Res. (1997) 25:3389-3402; Zhang, J., *et al.*, Genome Res. (1997) 7:649-656; Wootton, J.C., *et al.*, Comput. Chem. (1993) 17:149-163; Hancock, J.M., *et al.*, Comput. Appl. Biosci. (1994) 10:67-70; **ALIGNMENT SCORING SYSTEMS**: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, Natl. Biomed. Res. Found., Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3." M.O. Dayhoff (ed.), pp. 353-358, Natl. Biomed. Res. Found., Washington, DC; Altschul, S.F., J. Mol. Biol. (1991) 219:555-565; States, D.J., *et al.*, Methods (1991) 3:66-70; Henikoff, S., *et al.*, Proc. Natl. Acad. Sci. USA (1992)89:10915-10919; Altschul, S.F., *et al.*, J. Mol. Evol. (1993) 36:290-300; **ALIGNMENT STATISTICS**: Karlin, S., *et al.*, Proc. Natl. Acad. Sci. USA (1990) 87:2264-2268; Karlin, S., *et al.*, Proc. Natl. Acad. Sci. USA (1993) 90:5873-5877; Dembo, A., *et al.*, Ann. Prob. (1994) 22:2022-2039; and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in Theoretical and Computational Methods in Genome Research (S. Suhai, ed.), (1997) pp. 1-14, Plenum, New York.

Host Cells

[0001] The present invention encompasses any isolated *Pichia* sp. host cell (*e.g.*, such as *Pichia pastoris*) comprising a modified, truncated, or deleted form of the *XRN1* gene, including host cells comprising a promoter *e.g.*, operably linked to a polynucleotide encoding a heterologous polypeptide (*e.g.*, a reporter or immunoglobulin heavy and/or light chain; *e.g.*, optionally, wherein the immunoglobulin heavy chain or light chain is linked to an immunoglobulin constand domain) as well as methods of use thereof, *e.g.*, methods for expressing the heterologous polypeptide in the host cell. Host cells of the present invention, may be also genetically engineered so as to express particular glycosylation patterns on polypeptides that are expressed in such cells. Host cells of the present invention are discussed in detail herein. Any engineered *Pichia* host cell comprising a modified, truncated, or deleted form of the *XRN1* gene forms part of the present invention. In an embodiment of the invention, the host cell is selected from the group consisting of any *Pichia* cell, such as *Pichia pastoris, Pichia angusta (Hansenula polymorpha), Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia minuta* (*Ogataea minuta, Pichia lindneri*), *Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia.*

As used herein, the terms "N-glycan" and "glycoform" are used interchangeably and refer to an N-linked oligosaccharide, *e.g.*, one that is attached by an asparagine-N-acetylglucosamine linkage to an asparagine residue of a polypeptide. N-linked glycoproteins contain an N-acetylglucosamine residue linked to the amide nitrogen of an asparagine residue in the protein. Predominant sugars found on glycoproteins are glucose, galactose, mannose, fucose, N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc) and sialic acid (*e.g.*, N-acetyl-neuraminic acid (NANA)).

N-glycans have a common pentasaccharide core of $Man_3GlcNAc_2$ ("Man" refers to mannose; "Glc" refers to glucose; and "NAc" refers to N-acetyl; GlcNAc refers to N-acetylglucosamine). N-glycans differ with respect to the number of branches (antennae) comprising peripheral sugars (*e.g.*, GlcNAc, galactose, fucose and sialic acid) that are added to the $Man_3GlcNAc_2$ ("Man_3") core structure which is also referred to as the "trimannose core", the "pentasaccharide core" or the "paucimannose core". N-glycans are classified according to their branched constituents (*e.g.*, high mannose, complex or hybrid). A "high mannose" type N-glycan has five or more mannose residues. A "complex" type N-glycan typically has at least one GlcNAc attached to the 1,3 mannose arm and at least one GlcNAc attached to the 1,6 mannose arm of a "trimannose" core. Complex N-glycans may also have galactose ("Gal") or N- acetylgalactosamine ("GalNAc") residues that are optionally modified with sialic acid or derivatives (*e.g.*, "NANA" or "NeuAc", where "Neu" refers to neuraminic acid and "Ac" refers to acetyl). Complex N-glycans may also have intrachain substitutions comprising "bisecting" GlcNAc and core fucose ("Fuc"). Complex N-glycans may also have multiple antennae on the "trimannose core," often referred to as "multiple antennary glycans." A "hybrid" N-glycan has at least one GlcNAc on the terminal of the 1,3 mannose arm of the trimannose core and zero or more mannoses on the 1,6 mannose

arm of the trimannose core. The various N-glycans are also referred to as "glycoforms." "PNGase", or "glycanase" or "glucosidase" refer to peptide N-glycosidase F (EC 3.2.2.18).

Thus, the present invention includes isolated *Pichia* host cells comprising a modified, truncated, or deleted form of the *XRN1* gene, optionally further comprising an expression construct (*e.g.*, a promoter operably linked to a heterologous polynucleotide encoding a heterologous polypeptide) and further comprising a deletion of one or more of the genes encoding PMTs, and/or, e.g., wherein the host cell can be cultivated in a medium that includes one or more Pmtp inhibitors. Pmtp inhibitors include but are not limited to a benzylidene thiazolidinedione. Examples of benzylidene thiazolidinediones are 5-[[3,4bis(phenylmethoxy) phenyl]methylene]-4-oxo-2-thioxo-3-thiazolidineacetic Acid; 5-[[3-(1-Phenylethoxy)-4-(2-phenylethoxy)]phenyl]methylene]-4-oxo-2-thioxo-3-thiazolidineacetic Acid; and 5-[[3-(l-Phenyl-2-hydroxy)ethoxy)-4-(2-phenylethoxy)]phenyl]methylene]-4-oxo-2-thioxo3-thiazolidineacetic acid.

In an embodiment of the invention, a *Pichia* host cell (*e.g.*, *Pichia pastoris*) comprising a modified, truncated, or deleted form of the *XRN1* gene is, in an embodiment of the invention, genetically engineered to include a nucleic acid that encodes an alpha-l,2-mannosidase that has a signal peptide that directs it for secretion. For example, in an embodiment of the invention, the host cell is engineered to express an exogenous alpha-1,2-mannosidase enzyme having an optimal pH between 5.1 and 8.0, preferably between 5.9 and 7.5. In an embodiment of the invention, the exogenous enzyme is targeted to the endoplasmic reticulum or Golgi apparatus of the host cell, where it trims N-glycans such as $Man_8GlcNAc_2$ to yield $Man_5GlcNAc_2$. See U.S. Patent No. 7,029,872.

Pichia host cells (*e.g.*, *Pichia pastoris*) comprising a modified, truncated, or deleted form of the *XRN1* gene are, in an embodiment of the invention, genetically engineered to eliminate glycoproteins having alpha-mannosidase-resistant N-glycans by deleting or disrupting one or more of the beta-mannosyltransferasegenes (*e.g.*, *BMTl*, *BMT2*, *BMT3*, and *BMT4*)(See, U.S. Patent No. 7,465,577) or abrogating translation of RNAs encoding one or more of the beta-mannosyltransferasesusinginterfering RNA, antisense RNA, or the like. The scope of the present invention includes such an engineered *Pichia* host cell (*e.g.*, *Pichia pastoris*) comprising an expression cassette (*e.g.*, a promoter operably linked to a heterologous polynucleotide encoding a heterologous polypeptide).

Engineered host cells (*e.g.*, *Pichia pastoris*) of the present invention also include those that are genetically engineered to eliminate glycoproteins having phosphomannose residues, *e.g.*, by deleting or disrupting one or both of the phosphomannosyl transferase genes *PNO1* and *MNN4B* (See for example, U.S. Patent Nos. 7,198,921 and 7,259,007), which can include deleting or disrupting the *MNN4A* gene or abrogating translation of RNAs encoding one or more of the phosphomannosyltransferases using interfering RNA, antisense RNA, or the like. In an embodiment of the invention, an engineered *Pichia* host cell has been genetically modified to produce glycoproteins that have predominantly an N-glycan selected from the group consisting of complex N-glycans, hybrid N-glycans, and high mannose N-glycans wherein complex N-glycans are, in an embodiment of the invention, selected from the group

consisting of $Man_3GlcNAc_2$, $GlcNAC_{(1-4)}Man_3GlcNAc_2$, $NANA_{(1-4)}GlcNAc_{(1-4)}Man_3GlcNAc_2$, and $NANA_{(1-4)}Gal_{(1-4)}Man_3GlcNAc_2$; hybrid N-glycans are, in an embodiment of the invention, selected from the group consisting of $Man_5GlcNAc_2$, $GlcNAcMan_5GlcNAc_2$, $GalGlcNAcMan_5GlcNAc_2$, and $NANAGalGlcNAcMan_5GlcNAc_2$; and high mannose N-glycans are, in an embodiment of the invention, selected from the group consisting of $Man_6GlcNAc_2$, $Man_7GlcNAc_2$, $Mang_81cNAc_2$, and $Man_9GlcNAc_2$. The scope of the present invention includes such engineered *Pichia* host cells (*e.g.*, *Pichia pastoris*) comprising g a modified, truncated, or deleted form of the *XRN1* gene.

[0002] Additionally, engineered *Pichia* host cells (*e.g.*, *Pichia pastoris*) of the present invention also include those that are genetically engineered to include a nucleic acid that encodes the *Leishmania sp.* single-subunit oligosaccharyltransferase *STT3A* protein, *STT3B* protein, *STT3C* protein, *STT3D* protein, or combinations thereof such as those described in WO2011/06389. Additionally, engineered host cells (*e.g.*, *Pichia pastoris*) of the present invention also include those that are genetically engineered to eliminate nucleic acids encoding Dolichol-P-Man dependent alpha(1-3) mannosyltransferase, *e.g.*, Alg3, such as described in US Patent Publication No. US2005/0170452. The scope of the present invention includes any such engineered *Pichia* host cells (*e.g.*, *Pichia pastoris*) further comprising a modified, truncated, deleted form of the *XRN1* gene.

As used herein, the term "essentially free of" as it relates to lack of a particular sugar residue, such as fucose, or galactose or the like, on a glycoprotein, is used to indicate that the glycoprotein composition is substantially devoid of N-glycans which contain such residues. Expressed in terms of purity, essentially free means that the amount of N-glycan structures containing such sugar residues does not exceed 10%, and preferably is below 5%, more preferably below 1%, most preferably below 0.5%, wherein the percentages are by weight or by mole percent.

As used herein, a glycoprotein composition "lacks" or "is lacking" a particular sugar residue, such as fucose or galactose, when no detectable amount of such sugar residue is present on the N-glycan structures. For example, in an embodiment of the present invention, glycoprotein compositions produced by host cells of the invention will "lack fucose," because the cells do not have the enzymes needed to produce fucosylated N-glycan structures. Thus, the term "essentially free of fucose" encompasses the term "lacking fucose." However, a composition may be "essentially free of fucose" even if the composition at one time contained fucosylated N-glycan structures or contains limited, but detectable amounts of fucosylated N-glycan structures as described above.

CHARACTERIZATION OF *PICHIA PASTORIS* XRN1

The *Pichia pastoris* gene *XRN1* (SEQ ID NO:75, GenBank Accession No.: 002492616.1, amino acid sequence: SEQ ID NO:76) (is homologous to Kem1 in yeast *Saccharomyces cerevisiae*), part of a family of evolutionarily conserved cytoplasmic 5' to 3' exoribonucleases. *XRN1* is a member of a large family of conserved exonucleases, although little is known about the catalytic mechanism of its

members. Capped RNA is resistant to *Xrn1*, and *Xrn1* strongly prefers mRNA with a 5' monophosphate as substrate over RNA with a 5' hydroxyl end. Eukaryotic cells also contain a related exonuclease, Rat1, which is localized to the nucleus and seems to carry out the relevant 5′ to 3′ degradation and processing reactions in the nucleus.

To broadly improve protein quality produced by engineered host strains, several mutant *XRN1* knock-out strains were produced from a series of *Pichia* host strains. While non-mutagenized glyco-engineered parental strains typically produce heterologous proteins with a variety of N-glycosylation patterns, the engineered *Pichia* host strains with *XRN1* deletions produced heterologous protein products with decreased proteolytic degradation as well as desired glycosylation patterns. These engineered *Pichia* host strains produced glycoproteins with predominant complex N-glycans typically seen of the therapeutic proteins produced from mammalian cells (shown in Tables 7-11).

[0003] Such mutations in *XRN1* when engineered into any *Pichia* host strain would serve to increase fermentation robustness, improve recombinant protein yield, and reduce protein product proteolytic degradation. The mRNA stabilization in the engineered *Pichia XRN1* knockouts described herein provides useful strains and methods to improve protein fermentation titer and protein glycosylation quality simultaneously. Inhibition of global mRNA turnover by *XRN1* knockout increases mRNA abundance of both target protein and corresponding glycosyltransferases. This leads to a yeast host strain with high protein productivity and enhanced complex N-glycan profile. Moreover, mutation of *XRN1* may affect translation initiation to prevent stress-induced translation regulation and further improve the titer in these engineered *Pichia* host strains.

EXAMPLE 1

XRN1 Knock-out plasmids

Plasmid pGLY7392 (**Figure 3**) is an integration vector that targets the *XRN1/KEM1* loci and contains a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit flanked by nucleic acid molecules comprising *lacZ* repeats which in turn is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *XRN1* gene (SEQ ID NO:1) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *XRN1* gene (SEQ ID NO: 2).

Plasmid pGLY7392 was linearized with *Sfi*I and the linearized plasmid was transformed into a number of *P. pastoris* strains in which the *URA5* gene flanked by the *lacZ* repeats has been inserted into the *XRN1/KEM1* loci by double-crossover homologous recombination to generate the *XRN1 knock-out strains* as shown in the following examples.

EXAMPLE 2

Engineered *Pichia pastoris* strains with humanized glycosylation pathway for producing recombinant human antibodies

Genetically engineered *Pichia pastoris* strain **YGLY12501, YGLY13992, and YGLY14836** are strains that produce recombinant human anti-Her2 antibodies. Construction of

the strains is illustrated schematically in **Figures 1A-1H**. Briefly, the strains were constructed as follows.

The strain **YGLY8316** was constructed from wild-type *Pichia pastoris* strain **NRRL-Y 11430** using methods described earlier (*See* for example, U.S. Patent No. 7,449,308; U.S. Patent No. 7,479,389; U.S. Published Application No. 20090124000; Published PCT Application No. WO2009085135; Nett and Gerngross, Yeast 20:1279 (2003); Choi *et al.*, Proc. Natl. Acad. Sci. USA 100:5022 (2003); Hamilton *et al.*, Science 301:1244 (2003)). All plasmids were made in a pUC19 plasmid using standard molecular biology procedures. For nucleotide sequences that were optimized for expression in *P. pastoris*, the native nucleotide sequences were analyzed by the GENEOPTIMIZER software (GeneArt, Regensburg, Germany) and the results used to generate nucleotide sequences in which the codons were optimized for *P. pastoris* expression. Yeast strains were transformed by electroporation (using standard techniques as recommended by BioRad, Hercules, CA).

Plasmid pGLY6 (**Figure 4**) is an integration vector that targets the *URA5* locus. It contains a nucleic acid molecule comprising the *S. cerevisiae* invertase gene or transcription unit (*ScSUC2*; SEQ ID NO:3) flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *P. pastoris URA5* gene (SEQ ID NO:4) and on the other side by a nucleic acid molecule comprising the nucleotide sequence from the 3' region of the *P. pastoris URA5* gene (SEQ ID NO:5). Plasmid pGLY6 was linearized and the linearized plasmid transformed into wild-type strain **NRRL-Y 11430** to produce a number of strains in which the *ScSUC2* gene was inserted into the *URA5* locus by double-crossover homologous recombination. Strain **YGLY1-3** was selected from the strains produced and is auxotrophic for uracil.

Plasmid pGLY40 (**Figure 5**) is an integration vector that targets the *OCH1* locus and contains a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit (SEQ ID NO:6) flanked by nucleic acid molecules comprising *lacZ* repeats (SEQ ID NO:7) which in turn is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *OCH1* gene (SEQ ID NO:8) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *OCH1* gene (SEQ ID NO:9). Plasmid pGLY40 was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY1-3** to produce a number of strains in which the *URA5* gene flanked by the *lacZ* repeats has been inserted into the *OCH1* locus by double-crossover homologous recombination. Strain **YGLY2-3** was selected from the strains produced and is prototrophic for *URA5*. Strain **YGLY2-3** was counterselected in the presence of 5-fluoroorotic acid (5-FOA) to produce a number of strains in which the *URA5* gene has been lost and only the *lacZ* repeats remain in the *OCH1* locus. This renders the strain auxotrophic for uracil. Strain **YGLY4-3** was selected.

Plasmid pGLY43a (**Figure 6**) is an integration vector that targets the *BMT2* locus and contains a nucleic acid molecule comprising the *K. lactis* UDP-*N*-acetylglucosamine (UDP-GlcNAc) transporter gene or transcription unit (*KlMNN2-2*, SEQ ID NO:10) adjacent to a nucleic

acid molecule comprising the *P. pastoris URA5* gene or transcription unit flanked by nucleic acid molecules comprising *lacZ* repeats. The adjacent genes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *BMT2* gene (SEQ ID NO:11) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *BMT2* gene (SEQ ID NO:12). Plasmid pGLY43a was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY4-3** to produce to produce a number of strains in which the *KlMNN2-2* gene and *URA5* gene flanked by the *lacZ* repeats has been inserted into the *BMT2* locus by double-crossover homologous recombination. The *BMT2* gene was described in Mille *et al.*, J. Biol. Chem. 283: 9724-9736 (2008) and U.S. Patent No.7,465,557. Strain **YGLY6-3** was selected from the strains produced and is prototrophic for uracil. Strain **YGLY6-3** was counterselected in the presence of 5-FOA to produce strains in which the *URA5* gene has been lost and only the *lacZ* repeats remain. This renders the strain auxotrophic for uracil. Strain **YGLY8-3** was selected.

Plasmid pGLY48 (**Figure 7**) is an integration vector that targets the *MNN4L1* locus and contains an expression cassette comprising a nucleic acid molecule encoding the mouse homologue of the UDP-GlcNAc transporter (SEQ ID NO:13) open reading frame (ORF) operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GAPDH* promoter (SEQ ID NO:14) and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* termination sequences (SEQ ID NO:15) adjacent to a nucleic acid molecule comprising the *P. pastoris URA5* gene flanked by *lacZ* repeats and in which the expression cassettes together are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *P. pastoris MNN4L1* gene (SEQ ID NO:16) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *MNN4L1* gene (SEQ ID NO:17). Plasmid pGLY48 was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY8-3** to produce a number of strains in which the expression cassette encoding the mouse UDP-GlcNAc transporter and the *URA5* gene have been inserted into the *MNN4L1* locus by double-crossover homologous recombination. The *MNN4L1* gene (also referred to as *MNN4B*) has been disclosed in U.S. Patent No. 7,259,007. Strain **YGLY10-3** was selected from the strains produced and then counterselected in the presence of 5-FOA to produce a number of strains in which the *URA5* gene has been lost and only the *lacZ* repeats remain. Strain **YGLY12-3** was selected.

Plasmid pGLY45 (**Figure 8**) is an integration vector that targets the *PNO1/MNN4* loci and contains a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit flanked by nucleic acid molecules comprising *lacZ* repeats which in turn is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *PNO1* gene (SEQ ID NO:18) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *MNN4* gene (SEQ ID NO:19). Plasmid pGLY45 was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY12-3** to produce a

number of strains in which the *URA5* gene flanked by the *lacZ* repeats has been inserted into the *PNO1/MNN4* loci by double-crossover homologous recombination. The *PNO1* gene has been disclosed in U.S. Patent No. 7,198,921 and the *MNN4* gene (also referred to as *MNN4B*) has been disclosed in U.S. Patent No. 7,259,007. Strain **YGLY14-3** was selected from the strains produced and then counterselected in the presence of 5-FOA to produce a number of strains in which the *URA5* gene has been lost and only the *lacZ* repeats remain. Strain **YGLY16-3** was selected.

Plasmid pGLY1430 (**Figure 9**) is a KINKO integration vector that targets the *ADE1* locus without disrupting expression of the locus and contains in tandem four expression cassettes encoding (1) the human GlcNAc transferase I catalytic domain (NA) fused at the *N*-terminus to *P. pastoris SEC12* leader peptide (10) to target the chimeric enzyme to the ER or Golgi, (2) mouse homologue of the UDP-GlcNAc transporter (MmTr), (3) the mouse mannosidase IA catalytic domain (FB) fused at the *N*-terminus to *S. cerevisiae SEC12* leader peptide (8) to target the chimeric enzyme to the ER or Golgi, and (4) the *P. pastoris URA5* gene or transcription unit. KINKO (Knock-In with little or No Knock-Out) integration vectors enable insertion of heterologous DNA into a targeted locus without disrupting expression of the gene at the targeted locus and have been described in U.S. Published Application No. 20090124000. The expression cassette encoding the NA10 comprises a nucleic acid molecule encoding the human GlcNAc transferase I catalytic domain codon-optimized for expression in *P. pastoris* (SEQ ID NO:20) fused at the 5' end to a nucleic acid molecule encoding the *SEC12* leader 10 (SEQ ID NO:21), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris PMA1* promoter and at the 3' end to a nucleic acid molecule comprising the *P. pastoris PMA1* transcription termination sequence. The expression cassette encoding MmTr comprises a nucleic acid molecule encoding the mouse homologue of the UDP-GlcNAc transporter ORF operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris SEC4* promoter (SEQ ID NO:22) and at the 3' end to a nucleic acid molecule comprising the *P. pastoris OCH1* termination sequences (SEQ ID NO:23). The expression cassette encoding the FB8 comprises a nucleic acid molecule encoding the mouse mannosidase IA catalytic domain (SEQ ID NO:24) fused at the 5' end to a nucleic acid molecule encoding the *SEC12-m* leader 8 (SEQ ID NO:25), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GADPH* promoter and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* transcription termination sequence. The *URA5* expression cassette comprises a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit flanked by nucleic acid molecules comprising *lacZ* repeats. The four tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region and complete ORF of the *ADE1* gene (SEQ ID NO:26) followed by a *P. pastoris ALG3* termination sequence (SEQ ID NO:27) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *ADE1* gene (SEQ ID NO:28). Plasmid pGLY1430

was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY16-3** to produce a number of strains in which the four tandem expression cassette have been inserted into the *ADE1* locus immediately following the *ADE1* ORF by double-crossover homologous recombination. The strain **YGLY2798** was selected from the strains produced and is auxotrophic for arginine and now prototrophic for uridine, histidine, and adenine. The strain was then counterselected in the presence of 5-FOA to produce a number of strains now auxotrophic for uridine. Strain **YGLY3794** was selected and is capable of making glycoproteins that have predominantly galactose terminated *N*-glycans.

Plasmid pGLY582 (**Figure 10**) is an integration vector that targets the *HIS1* locus and contains in tandem four expression cassettes encoding (1) the *S. cerevisiae* UDP-glucose epimerase (*ScGAL10*), (2) the human galactosyltransferase I (*hGalT*) catalytic domain fused at the N-terminus to the *S. cerevisiae KRE2-s* leader peptide (33) to target the chimeric enzyme to the ER or Golgi, (3) the *P. pastoris URA5* gene or transcription unit flanked by lacZ repeats, and (4) the *D. melanogaster* UDP-galactose transporter (*DmUGT*). The expression cassette encoding the *ScGAL10* comprises a nucleic acid molecule encoding the *ScGAL10* ORF (SEQ ID NO:29) operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris PMA1* promoter (SEQ ID NO:30) and operably linked at the 3' end to a nucleic acid molecule comprising the *P. pastoris PMA1* transcription termination sequence (SEQ ID NO:31). The expression cassette encoding the chimeric galactosyltransferase I comprises a nucleic acid molecule encoding the *hGalT* catalytic domain codon optimized for expression in *P. pastoris* (SEQ ID NO:32) fused at the 5' end to a nucleic acid molecule encoding the *KRE2-s* leader 33 (SEQ ID NO:33), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GAPDH* promoter and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* transcription termination sequence. The *URA5* expression cassette comprises a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit flanked by nucleic acid molecules comprising *lacZ* repeats. The expression cassette encoding the *DmUGT* comprises a nucleic acid molecule encoding the *DmUGT* ORF (SEQ ID NO:34) operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris OCH1* promoter (SEQ ID NO:35) and operably linked at the 3' end to a nucleic acid molecule comprising the *P. pastoris ALG12* transcription termination sequence (SEQ ID NO:36). The four tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *HIS1* gene (SEQ ID NO:37) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *HIS1* gene (SEQ ID NO:38). Plasmid pGLY582 was linearized and the linearized plasmid transformed into strain **YGLY3794** to produce a number of strains in which the four tandem expression cassette have been inserted into the *HIS1* locus by homologous recombination. Strain **YGLY3853** was selected and is auxotrophic for histidine and prototrophic for uridine.

Plasmid pGLY167b (**Figure 11**) is an integration vector that targets the *ARG1* locus and contains in tandem three expression cassettes encoding (1) the *D. melanogaster* mannosidase II catalytic domain (KD) fused at the *N*-terminus to *S. cerevisiae MNN2* leader peptide (53) to target the chimeric enzyme to the ER or Golgi, (2) the *P. pastoris HIS1* gene or transcription unit, and (3) the rat *N*-acetylglucosamine (GlcNAc) transferase II catalytic domain (TC) fused at the *N*-terminus to *S. cerevisiae MNN2* leader peptide (54) to target the chimeric enzyme to the ER or Golgi. The expression cassette encoding the KD53 comprises a nucleic acid molecule encoding the *D. melanogaster* mannosidase II catalytic domain codon-optimized for expression in *P. pastoris* (SEQ ID NO:39) fused at the 5' end to a nucleic acid molecule encoding the *MNN2* leader 53 (SEQ ID NO:40), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GAPDH* promoter and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* transcription termination sequence. The *HIS1* expression cassette comprises a nucleic acid molecule comprising the *P. pastoris HIS1* gene or transcription unit (SEQ ID NO:41). The expression cassette encoding the TC54 comprises a nucleic acid molecule encoding the rat GlcNAc transferase II catalytic domain codon-optimized for expression in *P. pastoris* (SEQ ID NO:42) fused at the 5' end to a nucleic acid molecule encoding the *MNN2* leader 54 (SEQ ID NO:43), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris PMA1* promoter and at the 3' end to a nucleic acid molecule comprising the *P. pastoris PMA1* transcription termination sequence. The three tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *ARG1* gene (SEQ ID NO:44) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *ARG1* gene (SEQ ID NO:45). Plasmid pGLY167b was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY3853** to produce a number of strains (in which the three tandem expression cassette have been inserted into the *ARG1* locus by double-crossover homologous recombination. The strain **YGLY4754** was selected from the strains produced and is auxotrophic for arginine and prototrophic for uridine and histidine. The strain was then counterselected in the presence of 5-FOA to produce a number of strains now auxotrophic for uridine. Strain **YGLY4799** was selected.

Plasmid pGLY3411 (**Figure 12**) is an integration vector that contains the expression cassette comprising the *P. pastoris URA5* gene flanked by *lacZ* repeats flanked on one side with the 5' nucleotide sequence of the *P. pastoris BMT4* gene (SEQ ID NO:46) and on the other side with the 3' nucleotide sequence of the *P. pastoris BMT4* gene (SEQ ID NO:47). Plasmid pGLY3411 was linearized and the linearized plasmid transformed into **YGLY4799** to produce a number of strains in which the *URA5* expression cassette has been inserted into the *BMT4* locus by double-crossover homologous recombination. Strain **YGLY6903** was selected from the strains produced and is prototrophic for uracil, adenine, histidine, proline, arginine, and

tryptophan. The strain was then counterselected in the presence of 5-FOA to produce a number of strains now auxotrophic for uridine. Strains **YGLY7432** and **YGLY7433** were selected.

Plasmid pGLY3419 (**Figure 13**) is an integration vector that contains an expression cassette comprising the *P. pastoris URA5* gene flanked by *lacZ* repeats flanked on one side with the 5' nucleotide sequence of the *P. pastoris BMT1* gene (SEQ ID NO:48) and on the other side with the 3' nucleotide sequence of the *P. pastoris BMT1* gene (SEQ ID NO:49). Plasmid pGLY3419 was linearized and the linearized plasmid transformed into strain **YGLY7432** and **YGLY7433** to produce a number of strains in which the *URA5* expression cassette has been inserted into the *BMT1* locus by double-crossover homologous recombination. The strains **YGLY7656** and **YGLY7651** were selected from the strains produced and are prototrophic for uracil, adenine, histidine, proline, arginine, and tryptophan. The strains were then counterselected in the presence of 5-FOA to produce a number of strains now auxotrophic for uridine. Strains **YGLY7930** and **YGLY7940** were selected.

Plasmid pGLY3421 (**Figure 14**) is an integration vector that contains an expression cassette comprising the *P. pastoris URA5* gene flanked by *lacZ* repeats flanked on one side with the 5' nucleotide sequence of the *P. pastoris BMT3* gene (SEQ ID NO:50) and on the other side with the 3' nucleotide sequence of the *P. pastoris BMT3* gene (SEQ ID NO:51). Plasmid pGLY3419 was linearized and the linearized plasmid transformed into strain **YGLY7930** and **YGLY7940** to produce a number of strains in which the *URA5* expression cassette has been inserted into the *BMT1* locus by double-crossover homologous recombination. The strains **YGLY7965** and **YGLY7961** were selected from the strains produced and are prototrophic for uracil, adenine, histidine, proline, arginine, and tryptophan.

Plasmid pGLY3673 (**Figure 15**) is a KINKO integration vector that targets the *PRO1* locus without disrupting expression of the locus and contains expression cassettes encoding the *T. reesei* α-1,2-mannosidase catalytic domain fused at the *N*-terminus to *S. cerevisiae* αMATpre signal peptide (aMATTrMan) to target the chimeric protein to the secretory pathway and secretion from the cell. The expression cassette encoding the aMATTrMan comprises a nucleic acid molecule encoding the *T. reesei* catalytic domain (SEQ ID NO:52) fused at the 5' end to a nucleic acid molecule encoding the *S. cerevisiae* αMATpre signal peptide (SEQ ID NO:53, 54), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris AOX1* promoter (SEQ ID NO:55) and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). The cassette is flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region and complete ORF of the *ARG1* gene (SEQ ID NO:56) followed by a *P. pastoris ALG3* termination sequence and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *ARG1* gene (SEQ ID NO:57). Plasmid pGLY3673 was linearized and the linearized plasmid transformed into strains **YGLY7965** and **YGLY7961** to produce a number of strains in which the *URA5* expression cassette has been inserted into the *BMT1* locus

by double-crossover homologous recombination. The strains **YGLY78316** and **YGLY8323** were selected from the strains produced and are prototrophic for uracil, adenine, histidine, proline, arginine, and tryptophan.

Plasmid p GLY5883 (**Figure 16**) is a roll-in integration plasmid encoding the light and heavy chains of an anti-Her2 antibody that targets the *TRP2* locus in *P. pastoris*. The expression cassette encoding the anti-Her2 heavy chain comprises a nucleic acid molecule encoding the heavy chain ORF codon-optimized for effective expression in *P. pastoris* (SEQ ID NO:58) operably linked at the 5' end to a nucleic acid molecule encoding the *Saccharomyces cerevisiae* mating factor pre-signal sequence (SEQ ID NO:53) which in turn is fused at its *N*-terminus to a nucleic acid molecule that has the inducible *P. pastoris AOX1* promoter sequence (SEQ ID NO:55) and at the 3' end to a nucleic acid molecule that has the *S. cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). The expression cassette encoding the anti-Her2 light chain comprises a nucleic acid molecule encoding the light chain ORF codon-optimized for effective expression in *P. pastoris* (SEQ ID NO:59) operably linked at the 5' end to a nucleic acid molecule encoding the *Saccharomyces cerevisiae* mating factor pre-signal sequence (SEQ ID NO:53) which in turn is fused at its *N*-terminus to a nucleic acid molecule that has the inducible *P. pastoris AOX1* promoter sequence (SEQ ID NO:55) and at the 3' end to a nucleic acid molecule that has the *S. cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). For selecting transformants, the plasmid comprises an expression cassette encoding the Zeocin ORF in which the nucleic acid molecule encoding the ORF (SEQ ID NO:60) is operably linked at the 5' end to a nucleic acid molecule having the *S. cerevisiae TEF* promoter sequence (SEQ ID NO:61) and at the 3' end to a nucleic acid molecule having the *S. cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). The plasmid further includes a nucleic acid molecule for targeting the *TRP2* locus (SEQ ID NO:62).

Plasmid p GLY6833 (**Figure 17**) is a roll-in integration plasmid encoding the light and heavy chains of an anti-Her2 antibody that targets the *TRP2* locus in *P. pastoris*. The expression cassette encoding the anti-Her2 heavy chain comprises a nucleic acid molecule encoding the heavy chain ORF codon-optimized for effective expression in *P. pastoris* (SEQ ID NO:58) operably linked at the 5' end to a nucleic acid molecule encoding the *Saccharomyces cerevisiae* mating factor pre-signal sequence (SEQ ID NO:53) which in turn is fused at its *N*-terminus to a nucleic acid molecule that has the inducible *P. pastoris AOX1* promoter sequence (SEQ ID NO:55) and at the 3' end to a nucleic acid molecule that has the *P. pastoris CIT1* transcription termination sequence (SEQ ID NO:63). The expression cassette encoding the anti-Her2 light chain comprises a nucleic acid molecule encoding the light chain ORF codon-optimized for effective expression in *P. pastoris* (SEQ ID NO:59) operably linked at the 5' end to a nucleic acid molecule encoding the *Saccharomyces cerevisiae* mating factor pre-signal sequence (SEQ ID NO:53) which in turn is fused at its *N*-terminus to a nucleic acid molecule that has the inducible *P. pastoris AOX1* promoter sequence (SEQ ID NO:55) and at the 3' end to a nucleic acid

molecule that has the *P. pastoris CIT1* transcription termination sequence (SEQ ID NO:63). For selecting transformants, the plasmid comprises an expression cassette encoding the Zeocin ORF in which the nucleic acid molecule encoding the ORF (SEQ ID NO:60) is operably linked at the 5' end to a nucleic acid molecule having the *S. cerevisiae TEF* promoter sequence (SEQ ID NO:61) and at the 3' end to a nucleic acid molecule having the *S. cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). The plasmid further includes a nucleic acid molecule for targeting the *TRP2* locus (SEQ ID NO:62).

Plasmid pGLY3714 (**Figure 18**) is a KINKO integration vector that targets the *TRP1* locus without disrupting expression of the locus and contains expression cassettes encoding the mouse mannosidase IB catalytic domain (GD) fused at the *N*-terminus to *S. cerevisiae SEC12* leader peptide (GD9) to target the chimeric enzyme to the ER or Golgi. For selecting transformants, the plasmid comprises an expression cassette encoding the Nourseothricin resistance (NAT^R) ORF (originally from pAG25 from EROSCARF, Scientific Research and Development GmbH, Daimlerstrasse 13a, D-61352 Bad Homburg, Germany, *See* Goldstein *et al.*, Yeast 15: 1541 (1999)); wherein the nucleic acid molecule encoding the ORF (SEQ ID NO:64) is operably linked to at the 5' end to a nucleic acid molecule having the *Ashbya gossypii TEF1* promoter sequence (SEQ ID NO:65) and at the 3' end to a nucleic acid molecule that has the *Ashbya gossypii TEF1* termination sequence (SEQ ID NO:66). The two expression cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the ORF encoding Trp1p ending at the stop codon (SEQ ID NO:67 linked to a nucleic acid molecule having the *P. pastoris ALG3* termination sequence (SEQ ID NO:27) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *TRP1* gene (SEQ ID NO:68). Plasmid pGLY3714 was constructed by cloning the DNA fragment encoding the GD9 ORF flanked by a *Not*I site at the 5' end and a *Pac*I site at the 3' end into plasmid pGLY597. An expression cassette comprising a nucleic acid molecule encoding the Nourseothricin resistance ORF (NAT) operably linked to the *Ashbya gossypii TEF1* promoter (PTEF) and *Ashbya gossypii TEF1* termination sequence (TTEF).

EXAMPLE 3

Engineered *Pichia pastoris* host strains expressing heterologous proteins

Strain **YGLY12501** was generated by transforming pGLY5883, which encodes the anti-Her2 antibody, into **YGLY8316**. The strain **YGLY12501** was selected from the strains produced. In this strain, the expression cassettes encoding the anti-Her2 heavy and light chains are targeted to the *Pichia pastoris TRP2* locus (PpTRP2). This strain contains the wild-type *XRN1* sequence.

Strain **YGLY13992** was generated by transforming pGLY6833, which encodes the anti-Her2 antibody, into **YGLY8316**. The strain **YGLY13992** was selected from the strains produced. In this strain, the expression cassettes encoding the anti-Her2 heavy and light chains

are targeted to the *Pichia pastoris TRP2* locus (PpTRP2). This strain contains the wild-type *XRN1* sequence.

Strain **YGLY12511** was generated by transforming pGLY5883, which encodes the anti-Her2 antibody, into **YGLY8316**. The strain **YGLY12511** was selected from the strains produced. Strain **YGLY14836** was generated by transforming pGLY3714, which encodes the GD9, into **YGLY12511**. The strain **YGLY14836** was selected from the strains produced. In this strain, the expression cassettes encoding the anti-Her2 heavy and light chains are targeted to the *Pichia pastoris TRP2* locus (PpTRP2). This strain contains the wild-type *XRN1* sequence.

Transformation of the appropriate strains disclosed herein with pGLY7392 *XRN1* knock-out plasmid vector was performed essentially as follows. Appropriate *Pichia pastoris* strains were grown in 50 mL YPD media (yeast extract (1%), peptone (2%), and dextrose (2%)) overnight to an OD of about 0.2 to 6. After incubation on ice for 30 minutes, cells were pelleted by centrifugation at 2500-3000 rpm for five minutes. Media was removed and the cells washed three times with ice cold sterile 1 M sorbitol before resuspension in 0.5 mL ice cold sterile 1 M sorbitol. Ten μL linearized DNA (5-20 μg) and 100 μL cell suspension was combined in an electroporation cuvette and incubated for 5 minutes on ice. Electroporation was in a Bio-Rad GenePulser Xcell following the preset *Pichia pastoris* protocol (2 kV, 25 μF, 200 Ω), immediately followed by the addition of 1 mL YPDS recovery media (YPD media plus 1 M sorbitol). The transformed cells were allowed to recover for four hours to overnight at room temperature (24° C) before plating the cells on selective media.

Strains **YGLY13992, YGLY12501** and **YGLY14836** were each then transformed with pGLY7392 as described above to produce the strains described in Example 4.

EXAMPLE 4

Engineered *Pichia pastoris Xrn1*Δ strains for improved fermentation yield and N-glycosylation quality

The *XRN1* knock-out integration plasmid pGLY7392 was linearized with *Sfi*I and the linearized plasmid was transformed into each of the *Pichia pastoris* strains **YGLY12501, YGLY13992,** and **YGLY14836** to produce respective *Δxrn1* strains (*i.e.*, *xrn1* deletion strains) used in the following examples. Transformations were performed essentially as described in Example 3.

The genomic integration of pGLY7392 at the *XRN1* locus was confirmed by cPCR using the primers, PpXRN1-5'out/UP (5'- GTTAAATGACTCTAACACCTTGCACTTGA -3'; SEQ ID NO:69) and PpALG3TT/LP (5'-CCTCCCACTGGAACCGATGATATGGAA-3'; SEQ ID NO:70) or PpTEFTT/UP (5'-GATGCGAAGTTAAGTGCGCAGAAAGTAATATCA-3'; SEQ ID NO:71) and PpXRN1-3'out/LP (5'- TTGCAAAAACCAGTGAGGAATAGC-3'; SEQ ID NO:72). Loss of genomic *XRN1* sequences was confirmed using cPCR primers, PpXRN1/iUP (5'- GAATGCTGAAGAACGTCAAAGAAACT-3' (SEQ ID NO:73) and

PpXRN1/iLP (5'- TGAGACTTCAGAGCTTTCCATACGA-3' (SEQ ID NO:74). The PCR conditions were one cycle of 95 °C for two minutes, 35 cycles of 95° C for 20 seconds, 52° C for 20 seconds, and 72° C for one minute; followed by one cycle of 72° C for 10 minutes.

The strains were cultivated in either a DasGip 1 Liter or Micro24 5mL fermentor to produce the antibodies for titer and protein N-glycosylation analyses.

Cell growth conditions of the transformed strains for antibody production in the Micro24 5 mL fermentor were generally as follows. Protein expression for the transformed yeast strain seed cultures were prepared by adding *Pichia pastoris* cells from YSD plates to each well of a Whatman 24-well Uniplate (10 ml, natural polypropylene) containing 3.5 ml of 4% BMGY medium buffered to pH 6.0 with potassium phosphate buffer. The seed cultures were grown for approximately 65-72 hours in a temperature controlled shaker at 24°C and 650 rpm agitation. 1.0 mL of the 24 well plate grown seed culture and 4.0ml of 4% BMGY medium was then used to inoculate each well of a Micro24 plate (Type : REG2). 30 µl of Antifoam 204 (1:25 dilution, Sigma Aldrich) was added to each well. The Micro24 was operated in Microaerobic1 mode and the fermentations were controlled at 200% dissolved oxygen, pH at 6.5, temperature at 24°C and agitation at 800rpm. The induction phase was initiated upon observance of a DO spike after the growth phase by adding bolus shots of methanol feed solution (100% [w/w] methanol, 5 mg/l biotin and 12.5 ml/l PTM1 salts).

Cell growth conditions of the transformed strains for antibody production in the DasGip fermentor were generally as follows. Protein expression for the transformed yeast strains was carried out in shake flasks at 24° C with buffered glycerol-complex medium (BMGY) consisting of 1% yeast extract, 2% peptone, 100 mM potassium phosphate buffer pH 6.0, 1.34% yeast nitrogen base, 4 x 10^{-5}% biotin, and 4% glycerol. The induction medium for protein expression was buffered methanol-complex medium (BMMY) consisting of 1% methanol instead of glycerol in BMGY. Pmt inhibitor Pmti-3 (5-[[3-(1-Phenyl-2-hydroxy)ethoxy)-4-(2-phenylethoxy)]phenyl]methylene]-4-oxo-2-thioxo-3-thiazolidineacetic Acid) (*See* Published International Application No. WO 2007061631) in methanol was added to the growth medium to a final concentration of 18.3 µM at the time the induction medium was added. Cells were harvested and centrifuged at 2,000 rpm for five minutes.

DasGip Fermentor Screening Protocol followed the parameters shown in **Table 2.**

Table 2
DasGip Fermentor Parameters

Parameter	Set-point	Actuated Element
pH	6.5 ± 0.1	30% NH_4OH
Temperature	24 ± 0.1	Cooling Water & Heating Blanket

Dissolved O2	n/a	Initial impeller speed of 550 rpm is ramped to 1200 rpm over first 10 hr, then fixed at 1200 rpm for remainder of run

At time of about 18 hours post-inoculation, DasGip vessels containing 350 mL media A (*See* **Table 3** below) plus 4% glycerol were inoculated with strain of interest. A small dose (0.3 mL of 0.2 mg/mL in 100% methanol) of Pmti-3 was added with inoculum. At time about 20 hour, a bolus of 17 mL 50% glycerol solution (Glycerol Fed-Batch Feed, *See* Table 4 below) plus a larger dose (0.3 mL of 4 mg/mL) of Pmti-3 was added per vessel. At about 26 hours, when the glycerol was consumed, as indicated by a positive spike in the dissolved oxygen (DO) concentration, a methanol feed (*See* Table 5 below) was initiated at 0.7 mL/hr continuously. At the same time, another dose of Pmti-3 (0.3 mL of 4 mg/mL stock) was added per vessel. At time about 48 hours, another dose (0.3 mL of 4 mg/mL) of Pmti-3 was added per vessel. Cultures were harvested and processed at about 60 hours post-inoculation.

Table 3
Composition of Media A

Soytone L-1	20	g/L
Yeast Extract	10	g/L
KH_2PO4	11.9	g/L
K_2HPO_4	2.3	g/L
Sorbitol	18.2	g/L
Glycerol	40	g/L
Antifoam Sigma 204	8	drops/L
10X YNB w/Ammonium Sulfate w/o Amino Acids (134 g/L)	100	mL/L
250X Biotin (0.4 g/L)	10	mL/L
500X Chloramphenicol (50 g/L)	2	mL/L
500X Kanamycin (50 g/L)	2	mL/L

Table 4
Glycerol Fed-Batch Feed

Glycerol	50	% m/m
PTM1 Salts (see Table IV-E below)	12.5	mL/L
250X Biotin (0.4 g/L)	12.5	mL/L

Table 5 Methanol Feed		
Methanol	100	% m/m
PTM1 Salts (*See* Table 6)	12.5	mL/L
250X Biotin (0.4 g/L)	12.5	mL/L

Table 6 PTM1 Salts		
$CuSO_4$-$5H_2O$	6	g/L
NaI	80	mg/L
$MnSO_4$-$7H_2O$	3	g/L
$NaMoO_4$-$2H_2O$	200	mg/L
H_3BO_3	20	mg/L
$CoCl_2$-$6H_2O$	500	mg/L
$ZnCl_2$	20	g/L
$FeSO_4$-$7H_2O$	65	g/L
Biotin	200	mg/L
H_2SO_4 (98%)	5	mL/L

The quality of *N*-glycan composition of the anti-Her2 antibodies was determined as follows. The antibodies were recovered from the cell culture medium and purified by protein A column chromatography. The *N*-glycans from protein A-purified antibodies were analyzed with 2AB labeling. The high performance liquid chromatography (HPLC) system used consisted of an Agilent 1200 equipped with autoinjector, a column-heating compartment and a UV detector detecting at 210 and 280 nm. All LC–MS experiments performed with this system were running at 1 mL/min. The flow rate was not split for MS detection. Mass spectrometric analysis was carried out in positive ion mode on Accurate-Mass Q-TOF LC/MS 6520 (Agilent technology). The temperature of dual ESI source was set at 350 °C. The nitrogen gas flow rates were set at 13 L/h for the cone and 350 l/h and nebulizer was set at 45 psig with 4500 volt applied to the capillary. Reference mass of 922.009 was prepared from HP-0921 according to API-TOF reference mass solution kit for mass calibration and the protein mass measurements. The data for ion spectrum range from 300-3000 m/z were acquired and processed using Agilent Masshunter.

Sample preparation was as follows. An intact antibody sample (50 μg) was prepared 50 μL 25 mM NH_4HCO_3, pH 7.8. For deglycosylated antibody, a 50 μL aliquot of

intact antibody sample was treated with PNGase F (10 units) for 18 hours at 37° C. Reduced antibody was prepared by adding 1 M DTT to a final concentration of 10 mM to an aliquot of either intact antibody or deglycosylated antibody and incubated for 30 min at 37° C.

Three micrograms of intact or deglycosylated antibody sample was loaded onto a Poroshell 300SB-C3 column (2.1 mm × 75 mm, 5 μm) (Agilent Technologies) maintained at 70° C. The protein was first rinsed on the cartridge for 1 minute with 90% solvent A (0.1% HCOOH), 5% solvent B (90% Acetonitrile in 0.1% HCOOH). Elution was then performed using a gradient of 5-100% of B over 26 minutes followed by a three-minute regeneration at 100% B and by a final equilibration period of 10 minute at 5% B.

For reduced antibody, a three microgram sample was loaded onto a Poroshell 300SB-C3 column (2.1 mm × 75 mm, 5 μm) (Agilent Technologies) maintained at 40°C. The protein was first rinsed on the cartridge for three minutes with 90% solvent A, 5% solvent B. Elution was then performed using a gradient of 5-80% of B over 20 minutes followed by a seven-minute regeneration at 80% B and by a final equilibration period of 10 minutes at 5% B.

EXAMPLE 5

Production of *Pichia pastoris* strains for GlycoInsulin production

This example describes construction of strain **YGLY21058**. Genetically engineered *Pichia pastoris* strain **YGLY21058** produces recombinant human glycoinsulin molecules. The strain produces glycoproteins having sialylated *N*-glycans and expressing the insulin analogue comprising an *N*-glycosylation site on the B-chain at position 28 encoded by the expression cassette in plasmid pGLY4362. Construction of the strains is illustrated schematically in **Figures 2A-2D**. Briefly, the strain **YGLY21058** was constructed from glycoengineered *Pichia pastoris* strain **YGLY7961** from Example 1 using methods described as follows:

Figure 19 shows as map of plasmid pGLY2456. Plasmid pGLY2456 is a KINKO integration vector that targets the *TRP2* locus without disrupting expression of the locus and contains six expression cassettes encoding (1) the mouse CMP-sialic acid transporter (mCMP-Sia Transp), (2) the human UDP-GlcNAc 2-epimerase/*N*-acetylmannosamine kinase (hGNE), (3) the *Pichia pastoris ARG1* gene or transcription unit, (4) the human CMP-sialic acid synthase (hCSS), (5) the human N-acetylneuraminate-9-phosphate synthase (hSPS), (6) the mouse α-2,6-sialyltransferase catalytic domain (mST6) fused at the *N*-terminus to *S. cerevisiae KRE2* leader peptide (33) to target the chimeric enzyme to the ER or Golgi, and the *P. pastoris ARG1* gene or transcription unit. The expression cassette encoding the mouse CMP-sialic acid transporter comprises a nucleic acid molecule encoding the mCMP Sia Transp ORF codon optimized for expression in *P. pastoris* (SEQ ID NO: 77), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris PMA1* promoter and at the 3' end to a nucleic acid molecule comprising the *P. pastoris PMA1* transcription termination sequence. The expression cassette encoding the human UDP-GlcNAc 2-epimerase/*N*-acetylmannosamine kinase comprises a nucleic acid molecule encoding the hGNE ORF codon optimized for expression in *P. pastoris*

(SEQ ID NO: 78), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GAPDH* promoter and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* transcription termination sequence. The expression cassette encoding the *P. pastoris ARG1* gene comprises (SEQ ID NO: 79). The expression cassette encoding the human CMP-sialic acid synthase comprises a nucleic acid molecule encoding the hCSS ORF codon optimized for expression in *P. pastoris* (SEQ ID NO: 80), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris GAPDH* promoter and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae CYC* transcription termination sequence. The expression cassette encoding the human N-acetylneuraminate-9-phosphate synthase comprises a nucleic acid molecule encoding the hSIAP S ORF codon optimized for expression in *P. pastoris* (SEQ ID NO: 81), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris PMA1* promoter and at the 3' end to a nucleic acid molecule comprising the *P. pastoris PMA1* transcription termination sequence. The expression cassette encoding the chimeric mouse α-2,6-sialyltransferase comprises a nucleic acid molecule encoding the mST6 catalytic domain codon optimized for expression in *P. pastoris* (SEQ ID NO:82) fused at the 5' end to a nucleic acid molecule encoding the *S. cerevisiae KRE2* signal peptide, which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris TEF* promoter and at the 3' end to a nucleic acid molecule comprising the *P. pastoris TEF* transcription termination sequence. The six tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region and ORF of the *TRP2* gene ending at the stop codon (SEQ ID NO: 83) followed by a *P. pastoris ALG3* termination sequence and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *TRP2* gene (SEQ ID NO: 84). Plasmid pGLY2456 was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY7961** to produce a number of strains in which the six expression cassette have been inserted into the *TRP2* locus immediately following the *TRP2* ORF by double-crossover homologous recombination. The strain **YGLY8146** was selected from the strains produced. The strain was then counterselected in the presence of 5-FOA to produce a number of strains now auxotrophic for uridine. Strain **YGLY9296** was selected.

Figure 20 shows as map of plasmid pGLY5048. Plasmid pGLY5048 is an integration vector that targets the STE13 locus and contains expression cassettes encoding (1) the T. reesei α-1,2-mannosidase catalytic domain fused at the N-terminus to S. cerevisiae αMATpre signal peptide (aMATTrMan) to target the chimeric protein to the secretory pathway and secretion from the cell and (2) the *P. pastoris URA5* gene or transcription unit. The expression cassette encoding the aMATTrMan comprises a nucleic acid molecule encoding the *T. reesei* catalytic domain (SEQ ID NO:52) fused at the 5' end to a nucleic acid molecule encoding the *S. cerevisiae* αMATpre signal peptide (SEQ ID NO:53 encoding amino acid sequence SEQ ID NO:54), which is operably linked at the 5' end to a nucleic acid molecule comprising the *P. pastoris AOX1* promoter and at the 3' end to a nucleic acid molecule comprising the *S. cerevisiae*

CYC transcription termination sequence. The *URA5* expression cassette comprises a nucleic acid molecule comprising the *P. pastoris URA5* gene or transcription unit flanked by nucleic acid molecules comprising *lacZ* repeats. The two tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region of the *STE13* gene (SEQ ID NO: 85) and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *STE13* gene (SEQ ID NO: 86). Plasmid pGLY5048 was linearized with *Sfi*I and the linearized plasmid transformed into strain **YGLY9296** to produce a number of strains. The strains **YGLY9469** was selected from the strains produced. The strain is capable of producing glycoproteins that have single-mannose *O*-glycosylation (*See* Published U.S. Application No. 20090170159).

Figure 21 shows as map of plasmid pGLY5019. Plasmid pGLY5019 is an integration vector that targets the *DAP2* locus and contains an expression cassette comprising a nucleic acid molecule encoding the Nourseothricin resistance (NATR) expression cassette (originally from pAG25 from EROSCARF, Scientific Research and Development GmbH, Daimlerstrasse 13a, D-61352 Bad Homburg, Germany, *See* Goldstein *et al.*, Yeast 15: 1541 (1999)) . The NAT^R expression cassette (SEQ ID NO:64) is operably regulated to the *Ashbya gossypii TEF1* promoter (SEQ ID NO:65) and *A. gossypii TEF1* termination sequence (SEQ ID NO:66) flanked one side with the 5' nucleotide sequence of the *P. pastoris DAP2* gene (SEQ ID NO:87) and on the other side with the 3' nucleotide sequence of the *P. pastoris DAP2* gene (SEQ ID NO:88). Plasmid pGLY5019 was linearized and the linearized plasmid transformed into strain **YGLY9469** to produce a number of strains in which the NATR expression cassette has been inserted into the *DAP2* locus by double-crossover homologous recombination. The strain **YGLY9797** was selected from the strains produced.

Figure 22 shows as map of plasmid pGLY5085. Plasmid pGLY5085 is a KINKO plasmid for introducing a second set of the genes involved in producing sialylated *N*-glycans into *P. pastoris*. The plasmid is similar to plasmid YGLY2456 except that the *P. pastoris ARG1* gene has been replaced with an expression cassette encoding hygromycin resistance (HygR) and the plasmid targets the *P. pastoris TRP5* locus. The HYG^R resistance cassette is SEQ ID NO:89. The HYG^R expression cassette (SEQ ID NO:89) is operably regulated to the *Ashbya gossypii TEF1* promoter and *A. gossypii TEF1* termination sequences (*See* Goldstein *et al.*, Yeast 15: 1541 (1999)). The six tandem cassettes are flanked on one side by a nucleic acid molecule comprising a nucleotide sequence from the 5' region and ORF of the *TRP5* gene ending at the stop codon (SEQ ID NO:90) followed by a *P. pastoris ALG3* termination sequence and on the other side by a nucleic acid molecule comprising a nucleotide sequence from the 3' region of the *TRP5* gene (SEQ ID NO:91). Plasmid pGLY5085 was transformed into strain **YGLY9797** to produce a number of strains of which strain **YGLY12900** is selected.

Figure 23 shows as map of plasmid pGLY4362.Plamsid pGLY4362 is a roll-in integration plasmid that targets the *TRP2* locus or *AOX1* locus and includes an expression

cassette encoding a pre-proinsulin analogue precursor comprising a Yps1ss peptide (SEQ ID NO:92) fused to a TA57 propeptide (SEQ ID NO:93) fused to an *N*-terminal spacer (SEQ ID NO:94) fused to the human insulin B-chain with a P28N substitution (SEQ ID NO:95) fused to a C-peptide consisting of the amino acid sequence AAK fused to the human insulin insulin A-chain (SEQ ID NO:96). The pre-proinsulin analogue precursor has the amino acid sequence shown in SEQ ID NO:97 and is encoded by the nucleotide sequence shown in SEQ ID NO:98. The expression cassette comprises a nucleic acid molecule encoding the fusion protein (SEQ ID NO:98) operably linked at the 5' end to a nucleic acid molecule that has the inducible *P. pastoris AOX1* promoter sequence (SEQ ID NO:55) and at the 3' end to a nucleic acid molecule that has the *Saccharomyces cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). For selecting transformants, the plasmid comprises an expression cassette encoding the Zeocin ORF in which the nucleic acid molecule encoding the ORF (SEQ ID NO:60) is operably linked at the 5' end to a nucleic acid molecule having the *S. cerevisiae TEF* promoter sequence (SEQ ID NO:61) and at the 3' end to a nucleic acid molecule having the *S. cerevisiae CYC* transcription termination sequence (SEQ ID NO:15). The plasmid further includes a nucleic acid molecule for targeting the *TRP2* locus.

Strain **YGLY12900** was transformed with plasmid **pGLY4362**, which is an expression plasmid that in *Pichia pastoris* enables expression of a glycosylated insulin analogue precursor molecule comprising the Yps1ss domain fused to the TA57 propeptide domain fused to an N-terminal spacer fused to the human insulin B-chain having a P28N substitution fused to a C-peptide having the amino acid sequence AAK fused to the human insulin A-chain, to produce a number of strains of which strain **YGLY21058** was selected. The strain is capable of producing an *N*-glycosylated insulin analogue precursor comprising an *N*-terminal spacer fused to the human insulin B-chain having a P28N substitution fused to a C-peptide having the amino acid sequence AAK fused to the human insulin A-chain. The analysis of the *N*-glycosylated insulin analogue precursor expression from this engineered Pichia pastoris XRN1 knock-out strain is shown in Table 11.

EXAMPLE 6:

Production of Pichia pastoris strains for human erythropoietin (EPO) production

This example describes construction of strain YGLY7117. Genetically engineered Pichia pastoris strain YGLY7117 produces recombinant human erythropoietin molecules. The strain produces glycoproteins having sialylated N-glycans. The strain YGLY7117 was constructed from wild-type Pichia pastoris strain NRRL-Y 11430 described earlier ("Add Reference here: J Biotechnol. 2012 Jan; 157(1):198-206. Nett et al. "Optimization of erythropoietin production with controlled glycosylation-PEGylated erythropoietin produced in glycoengineered Pichia pastoris"). The analysis of the N-glycosylated insulin analogue precursor expression from this engineered Pichia pastoris XRN1 knock-out strain is shown in Table 7.

Summary: *XRN1* Knock-out Mutants are Resistant to Stress-induced Translational Inhibition

Analysis of *xrn1Δ Pichia* mutants illustrate that mRNA degradation enzymes are involved in regulating general translation repression in response to a variety of nutritional and environmental stresses. In two well-characterized examples, global protein synthesis is rapidly inhibited upon glucose deprivation or severe amino acid starvation. In general, the stress-induced translation inhibition is a rapid response mediated by a well-described pathway involving Gcn2 protein kinase and its subsequent phosphorylation of translation initiation factor eIF2. mRNA degradation enzymes have not been described to be involved in this Gcn2 protein phosphorylation process. However, *Saccharomyces* mutants effecting 5' to 3' mRNA decay such as Δ*dcp1* and Δ*xrn1* are generally resistant to this stress-induced translation repression. Thus, it has been surprisingly found that Δ*xrn1 Pichia* strains of the present invention continue to translate at a rate typical of that seen with glucose-containing medium, even in glucose deprivation or amino acid starvation conditions. Because current high cell-density fermentors usually operate at oxygen-limited or carbon-source limited processes, it is likely that part of the yield improvement result of Δ*xrn1 Pichia* cells can be attributed to this Δ*xrn1* translation derepression during fermentation process.

Tables 7-11 summarize yield improvement and N-Glycan quality improvement results with engineered *Pichia xrn1* knockout host cells expressing exemplary heterologous proteins, in this case three different therapeutic proteins, as described in Examples 2-5.

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Table 7	**yGLY7117 human EPO XRN1 Knockout Yield Improvement**
Strain ID	yGLY7117
Protein ID	Human EPO
Fermentation Platform	Micro-24 5 mL Reactor
Genotype	Average Titer (μg/L)
XRN1-wt (n=3)	**32.5**
Δxrn1 (n=4)	**58.1**

Table 8	**yGLY12501 Herceptin mAb XRN1 Knockout Yield Improvement**
Strain ID	yGLY12501
Protein ID	Herceptin mAb
Fermentation Platform	Micro-24 5 mL Reactor
Genotype	Average Titer (mg/L)
XRN1-wt (n=4)	**504**
Δxrn1 (n=6)	**600**

Table 9	yGLY13992 Herceptin mAb XRN1 Knockout N-Glycan Quality and Yield Improvement								
Strain ID	yGLY13992								
Protein ID	Herceptin mAb								
Fermentation Platform	DasGip 1 Liter Reactor								
Genotype	Man5 %	G0 %	G1 %	G2 %	Complex %	WCW (g/L)	Supernatant Titer (mg/L)	Broth Titer (mg/L)	Induction Hours
XRN1-wt (n=2)	15.0	65.9	15.5	1.2	**85.0**	319	1112	**757.2**	105.6
Δxrn1 (n=5)	10.5	55.5	23.8	4.7	**89.5**	234	1061	**812.7**	89.4

Table 10	yGLY14836 Herceptin mAb XRN1 Knockout N-Glycan Quality Improvement								
Strain ID	yGLY14836								
Protein ID	Herceptin mAb								
Fermentation Platform	DasGip 1 Liter Reactor								
Genotype	Man5 %	G0 %	G1 %	G2 %	Complex %	WCW (g/L)	Supernatant Titer (mg/L)	Broth Titer (mg/L)	Induction Hours
XRN1-wt (n=3)	23.0	42.7	16.3	4.7	**77.0**	285	1525	**1090**	103.0
Δxrn1 (n=3)	12.6	55.2	18.7	5.1	**87.4**	203	1067	**850**	94.3

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Table 11	yGLY21080 P28N Glyco-Insulin XRN1 Knockout N-Glycan Quality and Yield Improvement
Strain ID	yGLY21080
Protein ID	P28N Glyco-Insulin
Fermentation Platform	DasGip 1 Liter Reactor

Genotype	Man5 %	G0 %	A1 %	A2 %	Complex %	WCW (g/L)	Supernatant Titer (mg/L)	Broth Titer (mg/L)	Induction Hours
XRN1-wt (n=2)	17.4	0	21.3	54.1	**82.6**	329	53	**35.5**	86.9
Δxrn1 (n=4)	8.3	7.8	48.4	32.0	**91.7**	388	123	**75.2**	80.7

In summary, the mRNA stabilization technique presented herein provides a powerful and flexible method to improve protein fermentation titer and protein glycosylation quality simultaneously. Inhibition of global mRNA turnover by XRN1 knockout increases mRNA abundance of both target protein and corresponding glycosyltransferases. Moreover, mutation of XRN1 may affect translation initiation to prevent stress-induced translation regulation and further improve the titer.

Glossary:

ScSUC2	S. cerevisiae Invertase
OCH1	Alpha-1,6-mannosyltransferase
KlMNN2-2:	K. lactis UDP-GlcNAc transporter
BMT1:	Beta-mannose-transfer (beta-mannose elimination)
BMT2:	Beta-mannose-transfer (beta-mannose elimination)
BMT3:	Beta-mannose-transfer (beta-mannose elimination)
BMT4:	Beta-mannose-transfer (beta-mannose elimination)
MNN4L1:	MNN4-like 1 (charge elimination)
MmSLC35A3	Mouse homologue of UDP-GlcNAc transporter
PNO1:	Phosphomannosylation of N-glycans (charge elimination)
MNN4:	Mannosyltransferase (charge elimination)
ScGAL10	UDP-glucose 4-epimerase
XB33	Truncated HsGalT1 fused to ScKRE2 leader
DmUGT	UDP-Galactose transporter
KD53	Truncated DmMNSII fused to ScMNN2 leader
TC54	Truncated RnGNTII fused to ScMNN2 leader
NA10	Truncated HsGNTI fused to PpSEC12 leader
FB8:	Truncated MmMNS1A fused to ScSEC12 leader
TrMDS1:	Secreted T. reseei MNS1
Sh ble:	Zeocin resistance marker
Nat:	*Streptomyces noursei* nourseothricin acetyltransferase
GD9:	Truncated MmMNS1B fused to ScSEC12 leader
MmCST	Mouse CMP-sialic acid transporter
HsGNE	Human UDP-GlcNAc 2-epimerase/N-acetylmannosamine kinase

HsCSS	Human CMP-sialic acid synthase
HsSPS	Human N-acetylneuraminate-9-phosphate synthase
MmST6-33	Truncated Mouse alpha-2,6-sailyl transferase fused to ScKRE2 leader
TrMDS1:	Secreted T. reseei MNS1
STE13	Golgi dipeptidyl aminopeptidase
DAP2	Vacuolar dipeptidyl aminopeptidase
NatR	Nourseothricin resistance marker
HygR	Hygromycin resistance marker
TRP2	Tryptophan biosynthesis
Sh ble:	Zeocin resistance marker

Insulin precursor variant: YPS1ss+TA57propeptide+N-spacer+Bchain(P28N)+C-peptide(AAK)+Achain insulin precursor

Table 12
BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:	Description	Sequence
1	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpXRN1:	TGAATGGCCTGACTAACAGAAGAATATTTGTTTTCCA GGAAGTTAATGTCTCTTGGAACTGATTCAATTAGTTCT TTGTGGTTTGCTTGATCGTCTTTCAAGCTCTCTGCGAT GTCCATTTGCTGCTGAATGTACTGATCAAGTTGTTCTA TTTCTTTTTGATAGGCATCTGGTAGATCCGTGACTCTC GTAAGGGATGTTATCTGTGGTTGAGAAGCATTGTTAG GGTTGTTGGGTGCTGCATTGTTGCCCAGTAAACTATTA TTGTTATTACTTGAATTGAAAAGCCCACCAGCATTACT GTTAGTATTGTTTCCAAATAGACTCCCTGTAGTTGTAT TAGCGTTCGTATTATTGCTCCCAAAAAGACCTCCAGTG TTACCACTCGGATTATTTGAGCCTGAGAAACCACTTTG TGCAGGTTTATTGGCATTTCCAAACAACCCCCCTCCAG TTTGGGCATTACTATTTGCAGTATTGCTGCCTCCAAAA AGACCACCCGATTGTGTGTTATTTGAATTGGTTCCAAA TAGCCCTCCTGATTGTGTGTTACCAGAATTTCCTCCAA ATAATCCTCCCTGTTGAGTGTTAGAGTTAGTGTTGGTG TTACCTCCAAAGAGTCCTTTCGATTGAGTATTACTAGC ATTTCCCCCAAACAACCCTCCTGATTGATTGCTGTTGC TAGCAGTATTCCCACCAAACAATCCTTGCGATTGAGT ATTTCCCGTGTTGGTGTTGGCGCCAAATAAGCTACCTG ACTGATTCGTGTTGGTATTACCAGAATTGTTTCCAAAC ATACCGCCTGTATTGGTACTACCTGAAGCGTTCGTACT GCCAAACAATCCTCCGCTATTGCCTCCAGAATTTGTAC TAGCATTATTTCCAAACAAACCTCCTGTGTTATTCGTA TTCGTAGCAGGCTTTGCTCCAAACAAGCCTCCAGTGTT ACCAGAGGAGCTACCTCCAAAAGCCCCAACATTGCTA CCTGAGGCGTTTCCAAACATACCGCCTGAATTGGCGG GGTTCTTGTTGGAATTTCCAAACATTTGACTTTAAGGT TTTAAAGAACGTTGTTTTGACAAGGGAAACAAAAGTT CCCACTAAATTTTTCGATGTAAGGACTTGGGAGGAGC AACTGCTTACATGAACTCCCTCTAACTTTCCTCATAAA AATCCTTCCAAGCTCGCGAGGTCCCTTCCAACTAAGTC GGGTAAGTTT

2	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpXRN1:	TGCCCGGACTTCTATCCAGCAAATTTACGGAACGGTG TTCAATCAAGTGTTGAGCGCTCAACCTCAGTTGCAGCC TGTCAGAGGCTTCTCAAATCCGGTACCTGAAACCCCT GTAAATGGAGTCCAAGCGAATGAACAACACTCTGATT CTACCCCTCAAAATCATTCTAGGGATGAAAACCAAGG AAGAGGTCGTGGTAGAGGCAGAGGAAATAGAAGAGG AAGAGGTCGAGGTAGAGGCAAAGGAGGACAGTAAAT CAGAATGAAGGTGTCCCTCCGATTGAATAGAATTGTG TTGTAATATTAGTGTATTGCGTTAATGGTACTAATAAT CATCCTGCATTGTATAACTAAGAACTTTCTTCTCCTGC CTTAGAGCAGTCGTCTTGAAAACTTTTTGCTCCCAGCC ATTAGACCTATCAATTCCGTCCCATCTATACCCTGGCA TTATAGAGAAACGATTGTCCGGGAAAGAAGATTTGTA TAGTTTACGGCCTGTCTGTGAGATGTAACGGTCTTTTT GCAGCGACTTGACTTTATCTGGAGAAAATGCTAGCAA CGGGTCATCTACATAGCTTGGCGGAGCTACTCTTTTAG TGGCCGTTTGAGTAACCTCGGGGAGATTCATATTGCGT AATTTTTCAGATGCTTCTTTGGCTTTCTGTTTTTCTTGC TGTGATTTCTGTCTCTGTTGTTCTAGCATTTCCTCATGA GAGAGGACGTTTCCTGATAAGTCTCTATAAATAGTGG CATTCTCGGGTTTTGCAGGTTTTGTGGGTTTTTGTATC GACGACTTGGGTTTCGGCTTCAGTGATTTATCCCTTTT CTTGGACTTGGATTTGCCATATTTGGAATCCAGGTAGT CCTGTAGTGACATTCGTTTCGATACTAGGTGCGATGGT TCATTCGATACGATATATAATGCTTACATAAGCTAATG GTACTGGGAACATCACTTATACTATCCGTTCAGATCAA CAGGAGAATTCATTCATACAACATGCCAAATTCATTG AAGACCCAGTCATCATTCCATCAGACTGCCCTTGGTTA AAGGTGATAAGGAATTAATTGAGGTTTATCGGGGTTT AAAGTGAGGCGGGCATCAAGAAAAAAAAAAAGAGGG CAGGAGCAGTGGAACTTTCAAAACAAGAAAGAGATA AATCTTATCTCGTGACCCCTATCTTAGCAAATAACGTT TACGTTTGAAGGTAATAGATTAAGCAACCAATTACCT CATCCTAACTTACGAGTAATATCCCGTTTCATCTCATC ATCAATGAGGGACTTGATTTTATACCGACATTGTTGGC TCCCCACATTAACCCTTTAAAGCAGGAAGATCCAATT CCCCGAGGGACAAACTTGACACCCTAACTTTCCCGGG GTTCACGAAATATTCATGAACCCCCCCCCTTGATACGA ACATCTGCGCCGTATGCACCCTTCTGGGACATACCGCC TGAGGCCACCTC

3	*S. cerevisiae* invertase gene (ScSUC2) ORF underlined (909-2507 bp) -	AGGCCTCGCAACAACCTATAATTGAGTTAAGTGCCTTT CCAAGCTAAAAAGTTTGAGGTTATAGGGGCTTAGCAT CCACACGTCACAATCTCGGGTATCGAGTATAGTATGT AGAATTACGGCAGGAGGTTTCCCAATGAACAAAGGAC AGGGGCACGGTGAGCTGTCGAAGGTATCCATTTTATC ATGTTTCGTTTGTACAAGCACGACATACTAAGACATTT ACCGTATGGGAGTTGTTGTCCTAGCGTAGTTCTCGCTC CCCCAGCAAAGCTCAAAAAAGTACGTCATTTAGAATA GTTTGTGAGCAAATTACCAGTCGGTATGCTACGTTAG AAAGGCCCACAGTATTCTTCTACCAAAGGCGTGCCTTT GTTGAACTCGATCCATTATGAGGGCTTCCATTATTCCC CGCATTTTTATTACTCTGAACAGGAATAAAAAGAAAA AACCCAGTTTAGGAAATTATCCGGGGGCGAAGAAATA CGCGTAGCGTTAATCGACCCCACGTCCAGGGTTTTTCC ATGGAGGTTTCTGGAAAAACTGACGAGGAATGTGATT ATAAATCCCTTTATGTGATGTCTAAGACTTTTAAGGTA CGCCCGATGTTTGCCTATTACCATCATAGAGACGTTTC TTTTCGAGGAATGCTTAAACGACTTTGTTTGACAAAAA TGTTGCCTAAGGGCTCTATAGTAAACCATTTGGAAGA AAGATTTGACGACTTTTTTTTTTTGGATTTCGATCCTAT AATCCTTCCTCCTGAAAAGAAACATATAAATAGATAT GTATTATTCTTCAAAACATTCTCTTGTTCTTGTGCTTTT TTTTTACCATATATCTTACTTTTTTTTTTCTCTCAGAGA AACAAGCAAAACAAAAAGCTTTTCTTTTCACTAACGT ATATG**ATG**CTTTTGCAAGCTTTCCTTTTCCTTTTGGCTG GTTTTGCAGCCAAAATATCTGCATCAATGACAAACGA AACTAGCGATAGACCTTTGGTCCACTTCACACCCAAC AAGGGCTGGATGAATGACCCAAATGGGTTGTGGTACG ATGAAAAAGATGCCAAATGGCATCTGTACTTTCAATA CAACCCAAATGACACCGTATGGGGTACGCCATTGTTT TGGGGCCATGCTACTTCCGATGATTTGACTAATTGGGA AGATCAACCCATTGCTATCGCTCCCAAGCGTAACGAT TCAGGTGCTTTCTCTGGCTCCATGGTGGTTGATTACAA CAACACGAGTGGGTTTTTCAATGATACTATTGATCCAA GACAAAGATGCGTTGCGATTTGGACTTATAACACTCC TGAAAGTGAAGAGCAATACATTAGCTATTCTCTTGAT GGTGGTTACACTTTTACTGAATACCAAAAGAACCCTG TTTTAGCTGCCAACTCCACTCAATTCAGAGATCCAAAG GTGTTCTGGTATGAACCTTCTCAAAAATGGATTATGAC GGCTGCCAAATCACAAGACTACAAAATTGAAATTTAC TCCTCTGATGACTTGAAGTCCTGGAAGCTAGAATCTGC ATTTGCCAATGAAGGTTTCTTAGGCTACCAATACGAAT GTCCAGGTTTGATTGAAGTCCCAACTGAGCAAGATCC TTCCAAATCTTATTGGGTCATGTTTATTTCTATCAACC CAGGTGCACCTGCTGGCGGTTCCTTCAACCAATATTTT GTTGGATCCTTCAATGGTACTCATTTTGAAGCGTTTGA CAATCAATCTAGAGTGGTAGATTTTGGTAAGGACTAC TATGCCTTGCAAACTTTCTTCAACACTGACCCAACCTA CGGTTCAGCATTAGGTATTGCCTGGGCTTCAAACTGG GAGTACAGTGCCTTTGTCCCAACTAACCCATGGAGAT CATCCATGTCTTTGGTCCGCAAGTTTTCTTTGAACACT GAATATCAAGCTAATCCAGAGACTGAATTGATCAATT TGAAAGCCGAACCAATATTGAACATTAGTAATGCTGG

		TCCCTGGTCTCGTTTTGCTACTAACACAACTCTAACTA AGGCCAATTCTTACAATGTCGATTTGAGCAACTCGACT GGTACCCTAGAGTTTGAGTTGGTTTACGCTGTTAACAC CACACAAACCATATCCAAATCCGTCTTTGCCGACTTAT CACTTTGGTTCAAGGGTTTAGAAGATCCTGAAGAATA TTTGAGAATGGGTTTTGAAGTCAGTGCTTCTTCCTTCT TTTTGGACCGTGGTAACTCTAAGGTCAAGTTTGTCAAG GAGAACCCATATTTCACAAACAGAATGTCTGTCAACA ACCAACCATTCAAGTCTGAGAACGACCTAAGTTACTA TAAAGTGTACGGCCTACTGGATCAAAACATCTTGGAA TTGTACTTCAACGATGGAGATGTGGTTTCTACAAATAC CTACTTCATGACCACCGGTAACGCTCTAGGATCTGTGA ACATGACCACTGGTGTCGATAATTTGTTCTACATTGAC AAGTTCCAAGTAAGGGAAGTAAAA**TAG**AGGTTATAA AACTTATTGTCTTTTTTATTTTTTTCAAAAGCCATTCTA AAGGGCTTTAGCTAACGAGTGACGAATGTAAAACTTT ATGATTTCAAAGAATACCTCCAAACCATTGAAAATGT ATTTTTATTTTTATTTTCTCCCGACCCCAGTTACCTGGA ATTTGTTCTTTATGTACTTTATATAAGTATAATTCTCTT AAAAATTTTTACTACTTTGCAATAGACATCATTTTTTC ACGTAATAAACCCACAATCGTAATGTAGTTGCCTTAC ACTACTAGGATGGACCTTTTTGCCTTTATCTGTTTTGTT ACTGACACAATGAAACCGGGTAAAGTATTAGTTATGT GAAAATTTAAAAGCATTAAGTAGAAGTATACCATATT GTAAAAAAAAAAAGCGTTGTCTTCTACGTAAAAGTGT TCTCAAAAAGAAGTAGTGAGGGAAATGGATACCAAGC TATCTGTAACAGGAGCTAAAAAATCTCAGGGAAAAGC TTCTGGTTTGGGAAACGGTCGAC

4	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpURA5:	ATCGGCCTTTGTTGATGCAAGTTTTACGTGGATCATGG ACTAAGGAGTTTTATTTGGACCAAGTTCATCGTCCTAG ACATTACGGAAAGGGTTCTGCTCCTCTTTTTGGAAACT TTTTGGAACCTCTGAGTATGACAGCTTGGTGGATTGTA CCCATGGTATGGCTTCCTGTGAATTTCTATTTTTTCTAC ATTGGATTCACCAATCAAAACAAATTAGTCGCCATGG CTTTTTGGCTTTTGGGTCTATTTGTTTGGACCTTCTTGG AATATGCTTTGCATAGATTTTTGTTCCACTTGGACTAC TATCTTCCAGAGAATCAAATTGCATTTACCATTCATTT CTTATTGCATGGGATACACCACTATTTACCAATGGATA AATACAGATTGGTGATGCCACCTACACTTTTCATTGTA CTTTGCTACCCAATCAAGACGCTCGTCTTTTCTGTTCT ACCATATTACATGGCTTGTTCTGGATTTGCAGGTGGAT TCCTGGGCTATATCATGTATGATGTCACTCATTACGTT CTGCATCACTCCAAGCTGCCTCGTTATTTCCAAGAGTT GAAGAAATATCATTTGGAACATCACTACAAGAATTAC GAGTTAGGCTTTGGTGTCACTTCCAAATTCTGGGACAA AGTCTTTGGGACTTATCTGGGTCCAGACGATGTGTATC AAAAGACAAATTAGAGTATTTATAAAGTTATGTAAGC AAATAGGGGCTAATAGGGAAAGAAAAATTTTGGTTCT TTATCAGAGCTGGCTCGCGCGCAGTGTTTTTCGTGCTC CTTTGTAATAGTCATTTTTGACTACTGTTCAGATTGAA ATCACATTGAAGATGTCACTCGAGGGGTACCAAAAAA GGTTTTTGGATGCTGCAGTGGCTTCGC

5	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpURA5:	GGTCTTTTCAACAAAGCTCCATTAGTGAGTCAGCTGGC TGAATCTTATGCACAGGCCATCATTAACAGCAACCTG GAGATAGACGTTGTATTTGGACCAGCTTATAAAGGTA TTCCTTTGGCTGCTATTACCGTGTTGAAGTTGTACGAG CTCGGCGGCAAAAAATACGAAAATGTCGGATATGCGT TCAATAGAAAAGAAAAGAAAGACCACGGAGAAGGTG GAAGCATCGTTGGAGAAAGTCTAAAGAATAAAAGAGT ACTGATTATCGATGATGTGATGACTGCAGGTACTGCT ATCAACGAAGCATTTGCTATAATTGGAGCTGAAGGTG GGAGAGTTGAAGGTAGTATTATTGCCCTAGATAGAAT GGAGACTACAGGAGATGACTCAAATACCAGTGCTACC CAGGCTGTTAGTCAGAGATATGGTACCCCTGTCTTGA GTATAGTGACATTGGACCATATTGTGGCCCATTTGGGC GAAACTTTCACAGCAGACGAGAAATCTCAAATGGAAA CGTATAGAAAAAAGTATTTGCCCAAATAAGTATGAAT CTGCTTCGAATGAATGAATTAATCCAATTATCTTCTCA CCATTATTTTCTTCTGTTTCGGAGCTTTGGGCACGGCG GCGGGTGGTGCGGGCTCAGGTTCCCTTTCATAAACAG ATTTAGTACTTGGATGCTTAATAGTGAATGGCGAATGC AAAGGAACAATTTCGTTCATCTTTAACCCTTTCACTCG GGGTACACGTTCTGGAATGTACCCGCCCTGTTGCAACT CAGGTGGACCGGGCAATTCTTGAACTTTCTGTAACGTT GTTGGATGTTCAACCAGAAATTGTCCTACCAACTGTAT TAGTTTCCTTTTGGTCTTATATTGTTCATCGAGATACTT CCCACTCTCCTTGATAGCCACTCTCACTCTTCCTGGAT TACCAAAATCTTGAGGATGAGTCTTTTCAGGCTCCAG GATGCAAGGTATATCCAAGTACCTGCAAGCATCTAAT ATTGTCTTTGCCAGGGGGTTCTCCACACCATACTCCTT TTGGCGCATGC

6	*Pichia pastoris* Sequence of the PpURA5 auxotrophic marker:	TCTAGAGGGACTTATCTGGGTCCAGACGATGTGTATC AAAAGACAAATTAGAGTATTTATAAAGTTATGTAAGC AAATAGGGGCTAATAGGGAAAGAAAAATTTTGGTTCT TTATCAGAGCTGGCTCGCGCGCAGTGTTTTTCGTGCTC CTTTGTAATAGTCATTTTTGACTACTGTTCAGATTGAA ATCACATTGAAGATGTCACTGGAGGGGTACCAAAAAA GGTTTTTGGATGCTGCAGTGGCTTCGCAGGCCTTGAAG TTTGGAACTTTCACCTTGAAAAGTGGAAGACAGTCTC CATACTTCTTTAACATGGGTCTTTTCAACAAAGCTCCA TTAGTGAGTCAGCTGGCTGAATCTTATGCTCAGGCCAT CATTAACAGCAACCTGGAGATAGACGTTGTATTTGGA CCAGCTTATAAAGGTATTCCTTTGGCTGCTATTACCGT GTTGAAGTTGTACGAGCTGGGCGGCAAAAAATACGAA AATGTCGGATATGCGTTCAATAGAAAAGAAAAGAAAG ACCACGGAGAAGGTGGAAGCATCGTTGGAGAAAGTCT AAAGAATAAAAGAGTACTGATTATCGATGATGTGATG ACTGCAGGTACTGCTATCAACGAAGCATTTGCTATAA TTGGAGCTGAAGGTGGGAGAGTTGAAGGTTGTATTAT TGCCCTAGATAGAATGGAGACTACAGGAGATGACTCA AATACCAGTGCTACCCAGGCTGTTAGTCAGAGATATG GTACCCCTGTCTTGAGTATAGTGACATTGGACCATATT GTGGCCCATTTGGGCGAAACTTTCACAGCAGACGAGA AATCTCAAATGGAAACGTATAGAAAAAAGTATTTGCC CAAATAAGTATGAATCTGCTTCGAATGAATGAATTAA TCCAATTATCTTCTCACCATTATTTTCTTCTGTTTCGGA GCTTTGGGCACGGCGGCGGATCC
7	*Escherichia coli* Sequence of the part of the *E. coli* lacZ gene that was used to construct the PpURA5 blaster (recyclable auxotrophic marker)	CCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTG GCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAG GTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCC GGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTA GTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGC ACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAA CCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCC CGCATCTGACCACCAGCGAAATGGATTTTTGCATCGA GCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCA GGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAAC AACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGC ACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACC CGCATTGACCCTAACGCCTGGGTCGAACGCTGGAAGG CGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCA GTGCACGGCAGATACACTTGCTGATGCGGTGCTGATT ACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCT TATTTATCAGCCGGAAAACCTACCGGATTGATGGTAG TGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCG AGCGATACACCGCATCCGGCGCGGATTGGCCTGAACT GCCAG

8	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpOCH1:	AAAACCTTTTTTCCTATTCAAACACAAGGCATTGCTTC AACACGTGTGCGTATCCTTAACACAGATACTCCATACT TCTAATAATGTGATAGACGAATACAAAGATGTTCACT CTGTGTTGTGTCTACAAGCATTTCTTATTCTGATTGGG GATATTCTAGTTACAGCACTAAACAACTGGCGATACA AACTTAAATTAAATAATCCGAATCTAGAAAATGAACT TTTGGATGGTCCGCCTGTTGGTTGGATAAATCAATACC GATTAAATGGATTCTATTCCAATGAGAGAGTAATCCA AGACACTCTGATGTCAATAATCATTTGCTTGCAACAAC AAACCCGTCATCTAATCAAAGGGTTTGATGAGGCTTA CCTTCAATTGCAGATAAACTCATTGCTGTCCACTGCTG TATTATGTGAGAATATGGGTGATGAATCTGGTCTTCTC CACTCAGCTAACATGGCTGTTTGGGCAAAGGTGGTAC AATTATACGGAGATCAGGCAATAGTGAAATTGTTGAA TATGGCTACTGGACGATGCTTCAAGGATGTACGTCTA GTAGGAGCCGTGGGAAGATTGCTGGCAGAACCAGTTG GCACGTCGCAACAATCCCCAAGAAATGAAATAAGTGA AAACGTAACGTCAAAGACAGCAATGGAGTCAATATTG ATAACACCACTGGCAGAGCGGTTCGTACGTCGTTTTG GAGCCGATATGAGGCTCAGCGTGCTAACAGCACGATT GACAAGAAGACTCTCGAGTGACAGTAGGTTGAGTAAA GTATTCGCTTAGATTCCCAACCTTCGTTTTATTCTTTCG TAGACAAAGAAGCTGCATGCGAACATAGGGACAACTT TTATAAATCCAATTGTCAAACCAACGTAAAACCCTCT GGCACCATTTTCAACATATATTTGTGAAGCAGTACGC AATATCGATAAATACTCACCGTTGTTTGTAACAGCCCC AACTTGCATACGCCTTCTAATGACCTCAAATGGATAA GCCGCAGCTTGTGCTAACATACCAGCAGCACCGCCCG CGGTCAGCTGCGCCCACACATATAAAGGCAATCTACG ATCATGGGAGGAATTAGTTTTGACCGTCAGGTCTTCA AGAGTTTTGAACTCTTCTTCTTGAACTGTGTAACCTTT TAAATGACGGGATCTAAATACGTCATGGATGAGATCA TGTGTGTAAAAACTGACTCCAGCATATGGAATCATTC CAAAGATTGTAGGAGCGAACCCACGATAAAAGTTTCC CAACCTTGCCAAAGTGTCTAATGCTGTGACTTGAAATC TGGGTTCCTCGTTGAAGACCCTGCGTACTATGCCCAAA AACTTTCCTCCACGAGCCCTATTAACTTCTCTATGAGT TTCAAATGCCAAACGGACACGGATTAGGTCCAATGGG TAAGTGAAAAACACAGAGCAAACCCCAGCTAATGAG CCGGCCAGTAACCGTCTTGGAGCTGTTTCATAAGAGT CATTAGGGATCAATAACGTTCTAATCTGTTCATAACAT ACAAATTTTATGGCTGCATAGGGAAAAATTCTCAACA GGGTAGCCGAATGACCCTGATATAGACCTGCGACACC ATCATACCCATAGATCTGCCTGACAGCCTTAAAGAGC CCGCTAAAAGACCCGGAAAACCGAGAGAACTCTGGAT TAGCAGTCTGAAAAAGAATCTTCACTCTGTCTAGTGG AGCAATTAATGTCTTAGCGGCACTTCCTGCTACTCCGC CAGCTACTCCTGAATAGATCACATACTGCAAAGACTG CTTGTCGATGACCTTGGGGTTATTTAGCTTCAAGGGCA

		ATTTTTGGGACATTTTGGACACAGGAGACTCAGAAAC AGACACAGAGCGTTCTGAGTCCTGGTGCTCCTGACGT AGGCCTAGAACAGGAATTATTGGCTTTATTTGTTTGTC CATTTCATAGGCTTGGGGTAATAGATAGATGACAGAG AAATAGAGAAGACCTAATATTTTTTGTTCATGGCAAAT CGCGGGTTCGCGGTCGGGTCACACACGGAGAAGTAAT GAGAAGAGCTGGTAATCTGGGGTAAAAGGGTTCAAAA GAAGGTCGCCTGGTAGGGATGCAATACAAGGTTGTCT TGGAGTTTACATTGACCAGATGATTTGGCTTTTTCTCT GTTCAATTCACATTTTTCAGCGAGAATCGGATTGACGG AGAAATGGCGGGGTGTGGGGTGGATAGATGGCAGAA ATGCTCGCAATCACCGCGAAAGAAAGACTTTATGGAA TAGAACTACTGGGTGGTGTAAGGATTACATAGCTAGT CCAATGGAGTCCGTTGGAAAGGTAAGAAGAAGCTAAA ACCGGCTAAGTAACTAGGGAAGAATGATCAGACTTTG ATTTGATGAGGTCTGAAAATACTCTGCTGCTTTTTCAG TTGCTTTTTCCCTGCAACCTATCATTTTCCTTTTCATAA GCCTGCCTTTTCTGTTTTCACTTATATGAGTTCCGCCG AGACTTCCCCAAATTCTCTCCTGGAACATTCTCTATCG CTCTCCTTCCAAGTTGCGCCCCCTGGCACTGCCTAGTA ATATTACCACGCGACTTATATTCAGTTCCACAATTTCC AGTGTTCGTAGCAAATATCATCAGCCATGGCGAAGGC AGATGGCAGTTTGCTCTACTATAATCCTCACAATCCAC CCAGAAGGTATTACTTCTACATGGCTATATTCGCCGTT TCTGTCATTTGCGTTTTGTACGGACCCTCACAACAATT ATCATCTCCAAAAATAGACTATGATCCATTGACGCTCC GATCACTTGATTTGAAGACTTTGGAAGCTCCTTCACAG TTGAGTCCAGGCACCGTAGAAGATAATCTTCG

9	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpOCH1:	AAAGCTAGAGTAAAATAGATATAGCGAGATTAGAGA ATGAATACCTTCTTCTAAGCGATCGTCCGTCATCATAG AATATCATGGACTGTATAGTTTTTTTTTTGTACATATA ATGATTAAACGGTCATCCAACATCTCGTTGACAGATCT CTCAGTACGCGAAATCCCTGACTATCAAAGCAAGAAC CGATGAAGAAAAAAACAACAGTAACCCAAACACCAC AACAAACACTTTATCTTCTCCCCCCCAACACCAATCAT CAAAGAGATGTCGGAACCAAACACCAAGAAGCAAAA ACTAACCCCATATAAAAACATCCTGGTAGATAATGCT GGTAACCCGCTCTCCTTCCATATTCTGGGCTACTTCAC GAAGTCTGACCGGTCTCAGTTGATCAACATGATCCTC GAAATGGGTGGCAAGATCGTTCCAGACCTGCCTCCTC TGGTAGATGGAGTGTTGTTTTTGACAGGGGATTACAA GTCTATTGATGAAGATACCCTAAAGCAACTGGGGGAC GTTCCAATATACAGAGACTCCTTCATCTACCAGTGTTT TGTGCACAAGACATCTCTTCCCATTGACACTTTCCGAA TTGACAAGAACGTCGACTTGGCTCAAGATTTGATCAA TAGGGCCCTTCAAGAGTCTGTGGATCATGTCACTTCTG CCAGCACAGCTGCAGCTGCTGCTGTTGTTGTCGCTACC AACGGCCTGTCTTCTAAACCAGACGCTCGTACTAGCA AAATACAGTTCACTCCCGAAGAAGATCGTTTTATTCTT GACTTTGTTAGGAGAAATCCTAAACGAAGAAACACAC ATCAACTGTACACTGAGCTCGCTCAGCACATGAAAAA CCATACGAATCATTCTATCCGCCACAGATTTCGTCGTA ATCTTTCCGCTCAACTTGATTGGGTTTATGATATCGAT CCATTGACCAACCAACCTCGAAAAGATGAAAACGGGA ACTACATCAAGGTACAAGGCCTTCCA
10	*Kluyveromyces lactis* *K. lactis* UDP-GlcNAc transporter gene (KIMNN2-2) ORF underlined	AAACGTAACGCCTGGCACTCTATTTTCTCAAACTTCTG GGACGGAAGAGCTAAATATTGTGTTGCTTGAACAAAC CCAAAAAAAACAAAAAAAATGAACAAACTAAAACTACA CCTAAATAAACCGTGTGTAAAACGTAGTACCATATTA CTAGAAAAGATCACAAGTGTATCACACATGTGCATCT CATATTACATCTTTTATCCAATCCATTCTCTCTATCCCG TCTGTTCCTGTCAGATTCTTTTTCCATAAAAAGAAGAA GACCCCGAATCTCACCGGTACAATGCAAAACTGCTGA AAAAAAAAGAAAGTTCACTGGATACGGGAACAGTGC CAGTAGGCTTCACCACATGGACAAAACAATTGACGAT AAAATAAGCAGGTGAGCTTCTTTTTCAAGTCACGATC CCTTTATGTCTCAGAAACAATATATACAAGCTAAACC CTTTTGAACCAGTTCTCTCTTCATAGTTATGTTCACAT AAATTGCGGGAACAAGACTCCGCTGGCTGTCAGGTAC ACGTTGTAACGTTTTCGTCCGCCCAATTATTAGCACAA CATTGGCAAAAAGAAAAACTGCTCGTTTTCTCTACAG GTAAATTACAATTTTTTTCAGTAATTTTCGCTGAAAAA TTTAAAGGGCAGGAAAAAAAGACGATCTCGACTTTGC ATAGATGCAAGAACTGTGGTCAAAACTTGAAATAGTA ATTTTGCTGTGCGTGAACTAATAAATATATATATATAT

		ATATATATATATTTGTGTATTTTGTATATGTAATTGTGC ACGTCTTGGCTATTGGATATAAGATTTTCGCGGGTTGA TGACATAGAGCGTGTACTACTGTAATAGTTGTATATTC AAAAGCTGCTGCGTGGAGAAAGACTAAAATAGATAA AAAGCACACATTTTGACTTCGGTACCGTCAACTTAGTG GGACAGTCTTTTATATTTGGTGTAAGCTCATTTCTGGT ACTATTCGAAACAGAACAGTGTTTTCTGTATTACCGTC CAATCGTTTGTC**ATG**AGTTTTGTATTGATTTTGTCGTT AGTGTTCGGAGGATGTTGTTCCAATGTGATTAGTTTCG AGCACATGGTGCAAGGCAGCAATATAAATTTGGGAAA TATTGTTACATTCACTCAATTCGTGTCTGTGACGCTAA TTCAGTTGCCCAATGCTTTGGACTTCTCTCACTTTCCGT TTAGGTTGCGACCTAGACACATTCCTCTTAAGATCCAT ATGTTAGCTGTGTTTTTGTTCTTTACCAGTTCAGTCGCC AATAACAGTGTGTTTAAATTTGACATTTCCGTTCCGAT TCATATTATCATTAGATTTTCAGGTACCACTTTGACGA TGATAATAGGTTGGGCTGTTTGTAATAAGAGGTACTCC AAACTTCAGGTGCAATCTGCCATCATTATGACGCTTGG TGCGATTGTCGCATCATTATACCGTGACAAAGAATTTT CAATGGACAGTTTAAAGTTGAATACGGATTCAGTGGG TATGACCCAAAAATCTATGTTTGGTATCTTTGTTGTGC TAGTGGCCACTGCCTTGATGTCATTGTTGTCGTTGCTC AACGAATGGACGTATAACAAGTACGGGAAACATTGGA AAGAAACTTTGTTCTATTCGCATTTCTTGGCTCTACCG TTGTTTATGTTGGGGTACACAAGGCTCAGAGACGAAT TCAGAGACCTCTTAATTTCCTCAGACTCAATGGATATT CCTATTGTTAAATTACCAATTGCTACGAAACTTTTCAT GCTAATAGCAAATAACGTGACCCAGTTCATTTGTATC AAAGGTGTTAACATGCTAGCTAGTAACACGGATGCTT TGACACTTTCTGTCGTGCTTCTAGTGCGTAAATTTGTT AGTCTTTTACTCAGTGTCTACATCTACAAGAACGTCCT ATCCGTGACTGCATACCTAGGGACCATCACCGTGTTCC TGGGAGCTGGTTTGTATTCATATGGTTCGGTCAAAACT GCACTGCCTCGC**TGA**AACAATCCACGTCTGTATGATA CTCGTTTCAGAATTTTTTTGATTTTCTGCCGGATATGGT TTCTCATCTTTACAATCGCATTCTTAATTATACCAGAA CGTAATTCAATGATCCCAGTGACTCGTAACTCTTATAT GTCAATTTAAGC

11	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpBMT2:	GGCCGAGCGGGCCTAGATTTTCACTACAAATTTCAAA ACTACGCGGATTTATTGTCTCAGAGAGCAATTTGGCAT TTCTGAGCGTAGCAGGAGGCTTCATAAGATTGTATAG GACCGTACCAACAAATTGCCGAGGCACAACACGGTAT GCTGTGCACTTATGTGGCTACTTCCCTACAACGGAATG AAACCTTCCTCTTTCCGCTTAAACGAGAAAGTGTGTCG CAATTGAATGCAGGTGCCTGTGCGCCTTGGTGTATTGT TTTTGAGGGCCCAATTTATCAGGCGCCTTTTTTCTTGG TTGTTTTCCCTTAGCCTCAAGCAAGGTTGGTCTATTTC ATCTCCGCTTCTATACCGTGCCTGATACTGTTGGATGA GAACACGACTCAACTTCCTGCTGCTCTGTATTGCCAGT GTTTTGTCTGTGATTTGGATCGGAGTCCTCCTTACTTG GAATGATAATAATCTTGGCGGAATCTCCCTAAACGGA GGCAAGGATTCTGCCTATGATGATCTGCTATCATTGGG AAGCTTCAACGACATGGAGGTCGACTCCTATGTCACC AACATCTACGACAATGCTCCAGTGCTAGGATGTACGG ATTTGTCTTATCATGGATTGTTGAAAGTCACCCCAAAG CATGACTTAGCTTGCGATTTGGAGTTCATAAGAGCTCA GATTTTGGACATTGACGTTTACTCCGCCATAAAAGACT TAGAAGATAAAGCCTTGACTGTAAAACAAAAGGTTGA AAAACACTGGTTTACGTTTTATGGTAGTTCAGTCTTTC TGCCCGAACACGATGTGCATTACCTGGTTAGACGAGT CATCTTTTCGGCTGAAGGAAAGGCGAACTCTCCAGTA ACATC

12	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpBMT2:	CCATATGATGGGTGTTTGCTCACTCGTATGGATCAAAA TTCCATGGTTTCTTCTGTACAACTTGTACACTTATTTGG ACTTTTCTAACGGTTTTTCTGGTGATTTGAGAAGTCCT TATTTTGGTGTTCGCAGCTTATCCGTGATTGAACCATC AGAAATACTGCAGCTCGTTATCTAGTTTCAGAATGTGT TGTAGAATACAATCAATTCTGAGTCTAGTTTGGGTGGG TCTTGGCGACGGGACCGTTATATGCATCTATGCAGTGT TAAGGTACATAGAATGAAAATGTAGGGGTTAATCGAA AGCATCGTTAATTTCAGTAGAACGTAGTTCTATTCCCT ACCCAAATAATTTGCCAAGAATGCTTCGTATCCACAT ACGCAGTGGACGTAGCAAATTTCACTTTGGACTGTGA CCTCAAGTCGTTATCTTCTACTTGGACATTGATGGTCA TTACGTAATCCACAAAGAATTGGATAGCCTCTCGTTTT ATCTAGTGCACAGCCTAATAGCACTTAAGTAAGAGCA ATGGACAAATTTGCATAGACATTGAGCTAGATACGTA ACTCAGATCTTGTTCACTCATGGTGTACTCGAAGTACT GCTGGAACCGTTACCTCTTATCATTTCGCTACTGGCTC GTGAAACTACTGGATGAAAAAAAAAAAAGAGCTGAA AGCGAGATCATCCCATTTTGTCATCATACAAATTCACG CTTGCAGTTTTGCTTCGTTAACAAGACAAGATGTCTTT ATCAAAGACCCGTTTTTTCTTCTTGAAGAATACTTCCC TGTTGAGCACATGCAAACCATATTTATCTCAGATTTCA CTCAACTTGGGTGCTTCCAAGAGAAGTAAAATTCTTCC CACTGCATCAACTTCCAAGAAACCCGTAGACCAGTTT CTCTTCAGCCAAAAGAAGTTGCTCGCCGATCACCGCG GTAACAGAGGAGTCAGAAGGTTTCACACCCTTCCATC CCGATTTCAAAGTCAAAGTGCTGCGTTGAACCAAGGT TTTCAGGTTGCCAAAGCCCAGTCTGCAAAAACTAGTT CCAAATGGCCTATTAATTCCCATAAAAGTGTTGGCTAC GTATGTATCGGTACCTCCATTCTGGTATTTGCTATTGT TGTCGTTGGTGGGTTGACTAGACTGACCGAATCCGGT CTTTCCATAACGGAGTGGAAACCTATCACTGGTTCGGT TCCCCCACTGACTGAGGAAGACTGGAAGTTGGAATTT GAAAAATACAAACAAAGCCCTGAGTTTCAGGAACTAA ATTCTCACATAACATTGGAAGAGTTCAAGTTTATATTT TCCATGGAATGGGGACATAGATTGTTGGGAAGGGTCA TCGGCCTGTCGTTTGTTCTTCCCACGTTTTACTTCATTG CCCGTCGAAAGTGTTCCAAAGATGTTGCATTGAAACT GCTTGCAATATGCTCTATGATAGGATTCCAAGGTTTCA TCGGCTGGTGGATGGTGTATTCCGGATTGGACAAACA GCAATTGGCTGAACGTAACTCCAAACCAACTGTGTCT CCATATCGCTTAACTACCCATCTTGGAACTGCATTTGT TATTTACTGTTACATGATTTACACAGGGCTTCAAGTTT TGAAGAACTATAAGATCATGAAACAGCCTGAAGCGTA TGTTCAAATTTTCAAGCAAATTGCGTCTCCAAAATTGA AAACTTTCAAGAGACTCTCTTCAGTTCTATTAGGCCTG GTG

13	*Mus musculus* DNA encodes MmSLC35A3 UDP-GlcNAc transporter	ATGTCTGCCAACCTAAAATATCTTTCCTTGGGAATTTT GGTGTTTCAGACTACCAGTCTGGTTCTAACGATGCGGT ATTCTAGGACTTTAAAAGAGGAGGGGCCTCGTTATCT GTCTTCTACAGCAGTGGTTGTGGCTGAATTTTTGAAGA TAATGGCCTGCATCTTTTTAGTCTACAAAGACAGTAAG TGTAGTGTGAGAGCACTGAATAGAGTACTGCATGATG AAATTCTTAATAAGCCCATGGAAACCCTGAAGCTCGC TATCCCGTCAGGGATATATACTCTTCAGAACAACTTAC TCTATGTGGCACTGTCAAACCTAGATGCAGCCACTTAC CAGGTTACATATCAGTTGAAAATACTTACAACAGCAT TATTTTCTGTGTCTATGCTTGGTAAAAAATTAGGTGTG TACCAGTGGCTCTCCCTAGTAATTCTGATGGCAGGAGT TGCTTTTGTACAGTGGCCTTCAGATTCTCAAGAGCTGA ACTCTAAGGACCTTTCAACAGGCTCACAGTTTGTAGG CCTCATGGCAGTTCTCACAGCCTGTTTTTCAAGTGGCT TTGCTGGAGTTTATTTTGAGAAAATCTTAAAAGAAAC AAAACAGTCAGTATGGATAAGGAACATTCAACTTGGT TTCTTTGGAAGTATATTTGGATTAATGGGTGTATACGT TTATGATGGAGAATTGGTCTCAAAGAATGGATTTTTTC AGGGATATAATCAACTGACGTGGATAGTTGTTGCTCT GCAGGCACTTGGAGGCCTTGTAATAGCTGCTGTCATC AAATATGCAGATAACATTTTAAAAGGATTTGCGACCT CCTTATCCATAATATTGTCAACAATAATATCTTATTTT TGGTTGCAAGATTTTGTGCCAACCAGTGTCTTTTTCCT TGGAGCCATCCTTGTAATAGCAGCTACTTTCTTGTATG GTTACGATCCCAAACCTGCAGGAAATCCCACTAAAGC ATAG
14	*Pichia pastoris* PpGAPDH promoter	TTTTTGTAGAAATGTCTTGGTGTCCTCGTCCAATCAGG TAGCCATCTCTGAAATATCTGGCTCCGTTGCAACTCCG AACGACCTGCTGGCAACGTAAAATTCTCCGGGGTAAA ACTTAAATGTGGAGTAATGGAACCAGAAACGTCTCTT CCCTTCTCTCTCCTTCCACCGCCCGTTACCGTCCCTAG GAAATTTTACTCTGCTGGAGAGCTTCTTCTACGGCCCC CTTGCAGCAATGCTCTTCCCAGCATTACGTTGCGGGTA AAACGGAGGTCGTGTACCCGACCTAGCAGCCCAGGGA TGGAAAAGTCCCGGCCGTCGCTGGCAATAATAGCGGG CGGACGCATGTCATGAGATTATTGGAAACCACCAGAA TCGAATATAAAAGGCGAACACCTTTCCCAATTTTGGTT TCTCCTGACCCAAAGACTTTAAATTTAATTTATTTGTC CCTATTTCAATCAATTGAACAACTATCAAAACACA
15	*Saccharomyces cerevisiae* ScCYC TT	ACAGGCCCCTTTTCCTTTGTCGATATCATGTAATTAGT TATGTCACGCTTACATTCACGCCCTCCTCCCACATCCG CTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGT CTAGGTCCCTATTTATTTTTTTTAATAGTTATGTTAGTA TTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTT CTGTACAAACGCGTGTACGCATGTAACATTATACTGA AAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGC TTTAATTTGCAAGCTGCCGGCTCTTAAG

16	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpMNN4L1:	GATCTGGCCATTGTGAAACTTGACACTAAAGACAAAA CTCTTAGAGTTTCCAATCACTTAGGAGACGATGTTTCC TACAACGAGTACGATCCCTCATTGATCATGAGCAATTT GTATGTGAAAAAAGTCATCGACCTTGACACCTTGGAT AAAAGGGCTGGAGGAGGTGGAACCACCTGTGCAGGC GGTCTGAAAGTGTTCAAGTACGGATCTACTACCAAAT ATACATCTGGTAACCTGAACGGCGTCAGGTTAGTATA CTGGAACGAAGGAAAGTTGCAAAGCTCCAAATTTGTG GTTCGATCCTCTAATTACTCTCAAAAGCTTGGAGGAA ACAGCAACGCCGAATCAATTGACAACAATGGTGTGGG TTTTGCCTCAGCTGGAGACTCAGGCGCATGGATTCTTT CCAAGCTACAAGATGTTAGGGAGTACCAGTCATTCAC TGAAAAGCTAGGTGAAGCTACGATGAGCATTTTCGAT TTCCACGGTCTTAAACAGGAGACTTCTACTACAGGGC TTGGGGTAGTTGGTATGATTCATTCTTACGACGGTGAG TTCAAACAGTTTGGTTTGTTCACTCCAATGACATCTAT TCTACAAAGACTTCAACGAGTGACCAATGTAGAATGG TGTGTAGCGGGTTGCGAAGATGGGGATGTGGACACTG AAGGAGAACACGAATTGAGTGATTTGGAACAACTGCA TATGCATAGTGATTCCGACTAGTCAGGCAAGAGAGAG CCCTCAAATTTACCTCTCTGCCCCTCCTCACTCCTTTTG GTACGCATAATTGCAGTATAAAGAACTTGCTGCCAGC CAGTAATCTTATTTCATACGCAGTTCTATATAGCACAT AATCTTGCTTGTATGTATGAAATTTACCGCGTTTTAGT TGAAATTGTTTATGTTGTGTGCCTTGCATGAAATCTCT CGTTAGCCCTATCCTTACATTTAACTGGTCTCAAAACC TCTACCAATTCCATTGCTGTACAACAATATGAGGCGG CATTACTGTAGGGTTGGAAAAAAATTGTCATTCCAGC TAGAGATCACACGACTTCATCACGCTTATTGCTCCTCA TTGCTAAATCATTTACTCTTGACTTCGACCCAGAAAAG TTCGCC

17	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpMNN4L1:	GCATGTCAAACTTGAACACAACGACTAGATAGTTGTT TTTTCTATATAAAACGAAACGTTATCATCTTTAATAAT CATTGAGGTTTACCCTTATAGTTCCGTATTTTCGTTTCC AAACTTAGTAATCTTTTGGAAATATCATCAAAGCTGGT GCCAATCTTCTTGTTTGAAGTTTCAAACTGCTCCACCA AGCTACTTAGAGACTGTTCTAGGTCTGAAGCAACTTC GAACACAGAGACAGCTGCCGCCGATTGTTCTTTTTTGT GTTTTTCTTCTGGAAGAGGGGCATCATCTTGTATGTCC AATGCCCGTATCCTTTCTGAGTTGTCCGACACATTGTC CTTCGAAGAGTTTCCTGACATTGGGCTTCTTCTATCCG TGTATTAATTTTGGGTTAAGTTCCTCGTTTGCATAGCA GTGGATACCTCGATTTTTTTGGCTCCTATTTACCTGAC ATAATATTCTACTATAATCCAACTTGGACGCGTCATCT ATGATAACTAGGCTCTCCTTTGTTCAAAGGGGACGTCT TCATAATCCACTGGCACGAAGTAAGTCTGCAACGAGG CGGCTTTTGCAACAGAACGATAGTGTCGTTTCGTACTT GGACTATGCTAAACAAAAGGATCTGTCAAACATTTCA ACCGTGTTTCAAGGCACTCTTTACGAATTATCGACCAA GACCTTCCTAGACGAACATTTCAACATATCCAGGCTA CTGCTTCAAGGTGGTGCAAATGATAAAGGTATAGATA TTAGATGTGTTTGGGACCTAAAACAGTTCTTGCCTGAA GATTCCCTTGAGCAACAGGCTTCAATAGCCAAGTTAG AGAAGCAGTACCAAATCGGTAACAAAAGGGGGAAGC ATATAAAACCTTTACTATTGCGACAAAATCCATCCTTG AAAGTAAAGCTGTTTGTTCAATGTAAAGCATACGAAA CGAAGGAGGTAGATCCTAAGATGGTTAGAGAACTTAA CGGGACATACTCCAGCTGCATCCCATATTACGATCGCT GGAAGACTTTTTTCATGTACGTATCGCCCACCAACCTT TCAAAGCAAGCTAGGTATGATTTTGACAGTTCTCACA ATCCATTGGTTTTCATGCAACTTGAAAAAACCCAACTC AAACTTCATGGGGATCCATACAATGTAAATCATTACG AGAGGGCGAGGTTGAAAAGTTTCCATTGCAATCACGT CGCATCATGGCTACTGAAAGGCCTTAAC

18	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpPNO1 and PpMNN4:	TCATTCTATATGTTCAAGAAAAGGGTAGTGAAAGGAA AGAAAAGGCATATAGGCGAGGGAGAGTTAGCTAGCA TACAAGATAATGAAGGATCAATAGCGGTAGTTAAAGT GCACAAGAAAAGAGCACCTGTTGAGGCTGATGATAAA GCTCCAATTACATTGCCACAGAGAAACACAGTAACAG AAATAGGAGGGGATGCACCACGAGAAGAGCATTCAG TGAACAACTTTGCCAAATTCATAACCCCAAGCGCTAA TAAGCCAATGTCAAAGTCGGCTACTAACATTAATAGT ACAACAACTATCGATTTTCAACCAGATGTTTGCAAGG ACTACAAACAGACAGGTTACTGCGGATATGGTGACAC TTGTAAGTTTTTGCACCTGAGGGATGATTTCAAACAGG GATGGAAATTAGATAGGGAGTGGGAAAATGTCCAAA AGAAGAAGCATAATACTCTCAAAGGGGTTAAGGAGAT CCAAATGTTTAATGAAGATGAGCTCAAAGATATCCCG TTTAAATGCATTATATGCAAAGGAGATTACAAATCAC CCGTGAAAACTTCTTGCAATCATTATTTTTGCGAACAA TGTTTCCTGCAACGGTCAAGAAGAAAACCAAATTGTA TTATATGTGGCAGAGACACTTTAGGAGTTGCTTTACCA GCAAAGAAGTTGTCCCAATTTCTGGCTAAGATACATA ATAATGAAAGTAATAAAGTTTAGTAATTGCATTGCGTT GACTATTGATTGCATTGATGTCGTGTGATACTTTCACC GAAAAAAAACACGAAGCGCAATAGGAGCGGTTGCAT ATTAGTCCCCAAAGCTATTTAATTGTGCCTGAAACTGT TTTTTAAGCTCATCAAGCATAATTGTATGCATTGCGAC GTAACCAACGTTTAGGCGCAGTTTAATCATAGCCCAC TGCTAAGCC

19	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpPNO1 and PpMNN4:	CGGAGGAATGCAAATAATAATCTCCTTAATTACCCAC TGATAAGCTCAAGAGACGCGGTTTGAAAACGATATAA TGAATCATTTGGATTTTATAATAAACCCTGACAGTTTT TCCACTGTATTGTTTTAACACTCATTGGAAGCTGTATT GATTCTAAGAAGCTAGAAATCAATACGGCCATACAAA AGATGACATTGAATAAGCACCGGCTTTTTTGATTAGC ATATACCTTAAAGCATGCATTCATGGCTACATAGTTGT TAAAGGGCTTCTTCCATTATCAGTATAATGAATTACAT AATCATGCACTTATATTTGCCCATCTCTGTTCTCTCACT CTTGCCTGGGTATATTCTATGAAATTGCGTATAGCGTG TCTCCAGTTGAACCCCAAGCTTGGCGAGTTTGAAGAG AATGCTAACCTTGCGTATTCCTTGCTTCAGGAAACATT CAAGGAGAAACAGGTCAAGAAGCCAAACATTTTGATC CTTCCCGAGTTAGCATTGACTGGCTACAATTTTCAAAG CCAGCAGCGGATAGAGCCTTTTTTGGAGGAAACAACC AAGGGAGCTAGTACCCAATGGGCTCAAAAAGTATCCA AGACGTGGGATTGCTTTACTTTAATAGGATACCCAGA AAAAAGTTTAGAGAGCCCTCCCCGTATTTACAACAGT GCGGTACTTGTATCGCCTCAGGGAAAAGTAATGAACA ACTACAGAAAGTCCTTCTTGTATGAAGCTGATGAACA TTGGGGATGTTCGGAATCTTCTGATGGGTTTCAAACAG TAGATTTATTAATTGAAGGAAAGACTGTAAAGACATC ATTTGGAATTTGCATGGATTTGAATCCTTATAAATTTG AAGCTCCATTCACAGACTTCGAGTTCAGTGGCCATTGC TTGAAAACCGGTACAAGACTCATTTTGTGCCCAATGG CCTGGTTGTCCCCTCTATCGCCTTCCATTAAAAAGGAT CTTAGTGATATAGAGAAAAGCAGACTTCAAAAGTTCT ACCTTGAAAAAATAGATACCCCGGAATTTGACGTTAA TTACGAATTGAAAAAAGATGAAGTATTGCCCACCCGT ATGAATGAAACGTTGGAAACAATTGACTTTGAGCCTT CAAAACCGGACTACTCTAATATAAATTATTGGATACT AAGGTTTTTTCCCTTTCTGACTCATGTCTATAAACGAG ATGTGCTCAAAGAGAATGCAGTTGCAGTCTTATGCAA CCGAGTTGGCATTGAGAGTGATGTCTTGTACGGAGGA TCAACCACGATTCTAAACTTCAATGGTAAGTTAGCATC GACACAAGAGGAGCTGGAGTTGTACGGGCAGACTAAT AGTCTCAACCCCAGTGTGGAAGTATTGGGGGCCCTTG GCATGGGTCAACAGGGAATTCTAGTACGAGACATTGA ATTAACATAATATACAATATACAATAAACACAAATAA AGAATACAAGCCTGACAAAAATTCACAAATTATTGCC TAGACTTGTCGTTATCAGCAGCGACCTTTTTCCAATGC TCAATTTCACGATATGCCTTTTCTAGCTCTGCTTTAAG CTTCTCATTGGAATTGGCTAACTCGTTGACTGCTTGGT CAGTGATGAGTTTCTCCAAGGTCCATTTCTCGATGTTG TTGTTTTCGTTTTCCTTTAATCTCTTGATATAATCAACA GCCTTCTTTAATATCTGAGCCTTGTTCGAGTCCCCTGT TGGCAACAGAGCGGCCAGTTCCTTTATTCCGTGGTTTA TATTTTCTCTTCTACGCCTTTCTACTTCTTTGTGATTCT CTTTACGCATCTTATGCCATTCTTCAGAACCAGTGGCT GGCTTAACCGAATAGCCAGAGCCTGAAGAAGCCGCAC TAGAAGAAGCAGTGGCATTGTTGACTATGG

20	human DNA encodes human GnTI catalytic domain (NA) Codon-optimized	TCAGTCAGTGCTCTTGATGGTGACCCAGCAAGTTTGAC CAGAGAAGTGATTAGATTGGCCCAAGACGCAGAGGTG GAGTTGGAGAGACAACGTGGACTGCTGCAGCAAATCG GAGATGCATTGTCTAGTCAAAGAGGTAGGGTGCCTAC CGCAGCTCCTCCAGCACAGCCTAGAGTGCATGTGACC CCTGCACCAGCTGTGATTCCTATCTTGGTCATCGCCTG TGACAGATCTACTGTTAGAAGATGTCTGGACAAGCTG TTGCATTACAGACCATCTGCTGAGTTGTTCCCTATCAT CGTTAGTCAAGACTGTGGTCACGAGGAGACTGCCCAA GCCATCGCCTCCTACGGATCTGCTGTCACTCACATCAG ACAGCCTGACCTGTCATCTATTGCTGTGCCACCAGACC ACAGAAAGTTCCAAGGTTACTACAAGATCGCTAGACA CTACAGATGGGCATTGGGTCAAGTCTTCAGACAGTTT AGATTCCCTGCTGCTGTGGTGGTGGAGGATGACTTGG AGGTGGCTCCTGACTTCTTTGAGTACTTTAGAGCAACC TATCCATTGCTGAAGGCAGACCCATCCCTGTGGTGTGT CTCTGCCTGGAATGACAACGGTAAGGAGCAAATGGTG GACGCTTCTAGGCCTGAGCTGTTGTACAGAACCGACT TCTTTCCTGGTCTGGGATGGTTGCTGTTGGCTGAGTTG TGGGCTGAGTTGGAGCCTAAGTGGCCAAAGGCATTCT GGGACGACTGGATGAGAAGACCTGAGCAAAGACAGG GTAGAGCCTGTATCAGACCTGAGATCTCAAGAACCAT GACCTTTGGTAGAAAGGGAGTGTCTCACGGTCAATTC TTTGACCAACACTTGAAGTTTATCAAGCTGAACCAGC AATTTGTGCACTTCACCCAACTGGACCTGTCTTACTTG CAGAGAGAGGCCTATGACAGAGATTTCCTAGCTAGAG TCTACGGAGCTCCTCAACTGCAAGTGGAGAAAGTGAG GACCAATGACAGAAAGGAGTTGGGAGAGGTGAGAGT GCAGTACACTGGTAGGGACTCCTTTAAGGCTTTCGCTA AGGCTCTGGGTGTCATGGATGACCTTAAGTCTGGAGT TCCTAGAGCTGGTTACAGAGGTATTGTCACCTTTCAAT TCAGAGGTAGAAGAGTCCACTTGGCTCCTCCACCTAC TTGGGAGGGTTATGATCCTTCTTGGAATTAG
21	*Pichia pastoris* DNA encodes Pp SEC12 (10) The last 9 nucleotides are the linker containing the AscI restriction site used for fusion to proteins of interest.	ATGCCCAGAAAAATATTTAACTACTTCATTTTGACTGT ATTCATGGCAATTCTTGCTATTGTTTTACAATGGTCTA TAGAGAATGGACATGGGCGCGCC

22	*Pichia pastoris* Sequence of the PpSEC4 promoter:	GAAGTAAAGTTGGCGAAACTTTGGGAACCTTTGGTTA AAACTTTGTAATTTTTGTCGCTACCCATTAGGCAGAAT CTGCATCTTGGGAGGGGGATGTGGTGGCGTTCTGAGA TGTACGCGAAGAATGAAGAGCCAGTGGTAACAACAG GCCTAGAGAGATACGGGCATAATGGGTATAACCTACA AGTTAAGAATGTAGCAGCCCTGGAAACCAGATTGAAA CGAAAAACGAAATCATTTAAACTGTAGGATGTTTTGG CTCATTGTCTGGAAGGCTGGCTGTTTATTGCCCTGTTC TTTGCATGGGAATAAGCTATTATATCCCTCACATAATC CCAGAAAATAGATTGAAGCAACGCGAAATCCTTACGT ATCGAAGTAGCCTTCTTACACATTCACGTTGTACGGAT AAGAAAACTACTCAAACGAACAATC
23	*Pichia pastoris* Sequence of the PpOCH1 terminator:	AATAGATATAGCGAGATTAGAGAATGAATACCTTCTT CTAAGCGATCGTCCGTCATCATAGAATATCATGGACT GTATAGTTTTTTTTTTGTACATATAATGATTAAACGGT CATCCAACATCTCGTTGACAGATCTCTCAGTACGCGA AATCCCTGACTATCAAAGCAAGAACCGATGAAGAAAA AAACAACAGTAACCCAAACACCACAACAAACACTTTA TCTTCTCCCCCCCAACACCAATCATCAAAGAGATGTCG GAACACAAACACCAAGAAGCAAAAACTAACCCCATA TAAAAACATCCTGGTAGATAATGCTGGTAACCCGCTC TCCTTCCATATTCTGGGCTACTTCACGAAGTCTGACCG GTCTCAGTTGATCAACATGATCCTCGAAATGG

24	*Mus musculus* DNA encodes Mm ManI catalytic domain (FB)	GAGCCCGCTGACGCCACCATCCGTGAGAAGAGGGCAA AGATCAAAGAGATGATGACCCATGCTTGGAATAATTA TAAACGCTATGCGTGGGGCTTGAACGAACTGAAACCT ATATCAAAAGAAGGCCATTCAAGCAGTTTGTTTGGCA ACATCAAAGGAGCTACAATAGTAGATGCCCTGGATAC CCTTTTCATTATGGGCATGAAGACTGAATTTCAAGAA GCTAAATCGTGGATTAAAAAATATTTAGATTTTAATGT GAATGCTGAAGTTTCTGTTTTTGAAGTCAACATACGCT TCGTCGGTGGACTGCTGTCAGCCTACTATTTGTCCGGA GAGGAGATATTTCGAAAGAAAGCAGTGGAACTTGGGG TAAAATTGCTACCTGCATTTCATACTCCCTCTGGAATA CCTTGGGCATTGCTGAATATGAAAAGTGGGATCGGGC GGAACTGGCCCTGGGCCTCTGGAGGCAGCAGTATCCT GGCCGAATTTGGAACTCTGCATTTAGAGTTTATGCACT TGTCCCACTTATCAGGAGACCCAGTCTTTGCCGAAAA GGTTATGAAAATTCGAACAGTGTTGAACAAACTGGAC AAACCAGAAGGCCTTTATCCTAACTATCTGAACCCCA GTAGTGGACAGTGGGGTCAACATCATGTGTCGGTTGG AGGACTTGGAGACAGCTTTTATGAATATTTGCTTAAGG CGTGGTTAATGTCTGACAAGACAGATCTCGAAGCCAA GAAGATGTATTTTGATGCTGTTCAGGCCATCGAGACTC ACTTGATCCGCAAGTCAAGTGGGGGACTAACGTACAT CGCAGAGTGGAAGGGGGGCCTCCTGGAACACAAGAT GGGCCACCTGACGTGCTTTGCAGGAGGCATGTTTGCA CTTGGGGCAGATGGAGCTCCGGAAGCCCGGGCCCAAC ACTACCTTGAACTCGGAGCTGAAATTGCCCGCACTTGT CATGAATCTTATAATCGTACATATGTGAAGTTGGGAC CGGAAGCGTTTCGATTTGATGGCGGTGTGGAAGCTAT TGCCACGAGGCAAAATGAAAAGTATTACATCTTACGG CCCGAGGTCATCGAGACATACATGTACATGTGGCGAC TGACTCACGACCCCAAGTACAGGACCTGGGCCTGGGA AGCCGTGGAGGCTCTAGAAAGTCACTGCAGAGTGAAC GGAGGCTACTCAGGCTTACGGGATGTTTACATTGCCC GTGAGAGTTATGACGATGTCCAGCAAAGTTTCTTCCTG GCAGAGACACTGAAGTATTTGTACTTGATATTTTCCGA TGATGACCTTCTTCCACTAGAACACTGGATCTTCAACA CCGAGGCTCATCCTTTCCCTATACTCCGTGAACAGAAG AAGGAAATTGATGGCAAAGAGAAATGA

25	*Saccharomyces cerevisiae* DNA encodes ScSEC12 (8) The last 9 nucleotides are the linker containing the AscI restriction site used for fusion to proteins of interest	ATGAACACTATCCACATAATAAAATTACCGCTTAACT ACGCCAACTACACCTCAATGAAACAAAAAATCTCTAA ATTTTTCACCAACTTCATCCTTATTGTGCTGCTTTCTTA CATTTTACAGTTCTCCTATAAGCACAATTTGCATTCCA TGCTTTTCAATTACGCGAAGGACAATTTTCTAACGAAA AGAGACACCATCTCTTCGCCCTACGTAGTTGATGAAG ACTTACATCAAACAACTTTGTTTGGCAACCACGGTAC AAAAACATCTGTACCTAGCGTAGATTCCATAAAAGTG CATGGCGTGGGGCGCGCC
26	*Pichia pastoris* Sequence of the 5'-region that was used to knock into the PpADE1 locus:	GAGTCGGCCAAGAGATGATAACTGTTACTAAGCTTCT CCGTAATTAGTGGTATTTTGTAACTTTTACCAATAATC GTTTATGAATACGGATATTTTTCGACCTTATCCAGTGC CAAATCACGTAACTTAATCATGGTTTAAATACTCCACT TGAACGATTCATTATTCAGAAAAAAGTCAGGTTGGCA GAAACACTTGGGCGCTTTGAAGAGTATAAGAGTATTA AGCATTAAACATCTGAACTTTCACCGCCCCAATATACT ACTCTAGGAAACTCGAAAAATTCCTTTCCATGTGTCAT CGCTTCCAACACACTTTGCTGTATCCTTCCAAGT**ATG**T CCATTGTGAACACTGATCTGGACGGAATCCTACCTTTA ATCGCCAAAGGAAAGGTTAGAGACATTTATGCAGTCG ATGAGAACAACTTGCTGTTCGTCGCAACTGACCGTAT CTCCGCTTACGATGTGATTATGACAAACGGTATTCCTG ATAAGGGAAAGATTTTGACTCAGCTCTCAGTTTTCTGG TTTGATTTTTTGGCACCCTACATAAAGAATCATTTGGT TGCTTCTAATGACAAGGAAGTCTTTGCTTTACTACCAT CAAAACTGTCTGAAGAAAAaTACAAATCTCAATTAGA GGGACGATCCTTGATAGTAAAAAAGCACAGACTGATA CCTTTGGAAGCCATTGTCAGAGGTTACATCACTGGAA GTGCATGGAAAGAGTACAAGAACTCAAAAACTGTCCA TGGAGTCAAGGTTGAAAACGAGAACCTTCAAGAGAGC GACGCCTTTCCAACTCCGATTTTCACACCTTCAACGAA AGCTGAACAGGGTGAACACGATGAAAACATCTCTATT GAACAAGCTGCTGAGATTGTAGGTAAAGACATTTGTG AGAAGGTCGCTGTCAAGGCGGTCGAGTTGTATTCTGC TGCAAAAAACCTCGCCCTTTTGAAGGGGATCATTATT GCTGATACGAAATTCGAATTTGGACTGGACGAAAACA ATGAATTGGTACTAGTAGATGAAGTTTTAACTCCAGAT TCTTCTAGATTTTGGAATCAAAAGACTTACCAAGTGG GTAAATCGCAAGAGAGTTACGATAAGCAGTTTCTCAG AGATTGGTTGACGGCCAACGGATTGAATGGCAAAGAG GGCGTAGCCATGGATGCAGAAATTGCTATCAAGAGTA AAGAAAAGTATATTGAAGCTTATGAAGCAATTACTGG CAAGAAATGGGCTTGA

27	*Pichia pastoris* PpALG3 TT	ATTTACAATTAGTAATATTAAGGTGGTAAAAACATTC GTAGAATTGAAATGAATTAATATAGTATGACAATGGT TCATGTCTATAAATCTCCGGCTTCGGTACCTTCTCCCC AATTGAATACATTGTCAAAATGAATGGTTGAACTATT AGGTTCGCCAGTTTCGTTATTAAGAAAACTGTTAAAAT CAAATTCCATATCATCGGTTCCAGTGGGAGGACCAGT TCCATCGCCAAAATCCTGTAAGAATCCATTGTCAGAA CCTGTAAAGTCAGTTTGAGATGAAATTTTTCCGGTCTT TGTTGACTTGGAAGCTTCGTTAAGGTTAGGTGAAACA GTTTGATCAACCAGCGGCTCCCGTTTTCGTCGCTTAGT AG
28	*Pichia pastoris* Sequence of the 3'-region that was used to knock into the PpADE1 locus:	ATGATTAGTACCCTCCTCGCCTTTTTCAGACATCTGAA ATTTCCCTTATTCTTCCAATTCCATATAAAATCCTATTT AGGTAATTAGTAAACAATGATCATAAAGTGAAATCAT TCAAGTAACCATTCCGTTTATCGTTGATTTAAAATCAA TAACGAATGAATGTCGGTCTGAGTAGTCAATTTGTTGC CTTGGAGCTCATTGGCAGGGGGTCTTTTGGCTCAGTAT GGAAGGTTGAAAGGAAAACAGATGGAAAGTGGTTCG TCAGAAAAGAGGTATCCTACATGAAGATGAATGCCAA AGAGATATCTCAAGTGATAGCTGAGTTCAGAATTCTT AGTGAGTTAAGCCATCCCAACATTGTGAAGTACCTTC ATCACGAACATATTTCTGAGAATAAAACTGTCAATTT ATACATGGAATACTGTGATGGTGGAGATCTCTCCAAG CTGATTCGAACACATAGAAGGAACAAAGAGTACATTT CAGAAGAAAAAATATGGAGTATTTTTACGCAGGTTTT ATTAGCATTGTATCGTTGTCATTATGGAACTGATTTCA CGGCTTCAAAGGAGTTTGAATCGCTCAATAAAGGTAA TAGACGAACCCAGAATCCTTCGTGGGTAGACTCGACA AGAGTTATTATTCACAGGGATATAAAACCCGACAACA TCTTTCTGATGAACAATTCAAACCTTGTCAAACTGGGA GATTTTGGATTAGCAAAAATTCTGGACCAAGAAAACG ATTTTGCCAAAACATACGTCGGTACGCCGTATTACATG TCTCCTGAAGTGCTGTTGGACCAACCCTACTCACCATT ATGTGATATATGGTCTCTTGGGTGCGTCATGTATGAGC TATGTGCATTGAGGCCTCCTT

29	*Saccharomyces cerevisiae* DNA encodes ScGAL10	ATGACAGCTCAGTTACAAAGTGAAAGTACTTCTAAAA TTGTTTTGGTTACAGGTGGTGCTGGATACATTGGTTCA CACACTGTGGTAGAGCTAATTGAGAATGGATATGACT GTGTTGTTGCTGATAACCTGTCGAATTCAACTTATGAT TCTGTAGCCAGGTTAGAGGTCTTGACCAAGCATCACA TTCCCTTCTATGAGGTTGATTTGTGTGACCGAAAAGGT CTGGAAAAGGTTTTCAAAGAATATAAAATTGATTCGG TAATTCACTTTGCTGGTTTAAAGGCTGTAGGTGAATCT ACACAAATCCCGCTGAGATACTATCACAATAACATTT TGGGAACTGTCGTTTTATTAGAGTTAATGCAACAATAC AACGTTTCCAAATTTGTTTTTTCATCTTCTGCTACTGTC TATGGTGATGCTACGAGATTCCCAAATATGATTCCTAT CCCAGAAGAATGTCCCTTAGGGCCTACTAATCCGTAT GGTCATACGAAATACGCCATTGAGAATATCTTGAATG ATCTTTACAATAGCGACAAAAAAAGTTGGAAGTTTGC TATCTTGCGTTATTTTAACCCAATTGGCGCACATCCCT CTGGATTAATCGGAGAAGATCCGCTAGGTATACCAAA CAATTTGTTGCCATATATGGCTCAAGTAGCTGTTGGTA GGCGCGAGAAGCTTTACATCTTCGGAGACGATTATGA TTCCAGAGATGGTACCCCGATCAGGGATTATATCCAC GTAGTTGATCTAGCAAAAGGTCATATTGCAGCCCTGC AATACCTAGAGGCCTACAATGAAAATGAAGGTTTGTG TCGTGAGTGGAACTTGGGTTCCGGTAAAGGTTCTACA GTTTTTGAAGTTTATCATGCATTCTGCAAAGCTTCTGG TATTGATCTTCCATACAAAGTTACGGGCAGAAGAGCA GGTGATGTTTTGAACTTGACGGCTAAACCAGATAGGG CCAAACGCGAACTGAAATGGCAGACCGAGTTGCAGGT TGAAGACTCCTGCAAGGATTTATGGAAATGGACTACT GAGAATCCTTTTGGTTACCAGTTAAGGGGTGTCGAGG CCAGATTTTCCGCTGAAGATATGCGTTATGACGCAAG ATTTGTGACTATTGGTGCCGGCACCAGATTTCAAGCCA CGTTTGCCAATTTGGGCGCCAGCATTGTTGACCTGAAA GTGAACGGACAATCAGTTGTTCTTGGCTATGAAAATG AGGAAGGGTATTTGAATCCTGATAGTGCTTATATAGG CGCCACGATCGGCAGGTATGCTAATCGTATTTCGAAG GGTAAGTTTAGTTTATGCAACAAAGACTATCAGTTAA CCGTTAATAACGGCGTTAATGCGAATCATAGTAGTAT CGGTTCTTTCCACAGAAAAAGATTTTTGGGACCCATCA TTCAAAATCCTTCAAAGGATGTTTTTACCGCCGAGTAC ATGCTGATAGATAATGAGAAGGACACCGAATTTCCAG GTGATCTATTGGTAACCATACAGTATACTGTGAACGTT GCCCAAAAAAGTTTGGAAATGGTATATAAAGGTAAAT TGACTGCTGGTGAAGCGACGCCAATAAATTTAACAAA TCATAGTTATTTCAATCTGAACAAGCCATATGGAGAC ACTATTGAGGGTACGGAGATTATGGTGCGTTCAAAAA AATCTGTTGATGTCGACAAAAACATGATTCCTACGGG TAATATCGTCGATAGAGAAATTGCTACCTTTAACTCTA CAAAGCCAACGGTCTTAGGCCCCAAAAATCCCCAGTT TGATTGTTGTTTTGTGGTGGATGAAAATGCTAAGCCAA

		GTCAAATCAATACTCTAAACAATGAATTGACGCTTATT GTCAAGGCTTTTCATCCCGATTCCAATATTACATTAGA AGTTTTAAGTACAGAGCCAACTTATCAATTTTATACCG GTGATTTCTTGTCTGCTGGTTACGAAGCAAGACAAGG TTTTGCAATTGAGCCTGGTAGATACATTGATGCTATCA ATCAAGAGAACTGGAAAGATTGTGTAACCTTGAAAAA CGGTGAAACTTACGGGTCCAAGATTGTCTACAGATTTT CCTGA
30	*Pichia pastoris* Sequence of the PpPMA1 promoter:	AAATGCGTACCTCTTCTACGAGATTCAAGCGAATGAG AATAATGTAATATGCAAGATCAGAAAGAATGAAAGG AGTTGAAAAAAAAAAACCGTTGCGTTTTGACCTTGAAT GGGGTGGAGGTTTCCATTCAAAGTAAAGCCTGTGTCT TGGTATTTTCGGCGGCACAAGAAATCGTAATTTTCATC TTCTAAACGATGAAGATCGCAGCCCAACCTGTATGTA GTTAACCGGTCGGAATTATAAGAAAGATTTTCGATCA ACAAACCCTAGCAAATAGAAAGCAGGGTTACAACTTT AAACCGAAGTCACAAACGATAAACCACTCAGCTCCCA CCCAAATTCATTCCCACTAGCAGAAAGGAATTATTTA ATCCCTCAGGAAACCTCGATGATTCTCCCGTTCTTCCA TGGGCGGGTATCGCAAAATGAGGAATTTTTCAAATTT CTCTATTGTCAAGACTGTTTATTATCTAAGAAATAGCC CAATCCGAAGCTCAGTTTTGAAAAAATCACTTCCGCG TTTCTTTTTTACAGCCCGATGAATATCCAAATTTGGAA TATGGATTACTCTATCGGGACTGCAGATAATATGACA ACAACGCAGATTACATTTTAGGTAAGGCATAAACACC AGCCAGAAATGAAACGCCCACTAGCCATGGTCGAATA GTCCAATGAATTCAGATAGCTATGGTCTAAAAGCTGA TGTTTTTTATTGGGTAATGGCGAAGAGTCCAGTACGAC TTCCAGCAGAGCTGAGATGGCCATTTTTGGGGGTATT AGTAACTTTTTGAGCTCTTTTCACTTCGATGAAGTGTC CCATTCGGGATATAATCGGATCGCGTCGTTTTCTCGAA AATACAGCTTAGCGTCGTCCGCTTGTTGTAAAAGCAG CACCACATTCCTAATCTCTTATATAAACAAAACAACCC AAATTATCAGTGCTGTTTTCCCACCAGATATAAGTTTC TTTTCTCTTCCGCTTTTTGATTTTTTATCTCTTTCCTTTA AAAACTTCTTTACCTTAAAGGGCGGCC
31	*Pichia pastoris* Sequence of the PpPMA1 terminator:	TAAGCTTCACGATTTGTGTTCCAGTTTATCCCCCCTTT ATATACCGTTAACCCTTTCCCTGTTGAGCTGACTGTTG TTGTATTACCGCAATTTTTCCAAGTTTGCCATGCTTTTC GTGTTATTTGACCGATGTCTTTTTTCCCAAATCAAACT ATATTTGTTACCATTTAAACCAAGTTATCTTTTGTATT AAGAGTCTAAGTTTGTTCCCAGGCTTCATGTGAGAGT GATAACCATCCAGACTATGATTCTTGTTTTTTATTGGG TTTGTTTGTGTGATACATCTGAGTTGTGATTCGTAAAG TATGTCAGTCTATCTAGATTTTTAATAGTTAATTGGTA ATCAATGACTTGTTTGTTTTAACTTTTAAATTGTGGGT CGTATCCACGCGTTTAGTATAGCTGTTCATGGCTGTTA GAGGAGGGCGATGTTTATATACAGAGGACAAGAATGA GGAGGCGGCGTGTATTTTTAAAATGGAGACGCGACTC CTGTACACCTTATCGGTTGG

32	human hGalT codon optimized (XB)	GGTAGAGATTTGTCTAGATTGCCACAGTTGGTTGGTGT TTCCACTCCATTGCAAGGAGGTTCTAACTCTGCTGCTG CTATTGGTCAATCTTCCGGTGAGTTGAGAACTGGTGG AGCTAGACCACCTCCACCATTGGGAGCTTCCTCTCAAC CAAGACCAGGTGGTGATTCTTCTCCAGTTGTTGACTCT GGTCCAGGTCCAGCTTCTAACTTGACTTCCGTTCCAGT TCCACACACTACTGCTTTGTCCTTGCCAGCTTGTCCAG AAGAATCCCCATTGTTGGTTGGTCCAATGTTGATCGAG TTCAACATGCCAGTTGACTTGGAGTTGGTTGCTAAGCA GAACCCAAACGTTAAGATGGGTGGTAGATACGCTCCA AGAGACTGTGTTTCCCCACACAAAGTTGCTATCATCAT CCCATTCAGAAACAGACAGGAGCACTTGAAGTACTGG TTGTACTACTTGCACCCAGTTTTGCAAAGACAGCAGTT GGACTACGGTATCTACGTTATCAACCAGGCTGGTGAC ACTATTTTCAACAGAGCTAAGTTGTTGAATGTTGGTTT CCAGGAGGCTTTGAAGGATTACGACTACACTTGTTTC GTTTTCTCCGACGTTGACTTGATTCCAATGAACGACCA CAACGCTTACAGATGTTTCTCCCAGCCAAGACACATTT CTGTTGCTATGGACAAGTTCGGTTTCTCCTTGCCATAC GTTCAATACTTCGGTGGTGTTTCCGCTTTGTCCAAGCA GCAGTTCTTGACTATCAACGGTTTCCCAAACAATTACT GGGGATGGGGTGGTGAAGATGACGACATCTTTAACAG ATTGGTTTTCAGAGGAATGTCCATCTCTAGACCAAAC GCTGTTGTTGGTAGATGTAGAATGATCAGACACTCCA GAGACAAGAAGAACGAGCCAAACCCACAAAGATTCG ACAGAATCGCTCACACTAAGGAAACTATGTTGTCCGA CGGATTGAACTCCTTGACTTACCAGGTTTTGGACGTTC AGAGATACCCATTGTACACTCAGATCACTGTTGACAT CGGTACTCCATCCTAG
33	*Saccharomyces cerevisiae* DNA encodes ScMnt1 (Kre2) (33)	ATGGCCCTCTTTCTCAGTAAGAGACTGTTGAGATTTAC CGTCATTGCAGGTGCGGTTATTGTTCTCCTCCTAACAT TGAATTCCAACAGTAGAACTCAGCAATATATTCCGAG TTCCATCTCCGCTGCATTTGATTTTACCTCAGGATCTA TATCCCCTGAACAACAAGTCATCGGGCGCGCC

34	*Drosophila melanogaster* DNA encodes DmUGT	ATGAATAGCATACACATGAACGCCAATACGCTGAAGT ACATCAGCCTGCTGACGCTGACCCTGCAGAATGCCAT CCTGGGCCTCAGCATGCGCTACGCCCGCACCCGGCCA GGCGACATCTTCCTCAGCTCCACGGCCGTACTCATGGC AGAGTTCGCCAAACTGATCACGTGCCTGTTCCTGGTCT TCAACGAGGAGGGCAAGGATGCCCAGAAGTTTGTACG CTCGCTGCACAAGACCATCATTGCGAATCCCATGGAC ACGCTGAAGGTGTGCGTCCCCTCGCTGGTCTATATCGT TCAAAACAATCTGCTGTACGTCTCTGCCTCCCATTTGG ATGCGGCCACCTACCAGGTGACGTACCAGCTGAAGAT TCTCACCACGGCCATGTTCGCGGTTGTCATTCTGCGCC GCAAGCTGCTGAACACGCAGTGGGGTGCGCTGCTGCT CCTGGTGATGGGCATCGTCCTGGTGCAGTTGGCCCAA ACGGAGGGTCCGACGAGTGGCTCAGCCGGTGGTGCCG CAGCTGCAGCCACGGCCGCCTCCTCTGGCGGTGCTCC CGAGCAGAACAGGATGCTCGGACTGTGGGCCGCACTG GGCGCCTGCTTCCTCTCCGGATTCGCGGGCATCTACTT TGAGAAGATCCTCAAGGGTGCCGAGATCTCCGTGTGG ATGCGGAATGTGCAGTTGAGTCTGCTCAGCATTCCCTT CGGCCTGCTCACCTGTTTCGTTAACGACGGCAGTAGG ATCTTCGACCAGGGATTCTTCAAGGGCTACGATCTGTT TGTCTGGTACCTGGTCCTGCTGCAGGCCGGCGGTGGA TTGATCGTTGCCGTGGTGGTCAAGTACGCGGATAACA TTCTCAAGGGCTTCGCCACCTCGCTGGCCATCATCATC TCGTGCGTGGCCTCCATATACATCTTCGACTTCAATCT CACGCTGCAGTTCAGCTTCGGAGCTGGCCTGGTCATC GCCTCCATATTTCTCTACGGCTACGATCCGGCCAGGTC GGCGCCGAAGCCAACTATGCATGGTCCTGGCGGCGAT GAGGAGAAGCTGCTGCCGCGCGTCTAG

35	*Pichia pastoris* Sequence of the PpOCH1 promoter:	TGGACACAGGAGACTCAGAAACAGACACAGAGCGTT CTGAGTCCTGGTGCTCCTGACGTAGGCCTAGAACAGG AATTATTGGCTTTATTTGTTTGTCCATTTCATAGGCTTG GGGTAATAGATAGATGACAGAGAAATAGAGAAGACC TAATATTTTTTGTTCATGGCAAATCGCGGGTTCGCGGT CGGGTCACACACGGAGAAGTAATGAGAAGAGCTGGT AATCTGGGGTAAAAGGGTTCAAAAGAAGGTCGCCTGG TAGGGATGCAATACAAGGTTGTCTTGGAGTTTACATTG ACCAGATGATTTGGCTTTTTCTCTGTTCAATTCACATTT TTCAGCGAGAATCGGATTGACGGAGAAATGGCGGGGT GTGGGGTGGATAGATGGCAGAAATGCTCGCAATCACC GCGAAAGAAAGACTTTATGGAATAGAACTACTGGGTG GTGTAAGGATTACATAGCTAGTCCAATGGAGTCCGTT GGAAAGGTAAGAAGAAGCTAAAACCGGCTAAGTAAC TAGGGAAGAATGATCAGACTTTGATTTGATGAGGTCT GAAAATACTCTGCTGCTTTTTCAGTTGCTTTTTCCCTGC AACCTATCATTTTCCTTTTCATAAGCCTGCCTTTTCTGT TTTCACTTATATGAGTTCCGCCGAGACTTCCCCAAATT CTCTCCTGGAACATTCTCTATCGCTCTCCTTCCAAGTT GCGCCCCCTGGCACTGCCTAGTAATATTACCACGCGA CTTATATTCAGTTCCACAATTTCCAGTGTTCGTAGCAA ATATCATCAGCC
36	*Pichia pastoris* Sequence of the PpALG12 terminator:	AATATATACCTCATTTGTTCAATTTGGTGTAAAGAGTG TGGCGGATAGACTTCTTGTAAATCAGGAAAGCTACAA TTCCAATTGCTGCAAAAAATACCAATGCCCATAAACC AGTATGAGCGGTGCCTTCGACGGATTGCTTACTTTCCG ACCCTTTGTCGTTTGATTCTTCTGCCTTTGGTGAGTCA GTTTGTTTCGACTTTATATCTGACTCATCAACTTCCTTT ACGGTTGCGTTTTTAATCATAATTTTAGCCGTTGGCTT ATTATCCCTTGAGTTGGTAGGAGTTTTGATGATGCTG
37	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpHIS1:	TAACTGGCCCTTTGACGTTTCTGACAATAGTTCTAGAG GAGTCGTCCAAAAACTCAACTCTGACTTGGGTGACAC CACCACGGGATCCGGTTCTTCCGAGGACCTTGATGAC CTTGGCTAATGTAACTGGAGTTTTAGTATCCATTTTAA GATGTGTGTTTCTGTAGGTTCTGGGTTGGAAAAAAATT TTAGACACCAGAAGAGAGGAGTGAACTGGTTTGCGTG GGTTTAGACTGTGTAAGGCACTACTCTGTCGAAGTTTT AGATAGGGGTTACCCGCTCCGATGCATGGGAAGCGAT TAGCCCGGCTGTTGCCCGTTTGGTTTTTGAAGGGTAAT TTTCAATATCTCTGTTTGAGTCATCAATTTCATATTCA AAGATTCAAAAACAAAATCTGGTCCAAGGAGCGCATT TAGGATTATGGAGTTGGCGAATCACTTGAACGATAGA CTATTATTTGC

38	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpHIS1:	GTGACATTCTTGTCTTTGAGATCAGTAATTGTAGAGCA TAGATAGAATAATATTCAAGACCAACGGCTTCTCTTC GGAAGCTCCAAGTAGCTTATAGTGATGAGTACCGGCA TATATTTATAGGCTTAAAATTTCGAGGGTTCACTATAT TCGTTTAGTGGGAAGAGTTCCTTTCACTCTTGTTATCT ATATTGTCAGCGTGGACTGTTTATAACTGTACCAACTT AGTTTCTTTCAACTCCAGGTTAAGAGACATAAATGTCC TTTGATGCTGACAATAATCAGTGGAATTCAAGGAAGG ACAATCCCGACCTCAATCTGTTCATTAATGAAGAGTTC GAATCGTCCTTAAATCAAGCGCTAGACTCAATTGTCA ATGAGAACCCTTTCTTTGACCAAGAAACTATAAATAG ATCGAATGACAAAGTTGGAAATGAGTCCATTAGCTTA CATGATATTGAGCAGGCAGACCAAAATAAACCGTCCT TTGAGAGCGATATTGATGGTTCGGCGCCGTTGATAAG AGACGACAAATTGCCAAAGAAACAAAGCTGGGGGCT GAGCAATTTTTTTTCAAGAAGAAATAGCATATGTTTAC CACTACATGAAAATGATTCAAGTGTTGTTAAGACCGA AAGATCTATTGCAGTGGGAACACCCCATCTTCAATAC TGCTTCAATGGAATCTCCAATGCCAAGTACAATGCATT TACCTTTTTCCCAGTCATCCTATACGAGCAATTCAAAT TTTTTTTCAATTTATACTTTACTTTAGTGGCTCTCTCTC AAGCGATACCGCAACTTCGCATTGGATATCTTTCTTCG TATGTCGTCCCACTTTTGTTTGTACTCATAGTGACCAT GTCAAAAGAGGCGATGGATGATATTCAACGCCGAAGA AGGGATAGAGAACAGAACAATGAACCATATGAGGTTC TGTCCAGCCCATCACCAGTTTTGTCCAAAAACTTAAAA TGTGGTCACTTGGTTCGATTGCATAAGGGAATGAGAG TGCCCGCAGATATGGTTCTTGTCCAGTCAAGCGAATCC ACCGGAGAGTCATTTATCAAGACAGATCAGCTGGATG GTGAGACTGATTGGAAGCTTCGGATTGTTTCTCCAGTT ACACAATCGTTACCAATGACTGAACTTCAAAATGTCG CCATCACTGCAAGCGCACCCTCAAAATCAATTCACTC CTTTCTTGGAAGATTGACCTACAATGGGCAATCATATG GTCTTACGATAGACAACACAATGTGGTGTAATACTGT ATTAGCTTCTGGTTCAGCAATTGGTTGTATAATTTACA CAGGTAAAGATACTCGACAATCGATGAACACAACTCA GCCCAAACTGAAAACGGGCTTGTTAGAACTGGAAATC AATAGTTTGTCCAAGATCTTATGTGTTTGTGTGTTTGC ATTATCTGTCATCTTAGTGCTATTCCAAGGAATAGCTG ATGATTGGTACGTCGATATCATGCGGTTTCTCATTCTA TTCTCCACTATTATCCCAGTGTCTCTGAGAGTTAACCT TGATCTTGGAAAGTCAGTCCATGCTCATCAAATAGAA ACTGATAGCTCAATACCTGAAACCGTTGTTAGAACTA GTACAATACCGGAAGACCTGGGAAGAATTGAATACCT ATTAAGTGACAAAACTGGAACTCTTACTCAAAATGAT ATGGAAATGAAAAAACTACACCTAGGAACAGTCTCTT ATGCTGGTGATACCATGGATATTATTTCTGATCATGTT AAAGGTCTTAATAACGCTAAAACATCGAGGAAAGATC TTGGTATGAGAATAAGAGATTTGGTTACAACTCTGGC CATCTG

39	*Drosophila melanogaster* DNA encodes *Drosophila melanogaster* ManII codon-optimized (KD)	AGAGACGATCCAATTAGACCTCCATTGAAGGTTGCTA GATCCCCAAGACCAGGTCAATGTCAAGATGTTGTTCA GGACGTCCCAAACGTTGATGTCCAGATGTTGGAGTTG TACGATAGAATGTCCTTCAAGGACATTGATGGTGGTG TTTGGAAGCAGGGTTGGAACATTAAGTACGATCCATT GAAGTACAACGCTCATCACAAGTTGAAGGTCTTCGTT GTCCCACACTCCCACAACGATCCTGGTTGGATTCAGA CCTTCGAGGAATACTACCAGCACGACACCAAGCACAT CTTGTCCAACGCTTTGAGACATTTGCACGACAACCCA GAGATGAAGTTCATCTGGGCTGAAATCTCCTACTTCGC TAGATTCTACCACGATTTGGGTGAGAACAAGAAGTTG CAGATGAAGTCCATCGTCAAGAACGGTCAGTTGGAAT TCGTCACTGGTGGATGGGTCATGCCAGACGAGGCTAA CTCCCACTGGAGAAACGTTTTGTTGCAGTTGACCGAA GGTCAAACTTGGTTGAAGCAATTCATGAACGTCACTC CAACTGCTTCCTGGGCTATCGATCCATTCGGACACTCT CCAACTATGCCATACATTTTGCAGAAGTCTGGTTTCAA GAATATGTTGATCCAGAGAACCCACTACTCCGTTAAG AAGGAGTTGGCTCAACAGAGACAGTTGGAGTTCTTGT GGAGACAGATCTGGGACAACAAAGGTGACACTGCTTT GTTCACCCACATGATGCCATTCTACTCTTACGACATTC CTCATACCTGTGGTCCAGATCCAAAGGTTTGTTGTCAG TTCGATTTCAAAAGAATGGGTTCCTTCGGTTTGTCTTG TCCATGGAAGGTTCCACCTAGAACTATCTCTGATCAA AATGTTGCTGCTAGATCCGATTTGTTGGTTGATCAGTG GAAGAAGAAGGCTGAGTTGTACAGAACCAACGTCTTG TTGATTCCATTGGGTGACGACTTCAGATTCAAGCAGA ACACCGAGTGGGATGTTCAGAGAGTCAACTACGAAAG ATTGTTCGAACACATCAACTCTCAGGCTCACTTCAATG TCCAGGCTCAGTTCGGTACTTTGCAGGAATACTTCGAT GCTGTTCACCAGGCTGAAAGAGCTGGACAAGCTGAGT TCCCAACCTTGTCTGGTGACTTCTTCACTTACGCTGAT AGATCTGATAACTACTGGTCTGGTTACTACACTTCCAG ACCATACCATAAGAGAATGGACAGAGTCTTGATGCAC TACGTTAGAGCTGCTGAAATGTTGTCCGCTTGGCACTC CTGGGACGGTATGGCTAGAATCGAGGAAAGATTGGAG CAGGCTAGAAGAGAGTTGTCCTTGTTCCAGCACCACG ACGGTATTACTGGTACTGCTAAAACTCACGTTGTCGTC GACTACGAGCAAAGAATGCAGGAAGCTTTGAAAGCTT GTCAAATGGTCATGCAACAGTCTGTCTACAGATTGTTG ACTAAGCCATCCATCTACTCTCCAGACTTCTCCTTCTC CTACTTCACTTTGGACGACTCCAGATGGCCAGGTTCTG GTGTTGAGGACTCTAGAACTACCATCATCTTGGGTGA GGATATCTTGCCATCCAAGCATGTTGTCATGCACAAC ACCTTGCCACACTGGAGAGAGCAGTTGGTTGACTTCT ACGTCTCCTCTCCATTCGTTTCTGTTACCGACTTGGCT

		AACAATCCAGTTGAGGCTCAGGTTTCTCCAGTTTGGTC TTGGCACCACGACACTTTGACTAAGACTATCCACCCA CAAGGTTCCACCACCAAGTACAGAATCATCTTCAAGG CTAGAGTTCCACCAATGGGTTTGGCTACCTACGTTTTG ACCATCTCCGATTCCAAGCCAGAGCACACCTCCTACG CTTCCAATTTGTTGCTTAGAAAGAACCCAACTTCCTTG CCATTGGGTCAATACCCAGAGGATGTCAAGTTCGGTG ATCCAAGAGAGATCTCCTTGAGAGTTGGTAACGGTCC AACCTTGGCTTTCTCTGAGCAGGGTTTGTTGAAGTCCA TTCAGTTGACTCAGGATTCTCCACATGTTCCAGTTCAC TTCAAGTTCTTGAAGTACGGTGTTAGATCTCATGGTGA TAGATCTGGTGCTTACTTGTTCTTGCCAAATGGTCCAG CTTCTCCAGTCGAGTTGGGTCAGCCAGTTGTCTTGGTC ACTAAGGGTAAATTGGAGTCTTCCGTTTCTGTTGGTTT GCCATCTGTCGTTCACCAGACCATCATGAGAGGTGGT GCTCCAGAGATTAGAAATTTGGTCGATATTGGTTCTTT GGACAACACTGAGATCGTCATGAGATTGGAGACTCAT ATCGACTCTGGTGATATCTTCTACACTGATTTGAATGG ATTGCAATTCATCAAGAGGAGAAGATTGGACAAGTTG CCATTGCAGGCTAACTACTACCCAATTCCATCTGGTAT GTTCATTGAGGATGCTAATACCAGATTGACTTTGTTGA CCGGTCAACCATTGGGTGGATCTTCTTTGGCTTCTGGT GAGTTGGAGATTATGCAAGATAGAAGATTGGCTTCTG ATGATGAAAGAGGTTTGGGTCAGGGTGTTTTGGACAA CAAGCCAGTTTTGCATATTTACAGATTGGTCTTGGAGA AGGTTAACAACTGTGTCAGACCATCTAAGTTGCATCC AGCTGGTTACTTGACTTCTGCTGCTCACAAAGCTTCTC AGTCTTTGTTGGATCCATTGGACAAGTTCATCTTCGCT GAAAATGAGTGGATCGGTGCTCAGGGTCAATTCGGTG GTGATCATCCATCTGCTAGAGAGGATTTGGATGTCTCT GTCATGAGAAGATTGACCAAGTCTTCTGCTAAAACCC AGAGAGTTGGTTACGTTTTGCACAGAACCAATTTGAT GCAATGTGGTACTCCAGAGGAGCATACTCAGAAGTTG GATGTCTGTCACTTGTTGCCAAATGTTGCTAGATGTGA GAGAACTACCTTGACTTTCTTGCAGAATTTGGAGCACT TGGATGGTATGGTTGCTCCAGAAGTTTGTCCAATGGA AACCGCTGCTTACGTCTCTTCTCACTCTTCTTGA
40	*Saccharomyces cerevisiae* DNA encodes ScMnn2 leader (53)	ATGCTGCTTACCAAAAGGTTTTCAAAGCTGTTCAAGCT GACGTTCATAGTTTTGATATTGTGCGGGCTGTTCGTCA TTACAAACAAATACATGGATGAGAACACGTCG

41	*Pichia pastoris* Sequence of the PpHIS1 auxotrophic marker:	CAAGTTGCGTCCGGTATACGTAACGTCTCACGATGAT CAAAGATAATACTTAATCTTCATGGTCTACTGAATAAC TCATTTAAACAATTGACTAATTGTACATTATATTGAAC TTATGCATCCTATTAACGTAATCTTCTGGCTTCTCTCTC AGACTCCATCAGACACAGAATATCGTTCTCTCTAACTG GTCCTTTGACGTTTCTGACAATAGTTCTAGAGGAGTCG TCCAAAAACTCAACTCTGACTTGGGTGACACCACCAC GGGATCCGGTTCTTCCGAGGACCTTGATGACCTTGGCT AATGTAACTGGAGTTTTAGTATCCATTTTAAGATGTGT GTTTCTGTAGGTTCTGGGTTGGAAAAAAATTTTAGACA CCAGAAGAGAGGAGTGAACTGGTTTGCGTGGGTTTAG ACTGTGTAAGGCACTACTCTGTCGAAGTTTTAGATAG GGGTTACCCGCTCCGATGCATGGGAAGCGATTAGCCC GGCTGTTGCCCGTTTGGTTTTTGAAGGGTAATTTTCAA TATCTCTGTTTGAGTCATCAATTTCATATTCAAAGATT CAAAAACAAAATCTGGTCCAAGGAGCGCATTTAGGAT TATGGAGTTGGCGAATCACTTGAACGATAGACTATTA TTTGCTGTTCCTAAAGAGGGCAGATTGTATGAGAAAT GCGTTGAATTACTTAGGGGATCAGATATTCAGTTTCGA AGATCCAGTAGATTGGATATAGCTTTGTGCACTAACCT GCCCCTGGCATTGGTTTTCCTTCCAGCTGCTGACATTC CCACGTTTGTAGGAGAGGGTAAATGTGATTTGGGTAT AACTGGTATTGACCAGGTTCAGGAAAGTGACGTAGAT GTCATACCTTTATTAGACTTGAATTTCGGTAAGTGCAA GTTGCAGATTCAAGTTCCCGAGAATGGTGACTTGAAA GAACCTAAACAGCTAATTGGTAAAGAAATTGTTTCCT CCTTTACTAGCTTAACCACCAGGTACTTTGAACAACTG GAAGGAGTTAAGCCTGGTGAGCCACTAAAGACAAAA ATCAAATATGTTGGAGGGTCTGTTGAGGCCTCTTGTGC CCTAGGAGTTGCCGATGCTATTGTGGATCTTGTTGAGA GTGGAGAAACCATGAAAGCGGCAGGGCTGATCGATAT TGAAACTGTTCTTTCTACTTCCGCTTACCTGATCTCTTC GAAGCATCCTCAACACCCAGAACTGATGGATACTATC AAGGAGAGAATTGAAGGTGTACTGACTGCTCAGAAGT ATGTCTTGTGTAATTACAACGCACCTAGAGGTAACCTT CCTCAGCTGCTAAAACTGACTCCAGGCAAGAGAGCTG CTACCGTTTCTCCATTAGATGAAGAAGATTGGGTGGG AGTGTCCTCGATGGTAGAGAAGAAAGATGTTGGAAGA ATCATGGACGAATTAAAGAAACAAGGTGCCAGTGACA TTCTTGTCTTTGAGATCAGTAATTGTAGAGCATAGATA GAATAATATTCAAGACCAACGGCTTCTCTTCGGAAGC TCCAAGTAGCTTATAGTGATGAGTACCGGCATATATTT ATAGGCTTAAAATTTCGAGGGTTCACTATATTCGTTTA GTGGGAAGAGTTCCTTTCACTCTTGTTATCTATATTGT CAGCGTGGACTGTTTATAACTGTACCAACTTAGTTTCT TTCAACTCCAGGTTAAGAGACATAAATGTCCTTTGATG C

42	DNA encodes Rat GnT II (TC) Codon-optimized	TCCTTGGTTTACCAATTGAACTTCGACCAGATGTTGAG AAACGTTGACAAGGACGGTACTTGGTCTCCTGGTGAG TTGGTTTTGGTTGTTCAGGTTCACAACAGACCAGAGTA CTTGAGATTGTTGATCGACTCCTTGAGAAAGGCTCAA GGTATCAGAGAGGTTTTGGTTATCTTCTCCCACGATTT CTGGTCTGCTGAGATCAACTCCTTGATCTCCTCCGTTG ACTTCTGTCCAGTTTTGCAGGTTTTCTTCCCATTCTCCA TCCAATTGTACCCATCTGAGTTCCCAGGTTCTGATCCA AGAGACTGTCCAAGAGACTTGAAGAAGAACGCTGCTT TGAAGTTGGGTTGTATCAACGCTGAATACCCAGATTCT TTCGGTCACTACAGAGAGGCTAAGTTCTCCCAAACTA AGCATCATTGGTGGTGGAAGTTGCACTTTGTTTGGGAG AGAGTTAAGGTTTTGCAGGACTACACTGGATTGATCTT GTTCTTGGAGGAGGATCATTACTTGGCTCCAGACTTCT ACCACGTTTTCAAGAAGATGTGGAAGTTGAAGCAACA AGAGTGTCCAGGTTGTGACGTTTTGTCCTTGGGAACTT ACACTACTATCAGATCCTTCTACGGTATCGCTGACAAG GTTGACGTTAAGACTTGGAAGTCCACTGAACACAACA TGGGATTGGCTTTGACTAGAGATGCTTACCAGAAGTT GATCGAGTGTACTGACACTTTCTGTACTTACGACGACT ACAACTGGGACTGGACTTTGCAGTACTTGACTTTGGCT TGTTTGCCAAAAGTTTGGAAGGTTTTGGTTCCACAGGC TCCAAGAATTTTCCACGCTGGTGACTGTGGAATGCAC CACAAGAAAACTTGTAGACCATCCACTCAGTCCGCTC AAATTGAGTCCTTGTTGAACAACAACAAGCAGTACTT GTTCCCAGAGACTTTGGTTATCGGAGAGAAGTTTCCA ATGGCTGCTATTTCCCCACCAAGAAAGAATGGTGGAT GGGGTGATATTAGAGACCACGAGTTGTGTAAATCCTA CAGAAGATTGCAGTAG
43	*Saccharomyces cerevisiae* DNA encodes ScMnn2 leader (54) The last 9 nucleotides are the linker containing the AscI restriction site)	ATGCTGCTTACCAAAAGGTTTTCAAAGCTGTTCAAGCT GACGTTCATAGTTTTGATATTGTGCGGGCTGTTCGTCA TTACAAACAAATACATGGATGAGAACACGTCGGTCAA GGAGTACAAGGAGTACTTAGACAGATATGTCCAGAGT TACTCCAATAAGTATTCATCTTCCTCAGACGCCGCCAG CGCTGACGATTCAACCCCATTGAGGGACAATGATGAG GCAGGCAATGAAAAGTTGAAAAGCTTCTACAACAACG TTTTCAACTTTCTAATGGTTGATTCGCCCGGGCGCGCC

44	*Pichia pastoris* Sequence of the 5'-Region used for knock out of PpARG1:	GATCTGGCCTTCCCTGAATTTTTACGTCCAGCTATACG ATCCGTTGTGACTGTATTTCCTGAAATGAAGTTTCAAC CTAAAGTTTTGGTTGTACTTGCTCCACCTACCACGGAA ACTAATATCGAAACCAATGAAAAAGTAGAACTGGAAT CGTCAATCGAAATTCGCAACCAAGTGGAACCCAAAGA CTTGAATCTTTCTAAAGTCTATTCTAGTGACACTAATG GCAACAGAAGATTTGAGCTGACTTTTCAAATGAATCT CAATAATGCAATATCAACATCAGACAATCAATGGGCT TTGTCTAGTGACACAGGATCAATTATAGTAGTGTCTTC TGCAGGAAGAATAACTTCCCCGATCCTAGAAGTCGGG GCATCCGTCTGTGTCTTAAGATCGTACAACGAACACCT TTTGGCAATAACTTGTGAAGGAACATGCTTTTCATGGA ATTTAAAGAAGCAAGAATGTGTTCTAAACAGCATTTC ATTAGCACCTATAGTCAATTCACACATGCTAGTTAAG AAAGTTGGAGATGCAAGGAACTATTCTATTGTATCTG CCGAAGGAGACAACAATCCGTTACCCCAGATTCTAGA CTGCGAACTTTCCAAAAATGGCGCTCCAATTGTGGCTC TTAGCACGAAAGACATCTACTCTTATTCAAAGAAAAT GAAATGCTGGATCCATTTGATTGATTCGAAATACTTTG AATTGTTGGGTGCTGACAATGCACTGTTTGAGTGTGTG GAAGCGCTAGAAGGTCCAATTGGAATGCTAATTCATA GATTGGTAGATGAGTTCTTCCATGAAAACACTGCCGG TAAAAAACTCAAACTTTACAACAAGCGAGTACTGGAG GACCTTTCAAATTCACTTGAAGAACTAGGTGAAAATG CGTCTCAATTAAGAGAGAAACTTGACAAACTCTATGG TGATGAGGTTGAGGCTTCTTGACCTCTTCTCTCTATCT GCGTTTCTTTTTTTTTTTTTTTTTTTTTTTTTTCAGTTG AGCCAGACCGCGCTAAACGCATACCAATTGCCAAATC AGGCAATTGTGAGACAGTGGTAAAAAAGATGCCTGCA AAGTTAGATTCACACAGTAAGAGAGATCCTACTCATA AATGAGGCGCTTATTTAGTAGCTAGTGATAGCCACTG CGGTTCTGCTTTATGCTATTTGTTGTATGCCTTACTATC TTTGTTTGGCTCCTTTTTCTTGACGTTTTCCGTTGGAGG GACTCCCTATTCTGAGTCATGAGCCGCACAGATTATCG CCCAAAATTGACAAAATCTTCTGGCGAAAAAAGTATA AAAGGAGAAAAAAGCTCACCCTTTTCCAGCGTAGAAA GTATATATCAGTCATTGAAGAC

45	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpARG1:	GGGACTTTAACTCAAGTAAAAGGATAGTTGTACAATT ATATATACGAAGAATAAATCATTACAAAAAGTATTCG TTTCTTTGATTCTTAACAGGATTCATTTTCTGGGTGTCA TCAGGTACAGCGCTGAATATCTTGAAGTTAACATCGA GCTCATCATCGACGTTCATCACACTAGCCACGTTTCCG CAACGGTAGCAATAATTAGGAGCGGACCACACAGTGA CGACATCTTTCTCTTTGAAATGGTATCTGAAGCCTTCC ATGACCAATTGATGGGCTCTAGCGATGAGTTGCAAGT TATTAATGTGGTTGAACTCACGTGCTACTCGAGCACCG AATAACCAGCCAGCTCCACGAGGAGAAACAGCCCAA CTGTCGACTTCATCTGGGTCAGACCAAACCAAGTCAC AAAATCCTCCTTCATGAGGGACCTCTTGCGCTCGGCTG AGAACTCTGATTTGATCTAACATGCGAATATCGGGAG AGAGACCACCATGGATACATAATATTTTACCATCAAT GATGGCACTAAGGGTTAAAAAGTCGAACACCTGGCAA CAGTACTTCCAGACAGTGGTGGAACCATATTTATTGA GACATTCCTCATAAAATCCATAAACCTGAGTGATCTGT CTGGATTCATGATTTCCCCTTACCAATGTGATATGTTG AGGAAACTTAATTTTTAAAATCATGAGTAACGTGAAC GTCTCCAACGAGAAATAGCCTCTATCCACATAGTCTCC TAGGAAGATATAGTTCTGTTTTATTCCATTAGAGGAGG ATCCGGGAAACCCACCACTAATCTTGAAAAGTTCCAG TAGATCGTGAAATTGGCCGTGAATATCTCCGCATACT GTCACTGGACTCTGCACTGGCTGTATATTGGATTCCTC CATCAGCAAATCCTTCACCCGTTCGCAAAGATGCTTCA TATCATTTTCACTTAAAGCCTTGCAGCTTTTGACTTCTT CAAACCACTGATCTGGTCCTCTTTCTGGCATGATTAAG GTCTATAATATTTCTGAGCTGAGATGTAAAAAAAAAT AATAAAAATGGGGAGTGAAAAAGTGTGTAGCTTTTAG GAGTTTGGGATTGATACCCCAAAATGATCTTTATGAG AATTAAAAGGTAGATACGCTTTTAATAAGAACACCTA TCTATAGTACTTTGTGGTCTTGAGTAATTGAGATGTTC AGCTTCTGAGGTTTGCCGTTATTCTGGGATAGTAGTGC GCGACCAAACAACCCGCCAGGCAAAGTGTGTTGTGCT CGAAGACGATTGCCAGAAGAGTAAGTCCGTCCTGCCT CAGATGTTACACACTTTCTTCCCTAGACAGTCGATGCA TCATCGGATTTAAACCTGAAACTTTGATGCCATGATAC GCCTAGTCACGTCGACTGAGATTTTAGATAAGCCCCG ATCCCTTTAGTACATTCCTGTTATCCATGGATGGAATG GCCTGATA

46	*Pichia pastoris* Sequence of the 5'-Region used for knock out of BMT4	AAGCTTGTTCACCGTTGGGACTTTTCCGTGGACAATGT TGACTACTCCAGGAGGGATTCCAGCTTTCTCTACTAGC TCAGCAATAATCAATGCAGCCCCAGGCGCCCGTTCTG ATGGCTTGATGACCGTTGTATTGCCTGTCACTATAGCC AGGGGTAGGGTCCATAAAGGAATCATAGCAGGGAAA TTAAAAGGGCATATTGATGCAATCACTCCCAATGGCT CTCTTGCCATTGAAGTCTCCATATCAGCACTAACTTCC AAGAAGGACCCCTTCAAGTCTGACGTGATAGAGCACG CTTGCTCTGCCACCTGTAGTCCTCTCAAAACGTCACCT TGTGCATCAGCAAAGACTTTACCTTGCTCCAATACTAT GACGGAGGCAATTCTGTCAAAATTCTCTCTCAGCAATT CAACCAACTTGAAAGCAAATTGCTGTCTCTTGATGAT GGAGACTTTTTTCCAAGATTGAAATGCAATGTGGGAC GACTCAATTGCTTCTTCCAGCTCCTCTTCGGTTGATTG AGGAACTTTTGAAACCACAAAATTGGTCGTTGGGTCA TGTACATCAAACCATTCTGTAGATTTAGATTCGACGAA AGCGTTGTTGATGAAGGAAAAGGTTGGATACGGTTTG TCGGTCTCTTTGGTATGGCCGGTGGGGTATGCAATTGC AGTAGAAGATAATTGGACAGCCATTGTTGAAGGTAGA GAAAAGGTCAGGGAACTTGGGGGTTATTTATACCATT TTACCCCACAAATAACAACTGAAAAGTACCCATTCCA TAGTGAGAGGTAACCGACGGAAAAAGACGGGCCCAT GTTCTGGGACCAATAGAACTGTGTAATCCATTGGGAC TAATCAACAGACGATTGGCAATATAATGAAATAGTTC GTTGAAAAGCCACGTCAGCTGTCTTTTCATTAACTTTG GTCGGACACAACATTTTCTACTGTTGTATCTGTCCTAC TTTGCTTATCATCTGCCACAGGGCAAGTGGATTTCCTT CTCGCGCGGCTGGGTGAAAACGGTTAACGTGAA
47	*Pichia pastoris* Sequence of the 3'-Region used for knock out of BMT4	GCCTTGGGGGACTTCAAGTCTTTGCTAGAAACTAGAT GAGGTCAGGCCCTCTTATGGTTGTGTCCCAATTGGGCA ATTTCACTCACCTAAAAAGCATGACAATTATTTAGCG AAATAGGTAGTATATTTTCCCTCATCTCCCAAGCAGTT TCGTTTTTGCATCCATATCTCTCAAATGAGCAGCTACG ACTCATTAGAACCAGAGTCAAGTAGGGGTGAGCTCAG TCATCAGCCTTCGTTTCTAAAACGATTGAGTTCTTTTG TTGCTACAGGAAGCGCCCTAGGGAACTTTCGCACTTT GGAAATAGATTTTGATGACCAAGAGCGGGAGTTGATA TTAGAGAGGCTGTCCAAAGTACATGGGATCAGGCCGG CCAAATTGATTGGTGTGACTAAACCATTGTGTACTTGG ACACTCTATTACAAAAGCGAAGATGATTTGAAGTATT ACAAGTCCCGAAGTGTTAGAGGATTCTATCGAGCCCA GAATGAAATCATCAACCGTTATCAGCAGATTGATAAA CTCTTGGAAAGCGGTATCCCATTTTCATTATTGAAGAA CTACGATAATGAAGATGTGAGAGACGGCGACCCTCTG AACGTAGACGAAGAAACAAATCTACTTTTGGGGTACA ATAGAGAAAGTGAATCAAGGGAGGTATTTGTGGCCAT AATACTCAACTCTATCATTAATG

48	*Pichia pastoris* Sequence of the 5'-Region used for knock out of BMT1	CATATGGTGAGAGCCGTTCTGCACAACTAGATGTTTTC GAGCTTCGCATTGTTTCCTGCAGCTCGACTATTGAATT AAGATTTCCGGATATCTCCAATCTCACAAAAACTTATG TTGACCACGTGCTTTCCTGAGGCGAGGTGTTTTATATG CAAGCTGCCAAAAATGGAAAACGAATGGCCATTTTTC GCCCAGGCAAATTATTCGATTACTGCTGTCATAAAGA CAGTGTTGCAAGGCTCACATTTTTTTTTAGGATCCGAG ATAAAGTGAATACAGGACAGCTTATCTCTATATCTTGT ACCATTCGTGAATCTTAAGAGTTCGGTTAGGGGGACT CTAGTTGAGGGTTGGCACTCACGTATGGCTGGGCGCA GAAATAAAATTCAGGCGCAGCAGCACTTATCGATG
49	*Pichia pastoris* Sequence of the 3'-Region used for knock out of BMT1	GAATTCACAGTTATAAATAAAAACAAAAACTCAAAAA GTTTGGGCTCCACAAAATAACTTAATTTAAATTTTTGT CTAATAAATGAATGTAATTCCAAGATTATGTGATGCA AGCACAGTATGCTTCAGCCCTATGCAGCTACTAATGTC AATCTCGCCTGCGAGCGGGCCTAGATTTTCACTACAA ATTTCAAAACTACGCGGATTTATTGTCTCAGAGAGCA ATTTGGCATTTCTGAGCGTAGCAGGAGGCTTCATAAG ATTGTATAGGACCGTACCAACAAATTGCCGAGGCACA ACACGGTATGCTGTGCACTTATGTGGCTACTTCCCTAC AACGGAATGAAACCTTCCTCTTTCCGCTTAAACGAGA AAGTGTGTCGCAATTGAATGCAGGTGCCTGTGCGCCT TGGTGTATTGTTTTTGAGGGCCCAATTTATCAGGCGCC TTTTTTCTTGGTTGTTTTCCCTTAGCCTCAAGCAAGGTT GGTCTATTTCATCTCCGCTTCTATACCGTGCCTGATAC TGTTGGATGAGAACACGACTCAACTTCCTGCTGCTCTG TATTGCCAGTGTTTTGTCTGTGATTTGGATCGGAGTCC TCCTTACTTGGAATGATAATAATCTTGGCGGAATCTCC CTAAACGGAGGCAAGGATTCTGCCTATGATGATCTGC TATCATTGGGAAGCTT
50	*Pichia pastoris* Sequence of the 5'-Region used for knock out of BMT3	GATATCTCCCTGGGGACAATATGTGTTGCAACTGTTCG TTGTTGGTGCCCCAGTCCCCCAACCGGTACTAATCGGT CTATGTTCCCGTAACTCATATTCGGTTAGAACTAGAAC AATAAGTGCATCATTGTTCAACATTGTGGTTCAATTGT CGAACATTGCTGGTGCTTATATCTACAGGGAAGACGA TAAGCCTTTGTACAAGAGAGGTAACAGACAGTTAATT GGTATTTCTTTGGGAGTCGTTGCCCTCTACGTTGTCTC CAAGACATACTACATTCTGAGAAACAGATGGAAGACT CAAAAATGGGAGAAGCTTAGTGAAGAAGAGAAAGTT GCCTACTTGGACAGAGCTGAGAAGGAGAACCTGGGTT CTAAGAGGCTGGACTTTTTGTTCGAGAGTTAAACTGC ATAATTTTTTCTAAGTAAATTTCATAGTTATGAAATTT CTGCAGCTTAGTGTTTACTGCATCGTTTACTGCATCAC CCTGTAAATAATGTGAGCTTTTTTCCTTCCATTGCTTG GTATCTTCCTTGCTGCTGTTT

51	*Pichia pastoris* Sequence of the 3'-Region used for knock out of BMT3	ACAAAACAGTCATGTACAGAACTAACGCCTTTAAGAT GCAGACCACTGAAAAGAATTGGGTCCCATTTTTCTTG AAAGACGACCAGGAATCTGTCCATTTTGTTTACTCGTT CAATCCTCTGAGAGTACTCAACTGCAGTCTTGATAAC GGTGCATGTGATGTTCTATTTGAGTTACCACATGATTT TGGCATGTCTTCCGAGCTACGTGGTGCCACTCCTATGC TCAATCTTCCTCAGGCAATCCCGATGGCAGACGACAA AGAAATTTGGGTTTCATTCCCAAGAACGAGAATATCA GATTGCGGGTGTTCTGAAACAATGTACAGGCCAATGT TAATGCTTTTTGTTAGAGAAGGAACAAACTTTTTTGCT GAGC

52	*Trichoderma reesei* DNA encodes Tr ManI catalytic domain	CGCGCCGGATCTCCCAACCCTACGAGGGCGGCAGCAG TCAAGGCCGCATTCCAGACGTCGTGGAACGCTTACCA CCATTTTGCCTTTCCCCATGACGACCTCCACCCGGTCA GCAACAGCTTTGATGATGAGAGAAACGGCTGGGGCTC GTCGGCAATCGATGGCTTGGACACGGCTATCCTCATG GGGGATGCCGACATTGTGAACACGATCCTTCAGTATG TACCGCAGATCAACTTCACCACGACTGCGGTTGCCAA CCAAGGCATCTCCGTGTTCGAGACCAACATTCGGTAC CTCGGTGGCCTGCTTTCTGCCTATGACCTGTTGCGAGG TCCTTTCAGCTCCTTGGCGACAAACCAGACCCTGGTAA ACAGCCTTCTGAGGCAGGCTCAAACACTGGCCAACGG CCTCAAGGTTGCGTTCACCACTCCCAGCGGTGTCCCGG ACCCTACCGTCTTCTTCAACCCTACTGTCCGGAGAAGT GGTGCATCTAGCAACAACGTCGCTGAAATTGGAAGCC TGGTGCTCGAGTGGACACGGTTGAGCGACCTGACGGG AAACCCGCAGTATGCCCAGCTTGCGCAGAAGGGCGAG TCGTATCTCCTGAATCCAAAGGGAAGCCCGGAGGCAT GGCCTGGCCTGATTGGAACGTTTGTCAGCACGAGCAA CGGTACCTTTCAGGATAGCAGCGGCAGCTGGTCCGGC CTCATGGACAGCTTCTACGAGTACCTGATCAAGATGT ACCTGTACGACCCGGTTGCGTTTGCACACTACAAGGA TCGCTGGGTCCTTGCTGCCGACTCGACCATTGCGCATC TCGCCTCTCACCCGTCGACGCGCAAGGACTTGACCTTT TTGTCTTCGTACAACGGACAGTCTACGTCGCCAAACTC AGGACATTTGGCCAGTTTTGCCGGTGGCAACTTCATCT TGGGAGGCATTCTCCTGAACGAGCAAAAGTACATTGA CTTTGGAATCAAGCTTGCCAGCTCGTACTTTGCCACGT ACAACCAGACGGCTTCTGGAATCGGCCCCGAAGGCTT CGCGTGGGTGGACAGCGTGACGGGCGCCGGCGGCTCG CCGCCCTCGTCCCAGTCCGGGTTCTACTCGTCGGCAGG ATTCTGGGTGACGGCACCGTATTACATCCTGCGGCCG GAGACGCTGGAGAGCTTGTACTACGCATACCGCGTCA CGGGCGACTCCAAGTGGCAGGACCTGGCGTGGGAAGC GTTCAGTGCCATTGAGGACGCATGCCGCGCCGGCAGC GCGTACTCGTCCATCAACGACGTGACGCAGGCCAACG GCGGGGGTGCCTCTGACGATATGGAGAGCTTCTGGTT TGCCGAGGCGCTCAAGTATGCGTACCTGATCTTTGCG GAGGAGTCGGATGTGCAGGTGCAGGCCAACGGCGGG AACAAATTTGTCTTTAACACGGAGGCGCACCCCTTTA GCATCCGTTCATCATCACGACGGGGCGGCCACCTTGC TTAA
53	*Saccharomyces cerevisiae* mating factor pre-signal peptide (DNA)	ATGAGATTCCCATCCATCTTCACTGCTGTTTTGTTCGC TGCTTCTTCTGCTTTGGCT

54	*Saccharomyces cerevisiae* mating factor pre-signal peptide (protein)	MRFPSIFTAVLFAASSALA
55	*Pichia pastoris* Pp AOX1 promoter	AACATCCAAAGACGAAAGGTTGAATGAAACCTTTTTG CCATCCGACATCCACAGGTCCATTCTCACACATAAGT GCCAAACGCAACAGGAGGGGATACACTAGCAGCAGA CCGTTGCAAACGCAGGACCTCCACTCCTCTTCTCCTCA ACACCCACTTTTGCCATCGAAAAACCAGCCCAGTTATT GGGCTTGATTGGAGCTCGCTCATTCCAATTCCTTCTAT TAGGCTACTAACACCATGACTTTATTAGCCTGTCTATC CTGGCCCCCCTGGCGAGGTTCATGTTTGTTTATTTCCG AATGCAACAAGCTCCGCATTACACCCGAACATCACTC CAGATGAGGGCTTTCTGAGTGTGGGGTCAAATAGTTT CATGTTCCCCAAATGGCCCAAAACTGACAGTTTAAAC GCTGTCTTGGAACCTAATATGACAAAAGCGTGATCTC ATCCAAGATGAACTAAGTTTGGTTCGTTGAAATGCTA ACGGCCAGTTGGTCAAAAAGAAACTTCCAAAAGTCGG CATACCGTTTGTCTTGTTTGGTATTGATTGACGAATGC TCAAAAATAATCTCATTAATGCTTAGCGCAGTCTCTCT ATCGCTTCTGAACCCCGGTGCACCTGTGCCGAAACGC AAATGGGGAAACACCCGCTTTTTGGATGATTATGCAT TGTCTCCACATTGTATGCTTCCAAGATTCTGGTGGGAA TACTGCTGATAGCCTAACGTTCATGATCAAAATTTAAC TGTTCTAACCCCTACTTGACAGCAATATATAAACAGA AGGAAGCTGCCCTGTCTTAAACCTTTTTTTTTATCATC ATTATTAGCTTACTTTCATAATTGCGACTGGTTCCAAT TGACAAGCTTTTGATTTTAACGACTTTTAACGACAACT TGAGAAGATCAAAAAACAACTAATTATTCGAAACG

56	*Pichia pastoris* 5'ARG1 and ORF	TACCAATTGCCAAATCAGGCAATTGTGAGACAGTGGT AAAAAAGATGCCTGCAAAGTTAGATTCACACAGTAAG AGAGATCCTACTCATAAATGAGGCGCTTATTTAGTAG CTAGTGATAGCCACTGCGGTTCTGCTTTATGCTATTTG TTGTATGCCTTACTATCTTTGTTTGGCTCCTTTTTCTTG ACGTTTTCCGTTGGAGGGACTCCCTATTCTGAGTCATG AGCCGCACAGATTATCGCCCAAAATTGACAAAATCTT CTGGCGAAAAAAGTATAAAAGGAGAAAAAAGCTCAC CCTTTTCCAGCGTAGAAAGTATATATCAGTCATTGAAG ACTATTATTTAAATAACACAATGTCTAAAGGAAAAGT TTGTTTGGCCTACTCCGGTGGTTTGGATACCTCCATCA TCCTAGCTTGGTTGTTGGAGCAGGGATACGAAGTCGT TGCCTTTTTAGCCAACATTGGTCAAGAGGAAGACTTTG AGGCTGCTAGAGAGAAAGCTCTGAAGATCGGTGCTAC CAAGTTTATCGTCAGTGACGTTAGGAAGGAATTTGTTG AGGAAGTTTTGTTCCCAGCAGTCCAAGTTAACGCTATC TACGAGAACGTCTACTTACTGGGTACCTCTTTGGCCAG ACCAGTCATTGCCAAGGCCCAAATAGAGGTTGCTGAA CAAGAAGGTTGTTTTGCTGTTGCCCACGGTTGTACCGG AAAGGGTAACGATCAGGTTAGATTTGAGCTTTCCTTTT ATGCTCTGAAGCCTGACGTTGTCTGTATCGCCCCATGG AGAGACCCAGAATTCTTCGAAAGATTCGCTGGTAGAA ATGACTTGCTGAATTACGCTGCTGAGAAGGATATTCC AGTTGCTCAGACTAAAGCCAAGCCATGGTCTACTGAT GAGAACATGGCTCACATCTCCTTCGAGGCTGGTATTCT AGAAGATCCAAACACTACTCCTCCAAAGGACATGTGG AAGCTCACTGTTGACCCAGAAGATGCACCAGACAAGC CAGAGTTCTTTGACGTCCACTTTGAGAAGGGTAAGCC AGTTAAATTAGTTCTCGAGAACAAAACTGAGGTCACC GATCCGGTTGAGATCTTTTTGACTGCTAACGCCATTGC TAGAAGAAACGGTGTTGGTAGAATTGACATTGTCGAG AACAGATTCATCGGAATCAAGTCCAGAGGTTGTTATG AAACTCCAGGTTTGACTCTACTGAGAACCACTCACAT CGACTTGGAAGGTCTTACCGTTGACCGTGAAGTTAGA TCGATCAGAGACACTTTTGTTACCCCAACCTACTCTAA GTTGTTATACAACGGGTTGTACTTTACCCCAGAAGGTG AGTACGTCAGAACTATGATTCAGCCTTCTCAAAACAC CGTCAACGGTGTTGTTAGAGCCAAGGCCTACAAAGGT AATGTGTATAACCTAGGAAGATACTCTGAAACCGAGA AATTGTACGATGCTACCGAATCTTCCATGGATGAGTTG ACCGGATTCCACCCTCAAGAAGCTGGAGGATTTATCA CAACACAAGCCATCAGAATCAAGAAGTACGGAGAAA GTGTCAGAGAGAAGGGAAAGTTTTTGGGACTTTAACT CAAGTAAAAGGATAGTTGTACAATTATATATACGAAG AATAAATCATTACAAAAAGTATTCGTTTCTTTGATTCT TAACAGGATTCATTTTCTGGGTGTCATCAGGTACAGCG CTGAATATCTTGAAGTTAACATCGAGCTCATCATCGAC GTTCATCACACTAGCCACGTTTCCGCAACGGTAG

57	*Pichia pastoris* Sequence of the 3'-Region used for knock out of PpARG1:	GGGACTTTAACTCAAGTAAAAGGATAGTTGTACAATT ATATATACGAAGAATAAATCATTACAAAAAGTATTCG TTTCTTTGATTCTTAACAGGATTCATTTTCTGGGTGTCA TCAGGTACAGCGCTGAATATCTTGAAGTTAACATCGA GCTCATCATCGACGTTCATCACACTAGCCACGTTTCCG CAACGGTAGCAATAATTAGGAGCGGACCACACAGTGA CGACATCTTTCTCTTTGAAATGGTATCTGAAGCCTTCC ATGACCAATTGATGGGCTCTAGCGATGAGTTGCAAGT TATTAATGTGGTTGAACTCACGTGCTACTCGAGCACCG AATAACCAGCCAGCTCCACGAGGAGAAACAGCCCAA CTGTCGACTTCATCTGGGTCAGACCAAACCAAGTCAC AAAATCCTCCTTCATGAGGGACCTCTTGCGCTCGGCTG AGAACTCTGATTTGATCTAACATGCGAATATCGGGAG AGAGACCACCATGGATACATAATATTTTACCATCAAT GATGGCACTAAGGGTTAAAAAGTCGAACACCTGGCAA CAGTACTTCCAGACAGTGGTGGAACCATATTTATTGA GACATTCCTCATAAAATCCATAAACCTGAGTGATCTGT CTGGATTCATGATTTCCCCTTACCAATGTGATATGTTG AGGAAACTTAATTTTTAAAATCATGAGTAACGTGAAC GTCTCCAACGAGAAATAGCCTCTATCCACATAGTCTCC TAGGAAGATATAGTTCTGTTTTATTCCATTAGAGGAGG ATCCGGGAAACCCACCACTAATCTTGAAAAGTTCCAG TAGATCGTGAAATTGGCCGTGAATATCTCCGCATACT GTCACTGGACTCTGCACTGGCTGTATATTGGATTCCTC CATCAGCAAATCCTTCACCCGTTCGCAAAGATGCTTCA TATCATTTTCACTTAAAGCCTTGCAGCTTTTGACTTCTT CAAACCACTGATCTGGTCCTCTTTCTGGCATGATTAAG GTCTATAATATTTCTGAGCTGAGATGTAAAAAAAAAT AATAAAAATGGGGAGTGAAAAAGTGTGTAGCTTTTAG GAGTTTGGGATTGATACCCCAAAATGATCTTTATGAG AATTAAAAGGTAGATACGCTTTTAATAAGAACACCTA TCTATAGTACTTTGTGGTCTTGAGTAATTGAGATGTTC AGCTTCTGAGGTTTGCCGTTATTCTGGGATAGTAGTGC GCGACCAAACAACCCGCCAGGCAAAGTGTGTTGTGCT CGAAGACGATTGCCAGAAGAGTAAGTCCGTCCTGCCT CAGATGTTACACACTTTCTTCCCTAGACAGTCGATGCA TCATCGGATTTAAACCTGAAACTTTGATGCCATGATAC GCCTAGTCACGTCGACTGAGATTTTAGATAAGCCCCG ATCCCTTTAGTACATTCCTGTTATCCATGGATGGAATG GCCTGATA

58	human Anti-Her2 Heavy chain (VH + IgG1 constant region) (DNA)	GAGGTTCAGTTGGTTGAATCTGGAGGAGGATTGGTTC AACCTGGTGGTTCTTTGAGATTGTCCTGTGCTGCTTCC GGTTTCAACATCAAGGACACTTACATCCACTGGGTTA GACAAGCTCCAGGAAAGGGATTGGAGTGGGTTGCTAG AATCTACCCAACTAACGGTTACACAAGATACGCTGAC TCCGTTAAGGGAAGATTCACTATCTCTGCTGACACTTC CAAGAACACTGCTTACTTGCAGATGAACTCCTTGAGA GCTGAGGATACTGCTGTTTACTACTGTTCCAGATGGGG TGGTGATGGTTTCTACGCTATGGACTACTGGGGTCAA GGAACTTTGGTTACTGTTTCCTCCGCTTCTACTAAGGG ACCATCTGTTTTCCCATTGGCTCCATCTTCTAAGTCTA CTTCCGGTGGTACTGCTGCTTTGGGATGTTTGGTTAAA GACTACTTCCCAGAGCCAGTTACTGTTTCTTGGAACTC CGGTGCTTTGACTTCTGGTGTTCACACTTTCCCAGCTG TTTTGCAATCTTCCGGTTTGTACTCTTTGTCCTCCGTTG TTACTGTTCCATCCTCTTCCTTGGGTACTCAGACTTAC ATCTGTAACGTTAACCACAAGCCATCCAACACTAAGG TTGACAAGAAGGTTGAGCCAAAGTCCTGTGACAAGAC ACATACTTGTCCACCATGTCCAGCTCCAGAATTGTTGG GTGGTCCATCCGTTTTCTTGTTCCCACCAAAGCCAAAG GACACTTTGATGATCTCCAGAACTCCAGAGGTTACAT GTGTTGTTGTTGACGTTTCTCACGAGGACCCAGAGGTT AAGTTCAACTGGTACGTTGACGGTGTTGAAGTTCACA ACGCTAAGACTAAGCCAAGAGAAGAGCAGTACAACT CCACTTACAGAGTTGTTTCCGTTTTGACTGTTTTGCAC CAGGACTGGTTGAACGGTAAAGAATACAAGTGTAAGG TTTCCAACAAGGCTTTGCCAGCTCCAATCGAAAAGAC TATCTCCAAGGCTAAGGGTCAACCAAGAGAGCCACAG GTTTACACTTTGCCACCATCCAGAGAAGAGATGACTA AGAACCAGGTTTCCTTGACTTGTTTGGTTAAAGGATTC TACCCATCCGACATTGCTGTTGAGTGGGAATCTAACG GTCAACCAGAGAACAACTACAAGACTACTCCACCAGT TTTGGATTCTGATGGTTCCTTCTTCTTGTACTCCAAGTT GACTGTTGACAAGTCCAGATGGCAACAGGGTAACGTT TTCTCCTGTTCCGTTATGCATGAGGCTTTGCACAACCA CTACACTCAAAAGTCCTTGTCTTTGTCCCCTGGTTAA

59	human Anti-Her2 light chain (VL + Kappa constant region) (DNA)	GACATCCAAATGACTCAATCCCCATCTTCTTTGTCTGC TTCCGTTGGTGACAGAGTTACTATCACTTGTAGAGCTT CCCAGGACGTTAATACTGCTGTTGCTTGGTATCAACAG AAGCCAGGAAAGGCTCCAAAGTTGTTGATCTACTCCG CTTCCTTCTTGTACTCTGGTGTTCCATCCAGATTCTCTG GTTCCAGATCCGGTACTGACTTCACTTTGACTATCTCC TCCTTGCAACCAGAAGATTTCGCTACTTACTACTGTCA GCAGCACTACACTACTCCACCAACTTTCGGACAGGGT ACTAAGGTTGAGATCAAGAGAACTGTTGCTGCTCCAT CCGTTTTCATTTTCCCACCATCCGACGAACAGTTGAAG TCTGGTACAGCTTCCGTTGTTTGTTTGTTGAACAACTT CTACCCAAGAGAGGCTAAGGTTCAGTGGAAGGTTGAC AACGCTTTGCAATCCGGTAACTCCCAAGAATCCGTTA CTGAGCAAGACTCTAAGGACTCCACTTACTCCTTGTCC TCCACTTTGACTTTGTCCAAGGCTGATTACGAGAAGCA CAAGGTTTACGCTTGTGAGGTTACACATCAGGGTTTGT CCTCCCCAGTTACTAAGTCCTTCAACAGAGGAGAGTG TTAA
60	*Streptoalloteichus hindustanus* Sequence of the Sh ble ORF (Zeocin resistance marker):	ATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCG CGCGCGACGTCGCCGGAGCGGTCGAGTTCTGGACCGA CCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGAC TTCGCCGGTGTGGTCCGGGACGACGTGACCCTGTTCAT CAGCGCGGTCCAGGACCAGGTGGTGCCGGACAACACC CTGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGCTGT ACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTCCG GGACGCCTCCGGGCCGGCCATGACCGAGATCGGCGAG CAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGG CCGGCAACTGCGTGCACTTCGTGGCCGAGGAGCAGGA CTGA
61	*Saccharomyces cerevisiae* ScTEF1 promoter	GATCCCCCACACACCATAGCTTCAAAATGTTTCTACTC CTTTTTTACTCTTCCAGATTTTCTCGGACTCCGCGCATC GCCGTACCACTTCAAAACACCCAAGCACAGCATACTA AATTTCCCCTCTTTCTTCCTCTAGGGTGTCGTTAATTAC CCGTACTAAAGGTTTGGAAAAGAAAAAAGAGACCGC CTCGTTTCTTTTTCTTCGTCGAAAAAGGCAATAAAAAT TTTTATCACGTTTCTTTTTCTTGAAAATTTTTTTTTTG ATTTTTTTCTCTTTCGATGACCTCCCATTGATATTTAAG TTAATAAACGGTCTTCAATTTCTCAAGTTTCAGTTTCA TTTTTCTTGTTCTATTACAACTTTTTTTACTTCTTGCTC ATTAGAAAGAAAGCATAGCAATCTAATCTAAGTTTTA ATTACAAA

62	*Pichia pastoris* PpTRP2 Region	ATGAGTGTAAGTGATAGTCATCTTGCAACAGATTATTT TGGAACGCAACTAACAAAGCAGATACACCCTTCAGCA GAATCCTTTCTGGATATTGTGAAGAATGATCGCCAAA GTCACAGTCCTGAGACAGTTCCTAATCTTTACCCCATT TACAAGTTCATCCAATCAGACTTCTTAACGCCTCATCT GGCTTATATCAAGCTTACCAACAGTTCAGAAACTCCC AGTCCAAGTTTCTTGCTTGAAAGTGCGAAGAATGGTG ACACCGTTGACAGGTACACCTTTATGGGACATTCCCCC AGAAAAATAATCAAGACTGGGCCTTTAGAGGGTGCTG AAGTTGACCCCTTGGTGCTTCTGGAAAAAGAACTGAA GGGCACCAGACAAGCGCAACTTCCTGGTATTCCTCGT CTAAGTGGTGGTGCCATAGGATACATCTCGTACGATT GTATTAAGTACTTTGAACCAAAAACTGAAAGAAAACT GAAAGATGTTTTGCAACTTCCGGAAGCAGCTTTGATG TTGTTCGACACGATCGTGGCTTTTGACAATGTTTATCA AAGATTCCAGGTAATTGGAAACGTTTCTCTATCCGTTG ATGACTCGGACGAAGCTATTCTTGAGAAATATTATAA GACAAGAGAAGAAGTGGAAAAGATCAGTAAAGTGGT ATTTGACAATAAAACTGTTCCCTACTATGAACAGAAA GATATTATTCAAGGCCAAACGTTCACCTCTAATATTGG TCAGGAAGGGTATGAAAACCATGTTCGCAAGCTGAAA GAACATATTCTGAAAGGAGACATCTTCCAAGCTGTTC CCTCTCAAAGGGTAGCCAGGCCGACCTCATTGCACCC TTTCAACATCTATCGTCATTTGAGAACTGTCAATCCTT CTCCATACATGTTCTATATTGACTATCTAGACTTCCAA GTTGTTGGTGCTTCACCTGAATTACTAGTTAAATCCGA CAACAACAACAAAATCATCACACATCCTATTGCTGGA ACTCTTCCCAGAGGTAAAACTATCGAAGAGGACGACA ATTATGCTAAGCAATTGAAGTCGTCTTTGAAAGACAG GGCCGAGCACGTCATGCTGGTAGATTTGGCCAGAAAT GATATTAACCGTGTGTGTGAGCCCACCAGTACCACGG TTGATCGTTTATTGACTGTGGAGAGATTTTCTCATGTG ATGCATCTTGTGTCAGAAGTCAGTGGAACATTGAGAC CAAACAAGACTCGCTTCGATGCTTTCAGATCCATTTTC CCAGCAGGAACCGTCTCCGGTGCTCCGAAGGTAAGAG CAATGCAACTCATAGGAGAATTGGAAGGAGAAAAGA GAGGTGTTTATGCGGGGGCCGTAGGACACTGGTCGTA CGATGGAAAATCGATGGACACATGTATTGCCTTAAGA ACAATGGTCGTCAAGGACGGTGTCGCTTACCTTCAAG CCGGAGGTGGAATTGTCTACGATTCTGACCCCTATGA CGAGTACATCGAAACCATGAACAAAATGAGATCCAAC AATAACACCATCTTGGAGGCTGAGAAAATCTGGACCG ATAGGTTGGCCAGAGACGAGAATCAAAGTGAATCCGA AGAAAACGATCAATGA

63	*Pichia pastoris* PpCITI TT	CCGGCCATTTAAATATGTGACGACTGGGTGATCCGGG TTAGTGAGTTGTTCTCCCATCTGTATATTTTTCATTTAC GATGAATACGAAATGAGTATTAAGAAATCAGGCGTAG CAATATGGGCAGTGTTCAGTCCTGTCATAGATGGCAA GCACTGGCACATCCTTAATAGGTTAGAGAAAATCATT GAATCATTTGGGTGGTGAAAAAAAATTGATGTAAACA AGCCACCCACGCTGGGAGTCGAACCCAGAATCTTTTG ATTAGAAGTCAAACGCGTTAACCATTACGCTACGCAG GCATGTTTCACGTCCATTTTTGATTGCTTTCTATCATAA TCTAAAGATGTGAACTCAATTAGTTGCAATTTGACCA ATTCTTCCATTACAAGTCGTGCTTCCTCCGTTGATGCA AC
64	*Streptomyces noursei* NatR ORF	ATGGGTACCACTCTTGACGACACGGCTTACCGGTACC GCACCAGTGTCCCGGGGGACGCCGAGGCCATCGAGGC ACTGGATGGGTCCTTCACCACCGACACCGTCTTCCGCG TCACCGCCACCGGGGACGGCTTCACCCTGCGGGAGGT GCCGGTGGACCCGCCCCTGACCAAGGTGTTCCCCGAC GACGAATCGGACGACGAATCGGACGACGGGGAGGAC GGCGACCCGGACTCCCGGACGTTCGTCGCGTACGGGG ACGACGGCGACCTGGCGGGCTTCGTGGTCGTCTCGTA CTCCGGCTGGAACCGCCGGCTGACCGTCGAGGACATC GAGGTCGCCCCGGAGCACCGGGGGCACGGGGTCGGG CGCGCGTTGATGGGGCTCGCGACGGAGTTCGCCCGCG AGCGGGGCGCCGGGCACCTCTGGCTGGAGGTCACCAA CGTCAACGCACCGGCGATCCACGCGTACCGGCGGATG GGGTTCACCCTCTGCGGCCTGGACACCGCCCTGTACG ACGGCACCGCCTCGGACGGCGAGCAGGCGCTCTACAT GAGCATGCCCTGCCCCTAATCAGTACTG
65	*Ashbya gossypii TEF1* promoter	GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCG GCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACA GTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTG TCGCCCGTACATTTAGCCCATACATCCCCATGTATAAT CATTTGCATCCATACATTTTGATGGCCGCACGGCGCGA AGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGC AGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCC CCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAG GATTTGCCACTGAGGTTCTTCTTTCATATACTTCCTTTT AAAATCTTGCTAGGATACAGTTCTCACATCACATCCG AACATAAACAACC
66	*Ashbya gossypii TEF1* termination sequence	TAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAG AACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTT CTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTT CGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTG CGCAGAAAGTAATATCATGCGTCAATCGTATGTGAAT GCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC GCCATCCAGTGTCGAAAAC

67	*Pichia pastoris* PpTRP1 5' region and ORF	GCGGAAACGGCAGTAAACAATGGAGCTTCATTAGTGG GTGTTATTATGGTCCCTGGCCGGGAACGAACGGTGAA ACAAGAGGTTGCGAGGGAAATTTCGCAGATGGTGCGG GAAAAGAGAATTTCAAAGGGCTCAAAATACTTGGATT CCAGACAACTGAGGAAAGAGTGGGACGACTGTCCTCT GGAAGACTGGTTTGAGTACAACGTGAAAGAAATAAAC AGCAGTGGTCCATTTTTAGTTGGAGTTTTTCGTAATCA AAGTATAGATGAAATCCAGCAAGCTATCCACACTCAT GGTTTGGATTTCGTCCAACTACATGGGTCTGAGGATTT TGATTCGTATATACGCAATATCCCAGTTCCTGTGATTA CCAGATACACAGATAATGCCGTCGATGGTCTTACCGG AGAAGACCTCGCTATAAATAGGGCCCTGGTGCTACTG GACAGCGAGCAAGGAGGTGAAGGAAAAACCATCGAT TGGGCTCGTGCACAAAAATTTGGAGAACGTAGAGGAA AATATTTACTAGCCGGAGGTTTGACACCTGATAATGTT GCTCATGCTCGATCTCATACTGGCTGTATTGGTGTTGA CGTCTCTGGTGGGGTAGAAACAAATGCCTCAAAAGAT ATGGACAAGATCACACAATTTATCAGAAACGCTACAT AA
68	*Pichia pastoris* PpTRP1 3' region	AAGTCAATTAAATACACGCTTGAAAGGACATTACATA GCTTTCGATTTAAGCAGAACCAGAAATGTAGAACCAC TTGTCAATAGATTGGTCAATCTTAGCAGGAGCGGCTG GGCTAGCAGTTGGAACAGCAGAGGTTGCTGAAGGTGA GAAGGATGGAGTGGATTGCAAAGTGGTGTTGGTTAAG TCAATCTCACCAGGGCTGGTTTTGCCAAAAATCAACTT CTCCCAGGCTTCACGGCATTCTTGAATGACCTCTTCTG CATACTTCTTGTTCTTGCATTCACCAGAGAAAGCAAAC TGGTTCTCAGGTTTTCCATCAGGGATCTTGTAAATTCT GAACCATTCGTTGGTAGCTCTCAACAAGCCCGGCATG TGCTTTTCAACATCCTCGATGTCATTGAGCTTAGGAGC CAATGGGTCGTTGATGTCGATGACGATGACCTTCCAG TCAGTCTCTCCCTCATCCAACAAAGCCATAACACCGA GGACCTTGACTTGCTTGACCTGTCCAGTGTAACCTACG GCTTCACCAATTTCGCAAACGTCCAATGGATCATTGTC ACCCTTGGCCTTGGTCTCTGGATGAGTGACGTTAGGGT CTTCCCATGTCTGAGGGAAGGCACCGTAGTTGTGAAT GTATCCGTGGTGAGGGAAACAGTTACGAACGAAACGA AGTTTTCCCTTCTTTGTGTCCTGAAGAATTGGGTTCAG TTTCTCCTCCTTGGAAATCTCCAACTTGGCGTTGGTCC AACGGGGGACTTCAACAACCATGTTGAGAACCTTCTT GGATTCGTCAGCATAAAGTGGGATGTCGTGGAAAGGA GATACGACTT
69	*Pichia pastoris* PpXRN1-5'out/UP	GTTAAATGACTCTAACACCTTGCACTTGA
70	*Pichia pastoris* PpALG3TT/LP	CCTCCCACTGGAACCGATGATATGGAA

71	*Pichia pastoris* PpTEFTT/UP	GATGCGAAGTTAAGTGCGCAGAAAGTAATATCA
72	*Pichia pastoris* PpXRN1-3'out/LP	TTGCAAAAACCAGTGAGGAATAGC
73	*Pichia pastoris* PpXRN1/iUP	GAATGCTGAAGAACGTCAAAGAAACT
74	*Pichia pastoris* PpXRN1/iLP	TGAGACTTCAGAGCTTTCCATACGA
75	*Pichia pastoris* Sequence of the PpXRN1 (DNA)	ATGGGTATTCCAAAGTTTTTTCGGTACATCTCTGAGAG ATGGCCGATGATATCTGAGCCAATAGAAGACAGCCAA ATTGCCGAGTTTGATAACCTGTATCTGGACATGAACTC AATTCTTCATAATTGTACACATAGTAACGATGGATCA GTTGATTTAATGAAAGAAGAAGAGATGTTCAGTGCTA TTTTTGCTTACATTGAACATCTTTTCACTTTGATCAAAC CTGGAAAGACGTTTTTCATGGCCATTGATGGTGTTGCT CCTAGAGCAAAGATGAACCAGCAACGATCTAGAAGAT TCAGGACGGCCATTGAGGCAGAAAAAAGTGTAGAGAT AGCCCAAAAGAATGGACTCATTACTAGCAAAGACGAG AATTTTGACAGTAACTGTATCACTCCTGGAACGGAGTT TATGGCCAAGGTTACCACTAACTTGAAGTTTTTCATCC ACCAGAAAATCTCTTCAGATGCAAAATGGCAGAAAGT CCAGGTTATTCTCAGTGGACATGAAGTCCCCGGAGAA GGAGAGCACAAAATTATGGACTATATTCGTTTTTTGAA GGCTCAAGAGGGGTATGATCCGAATACGAGACATTGT ATTTACGGGCTGGATGCAGACTTGATCATGTTGGGATT GGTCATTCACGATCCTCATTTTGCCATCTTGAGAGAAG AGGTTGTGTTCGGAAGAGGATCCAAGGCCTCGTCAAC AGATGTGTCAGAACAGCAATTCTACCTTCTACATCTCT CCTTGTTACGTGAATACCTTGCTCTTGAGTTCAAAGAT TTAGAAGACCAGATAAAGTTTGATTATGACTTAGAGA GAATCTTGGACGATTTTATTTTTATCATGTATGTTATTG GAAATGATTTCTTACCCAACTTACCTGACTTGCATATC AACAAAGGTGCCTTCCCCAGACTCTTAGCAACGATCA AAGAAACCATGATAGATTCGGATGGATATCTCCAAGA AGGAGGTGTCATCAATATGGAACGATTCGGTCTGTGG CTTGACCACTTGTCACAATTTGAGCTTCAAAATTTTGA GCAGGTCGACGTGGATGTAGAATGGTTTAACAAGCAG CTAGAGAACATATCCCTAGATGGTGAGCGAAAGAGGG AAAGAGCTGGAAGAAAGTTGTTGCTTAGACGTCAACA GGAATTAATTTCAAAACTTAGACCATGGATTCTCGAG TTTTATTCATCAAGAGACAATATATATTCTGCTCATGA TGATGACTCCTTAATTCCAACTTTGCAATTGGACACCG AATTGATGGAAGAAGAAATCAAGTTACCCTTCATAAA GCAGTTTGCACTTGATGTTGGTTTCTTTATTGT

GCACAGCAAATCTCAGAATACGTACCATGCTAAGATA
GACATTGATGGTATAAATGTCAATGAATCTGATGAAG
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CAAAGAATATGAAAACTCAATTTTTGTTGAAGATGAA
AACACTTTAGAGGAACAAAAGAACATTTATGATACCA
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ACCGTATAATAGTAAGTTGAATGCTGAAGAACGTCAA
AGAAACTCTTTGGGCACGAATATCGAGTTTAGCTATC
ATCCTCAAGTGAATCAAGTTCATTCTTCCCCAATTCCA
ACAATATTCCCAGATATCACTGAGGATCACTGCTACG
AAATAGTGGTCGAATTTGACAAGCTGCACTCTTCAAC
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CTGACGGTTTGAACGTTTTTGAGCCTGGAAACAAGTCT
ATCCGAACGACTCGTATGGAAAGCTCTGAAGTCTCAG
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ATTCAAGAGAAAAGGTGTTTTACTGGTGAACTACACT
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CCCCTCACAATGACGATGCGATCCAAGGAATTGTGTT
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GATACACTAAAAGAAACGAGAAACATTGGTCATACAG
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CGTTTCCCATCTGTTTTTAAGATAATTTCTCTCCGA

		AATGATTCTTCAATTCCTGAAGCCAAAGAACTGTTTCC TCAAGTCCCCGCTAGCCAAGTGGATGAAAAAGTCAAT GAACTTGTGGCTTGGGTTAAAGAACAAAAGAAAAGCT TTGTTGTTGCAACATTGGAGTCTGAGTCTTTGACCAAG GTTAGTATCGGAAAGATTGAACAGGAAGTGATTAAGT TTGTTTCAAAACCACATGAACATATTCCCCAAAAGGG TTTGAAAGGAGTTCCTCGTGAGGCTACTCTGGATATCA GCAACTCTTCTCAATTTCTTTCCAAGCAGACTTTTAAT CTAGGAGACAGAGTGATCTACGTAGAAGATTCTGGTA AGGTACCCAACTTCAGTAAAGGTACAGTTATTGGGGT TCGAAGTGTCGGTACGAAAGTGACTTTGAACGTATTG TTTGACTTGCCATTGCTCTCTGGTAATACCTTTGATGG AAGACTTGCAACCCCCAGGGGTGTCACTGTTGATAGT TCATTGGTATTGAACTTAACAAAGCGTCAATTGATTTA CCACGATAGGAAACCCGCTAAGAAAGAAGGTAAGCC TGGAGTTAAGAAGACATCTCAGGACTCCAAAAAGCAA ACAGTGAAGTTGCAGAACGGTTCAGGTAAGGCCAAAC AGGCACAAGATGCTGAACTGTCAGTCACTACGACACC TGTTCCGGTTCCTGCCGCTAACACTGCACCAGTTACTG CATCTGTACAGGCTACCTCGGTGGTTACTGCTACCGTT CCAACTTCTAAACCTTCAAACAACTCTGAGGACGAAC ATGAATTACTTCGCTTACTGAAAGGTAACAAGGACAG TAGTGAATCGCAAGGCTCTCAAGAGCCTCTTGCCCGG ACTTCTATCCAGCAAATTTACGGAACGGTGTTCAATCA AGTGTTGAGCGCTCAACCTCAGTTGCAGCCTGTCAGA GGCTTCTCAAATCCGGTACCTGAAACCCCTGTAAATG GAGTCCAAGCGAATGAACAACACTCTGATTCTACCCC TCAAAATCATTCTAGGGATGAAAACCAAGGAAGAGGT CGTGGTAGAGGCAGAGGAAATAGAAGAGGAAGAGGT CGAGGTAGAGGCAAAGGAGGACAGTAA

76	*Pichia pastoris* Sequence of the PpXRN1 (protein)	MGIPKFFRYISERWPMISEPIEDSQIAEFDNLYLDMNSILH NCTHSNDGSVDLMKEEEMFSAIFAYIEHLFTLIKPGKTFF MAIDGVAPRAKMNQQRSRRFRTAIEAEKSVEIAQKNGLI TSKDENFDSNCITPGTEFMAKVTTNLKFFIHQKISSDAKW QKVQVILSGHEVPGEGEHKIMDYIRFLKAQEGYDPNTRH CIYGLDADLIMLGLVIHDPHFAILREEVVFGRGSKASSTD VSEQQFYLLHLSLLREYLALEFKDLEDQIKFDYDLERILD DFIFIMYVIGNDFLPNLPDLHINKGAFPRLLATIKETMIDS DGYLQEGGVINMERFGLWLDHLSQFELQNFEQVDVDVE WFNKQLENISLDGERKRERAGRKLLLRRQQELISKLRPWI LEFYSSRDNIYSAHDDDSLIPTLQLDTELMEEEIKLPFIKQF ALDVGFFIVHSKSQNTYHAKIDIDGINVNESDEEFEARVL YIRKKIKEYENSIFVEDENTLEEQKNIYDTKFVNWKNKY YKEKFGFTLSDTEDILKLTESYIQGLQWVLFYYYRGVPS WQWYYPYYYAPRISDIKLGIKACLEFDKGTPFKPFEQLM AVLPARSKQLVPACYRPLMTDPNSPIIEFYPDEVEIDKNG KTASWEAVVKINFVDEKRLLEALEPYNSKLNAEERQRNS LGTNIEFSYHPQVNQVHSSPIPTIFPDITEDHCYEIVVEFDK LHSSTYAKPMKGAKQGINLLAGFPTLKTIPFTSQLMLAEC HIFNQPTKSESMILSTQNPFEGLTVEQFAAQNLGQTIYVN WPYLKEAKVVAVSDGLNVFEPGNKSIRTTRMESSEVSEF ASDVRYISETLFKRKGVLLVNYTEEELQGTPEPRRSPHND DAIQGIVFVKKVNGVIRTRSGAYVKTYSDKIEKYPINLLV DDVVNKDRRLLEKPPVPLEEEFPKGTTLISLGAFAYGTPA TVVDHQNDLMTVRFVNQPIKHEFNYGEIQAQREAHTNV YYPSFKVAKIVGITALALSRITSSYRIVNGAKKTVNIGLDL KFEGRKLKVLGYTKRNEKHWSYSALAVNLLQQYQKRFP SVFKIISLRNDSSIPEAKELFPQVPASQVDEKVNELVAWV KEQKKSFVVATLESESLTKVSIGKIEQEVIKFVSKPHEHIP QKGLKGVPREATLDISNSSQFLSKQTFNLGDRVIYVEDSG KVPNFSKGTVIGVRSVGTKVTLNVLFDLPLLSGNTFDGR LATPRGVTVDSSLVLNLTKRQLIYHDRKPAKKEGKPGVK KTSQDSKKQTVKLQNGSGKAKQAQDAELSVTTTPVPVP AANTAPVTASVQATSVVTATVPTSKPSNNSEDEHELLRL LKGNKDSSESQGSQEPLARTSIQQIYGTVFNQVLSAQPQL QPVRGFSNPVPETPVNGVQANEQHSDSTPQNHSRDENQ GRGRGRGRGNRRGRGRGRGKGGQ

77	Mouse CMP-sialic acid transporter (MmCST) Codon optimized	ATGGCTCCAGCTAGAGAAAACGTTTCCTTGTTCTTCAA GTTGTACTGTTTGGCTGTTATGACTTTGGTTGCTGCTG CTTACACTGTTGCTTTGAGATACACTAGAACTACTGCT GAGGAGTTGTACTTCTCCACTACTGCTGTTTGTATCAC TGAGGTTATCAAGTTGTTGATCTCCGTTGGTTTGTTGG CTAAGGAGACTGGTTCTTTGGGAAGATTCAAGGCTTC CTTGTCCGAAAACGTTTTGGGTTCCCCAAAGGAGTTG GCTAAGTTGTCTGTTCCATCCTTGGTTTACGCTGTTCA GAACAACATGGCTTTCTTGGCTTTGTCTAACTTGGACG CTGCTGTTTACCAAGTTACTTACCAGTTGAAGATCCCA TGTACTGCTTTGTGTACTGTTTTGATGTTGAACAGAAC ATTGTCCAAGTTGCAGTGGATCTCCGTTTTCATGTTGT GTGGTGGTGTTACTTTGGTTCAGTGGAAGCCAGCTCA AGCTTCCAAAGTTGTTGTTGCTCAGAACCCATTGTTGG GTTTCGGTGCTATTGCTATCGCTGTTTTGTGTTCCGGTT TCGCTGGTGTTTACTTCGAGAAGGTTTTGAAGTCCTCC GACACTTCTTTGTGGGTTAGAAACATCCAGATGTACTT GTCCGGTATCGTTGTTACTTTGGCTGGTACTTACTTGT CTGACGGTGCTGAGATTCAAGAGAAGGGATTCTTCTA CGGTTACACTTACTATGTTTGGTTCGTTATCTTCTTGGC TTCCGTTGGTGGTTTGTACACTTCCGTTGTTGTTAAGT ACACTGACAACATCATGAAGGGATTCTCTGCTGCTGC TGCTATTGTTTTGTCCACTATCGCTTCCGTTTTGTTGTT CGGATTGCAGATCACATTGTCCTTTGCTTTGGGAGCTT TGTTGGTTTGTGTTTCCATCTACTTGTACGGATTGCCA AGACAAGACACTACTTCCATTCAGCAAGAGGCTACTT CCAAGGAGAGAATCATCGGTGTTTAGTAG

78	Human UDP-GlcNAc 2-epimerase/N-acetylmannosamine kinase (HsGNE) codon opitimized	ATGGAAAAGAACGGTAACAACAGAAAGTTGAGAGTTT GTGTTGCTACTTGTAACAGAGCTGACTACTCCAAGTTG GCTCCAATCATGTTCGGTATCAAGACTGAGCCAGAGT TCTTCGAGTTGGACGTTGTTGTTTTGGGTTCCCACTTG ATTGATGACTACGGTAACACTTACAGAATGATCGAGC AGGACGACTTCGACATCAACACTAGATTGCACACTAT TGTTAGAGGAGAGGACGAAGCTGCTATGGTTGAATCT GTTGGATTGGCTTTGGTTAAGTTGCCAGACGTTTTGAA CAGATTGAAGCCAGACATCATGATTGTTCACGGTGAC AGATTCGATGCTTTGGCTTTGGCTACTTCCGCTGCTTT GATGAACATTAGAATCTTGCACATCGAGGGTGGTGAA GTTTCTGGTACTATCGACGACTCCATCAGACACGCTAT CACTAAGTTGGCTCACTACCATGTTTGTTGTACTAGAT CCGCTGAGCAACACTTGATTTCCATGTGTGAGGACCA CGACAGAATTTTGTTGGCTGGTTGTCCATCTTACGACA AGTTGTTGTCCGCTAAGAACAAGGACTACATGTCCAT CATCAGAATGTGGTTGGGTGACGACGTTAAGTCTAAG GACTACATCGTTGCTTTGCAGCACCCAGTTACTACTGA CATCAAGCACTCCATCAAGATGTTCGAGTTGACTTTGG ACGCTTTGATCTCCTTCAACAAGAGAACTTTGGTTTTG TTCCCAAACATTGACGCTGGTTCCAAAGAGATGGTTA GAGTTATGAGAAAGAAGGGTATCGAACACCACCCAA ACTTCAGAGCTGTTAAGCACGTTCCATTCGACCAATTC ATCCAGTTGGTTGCTCATGCTGGTTGTATGATCGGTAA CTCCTCCTGTGGTGTTAGAGAAGTTGGTGCTTTCGGTA CTCCAGTTATCAACTTGGGTACTAGACAGATCGGTAG AGAGACTGGAGAAAACGTTTTGCATGTTAGAGATGCT GACACTCAGGACAAGATTTTGCAGGCTTTGCACTTGC AATTCGGAAAGCAGTACCCATGTTCCAAAATCTACGG TGACGGTAACGCTGTTCCAAGAATCTTGAAGTTTTTGA AGTCCATCGACTTGCAAGAGCCATTGCAGAAGAAGTT CTGTTTCCCACCAGTTAAGGAGAACATCTCCCAGGAC ATTGACCACATCTTGGAGACATTGTCCGCTTTGGCTGT TGATTTGGGTGGAACTAACTTGAGAGTTGCTATCGTTT CCATGAAGGGAGAGATCGTTAAGAAGTACACTCAGTT CAACCCAAAGACTTACGAGGAGAGAATCAACTTGATC TTGCAGATGTGTGTTGAAGCTGCTGCTGAGGCTGTTAA GTTGAACTGTAGAATCTTGGGTGTTGGTATCTCTACTG GTGGTAGAGTTAATCCAAGAGAGGGTATCGTTTTGCA CTCCACTAAGTTGATTCAGGAGTGGAACTCCGTTGATT TGAGAACTCCATTGTCCGACACATTGCACTTGCCAGTT TGGGTTGACAACGACGGTAATTGTGCTGCTTTGGCTG AGAGAAAGTTCGGTCAAGGAAAGGGATTGGAGAACTT CGTTACTTTGATCACTGGTACTGGTATTGGTGGTGGTA TCATTCACCAGCACGAGTTGATTCACGGTTCTTCCTTC TGTGCTGCTGAATTGGGACACTTGGTTGTTTCTTTGGA CGGTCCAGACTGTTCTTGTGGTTCCCACGGTTGTATTG AAGCTTACGCATCAGGAATGGCATTGCAGAGAGAGGC TAAGAAGTTGCACGACGAGGACTTGTTGTTGGTTGAG GGAATGTCTGTTCCAAAGGACGAGGCTGTTGGTGCTT TGCATTTGATCCAGGCTGCTAAGTTGGGTAATGCTAA GGCTCAGTCCATCTTGAGAACTGCTGGTACTGCTTTGG

		GATTGGGTGTTGTTAATATCTTGCACACTATGAACCCA TCCTTGGTTATCTTGTCCGGTGTTTTGGCTTCTCACTAC ATCCACATCGTTAAGGACGTTATCAGACAGCAAGCTT TGTCCTCCGTTCAAGACGTTGATGTTGTTGTTTCCGAC TTGGTTGACCCAGCTTTGTTGGGTGCTGCTTCCATGGT TTTGGACTACACTACTAGAAGAATCTACTAATAG

79	*Pichia pastoris* Sequence of the PpARG1 auxotrophic marker:	CAGTTGAGCCAGACCGCGCTAAACGCATACCAATTGC CAAATCAGGCAATTGTGAGACAGTGGTAAAAAAGATG CCTGCAAAGTTAGATTCACACAGTAAGAGAGATCCTA CTCATAAATGAGGCGCTTATTTAGTAGCTAGTGATAG CCACTGCGGTTCTGCTTTATGCTATTTGTTGTATGCCTT ACTATCTTTGTTTGGCTCCTTTTTCTTGACGTTTTCCGT TGGAGGGACTCCCTATTCTGAGTCATGAGCCGCACAG ATTATCGCCCAAAATTGACAAAATCTTCTGGCGAAAA AAGTATAAAAGGAGAAAAAAGCTCACCCTTTTCCAGC GTAGAAAGTATATATCAGTCATTGAAGACTATTATTTA AATAACACAATGTCTAAAGGAAAAGTTTGTTTGGCCT ACTCCGGTGGTTTGGATACCTCCATCATCCTAGCTTGG TTGTTGGAGCAGGGATACGAAGTCGTTGCCTTTTTAGC CAACATTGGTCAAGAGGAAGACTTTGAGGCTGCTAGA GAGAAAGCTCTGAAGATCGGTGCTACCAAGTTTATCG TCAGTGACGTTAGGAAGGAATTTGTTGAGGAAGTTTT GTTCCCAGCAGTCCAAGTTAACGCTATCTACGAGAAC GTCTACTTACTGGGTACCTCTTTGGCCAGACCAGTCAT TGCCAAGGCCCAAATAGAGGTTGCTGAACAAGAAGGT TGTTTTGCTGTTGCCCACGGTTGTACCGGAAAGGGTAA CGATCAGGTTAGATTTGAGCTTTCCTTTTATGCTCTGA AGCCTGACGTTGTCTGTATCGCCCCATGGAGAGACCC AGAATTCTTCGAAAGATTCGCTGGTAGAAATGACTTG CTGAATTACGCTGCTGAGAAGGATATTCCAGTTGCTC AGACTAAAGCCAAGCCATGGTCTACTGATGAGAACAT GGCTCACATCTCCTTCGAGGCTGGTATTCTAGAAGATC CAAACACTACTCCTCCAAAGGACATGTGGAAGCTCAC TGTTGACCCAGAAGATGCACCAGACAAGCCAGAGTTC TTTGACGTCCACTTTGAGAAGGGTAAGCCAGTTAAAT TAGTTCTCGAGAACAAAACTGAGGTCACCGATCCGGT TGAGATCTTTTTGACTGCTAACGCCATTGCTAGAAGAA ACGGTGTTGGTAGAATTGACATTGTCGAGAACAGATT CATCGGAATCAAGTCCAGAGGTTGTTATGAAACTCCA GGTTTGACTCTACTGAGAACCACTCACATCGACTTGG AAGGTCTTACCGTTGACCGTGAAGTTAGATCGATCAG AGACACTTTTGTTACCCCAACCTACTCTAAGTTGTTAT ACAACGGGTTGTACTTTACCCCAGAAGGTGAGTACGT CAGAACTATGATTCAGCCTTCTCAAAACACCGTCAAC GGTGTTGTTAGAGCCAAGGCCTACAAAGGTAATGTGT ATAACCTAGGAAGATACTCTGAAACCGAGAAATTGTA CGATGCTACCGAATCTTCCATGGATGAGTTGACCGGA TTCCACCCTCAAGAAGCTGGAGGATTTATCACAACAC AAGCCATCAGAATCAAGAAGTACGGAGAAAGTGTCA GAGAGAAGGGAAAGTTTTTGGGACTTTAACTCAAGTA AAAGGATAGTTGTACAATTATATATACGAAGAATAAA TCATTACAAAAAGTATTCGTTTCTTTGATTCTTAACAG GATTCATTTTCTGGGTGTCATCAGGTACAGCGCTGAAT ATCTTGAAGTTAACATCGAGCTCATCATCGACGTTCAT CACACTAGCCACGTTTCCGCAACGGTAGCAATAATTA GGAGCGGACCACACAGTGACGACATC

80	Human CMP-sialic acid synthase (HsCSS) codon optimized	ATGGACTCTGTTGAAAAGGGTGCTGCTACTTCTGTTTC CAACCCAAGAGGTAGACCATCCAGAGGTAGACCTCCT AAGTTGCAGAGAAACTCCAGAGGTGGTCAAGGTAGAG GTGTTGAAAAGCCACCACACTTGGCTGCTTTGATCTTG GCTAGAGGAGGTTCTAAGGGTATCCCATTGAAGAACA TCAAGCACTTGGCTGGTGTTCCATTGATTGGATGGGTT TTGAGAGCTGCTTTGGACTCTGGTGCTTTCCAATCTGT TTGGGTTTCCACTGACCACGACGAGATTGAGAACGTT GCTAAGCAATTCGGTGCTCAGGTTCACAGAAGATCCT CTGAGGTTTCCAAGGACTCTTCTACTTCCTTGGACGCT ATCATCGAGTTCTTGAACTACCACAACGAGGTTGACA TCGTTGGTAACATCCAAGCTACTTCCCCATGTTTGCAC CCAACTGACTTGCAAAAAGTTGCTGAGATGATCAGAG AAGAGGGTTACGACTCCGTTTTCTCCGTTGTTAGAAGG CACCAGTTCAGATGGTCCGAGATTCAGAAGGGTGTTA GAGAGGTTACAGAGCCATTGAACTTGAACCCAGCTAA AAGACCAAGAAGGCAGGATTGGGACGGTGAATTGTAC GAAAACGGTTCCTTCTACTTCGCTAAGAGACACTTGAT CGAGATGGGATACTTGCAAGGTGGAAAGATGGCTTAC TACGAGATGAGAGCTGAACACTCCGTTGACATCGACG TTGATATCGACTGGCCAATTGCTGAGCAGAGAGTTTT GAGATACGGTTACTTCGGAAAGGAGAAGTTGAAGGAG ATCAAGTTGTTGGTTTGTAACATCGACGGTTGTTTGAC TAACGGTCACATCTACGTTTCTGGTGACCAGAAGGAG ATTATCTCCTACGACGTTAAGGACGCTATTGGTATCTC CTTGTTGAAGAAGTCCGGTATCGAAGTTAGATTGATCT CCGAGAGAGCTTGTTCCAAGCAAACATTGTCCTCTTTG AAGTTGGACTGTAAGATGGAGGTTTCCGTTTCTGACA AGTTGGCTGTTGTTGACGAATGGAGAAAGGAGATGGG TTTGTGTTGGAAGGAAGTTGCTTACTTGGGTAACGAA GTTTCTGACGAGGAGTGTTTGAAGAGAGTTGGTTTGTC TGGTGCTCCAGCTGATGCTTGTTCCACTGCTCAAAAGG CTGTTGGTTACATCTGTAAGTGTAACGGTGGTAGAGGT GCTATTAGAGAGTTCGCTGAGCACATCTGTTTGTTGAT GGAGAAAGTTAATAACTCCTGTCAGAAGTAGTAG

81	Human N-acetylneuraminate-9-phosphate synthase (HsSPS) codon optimized	ATGCCATTGGAATTGGAGTTGTGTCCTGGTAGATGGGT TGGTGGTCAACACCCATGTTTCATCATCGCTGAGATCG GTCAAAACCACCAAGGAGACTTGGACGTTGCTAAGAG AATGATCAGAATGGCTAAGGAATGTGGTGCTGACTGT GCTAAGTTCCAGAAGTCCGAGTTGGAGTTCAAGTTCA ACAGAAAGGCTTTGGAAAGACCATACACTTCCAAGCA CTCTTGGGGAAAGACTTACGGAGAACACAAGAGACAC TTGGAGTTCTCTCACGACCAATACAGAGAGTTGCAGA GATACGCTGAGGAAGTTGGTATCTTCTTCACTGCTTCT GGAATGGACGAAATGGCTGTTGAGTTCTTGCACGAGT TGAACGTTCCATTCTTCAAAGTTGGTTCCGGTGACACT AACAACTTCCCATACTTGGAAAAGACTGCTAAGAAAG GTAGACCAATGGTTATCTCCTCTGGAATGCAGTCTATG GACACTATGAAGCAGGTTTACCAGATCGTTAAGCCAT TGAACCCAAACTTTTGTTTCTTGCAGTGTACTTCCGCT TACCCATTGCAACCAGAGGACGTTAATTTGAGAGTTA TCTCCGAGTACCAGAAGTTGTTCCCAGACATCCCAATT GGTTACTCTGGTCACGAGACTGGTATTGCTATTTCCGT TGCTGCTGTTGCTTTGGGTGCTAAGGTTTTGGAGAGAC ACATCACTTTGGACAAGACTTGGAAGGGTTCTGATCA CTCTGCTTCTTTGGAACCTGGTGAGTTGGCTGAACTTG TTAGATCAGTTAGATTGGTTGAGAGAGCTTTGGGTTCC CCAACTAAGCAATTGTTGCCATGTGAGATGGCTTGTA ACGAGAAGTTGGGAAAGTCCGTTGTTGCTAAGGTTAA GATCCCAGAGGGTACTATCTTGACTATGGACATGTTG ACTGTTAAAGTTGGAGAGCCAAAGGGTTACCCACCAG AGGACATCTTTAACTTGGTTGGTAAAAAGGTTTTGGTT ACTGTTGAGGAGGACGACACTATTATGGAGGAGTTGG TTGACAACCACGGAAAGAAGATCAAGTCCTAG

82	Mouse alpha-2,6-sialyl transferase catalytic domain (MmmST6) codon optimized	GTTTTTCAAATGCCAAAGTCCCAGGAGAAAGTTGCTG TTGGTCCAGCTCCACAAGCTGTTTTCTCCAACTCCAAG CAAGATCCAAAGGAGGGTGTTCAAATCTTGTCCTACC CAAGAGTTACTGCTAAGGTTAAGCCACAACCATCCTT GCAAGTTTGGGACAAGGACTCCACTTACTCCAAGTTG AACCCAAGATTGTTGAAGATTTGGAGAAACTACTTGA ACATGAACAAGTACAAGGTTTCCTACAAGGGTCCAGG TCCAGGTGTTAAGTTCTCCGTTGAGGCTTTGAGATGTC ACTTGAGAGACCACGTTAACGTTTCCATGATCGAGGC TACTGACTTCCCATTCAACACTACTGAATGGGAGGGA TACTTGCCAAAGGAGAACTTCAGAACTAAGGCTGGTC CATGGCATAAGTGTGCTGTTGTTTCTTCTGCTGGTTCC TTGAAGAACTCCCAGTTGGGTAGAGAAATTGACAACC ACGACGCTGTTTTGAGATTCAACGGTGCTCCAACTGA CAACTTCCAGCAGGATGTTGGTACTAAGACTACTATC AGATTGGTTAACTCCCAATTGGTTACTACTGAGAAGA GATTCTTGAAGGACTCCTTGTACACTGAGGGAATCTTG ATTTTGTGGGACCCATCTGTTTACCACGCTGACATTCC ACAATGGTATCAGAAGCCAGACTACAACTTCTTCGAG ACTTACAAGTCCTACAGAAGATTGCACCCATCCCAGC CATTCTACATCTTGAAGCCACAAATGCCATGGGAATT GTGGGACATCATCCAGGAAATTTCCCCAGACTTGATC CAACCAAACCCACCATCTTCTGGAATGTTGGGTATCAT CATCATGATGACTTTGTGTGACCAGGTTGACATCTACG AGTTCTTGCCATCCAAGAGAAAGACTGATGTTTGTTAC TACCACCAGAAGTTCTTCGACTCCGCTTGTACTATGGG AGCTTACCACCCATTGTTGTTCGAGAAGAACATGGTT AAGCACTTGAACGAAGGTACTGACGAGGACATCTACT TGTTCGGAAAGGCTACTTTGTCCGGTTTCAGAAACAA CAGATGTTAG

83	*Pichia pastoris* Pp TRP2: 5' and ORF	ACTGGGCCTTTAGAGGGTGCTGAAGTTGACCCCTTGG TGCTTCTGGAAAAAGAACTGAAGGGCACCAGACAAGC GCAACTTCCTGGTATTCCTCGTCTAAGTGGTGGTGCCA TAGGATACATCTCGTACGATTGTATTAAGTACTTTGAA CCAAAAACTGAAAGAAAACTGAAAGATGTTTTGCAAC TTCCGGAAGCAGCTTTGATGTTGTTCGACACGATCGTG GCTTTTGACAATGTTTATCAAAGATTCCAGGTAATTGG AAACGTTTCTCTATCCGTTGATGACTCGGACGAAGCTA TTCTTGAGAAATATTATAAGACAAGAGAAGAAGTGGA AAAGATCAGTAAAGTGGTATTTGACAATAAAACTGTT CCCTACTATGAACAGAAAGATATTATTCAAGGCCAAA CGTTCACCTCTAATATTGGTCAGGAAGGGTATGAAAA CCATGTTCGCAAGCTGAAAGAACATATTCTGAAAGGA GACATCTTCCAAGCTGTTCCCTCTCAAAGGGTAGCCA GGCCGACCTCATTGCACCCTTTCAACATCTATCGTCAT TTGAGAACTGTCAATCCTTCTCCATACATGTTCTATAT TGACTATCTAGACTTCCAAGTTGTTGGTGCTTCACCTG AATTACTAGTTAAATCCGACAACAACAACAAAATCAT CACACATCCTATTGCTGGAACTCTTCCCAGAGGTAAA ACTATCGAAGAGGACGACAATTATGCTAAGCAATTGA AGTCGTCTTTGAAAGACAGGGCCGAGCACGTCATGCT GGTAGATTTGGCCAGAAATGATATTAACCGTGTGTGT GAGCCCACCAGTACCACGGTTGATCGTTTATTGACTGT GGAGAGATTTTCTCATGTGATGCATCTTGTGTCAGAAG TCAGTGGAACATTGAGACCAAACAAGACTCGCTTCGA TGCTTTCAGATCCATTTTCCCAGCAGGTACCGTCTCCG GTGCTCCGAAGGTAAGAGCAATGCAACTCATAGGAGA ATTGGAAGGAGAAAAGAGAGGTGTTTATGCGGGGGCC GTAGGACACTGGTCGTACGATGGAAAATCGATGGACA CATGTATTGCCTTAAGAACAATGGTCGTCAAGGACGG TGTCGCTTACCTTCAAGCCGGAGGTGGAATTGTCTACG ATTCTGACCCCTATGACGAGTACATCGAAACCATGAA CAAAATGAGATCCAACAATAACACCATCTTGGAGGCT GAGAAAATCTGGACCGATAGGTTGGCCAGAGACGAG AATCAAAGTGAATCCGAAGAAAACGATCAATGA

84	*Pichia pastoris* PpTRP2 3' region	ACGGAGGACGTAAGTAGGAATTTATGTAATCATGCCA ATACATCTTTAGATTTCTTCCTCTTCTTTTTAACGAAAG ACCTCCAGTTTTGCACTCTCGACTCTCTAGTATCTTCC CATTTCTGTTGCTGCAACCTCTTGCCTTCTGTTTCCTTC AATTGTTCTTCTTTCTTCTGTTGCACTTGGCCTTCTTCC TCCATCTTTCGTTTTTTTTCAAGCCTTTTCAGCAGTTCT TCTTCCAAGAGCAGTTCTTTGATTTTCTCTCTCCAATCC ACCAAAAAAACTGGATGAATTCAACCGGGCATCATCAA TGTTCCACTTTCTTTCTCTTATCAATAATCTACGTGCTT CGGCATACGAGGAATCCAGTTGCTCCCTAATCGAGTC ATCCACAAGGTTAGCATGGGCCTTTTTCAGGGTGTCA AAAGCATCTGGAGCTCGTTTATTCGGAGTCTTGTCTGG ATGGATCAGCAAAGACTTTTTGCGGAAAGTCTTTCTTA TATCTTCCGGAGAACAACCTGGTTTCAAATCCAAGAT GGCATAGCTGTCCAATTTGAAAGTGGAAAGAATCCTG CCAATTTCCTTCTCTCGTGTCAGCTCGTTCTCCTCCTTT TGCAACAGGTCCACTTCATCTGGCATTTTTCTTTATGT TAACTTTAATTATTATTAATTATAAAGTTGATTATCGT TATCAAAATAATCATATTCGAGAAATAATCCGTCCAT GCAATATATAAATAAGAATTCATAATAATGTAATGAT AACAGTACCTCTGATGACCTTTGATGAACCGCAATTTT CTTTCCAATGACAAGACATCCCTATAATACAATTATAC AGTTTATATATCACAAATAATCACCTTTTTATAAGAAA ACCGTCCTCTCCGTAACAGAACTTATTATCCGCACGTT ATGGTTAACACACTACTAATACCGATATAGTGTATGA AGTCGCTACGAGATAGCCATCCAGGAAACTTACCAAT TCATCAGCACTTTCATGATCCGATTGTTGGCTTTATTC TTTGCGAGACAGATACTTGCCAATGAAATAACTGATC CCACAGATGAGAATCCGGTGCTCGT
85	*Pichia pastoris* Sequence of the 5'-Region used for knock out of STE13	TTGGGGGCCTCCAGGACTTGCTGAAATTTGCTGACTCA TCTTCGCCATCCAAGGATAATGAGTTAGCTAATGTGA CAGTTAATGAGTCGTCTTGACTAACGGGGAACATTTC ATTATTTATATCCAGAGTCAATTTGATAGCAGAGTTTG TGGTTGAAATACCTATGATTCGGGAGACTTTGTTGTAA CGACCATTATCCACAGTTTGGACCGTGAAAATGTCAT CGAAGAGAGCAGACGACATATTATCTATTGTGGTAAG TGATAGTTGGAAGTCCGACTAAGGCATGAAAATGAGA AGACTGAAAATTTAAAGTTTTTGAAAACACTAATCGG GTAATAACTTGGAAATTACGTTTACGTGCCTTTAGCTC TTGTCCTTACCCCTGATAATCTATCCATTTCCCGAGAG ACAATGACATCTCGGACAGCTGAGAACCCGTTCGATA TAGAGCTTCAAGAGAATCTAAGTCCACGTTCTTCCAAT TCGTCCATATTGGAAAACATTAATGAGTATGCTAGAA GACATCGCAATGATTCGCTTTCCCAAGAATGTGATAA TGAAGATGAGAACGAAAATCTCAATTATACTGATAAC TTGGCCAAGTTTTCAAAGTCTGGAGTATCAAGAAAGA GCTGTATGCTAATATTTGGTATTTGCTTTGTTATCTGG CTGTTTCTCTTTGCCTTGTATGCGAGGGACAATCGATT TTCCAATTTGAACGAGTACGTTCCAGATTCAAACAG

86	*Pichia pastoris* Sequence of the 3'-Region used for knock out of STE13	CTACTGGGAACCACGAGACATCACTGCAGTAGTTTCC AAGTGGATTTCAGATCACTCATTTGTGAATCCTGACAA AACTGCGATATGGGGGTGGTCTTACGGTGGGTTCACT ACGCTTAAGACATTGGAATATGATTCTGGAGAGGTTTT CAAATATGGTATGGCTGTTGCTCCAGTAACTAATTGGC TTTTGTATGACTCCATCTACACTGAAAGATACATGAAC CTTCCAAAGGACAATGTTGAAGGCTACAGTGAACACA GCGTCATTAAGAAGGTTTCCAATTTTAAGAATGTAAA CCGATTCTTGGTTTGTCACGGGACTACTGATGATAACG TGCATTTTCAGAACACACTAACCTTACTGGACCAGTTC AATATTAATGGTGTTGTGAATTACGATCTTCAGGTGTA TCCCGACAGTGAACATAGCATTGCCCATCACAACGCA AATAAAGTGATCTACGAGAGGTTATTCAAGTGGTTAG AGCGGGCATTTAACGATAGATTTTTGTAACATTCCGTA CTTCATGCCATACTATATATCCTGCAAGGTTTCCCTTT CAGACACAATAATTGCTTTGCAATTTTACATACCACCA ATTGGCAAAAATAATCTCTTCAGTAAGTTGAATGCTTT TCAAGCCAGCACCGTGAGAAATTGCTACAGCGCGCAT TCTAACATCACTTTAAAATTCCCTCGCCGGTGCTCACT GGAGTTTCCAACCCTTAGCTTATCAAAATCGGGTGAT AACTCTGAGTTTTTTTTTTCACTTCTATTCCTAAACCTT CGCCCAATGCTACCACCTCCAATCAACATCCCGAAAT GGATAGAAGAGAATGGACATCTCTTGCAACCTCCGGT TAATAATTACTGTCTCCACAGAGGAGGATTTACGGTA ATGATTGTAGGTGGGCCTAATG

87	*Pichia pastoris* Sequence of the 5'-Region used for knock out of DAP2	CACCTGGGCCTGTTGCTGCTGGTACTGCTGTTGGAACT GTTGGTATTGTTGCTGATCTAAGGCCGCCTGTTCCACA CCGTGTGTATCGAATGCTTGGGCAAAATCATCGCCTG CCGGAGGCCCCACTACCGCTTGTTCCTCCTGCTCTTGT TTGTTTTGCTCATTGATGATATCGGCGTCAATGAATTG ATCCTCAATCGTGTGGTGGTGGTGTCGTGATTCCTCTT CTTTCTTGAGTGCCTTATCCATATTCCTATCTTAGTGTA CCAATAATTTTGTTAAACACACGCTGTTGTTTATGAAA AGTCGTCAAAAGGTTAAAAATTCTACTTGGTGTGTGTC AGAGAAAGTAGTGCAGACCCCCAGTTTGTTGACTAGT TGAGAAGGCGGCTCACTATTGCGCGAATAGCATGAGA AATTTGCAAACATCTGGCAAAGTGGTCAATACCTGCC AACCTGCCAATCTTCGCGACGGAGGCTGTTAAGCGGG TTGGGTTCCCAAAGTGAATGGATATTACGGGCAGGAA AAACAGCCCCTTCCACACTAGTCTTTGCTACTGACATC TTCCCTCTCATGTATCCCGAACACAAGTATCGGGAGTA TCAACGGAGGGTGCCCTTATGGCAGTACTCCCTGTTG GTGATTGTACTGCTATACGGGTCTCATTTGCTTATCAG CACCATCAACTTGATACACTATAACCACAAAAATTAT CATGCACACCCAGTCAATAGTGGTATCGTTCTTAATGA GTTTGCTGATGACGATTCATTCTCTTTGAATGGCACTC TGAACTTGGAGAACTGGAGAAATGGTACCTTTTCCCC TAAATTTCATTCCATTCAGTGGACCGAAATAGGTCAG GAAGATGACCAGGGATATTACATTCTCTCTTCCAATTC CTCTTACATAGTAAAGTCTTTATCCGACCCAGACTTTG AATCTGTTCTATTCAACGAGTCTACAATCACTTACAAC G

88	*Pichia pastoris* Sequence of the 3'-Region used for knock out of DAP2	GGCAGCAAAGCCTTACGTTGATGAGAATAGACTGGCC ATTTGGGGTTGGTCTTATGGAGGTTACATGACGCTAAA GGTTTTAGAACAGGATAAAGGTGAAACATTCAAATAT GGAATGTCTGTTGCCCCTGTGACGAATTGGAAATTCTA TGATTCTATCTACACAGAAAGATACATGCACACTCCTC AGGACAATCCAAACTATTATAATTCGTCAATCCATGA GATTGATAATTTGAAGGGAGTGAAGAGGTTCTTGCTA ATGCACGGAACTGGTGACGACAATGTTCACTTCCAAA ATACACTCAAAGTTCTAGATTTATTTGATTTACATGGT CTTGAAAACTATGATATCCACGTGTTCCCTGATAGTGA TCACAGTATTAGATATCACAACGGTAATGTTATAGTGT ATGATAAGCTATTCCATTGGATTAGGCGTGCATTCAA GGCTGGCAAATAAATAGGTGCAAAAATATTATTAGAC TTTTTTTTTCGTTCGCAAGTTATTACTGTGTACCATACC GATCCAATCCGTATTGTAATTCATGTTCTAGATCCAAA ATTTGGGACTCTAATTCATGAGGTCTAGGAAGATGAT CATCTCTATAGTTTTCAGCGGGGGGCTCGATTTGCGGT TGGTCAAAGCTAACATCAAAATGTTTGTCAGGTTCAG TGAATGGTAACTGCTGCTCTTGAATTGGTCGTCTGACA AATTCTCTAAGTGATAGCACTTCATCTACAATCATTTG CTTCATCGTTTCTATATCGTCCACGACCTCAAACGAGA AATCGAATTTGGAAGAACAGACGGGCTCATCGTTAGG ATCATGCCAAACCTTGAGATATGGATGCTCTAAAGCC TCAGTAACTGTAATTCTGTGAGTGGGATCTACCGTGA GCATTCGATCCAGTAAGTCTATCGCTTCAGGGTTGGCA CCGGGAAATAACTGGCTGAATGGGATCTTGGGCATGA ATGGCAGGGAGCGAACATAATCCTGGGCACGCTCTGA TCTGATAGACTGAAGTGTCTCTTCCGAAACAGTACCC AGCGTACTCAAAATCAAGTTCAATTGATCCACATAGT CTCTTCCTCTAAAAATGGGTCGGCCACCTA

89	*Escherichia coli* HYG^R resistance cassette	GATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCG GCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACA GTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTG TCGCCCGTACATTTAGCCCATACATCCCCATGTATAAT CATTTGCATCCATACATTTTGATGGCCGCACGGCGCGA AGCAAAAATTACGGCTCCTCGCTGCGGACCTGCGAGC AGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCC CCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAG GATTTGCCACTGAGGTTCTTCTTTCATATACTTCCTTTT AAAATCTTGCTAGGATACAGTTCTCACATCACATCCG AACATAAACAACCATGGGTAAAAAGCCTGAACTCACC GCGACGTCTGTCGAGAAGTTTCTGATCGAAAAGTTCG ACAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGA AGAATCTCGTGCTTTCAGCTTCGATGTAGGAGGGCGT GGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTT TCTACAAAGATCGTTATGTTTATCGGCACTTTGCATCG GCCGCGCTCCCGATTCCGGAAGTGCTTGACATTGGGG AATTCAGCGAGAGCCTGACCTATTGCATCTCCCGCCGT GCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCG AACTGCCCGCTGTTCTGCAGCCGGTCGCGGAGGCCAT GGATGCGATCGCTGCGGCCGATCTTAGCCAGACGAGC GGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAAT ACACTACATGGCGTGATTTCATATGCGCGATTGCTGAT CCCCATGTGTATCACTGGCAAACTGTGATGGACGACA CCGTCAGTGCGTCCGTCGCGCAGGCTCTCGATGAGCT GATGCTTTGGGCCGAGGACTGCCCCGAAGTCCGGCAC CTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGAC GGACAATGGCCGCATAACAGCGGTCATTGACTGGAGC GAGGCGATGTTCGGGGATTCCCAATACGAGGTCGCCA ACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAG CAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAGC TTGCAGGATCGCCGCGGCTCCGGGCGTATATGCTCCG CATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACG GCAATTTCGATGATGCAGCTTGGGCGCAGGGTCGATG CGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGG CGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGA CCGATGGCTGTGTAGAAGTACTCGCCGATAGTGGAAA CCGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAA TCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAA CTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCT ATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCG CCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCG CAGAAAGTAATATCATGCGTCAATCGTATGTGAATGC TGGTCGCTATACTGCTGTCGATTCGATACTAACGCCGC CATCCAGTGTCGAAAACGAGCT

90	*Pichia pastoris* Sequence of *PpTRP5* 5' integration fragment	ACGACGGCCAAATTCATGATACACACTCTGTTTCAGCT GGTTTGGACTACCCTGGAGTTGGTCCTGAATTGGCTGC CTGGAAAGCAAATGGTAGAGCCCAATTTTCCGCTGTA ACTGATGCCCAAGCATTAGAGGGATTCAAAATCCTGT CTCAATTGGAAGGGATCATTCCAGCACTAGAGTCTAG TCATGCAATCTACGGCGCATTGCAAATTGCAAAGACT ATGTCTTCGGACCAGTCCTTAGTTATTAATGTATCTGG AAGGGGTGATAAGGACGTCCAGAGTGTAGCTGAGATT TTACCTAAATTGGGACCTCAAATTGGATGGGATTTGC GTTTCAGCGAAGACATTACTAAAGAGTGA
91	*Pichia pastoris* Sequence of *PpTRP5* 3' integration fragment	TCGATAGCACAATATTCAACTTGACTGGGTGTTAAGA ACTAAGAGCTCTGGGAAACTTTGTATTTATTACTACCA ACACAGTCAAATTATTGGATGTGTTTTTTTTTCCAGTA CATTTCACTGAGCAGTTTGTTATACTCGGTCTTTAATC TCCATATACATGCAGATTGTAATACAGATCTGAACAG TTTGATTCTGATTGATCTTGCCACCAATATTCTATTTTT GTATCAAGTAACAGAGTCAATGATCATTGGTAACGTA ACGGTTTTCGTGTATAGTAGTTAGAGCCCATCTTGTAA CCTCATTTCCTCCCATATTAAAGTATCAGTGATTCGCT GGAACGATTAACTAAGAAAAAAAAAATATCTGCACAT ACTCATCAGTCTGTAAATCTAAGTCAAAACTGCTGTAT CCAATAGAAATCGGGATATACCTGGATGTTTTTTCCAC ATAAACAAACGGGAGTTCAGCTTACTTATGGTGTTGA TGCAATTCAGTATGATCCTACCAATAAAACGAAACTT TGGGATTTTGGCTGTTTGAGGGATCAAAAGCTGCACC TTTACAAGATTGACGGATCGACCATTAGACCAAAGCA AATGGCCACCAA
92	*Saccharomyces cerecisiae* Yps1ss	MKLKTVRSAVLSSLFASQVLG
93	Synthetic construct TA57 pro	QPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGLI SMAKR
94	Synthetic construct N-terminal spacer	EEGEPK
95	Synthetic construct Glycosylated insulin B chain P28N	FVNQHLCGSHLVEALYLVCGERGFFYTNKT
96	Human insulin A chain	GIVEQCCTSICSLYQLENYCN

97	Synthetic construct Pre-proinsulin analogue: Yps1ss+TA57 propeptide+N-terminal spacer+B chain P28N+C-peptide "AAK"+ insulin A chain	MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMAD DTESAFATQTNSGGLDVVGLISMAKREEGEPKFVNQHLC GSHLVEALYLVCGERGFFYTNKTAAKGIVEQCCTSICSLY QLENYCN
98	Synthetic construct DNA encoding pre-proinsulin analogue: Yps1ss+TA57 propeptide+N-terminal spacer+B chain P28N+C-peptide "AAK"+ insulin A chain	ATGAAGTTGAAGACTGTTAGATCCGCTGTTTTGTCTTC TTTGTTTGCTTCTCAAGTTTTGGGTCAACCAATTGATG ATACTGAATCTCAAACTACTTCTGTTAACTTGATGGCT GATGATACTGAATCTGCTTTTGCTACTCAAACTAACTC TGGTGGTTTGGATGTTGTTGGTTTGATTTCTATGGCTA AGAGAGAAGAAGGTGAACCAAAGTTTGTTAACCAACA TTTGTGTGGTTCTCATTTGGTTGAAGCTTTGTACTTGGT TTGTGGTGAAAGAGGTTTTTTTTACACTAACAAGACTG CTGCTAAGGGTATTGTTGAACAATGTTGTACTTCTATT TGTTCTTTGTACCAATTGGAAAACTACTGTAACTAA

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes. All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

The present invention is not to be limited in scope by the specific embodiments described herein; the embodiments specifically set forth herein are not necessarily intended to be exhaustive. Indeed, various modifications of the invention in addition to those described herein will

become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated modified *Pichia* sp. host cell wherein the host cell has been modified to reduce or eliminate expression of a functional gene product of a nucleic acid sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:76.

2. The host cell of claim 1, wherein said modified host cell comprises a disruption or deletion in the nucleic acid sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:76.

3. The host cell of claim 1, which further comprises disruption or deletion of one or more of a functional gene products encoding an alpha-1,6-mannosyltransferase activity, mannosylphosphate transferase activity, β-mannosyltransferase activity, or a dolichol-P-Man dependent alpha(1-3) mannosyltransferaseactivity.

4. The host cell of claim 1, further comprising one or more nucleic acid sequences of interest.

5. The host cell of claim 4, wherein the nucleic acid sequences of interest encode one or more glycosylation enzymes or oligosaccharyltransferases.

6. The host cell of claim 5, wherein the glycosylation enzymes are selected from the group consisting of glycosidases, mannosidases, phosphomannosidases, phosphatases, nucleotide sugar transporters, mannosyltransferases, the N-acetylglucosaminyltransferases, the UDP-N-acetylglucosamine transporters, the galactosyltransferases, the sialyltransferases, the protein mannosyltransferases, and the oligosaccharyltransferases STT3A, STT3B, STT3C and STT3D.

7. The host cell of claim 6, wherein the nucleic acid sequences of interest encode one or more therapeutic proteins.

8. The host cell of claim 7, wherein the therapeutic proteins are selected from the group consisting of kringle domains of the human plasminogen, erythropoietin, cytokines, coagulation factors, soluble IgE receptor α-chain, IgG, IgG fragments, IgM, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α-1 antitrypsin, DNase II, α-feto proteins, insulin, Fc-fusions, and HSA-fusions.

9. The host cell of claim 7, wherein the cell is capable of expressing an increased amount of the therapeutic protein and wherein protein glycosylation quality of the therapeutic protein is improved compared with the XRN1 naïve parental host cell under similar culture conditions.

10. A *Pichia sp.* host cell comprising a disruption or deletion of the XRN1 gene in the genomic DNA of the host cell that encodes a protein having of a nucleic acid sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:76.

11. The host cell of claim 10, wherein the host cell further comprises disruption or deletion of one or more of a functional gene product encoding an alpha-1,6-mannosyltransferase activity, mannosylphosphate transferase activity, β-mannosyltransferase activity, or a dolichol-P-Man dependent alpha(1-3) mannosyltransferaseactivity.

12. The host cell of claim 10, further comprising one or more nucleic acid sequences of interest.

13. The host cell of claim 12, wherein the nucleic acid sequences of interest encode one or more glycosylation enzymes or oligosaccharyltransferases.

14. The host cell of claim 13, wherein the glycosylation enzymes or oligosaccharyltransferases are selected from the group consisting of glycosidases, mannosidases, phosphomannosidases, phosphatases, nucleotide sugar transporters, mannosyltransferases, the N-acetylglucosaminyltransferases, the UDP-N-acetylglucosamine transporters, the galactosyltransferases, the sialyltransferases, the protein mannosyltransferases, and the oligosaccharyltransferases STT3A, STT3B, STT3C and STT3D.

15. The host cell of claim 12, wherein the nucleic acid sequences of interest encode one or more therapeutic proteins.

16. The host cell of claim 15, wherein the therapeutic proteins are selected from the group consisting of kringle domains of the human plasminogen, erythropoietin, cytokines, coagulation factors, soluble IgE receptor α-chain, IgG, IgG fragments, IgM, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α-1 antitrypsin, DNase II, α-feto proteins, insulin, Fc-fusions, an immunoglobulin heavy chain, an immunoglobulin light chain, and HSA-fusions.

17. The host cell of claim 15, which is capable of expressing an increased amount of the therapeutic protein and wherein protein glycosylation quality of the therapeutic protein is improved compared with the XRN1 naïve parental host cell under similar culture conditions.

18. A method for producing a glycoprotein composition in an isolated *Pichia sp.* host cell, said method comprising growing said host cell of claim 1 under inducing conditions.

19. The method of claim 18, wherein said host cell is capable of expressing an increased amount of the therapeutic protein and wherein protein glycosylation quality of the therapeutic protein is improved compared with the XRN1 naïve parental host cell under similar culture conditions.

20. A method for producing glycoprotein compositions in *Pichia sp.* host cells, said method comprising growing said host cell of claim 10 under inducing conditions.

21. The method of claim 20, wherein said host cell is capable of expressing an increased amount of the therapeutic protein and wherein protein glycosylation quality of the therapeutic protein is improved compared with the XRN1 naïve parental host cell under similar culture conditions.

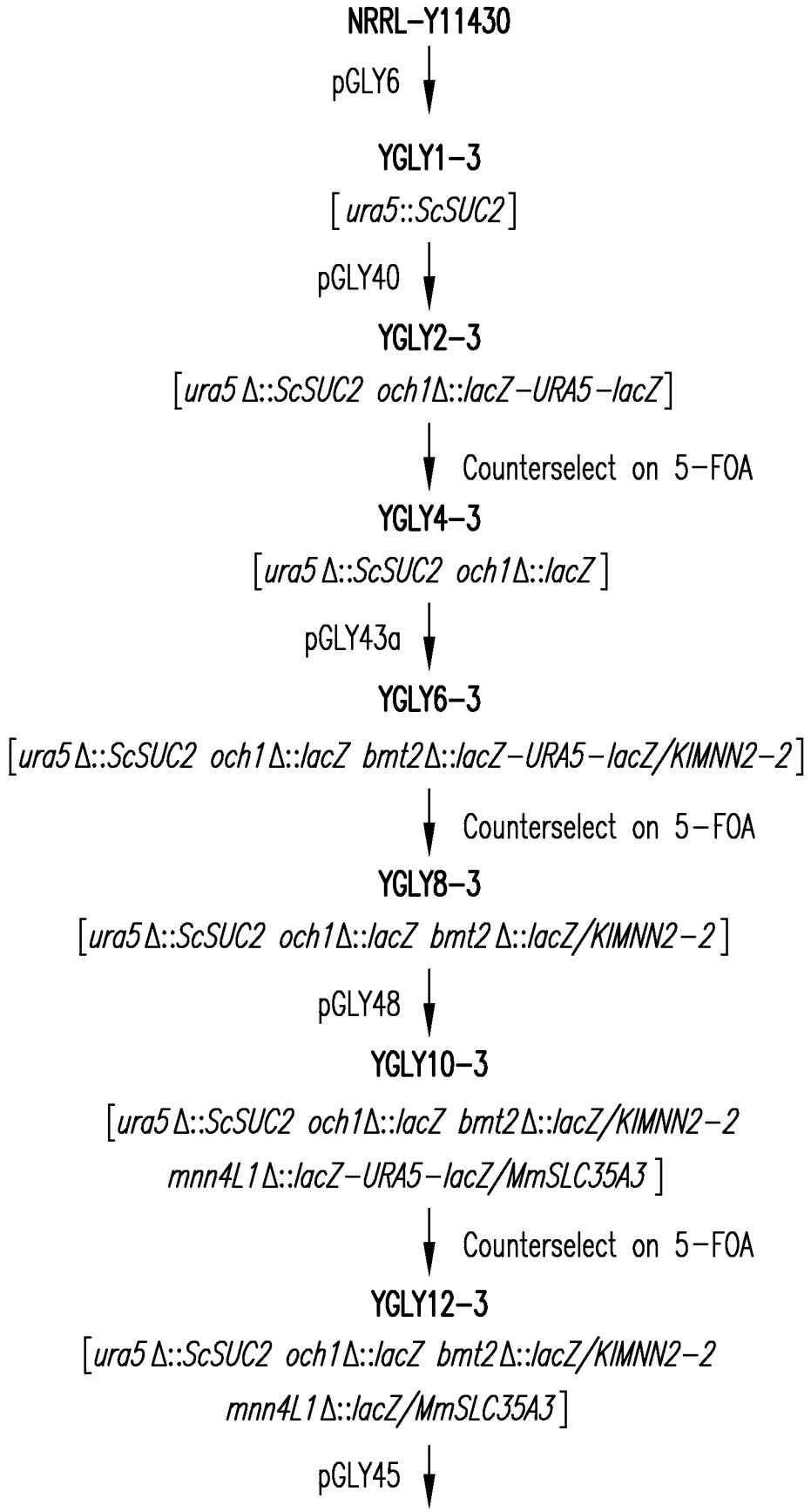

NRRL-Y11430
pGLY6
YGLY1-3
[ura5::ScSUC2]
pGLY40
YGLY2-3
[ura5Δ::ScSUC2 och1Δ::lacZ-URA5-lacZ]
Counterselect on 5-FOA
YGLY4-3
[ura5Δ::ScSUC2 och1Δ::lacZ]
pGLY43a
YGLY6-3
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ-URA5-lacZ/KlMNN2-2]
Counterselect on 5-FOA
YGLY8-3
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2]
pGLY48
YGLY10-3
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ-URA5-lacZ/MmSLC35A3]
Counterselect on 5-FOA
YGLY12-3
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3]
pGLY45

FIG.1A

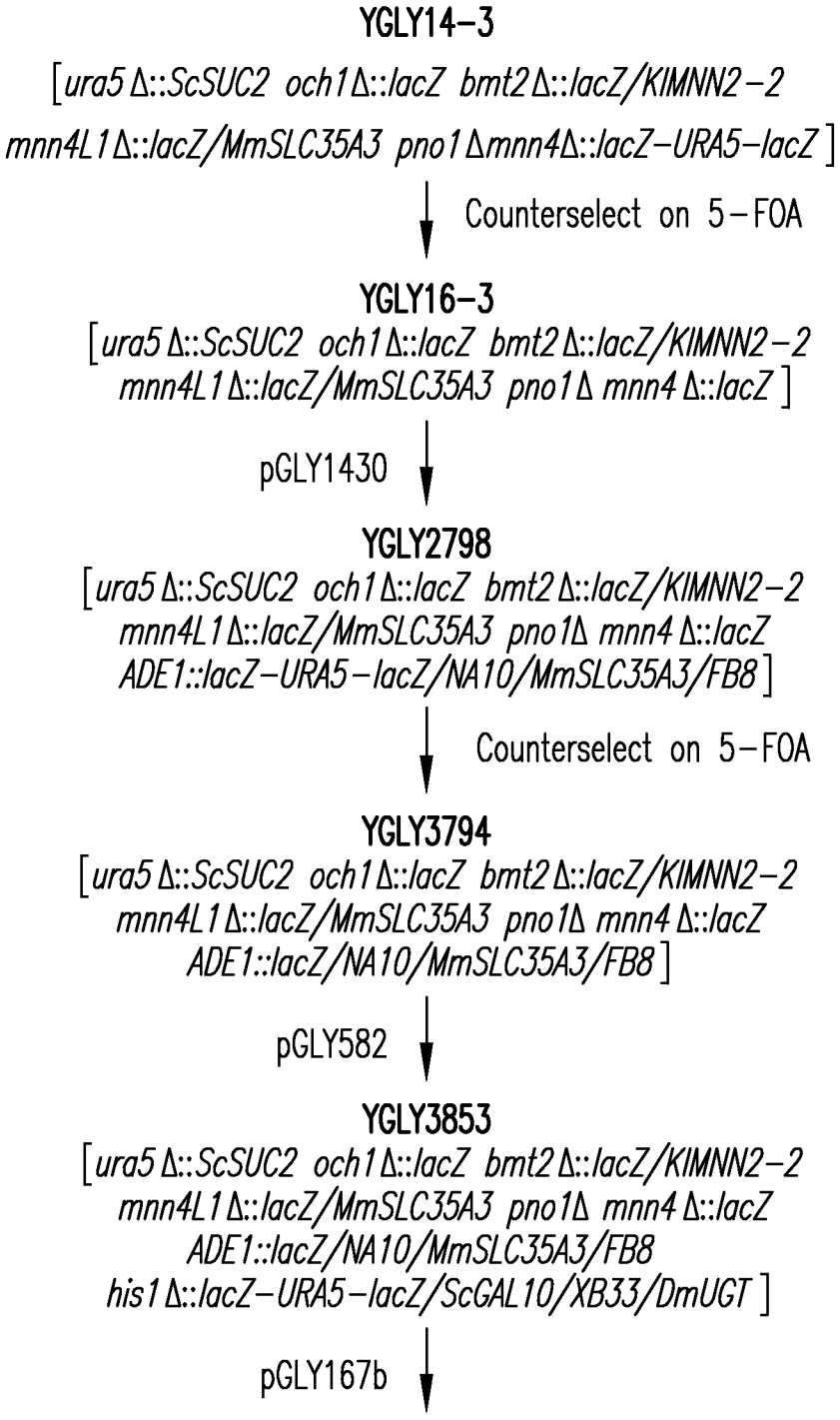

YGLY14-3
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δmnn4Δ::lacZ-URA5-lacZ]
Counterselect on 5-FOA
YGLY16-3
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ]
pGLY1430
YGLY2798
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ-URA5-lacZ/NA10/MmSLC35A3/FB8]
Counterselect on 5-FOA
YGLY3794
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8]
pGLY582
YGLY3853
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ-URA5-lacZ/ScGAL10/XB33/DmUGT]
pGLY167b

FIG.1B

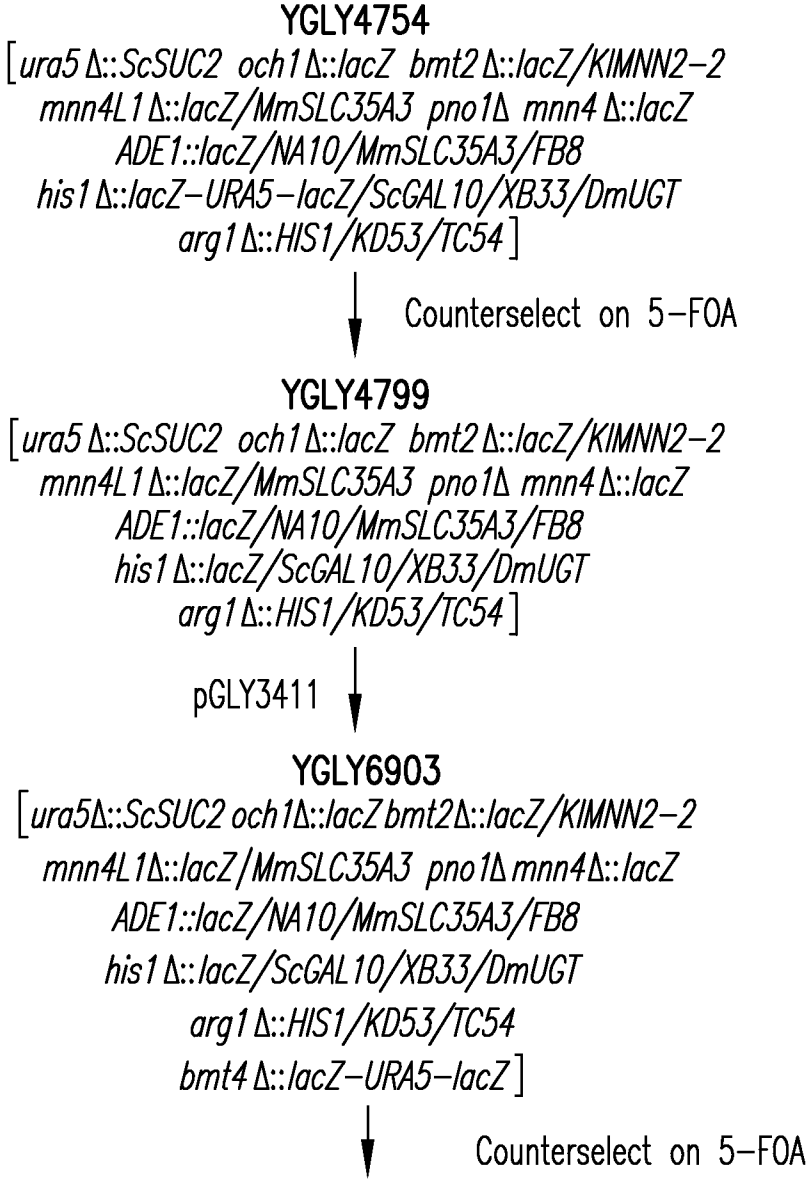

YGLY4754
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ-URA5-lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54]
Counterselect on 5-FOA
YGLY4799
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54]
pGLY3411
YGLY6903
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ-URA5-lacZ]
Counterselect on 5-FOA

FIG.1C

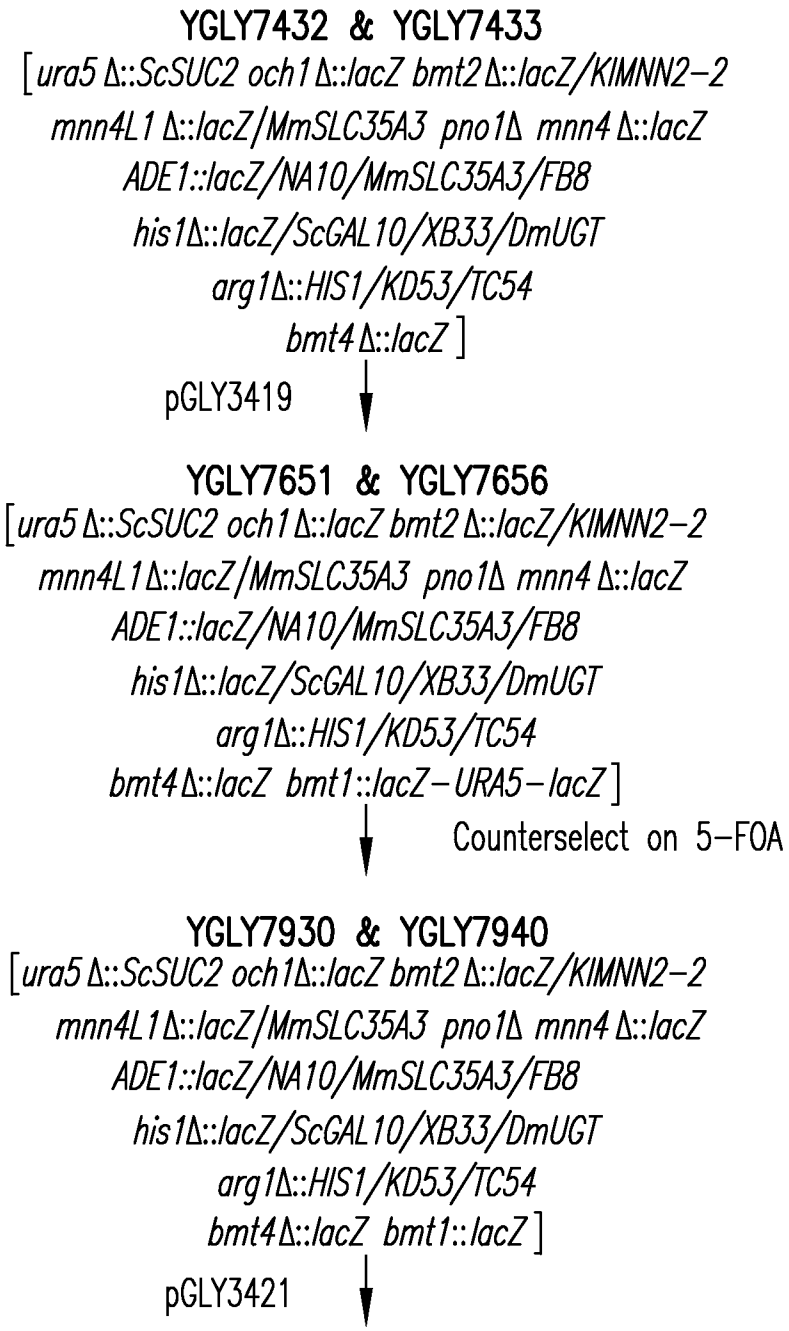

YGLY7432 & YGLY7433
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ]
pGLY3419
YGLY7651 & YGLY7656
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ-URA5-lacZ]
Counterselect on 5-FOA
YGLY7930 & YGLY7940
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ]
pGLY3421

FIG. 1D

YGLY7961 & YGLY7965

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KIMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ]

pGLY3673 ↓

YGLY8316 & YGLY8323

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KIMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]

FIG. 1E

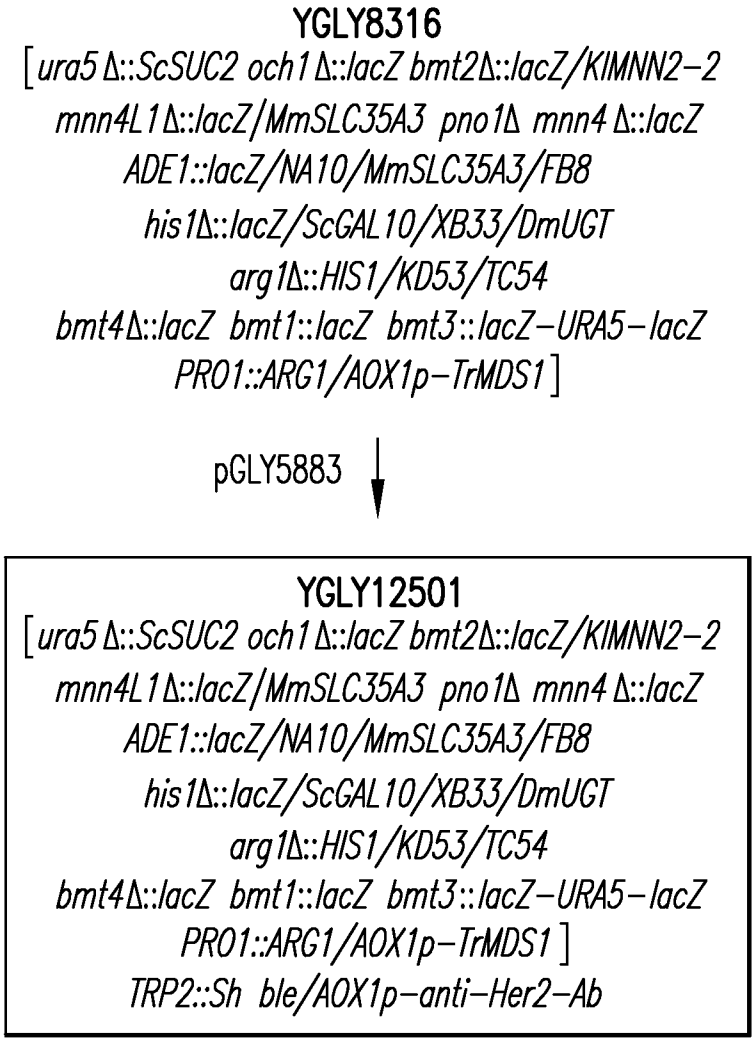

YGLY8316
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]
pGLY5883
YGLY12501
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]
TRP2::Sh ble/AOX1p-anti-Her2-Ab

FIG. 1F

YGLY8316
[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]

pGLY6833 ↓

YGLY13992
[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]
TRP2::Sh ble/AOX1p-anti-Her2-Ab

FIG.1G

YGLY8316
[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]

pGLY5883 ↓

YGLY12511
[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]
TRP2::Sh ble/AOX1p-anti-Her2-Ab

pGLY3714 ↓

YGLY14836
[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1::lacZ bmt3::lacZ-URA5-lacZ
PRO1::ARG1/AOX1p-TrMDS1]
TRP2::Sh ble/AOX1p-anti-Her2-Ab
TRP1::NAT/GD9

FIG. 1H

YGLY7961 (From Fig.1E)

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2 mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ ADE1::lacZ/NA10/MmSLC35A3/FB8 his1Δ::lacZ/ScGAL10/XB33/DmUGT arg1Δ::HIS1/KD53/TC54 bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ-URA5-lacZ*]

pGLY2456 ↓

YGLY8146

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2 mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ ADE1::lacZ/NA10/MmSLC35A3/FB8 his1Δ::lacZ/ScGAL10/XB33/DmUGT arg1Δ::HIS1/KD53/TC54 bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ-URA5-lacZ TRP2::ARG1/MmCST/HsGNE/HsCSS/HsSPS/MmST6-33*]

↓ Counterselect on 5-FOA

YGLY9296

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2 mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ ADE1::lacZ/NA10/MmSLC35A3/FB8 his1Δ::lacZ/ScGAL10/XB33/DmUGT arg1Δ::HIS1/KD53/TC54 bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ TRP2::ARG1/MmCST/HsGNE/HsCSS/HsSPS/MmST6-33*]

pGLY5048 ↓

FIG.2A


YGLY9469
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ
TRP2::ARG1/MmCST/HsGNE/HsCSS/HsSPS/MmST6-33
ste13Δ::lacZ-URA5-lacZ/TrMDS1]
pGLY5019
YGLY9797
[ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT
arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ
TRP2::ARG1/MmCST/HsGNE/HsCSS/HsSPS/MmST6-33
ste13Δ::lacZ-URA5-lacZ/TrMDS1 dap2Δ::NatR]
pGLY5085

FIG.2B

YGLY12900

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ
TRP2::ARG1/MmCST/HsGNE/HsCSS/HsSPS/MmST6-33
ste13Δ::lacZ-URA5-lacZ/TrMDS1 dap2Δ ::Nat^{R}
TRP5::Hyg^{R}MmCST/HsGNE/HsCSS/HsSPS/MmST6-33]

pGLY4362 ↓

YGLY21058

[*ura5Δ::ScSUC2 och1Δ::lacZ bmt2Δ::lacZ/KlMNN2-2*
mnn4L1Δ::lacZ/MmSLC35A3 pno1Δ mnn4Δ::lacZ
ADE1::lacZ/NA10/MmSLC35A3/FB8
his1Δ::lacZ/ScGAL10/XB33/DmUGT arg1Δ::HIS1/KD53/TC54
bmt4Δ::lacZ bmt1Δ::lacZ bmt3Δ::lacZ
TRP2::ARG1/MmCST/HsGNE/HsCSS/HsSPS/MmST6-33
ste13Δ::lacZ-URA5-lacZ/TrMDS1 dap2Δ ::Nat^{R}
TRP5::Hyg^{R}MmCST/HsGNE/HsCSS/HsSPS/MmST6-33
TRP2:Sh ble/AOX1p/insulin precursor variant]

FIG.2C

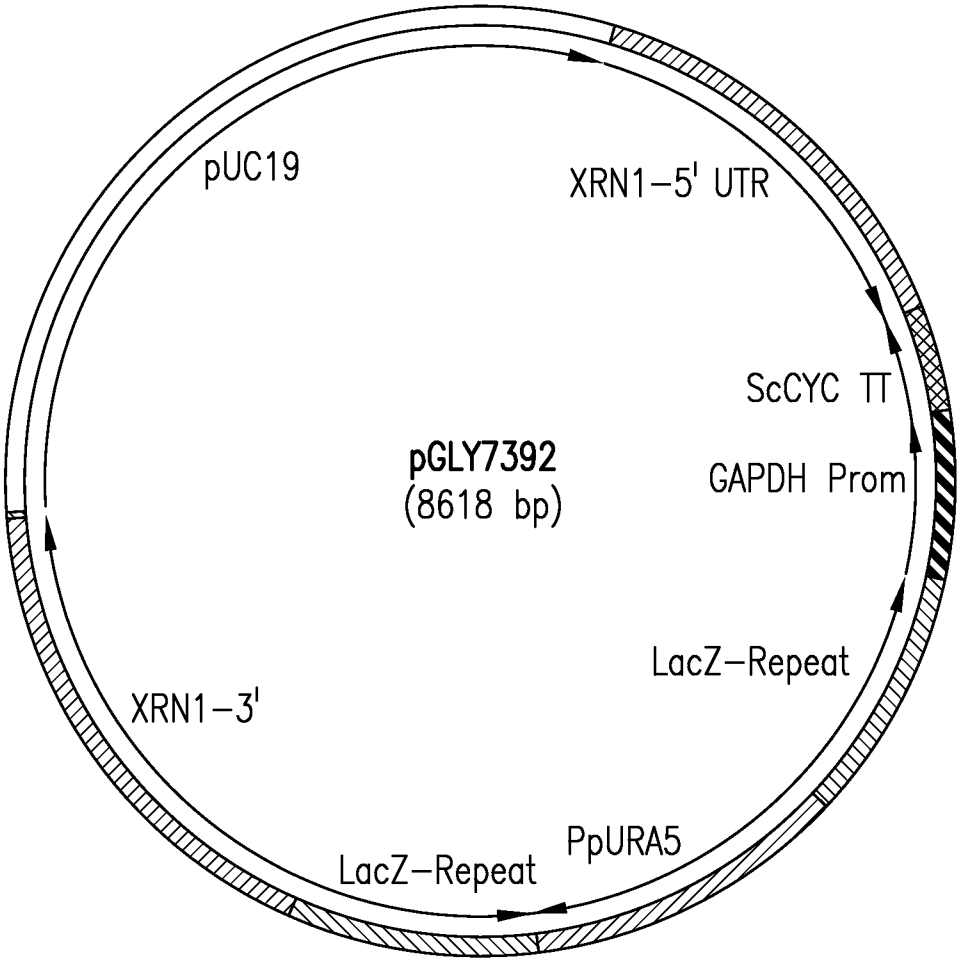

pUC19
XRN1–5' UTR
ScCYC TT
pGLY7392
(8618 bp)
GAPDH Prom
LacZ–Repeat
XRN1–3'
PpURA5
LacZ–Repeat

FIG.3

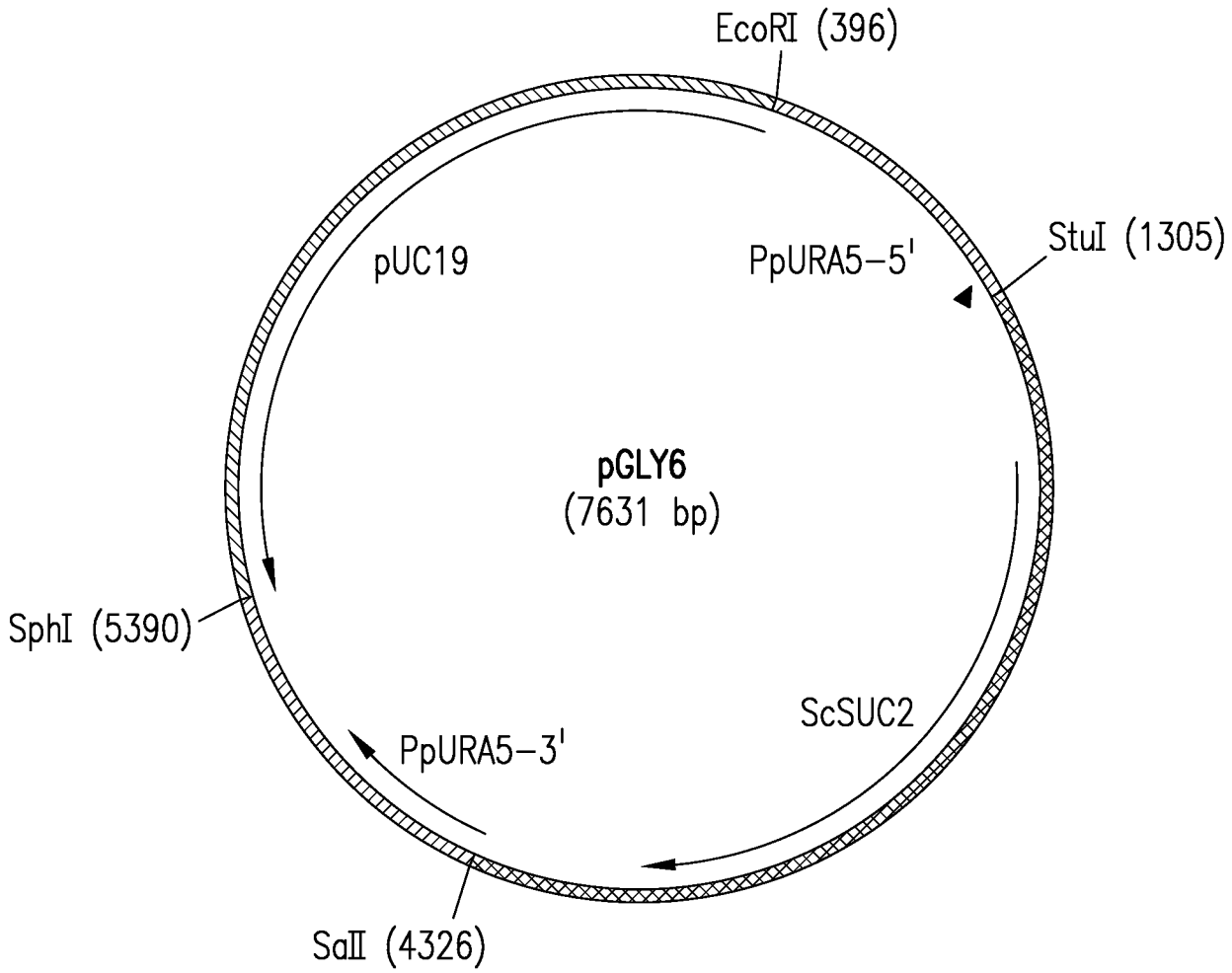

EcoRI (396)
StuI (1305)
pUC19
PpURA5-5'
pGLY6
(7631 bp)
SphI (5390)
ScSUC2
PpURA5-3'
SalI (4326)

FIG.4

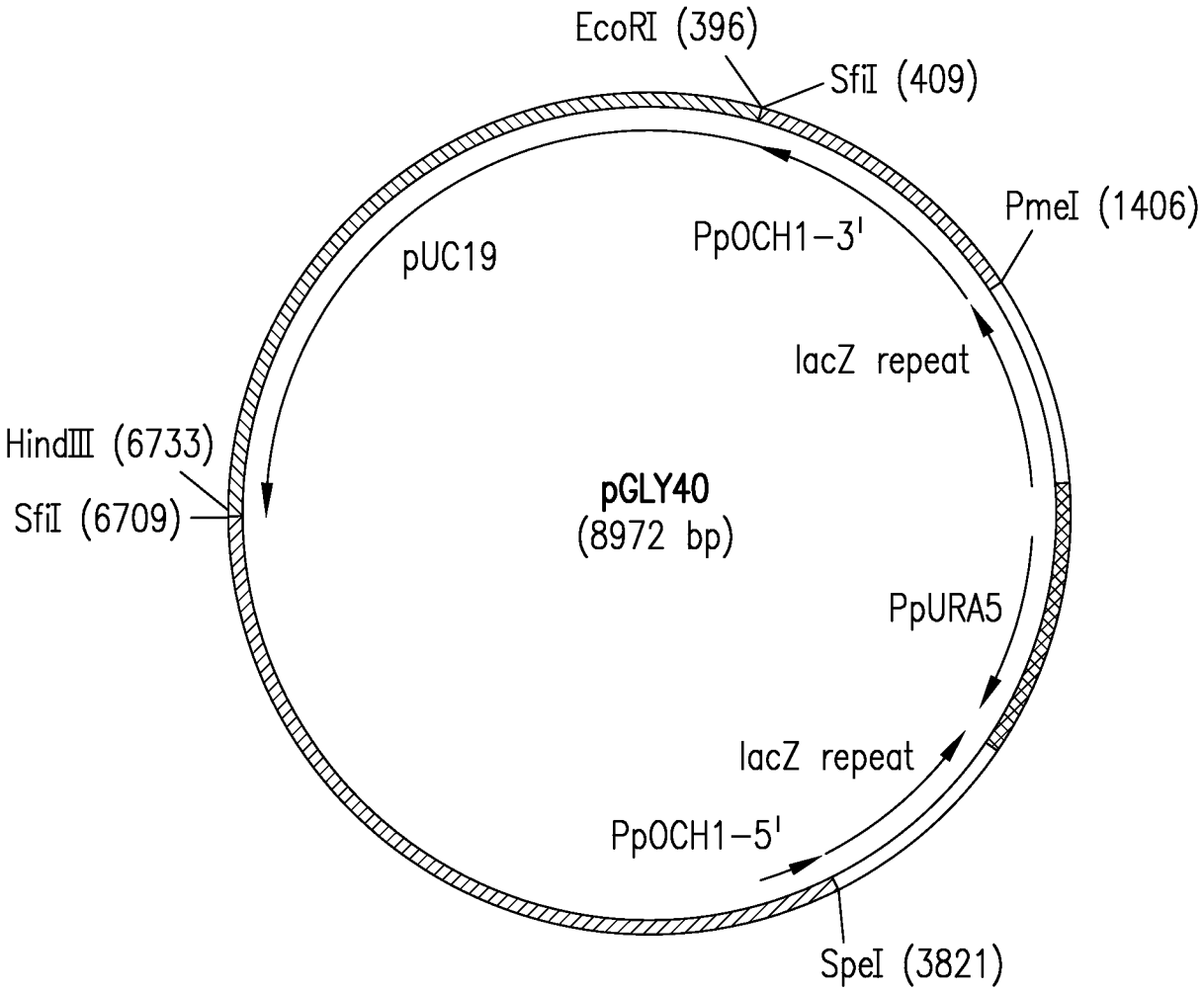

EcoRI (396)
SfiI (409)
PmeI (1406)
PpOCH1-3'
pUC19
lacZ repeat
HindIII (6733)
SfiI (6709)
pGLY40
(8972 bp)
PpURA5
lacZ repeat
PpOCH1-5'
SpeI (3821)

FIG.5

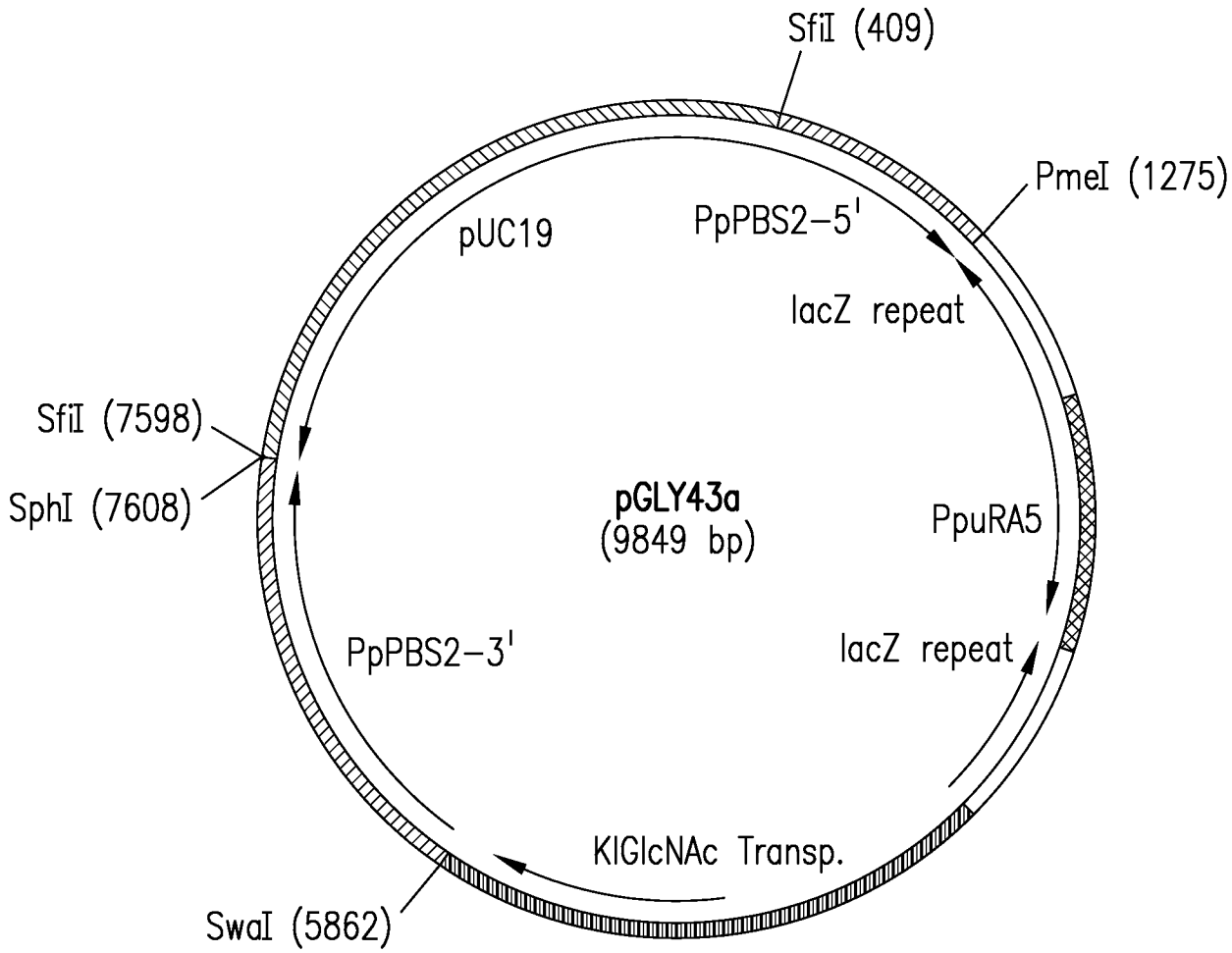

SfiI (409)
PmeI (1275)
pUC19
PpPBS2–5'
lacZ repeat
SfiI (7598)
SphI (7608)
pGLY43a
(9849 bp)
PpuRA5
PpPBS2–3'
lacZ repeat
KlGlcNAc Transp.
SwaI (5862)

FIG.6

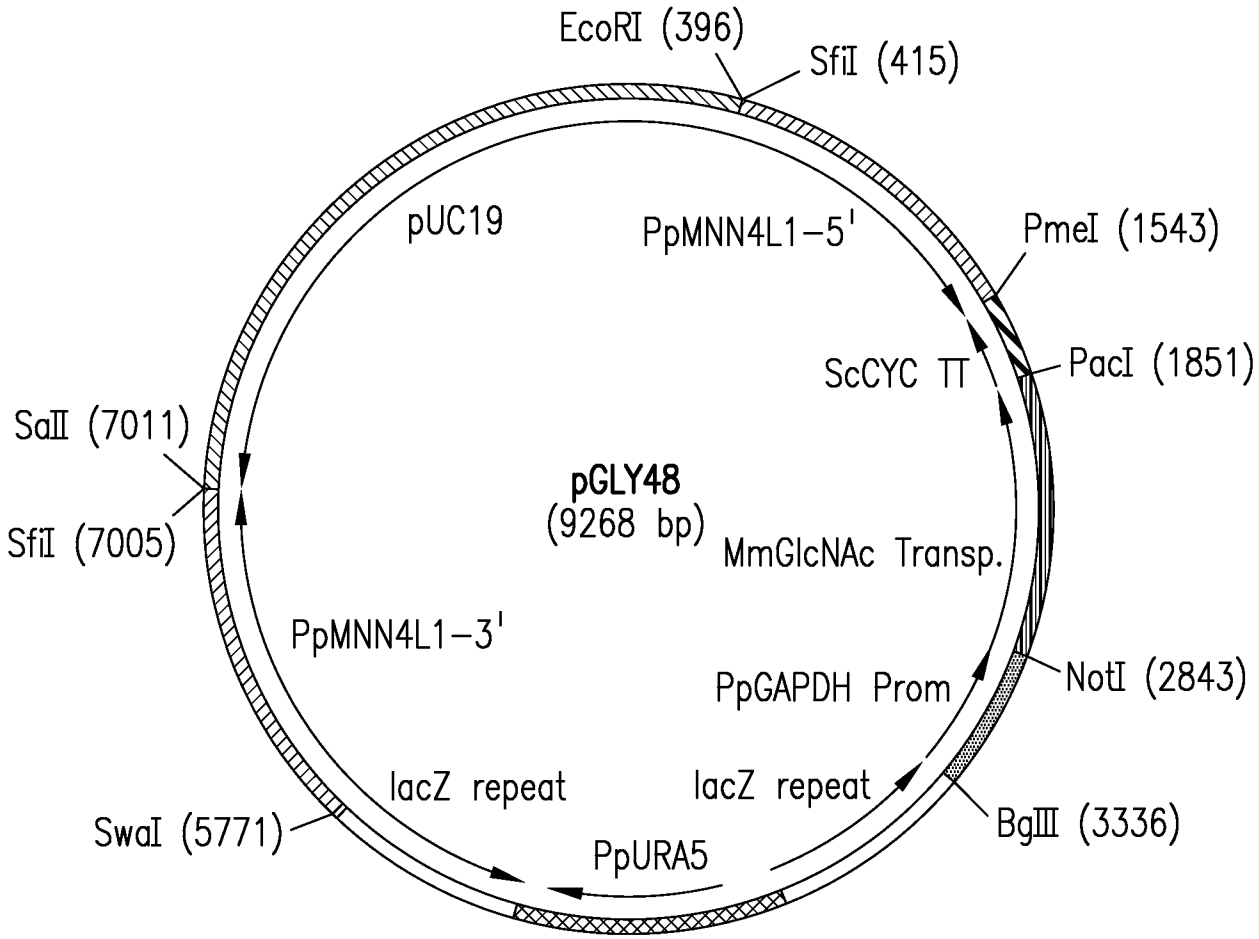

EcoRI (396)
SfiI (415)
pUC19
PpMNN4L1-5'
PmeI (1543)
ScCYC TT
PacI (1851)
SalI (7011)
pGLY48
(9268 bp)
SfiI (7005)
MmGlcNAc Transp.
PpMNN4L1-3'
PpGAPDH Prom
NotI (2843)
lacZ repeat
lacZ repeat
SwaI (5771)
BglII (3336)
PpURA5

FIG.7

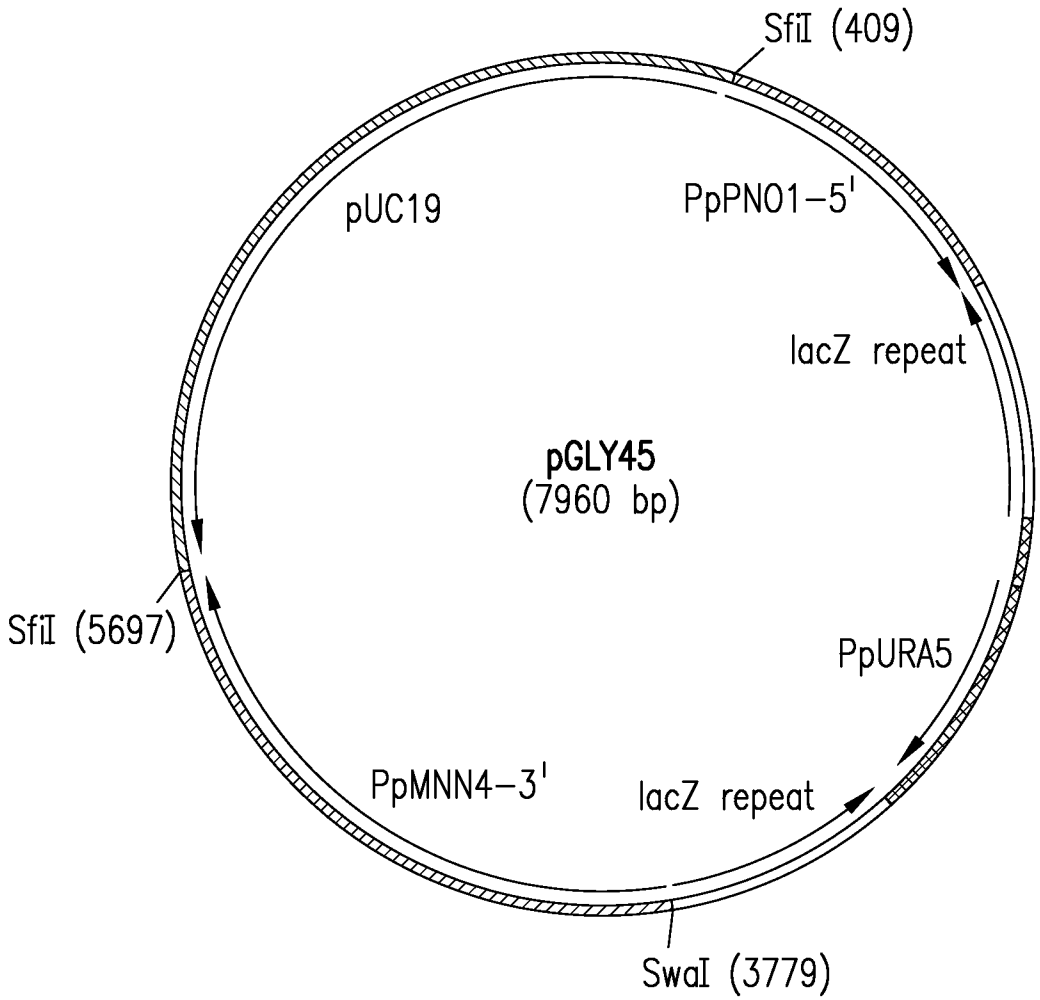

SfiI (409)
pUC19
PpPNO1–5'
lacZ repeat
pGLY45
(7960 bp)
SfiI (5697)
PpURA5
PpMNN4–3'
lacZ repeat
SwaI (3779)

FIG.8

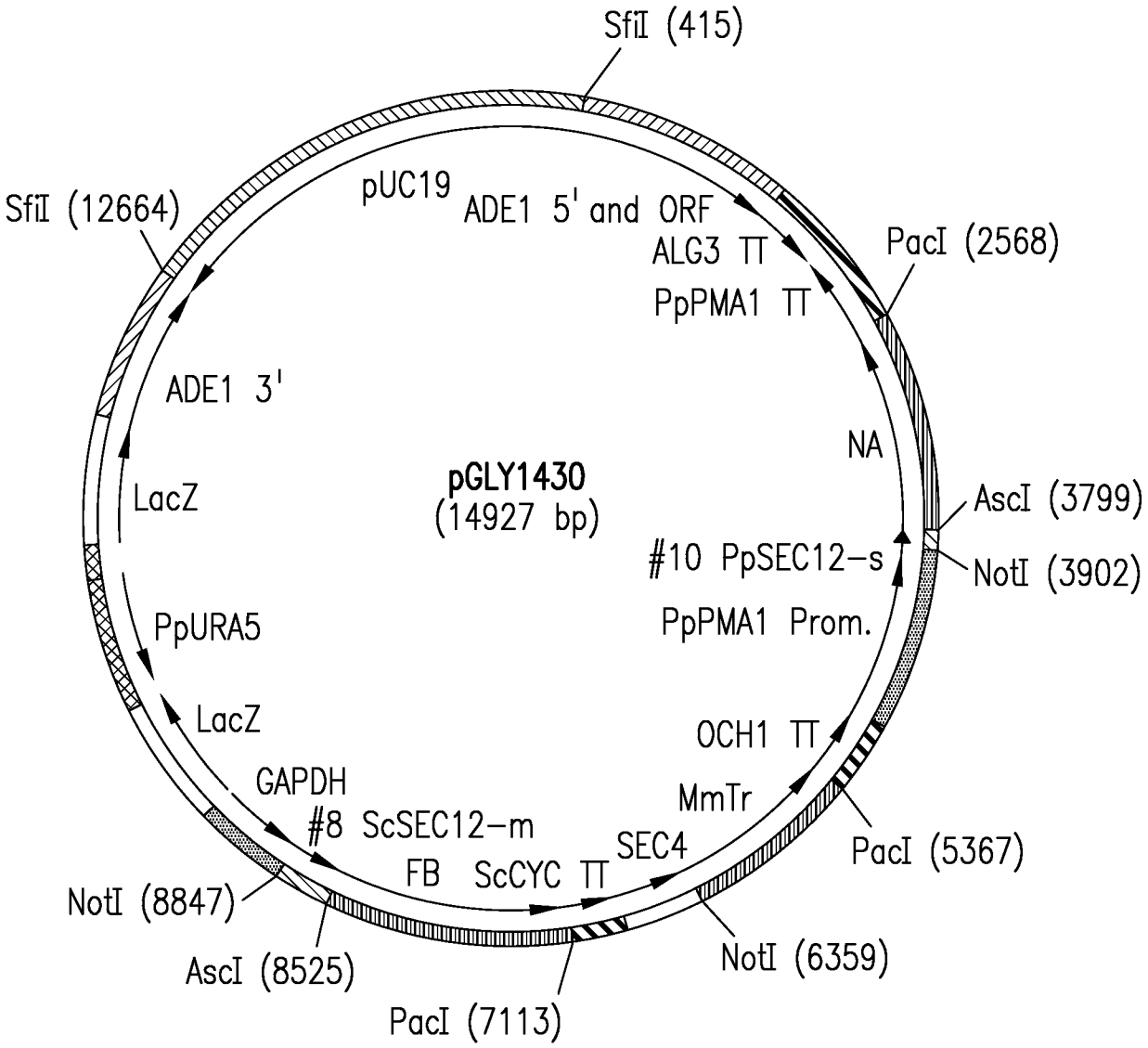

SfiI (415)
pUC19
ADE1 5' and ORF
ALG3 TT
PpPMA1 TT
PacI (2568)
SfiI (12664)
ADE1 3'
NA
pGLY1430
(14927 bp)
AscI (3799)
NotI (3902)
LacZ
#10 PpSEC12-s
PpPMA1 Prom.
PpURA5
LacZ
OCH1 TT
GAPDH
MmTr
#8 ScSEC12-m
SEC4
PacI (5367)
FB ScCYC TT
NotI (8847)
NotI (6359)
AscI (8525)
PacI (7113)

FIG.9

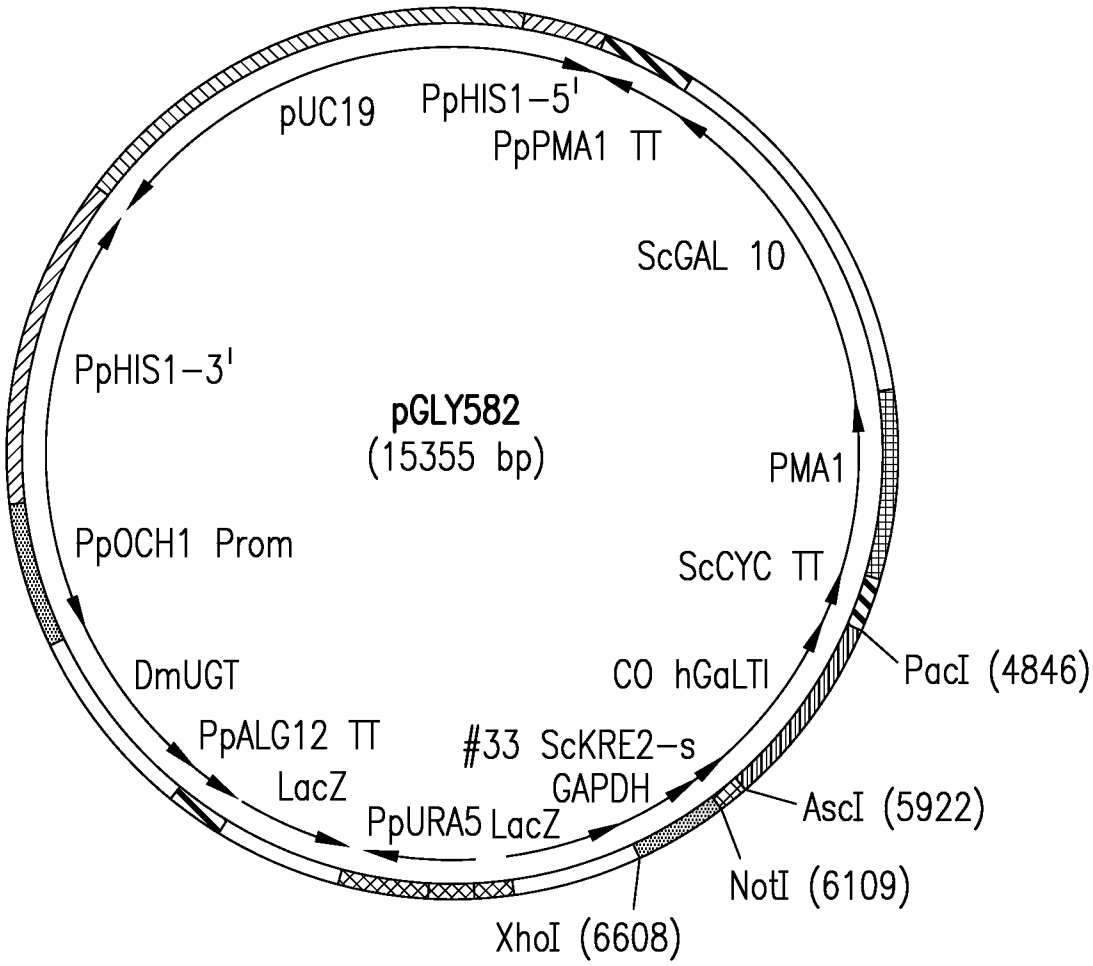

pUC19
PpHIS1–5'
PpPMA1 TT
ScGAL 10
PpHIS1–3'
pGLY582
(15355 bp)
PMA1
PpOCH1 Prom
ScCYC TT
DmUGT
CO hGaLTI
PacI (4846)
PpALG12 TT
#33 ScKRE2–s
LacZ
GAPDH
AscI (5922)
PpURA5
LacZ
NotI (6109)
XhoI (6608)

FIG.10

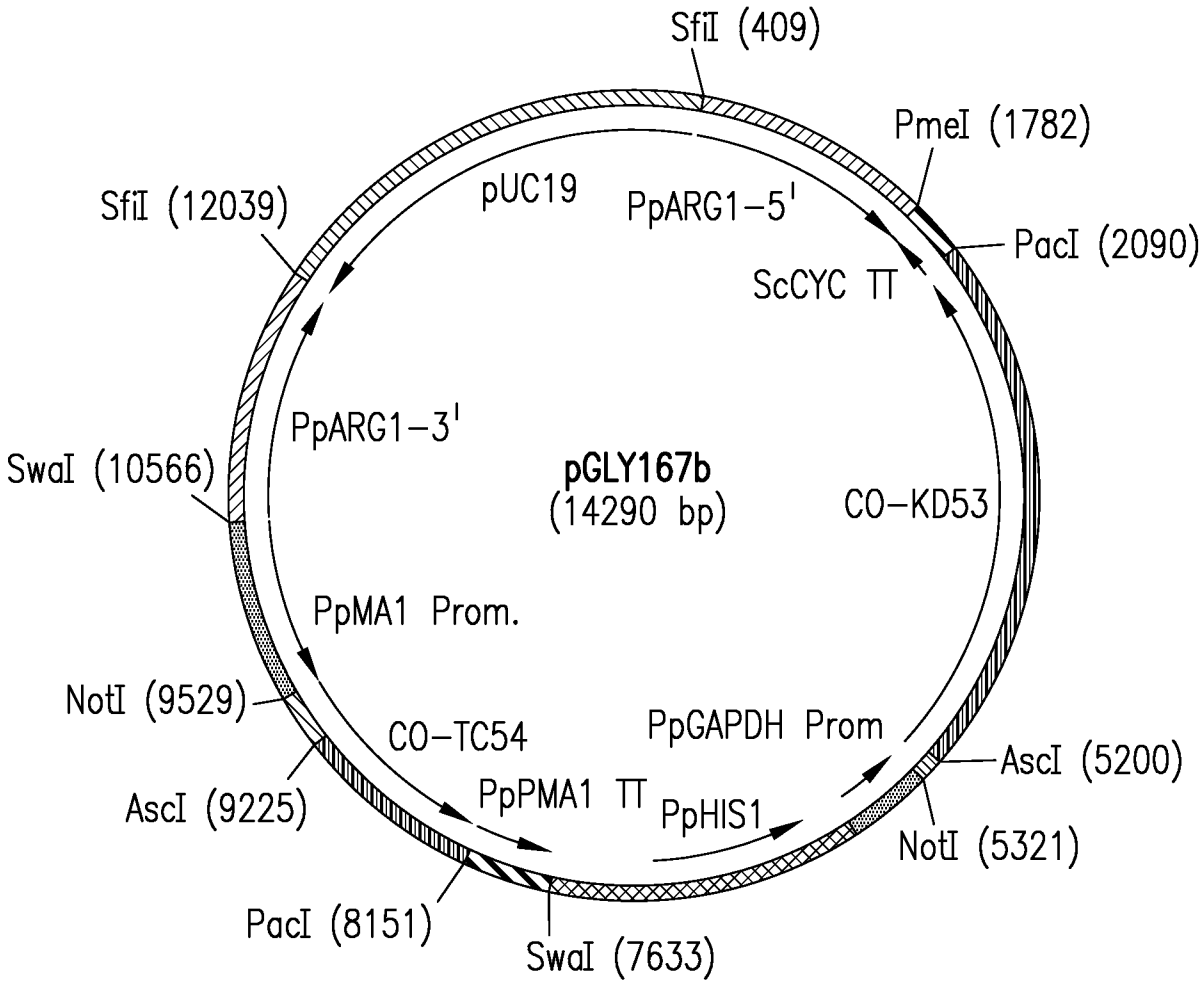

SfiI (409)
PmeI (1782)
PacI (2090)
ScCYC TT
PpARG1-5'
pUC19
SfiI (12039)
PpARG1-3'
SwaI (10566)
pGLY167b
(14290 bp)
CO-KD53
PpMA1 Prom.
NotI (9529)
CO-TC54
PpGAPDH Prom
AscI (5200)
AscI (9225)
PpPMA1 TT
PpHIS1
NotI (5321)
PacI (8151)
SwaI (7633)

FIG.11

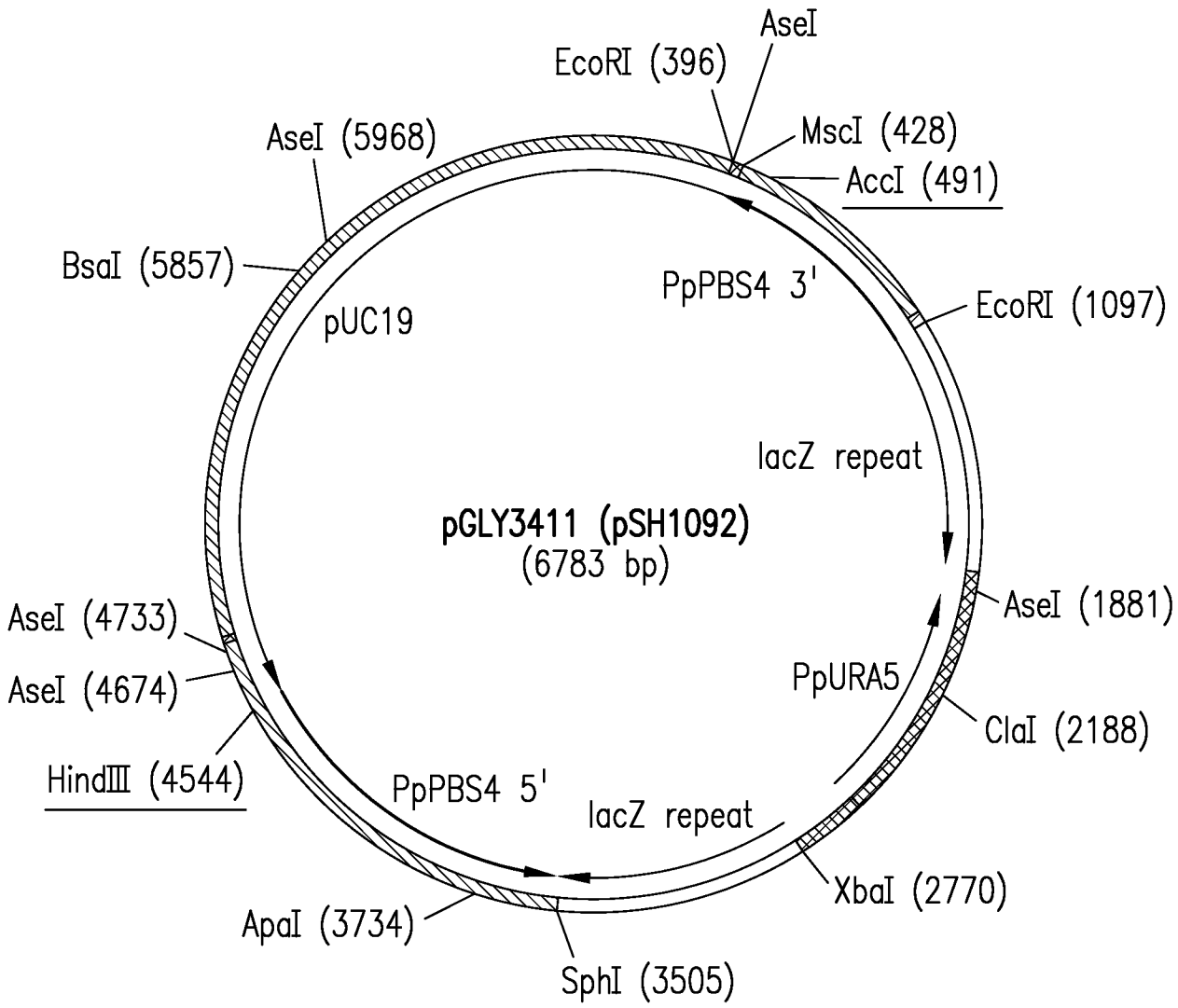

AseI
EcoRI (396)
MscI (428)
AccI (491)
AseI (5968)
BsaI (5857)
pUC19
PpPBS4 3'
EcoRI (1097)
lacZ repeat
pGLY3411 (pSH1092)
(6783 bp)
AseI (1881)
AseI (4733)
AseI (4674)
PpURA5
ClaI (2188)
HindIII (4544)
PpPBS4 5'
lacZ repeat
XbaI (2770)
ApaI (3734)
SphI (3505)

FIG.12

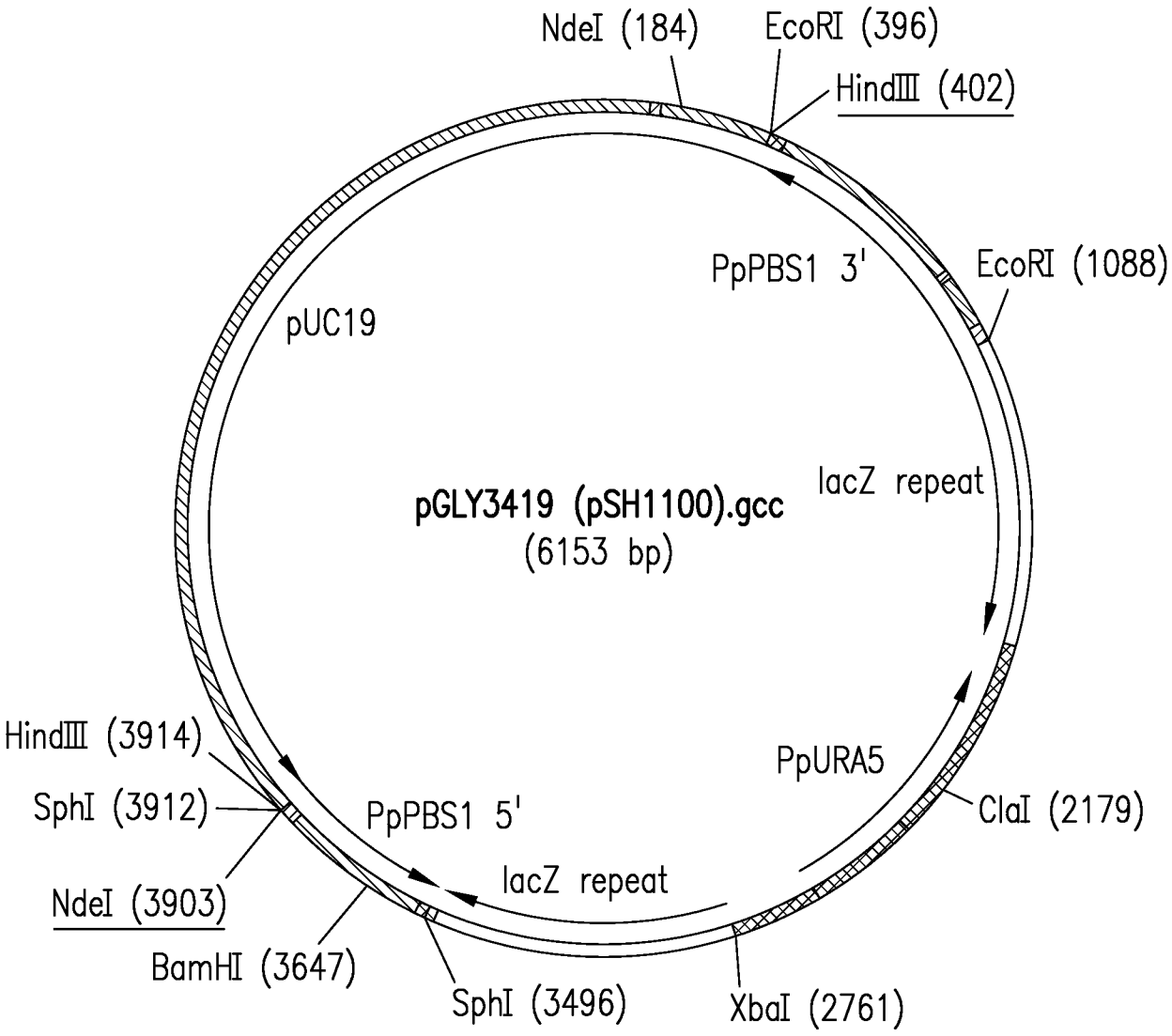

NdeI (184)
EcoRI (396)
HindIII (402)
PpPBS1 3'
EcoRI (1088)
pUC19
pGLY3419 (pSH1100).gcc
(6153 bp)
lacZ repeat
HindIII (3914)
SphI (3912)
PpPBS1 5'
PpURA5
ClaI (2179)
lacZ repeat
NdeI (3903)
BamHI (3647)
SphI (3496)
XbaI (2761)

FIG.13

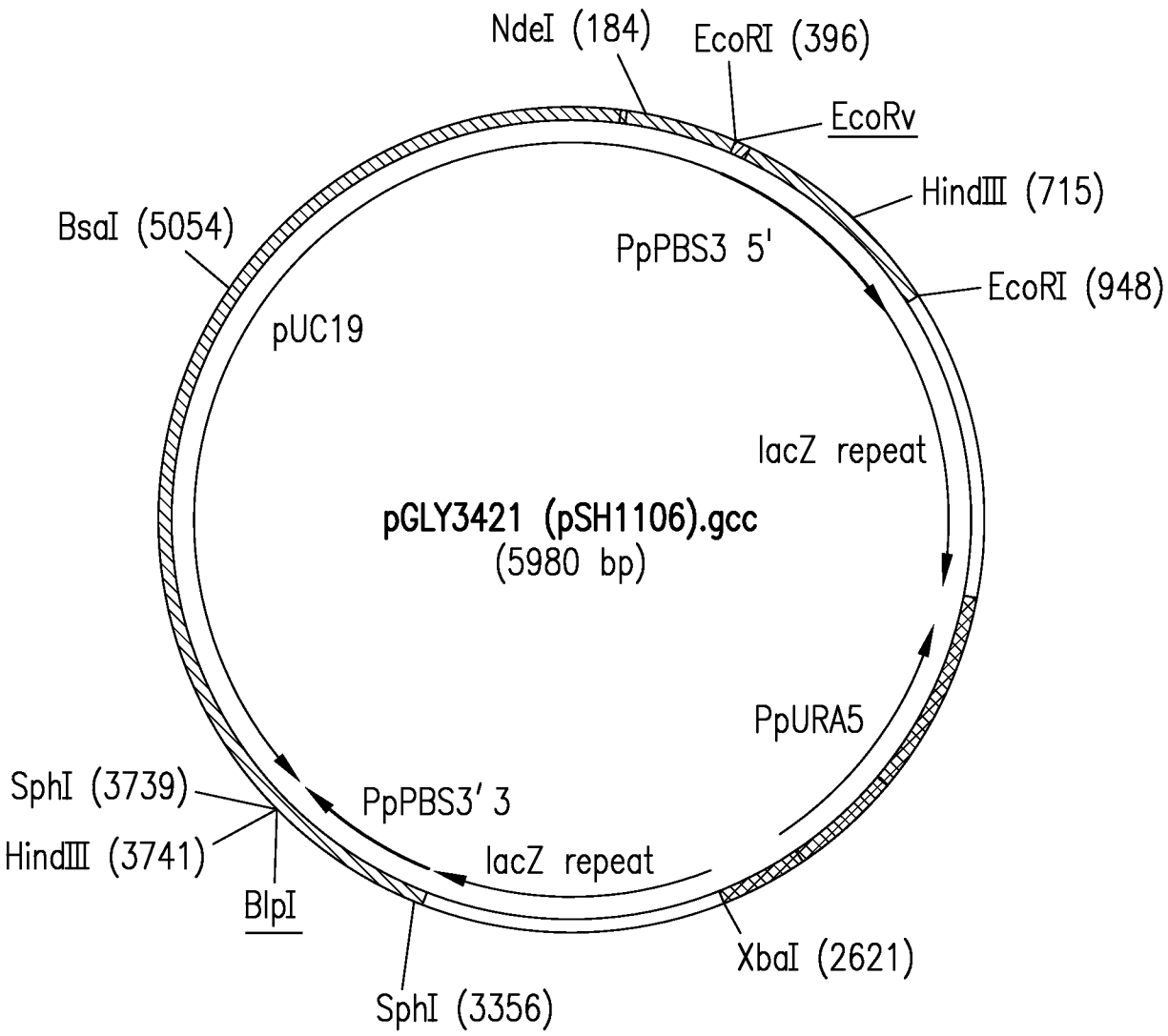

NdeI (184)
EcoRI (396)
EcoRv
HindIII (715)
EcoRI (948)
PpPBS3 5'
lacZ repeat
PpURA5
XbaI (2621)
SphI (3356)
BlpI
HindIII (3741)
SphI (3739)
PpPBS3' 3
lacZ repeat
BsaI (5054)
pUC19
pGLY3421 (pSH1106).gcc
(5980 bp)

FIG.14

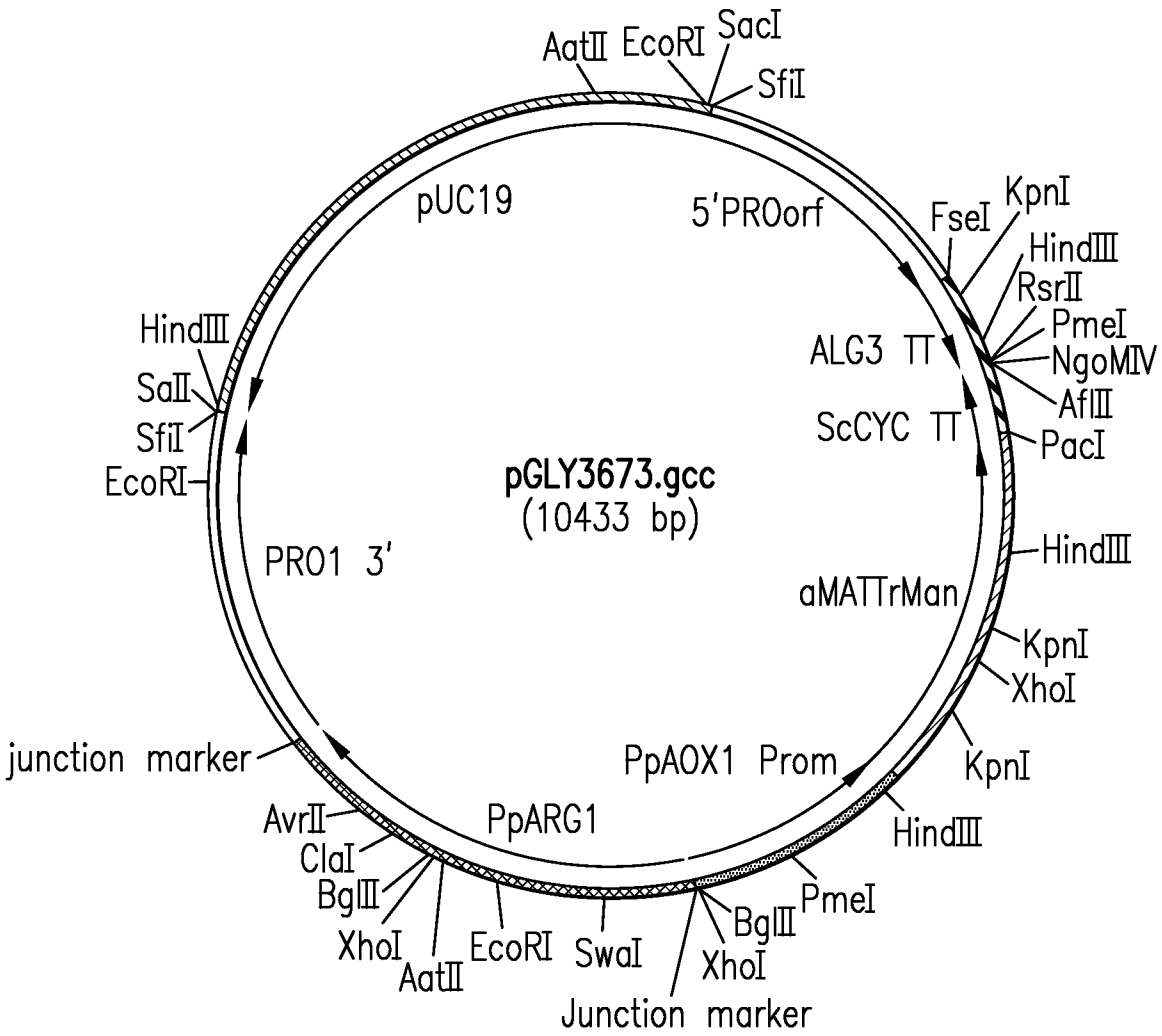

AatII
EcoRI
SacI
SfiI
pUC19
5'PROorf
FseI
KpnI
HindIII
RsrII
PmeI
NgoMIV
AflII
PacI
HindIII
KpnI
XhoI
KpnI
HindIII
PmeI
BglII
XhoI
Junction marker
SwaI
EcoRI
AatII
XhoI
BglII
ClaI
AvrII
junction marker
HindIII
SalI
SfiI
EcoRI
PRO1 3'
pGLY3673.gcc
(10433 bp)
ALG3 TT
ScCYC TT
aMATTrMan
PpAOX1 Prom
PpARG1

FIG.15

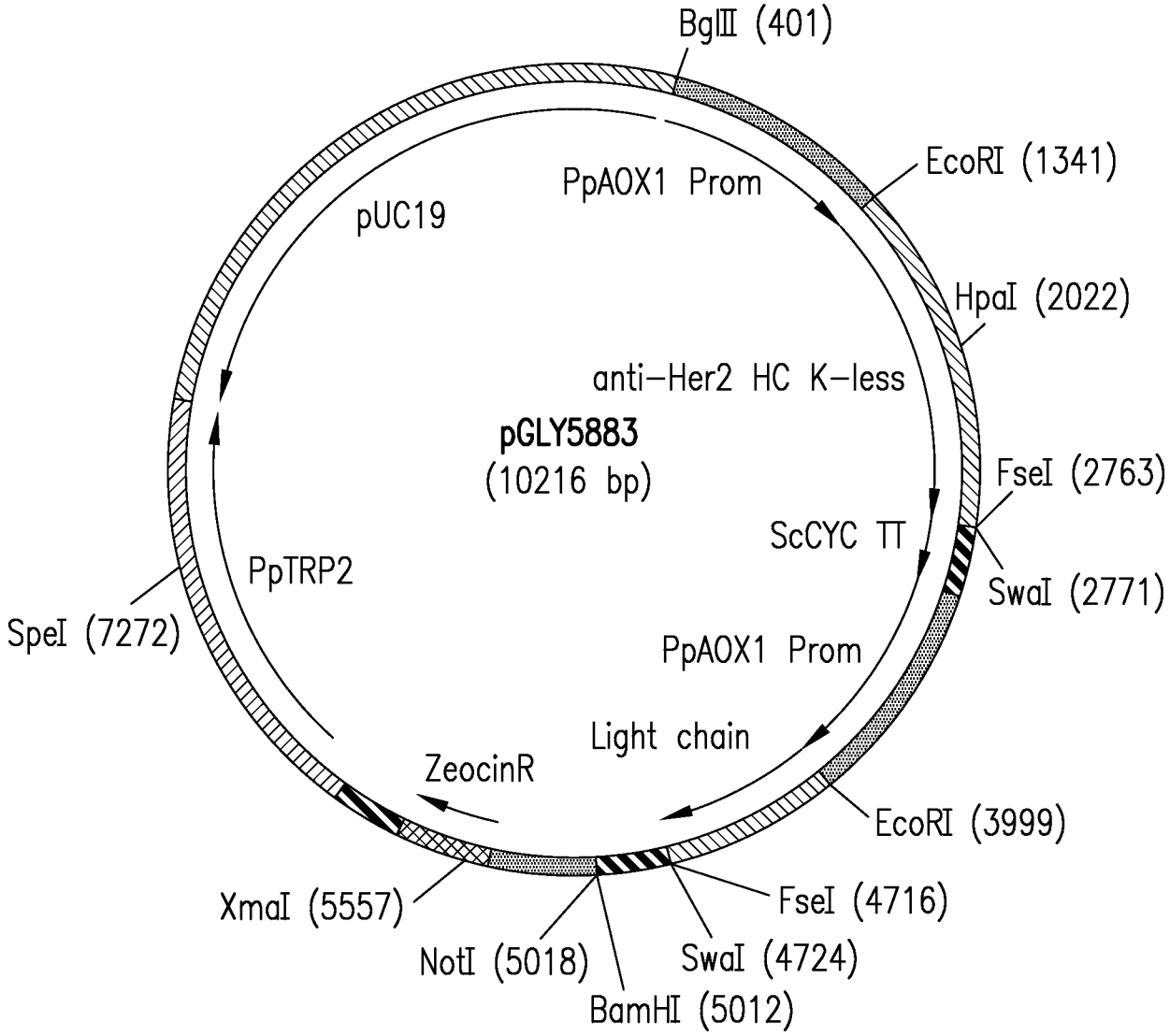

BglII (401)
EcoRI (1341)
HpaI (2022)
FseI (2763)
SwaI (2771)
PpAOX1 Prom
Light chain
ZeocinR
EcoRI (3999)
FseI (4716)
SwaI (4724)
BamHI (5012)
NotI (5018)
XmaI (5557)
SpeI (7272)
PpTRP2
ScCYC TT
anti-Her2 HC K-less
pGLY5883
(10216 bp)
PpAOX1 Prom
pUC19

FIG.16

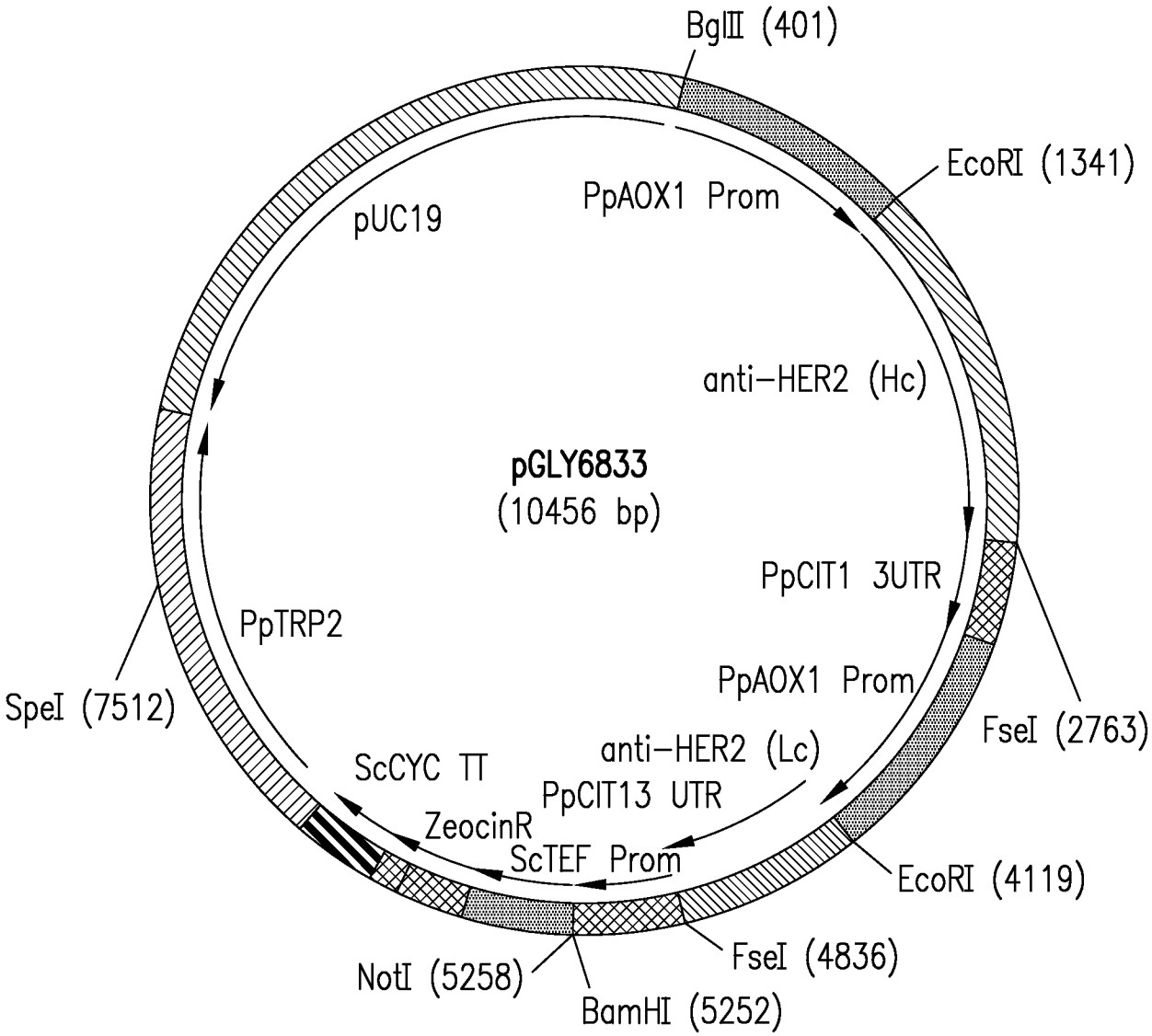

BglII (401)
EcoRI (1341)
PpAOX1 Prom
pUC19
anti-HER2 (Hc)
pGLY6833
(10456 bp)
PpCIT1 3UTR
PpTRP2
PpAOX1 Prom
SpeI (7512)
FseI (2763)
anti-HER2 (Lc)
ScCYC TT
PpCIT13 UTR
ZeocinR
ScTEF Prom
EcoRI (4119)
FseI (4836)
NotI (5258)
BamHI (5252)

FIG.17

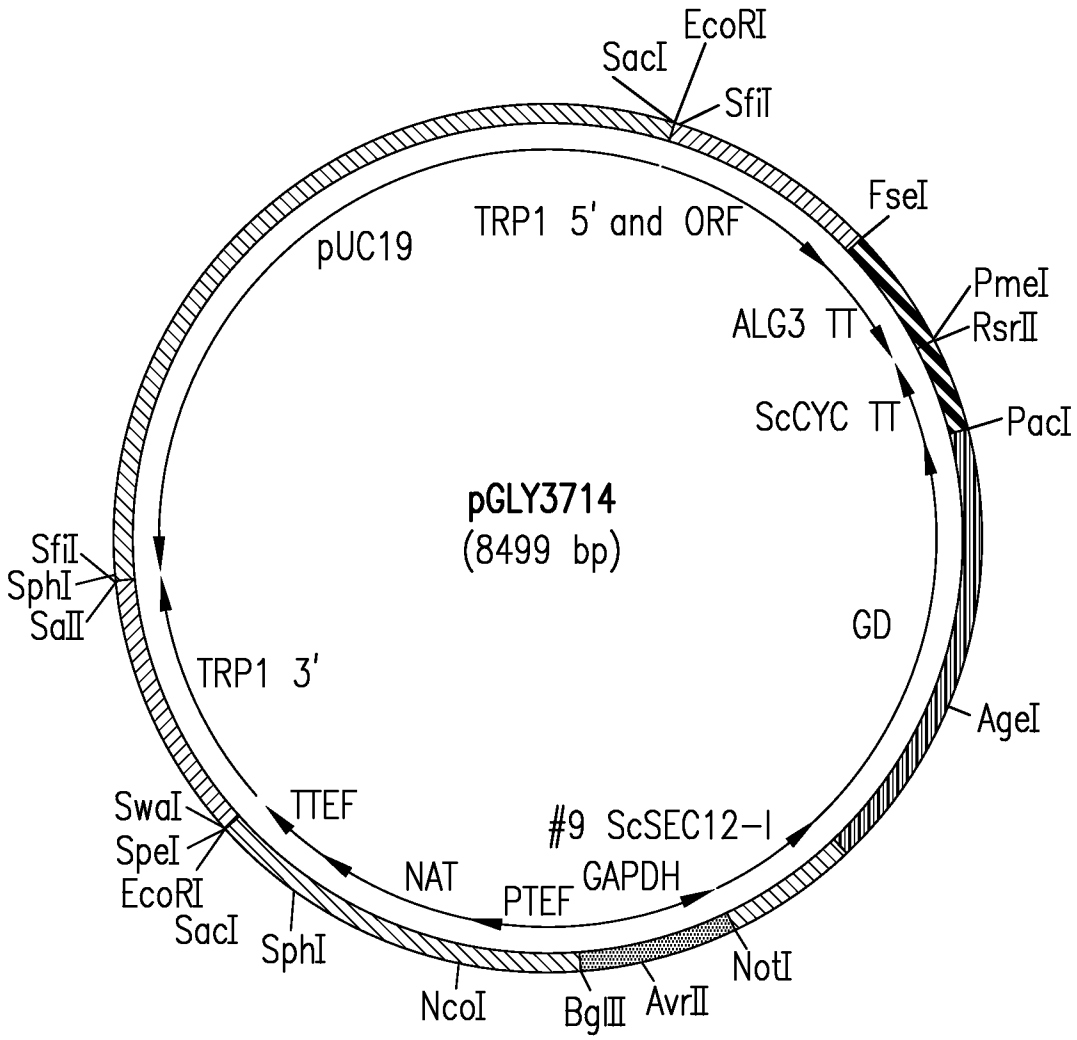

EcoRI
SacI
SfiI
FseI
TRP1 5' and ORF
pUC19
PmeI
RsrII
ALG3 TT
ScCYC TT
PacI
pGLY3714
(8499 bp)
SfiI
SphI
SalI
GD
TRP1 3'
AgeI
SwaI
SpeI
EcoRI
SacI
SphI
TTEF
#9 ScSEC12-I
NAT
PTEF
GAPDH
NotI
NcoI
BglII
AvrII

FIG.18

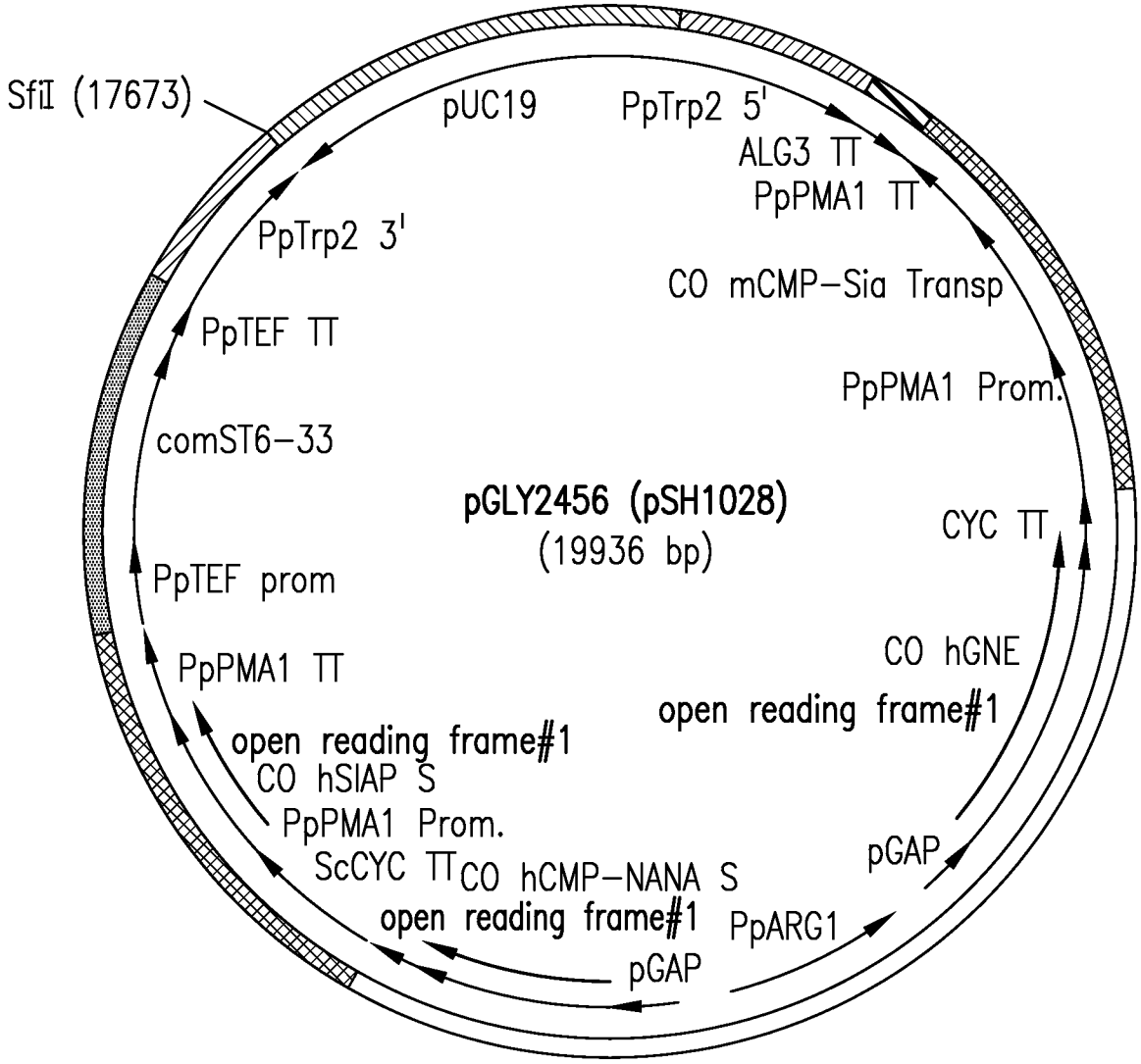

SfiI (17673)
pUC19
PpTrp2 5'
ALG3 TT
PpPMA1 TT
PpTrp2 3'
CO mCMP-Sia Transp
PpTEF TT
PpPMA1 Prom.
comST6-33
pGLY2456 (pSH1028)
(19936 bp)
CYC TT
PpTEF prom
CO hGNE
PpPMA1 TT
open reading frame#1
open reading frame#1
CO hSIAP S
PpPMA1 Prom.
pGAP
ScCYC TT
CO hCMP-NANA S
open reading frame#1
PpARG1
pGAP

FIG.19

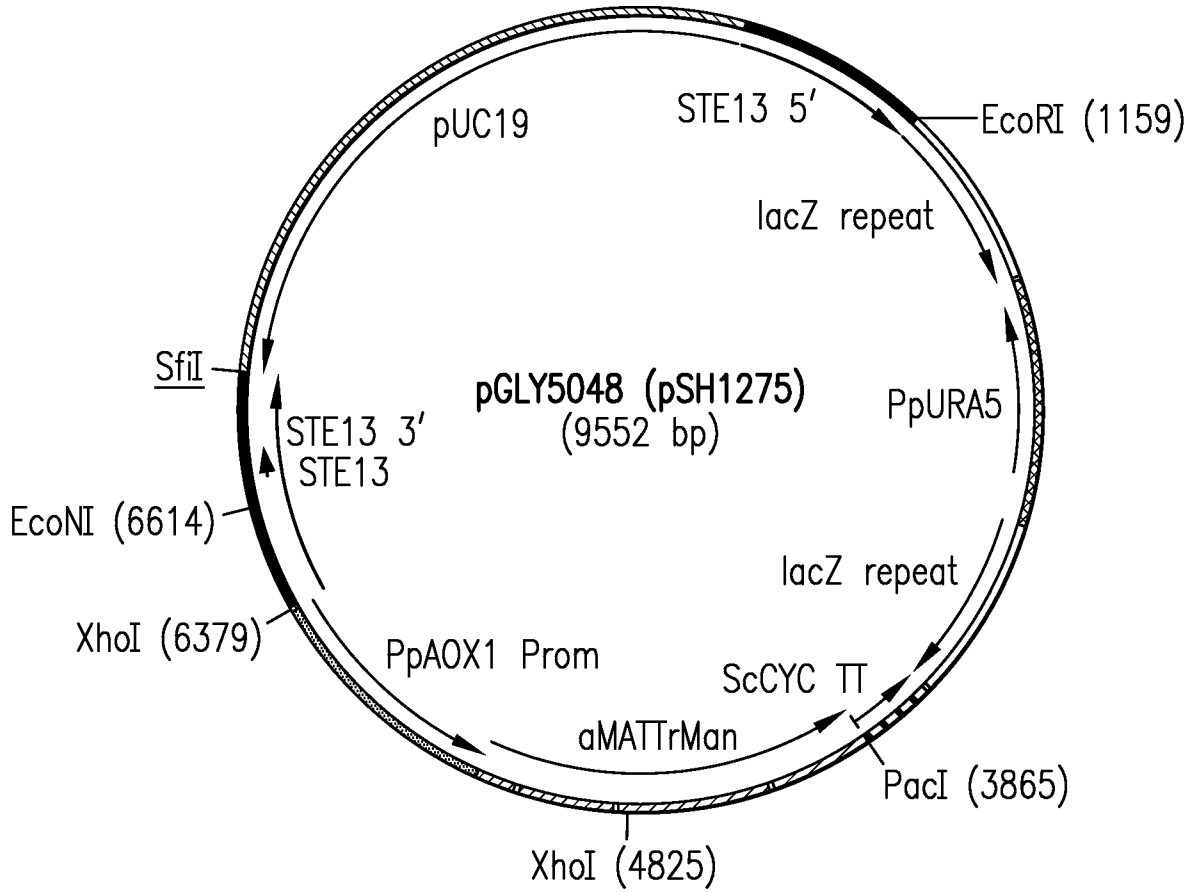

pUC19
STE13 5′
EcoRI (1159)
lacZ repeat
SfiI
pGLY5048 (pSH1275)
(9552 bp)
PpURA5
STE13 3′
STE13
EcoNI (6614)
lacZ repeat
XhoI (6379)
PpAOX1 Prom
ScCYC TT
aMATTrMan
PacI (3865)
XhoI (4825)

FIG.20

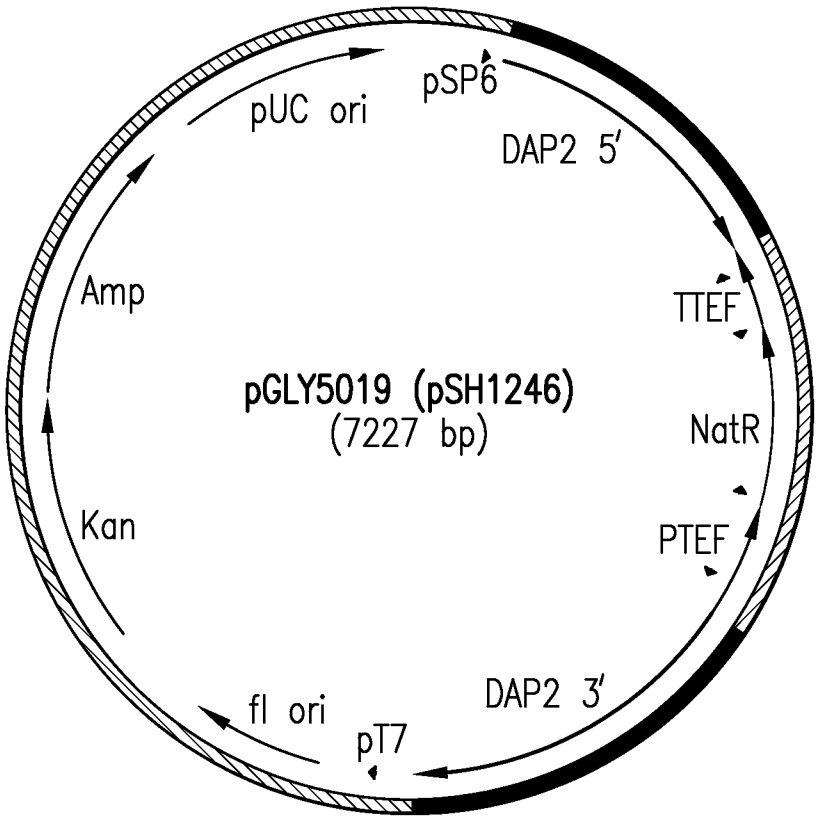

pSP6
pUC ori
DAP2 5′
Amp
TTEF
pGLY5019 (pSH1246)
(7227 bp)
NatR
Kan
PTEF
fl ori
DAP2 3′
pT7

FIG.21

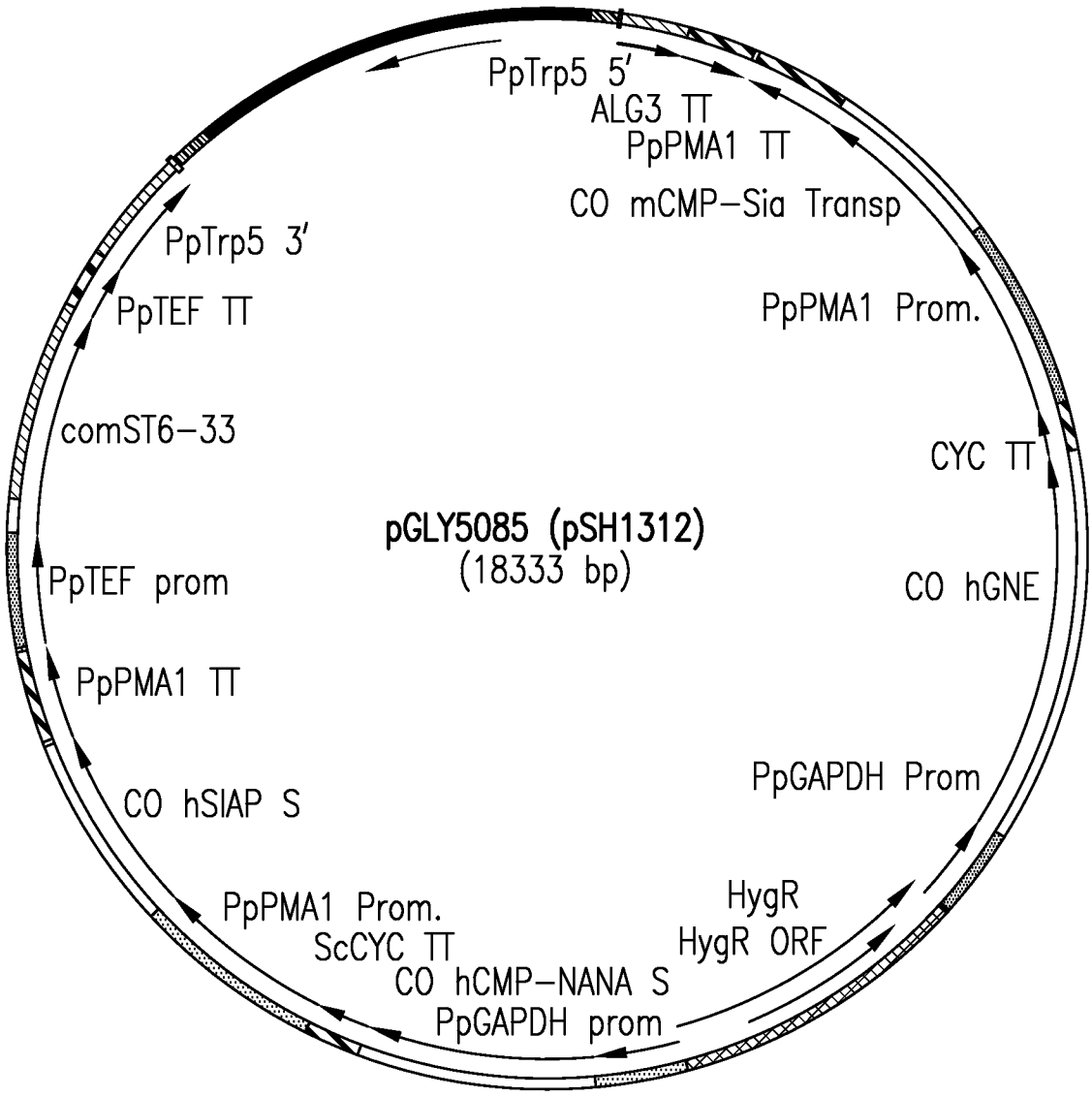

PpTrp5 5′
ALG3 TT
PpPMA1 TT
CO mCMP-Sia Transp
PpPMA1 Prom.
CYC TT
CO hGNE
PpGAPDH Prom
HygR
HygR ORF
PpGAPDH prom
CO hCMP-NANA S
ScCYC TT
PpPMA1 Prom.
CO hSIAP S
PpPMA1 TT
PpTEF prom
comST6-33
PpTEF TT
PpTrp5 3′
pGLY5085 (pSH1312)
(18333 bp)

FIG.22

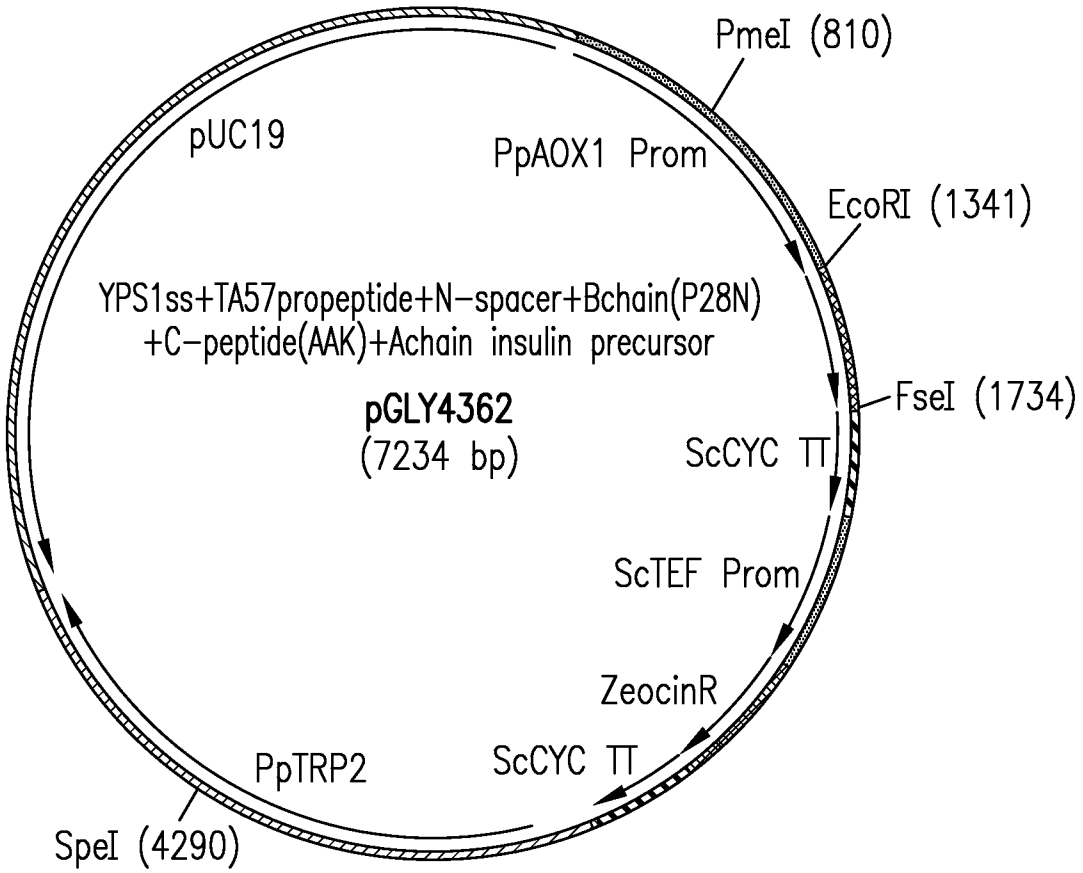

PmeI (810)
pUC19
PpAOX1 Prom
EcoRI (1341)
YPS1ss+TA57propeptide+N-spacer+Bchain(P28N)
+C-peptide(AAK)+Achain insulin precursor
pGLY4362
(7234 bp)
FseI (1734)
ScCYC TT
ScTEF Prom
ZeocinR
ScCYC TT
PpTRP2
SpeI (4290)

FIG.23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/61599

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 21/00; C12N 15/00 (2012.01)
USPC - 435/71.1, 440
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12P 21/00; C12N 15/00 (2012.01)
USPC - 435/71.1, 440

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/71.1, 435/440, 435/41, 435/69.1, 435/72

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubMed; Pichia pastoris, XRN1, KEM1, SKI1, Xrn1p, Pacman, expression, activity, reduce, eliminate, mutation, deletion, truncation, mannosyltransferase, glycosidase, mannosidase, glycosylation enzyme

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/053612 A1 (HAMILTON et al.) 05 May 2011 (05.05.2011) pg 1, ln 6-8; pg 2, ln 2-9; pg 4, ln 14-18; pg 8, ln 17-20; pg 14, ln 14-22; pg 16, ln 31 - pg 17, ln 12; pg 17, ln 13-19; pg 18, ln 9-20; pg 19, ln 1-20; pg 20, ln 3-9; pg 20, ln 17-32; pg 22, ln 1-10; pg 26, ln 11-24; pg 27, ln 14-19; pg 28, ln 12-24	1-21
Y	LARIMER et al., Characterization of the XRN1 gene encoding a 5'-3' exoribonuclease: sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells. Gene; 12 October 1992; Vol 120, No 1; pp 51-7; pg 52, col 2, para 3; pg 53, col 1, para 3; pg 54, col 1, para 2; Fig 1A, 2; Table II, III	1-21
Y	KUEBERL et al., High-quality genome sequence of Pichia pastoris CBS 7435. J. Biotechnol.; 20 July 2011 (published online 06 May 2011); Vol 154, No 4, pp 312-20; pg 314, col 1, para 1	1-21

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2012 (19.12.2012)	25 JAN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)