Claims

1. A method for producing a glycoprotein *in vitro*, the method comprising:

obtaining a mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase-deficient (MGAT1-deficient) cell, wherein the MGAT1-deficient cell comprises in its genome SEQ ID NO: 6 and is produced by a method comprising:

introducing into a parent cell an RNA-guided endonuclease together with a guide RNA (gRNA) comprising a sequence as set forth in GGAUGCGCAGACCUGAGCAG (SEQ ID NO: 2), GGUAGUGGAGGACGAUCUGG (SEQ ID NO: 3), UUUCUCCACCUGUAGCAGGG (SEQ ID NO: 4), or GAUCGCCAGGCACUACCGCU (SEQ ID NO: 5);

culturing the parent cell and expanding the parent cell;

isolating a plurality of daughter cells from the cell culture;

identifying a modified cell deficient in MGAT1 activity; and

isolating said modified cell deficient in MGAT1 activity;

expressing in the MGAT1-deficient cell a protein having an N-glycosylation site, whereby the protein is N-glycosylated at the N-glycosylation site with an oligomannose glycan; and

isolating the N-glycosylated protein from the MGAT1-deficient cell.

2. The method of claim 1, further comprising incubating the N-glycosylated protein with glycopeptide-D-mannosyl-N4-(N-acetyl-D-glucosaminyl)2-asparagine 1,4-N-acetyl-beta-glucosaminohydrolase (Endo H) to remove the oligomannose glycan, whereby an N-acetylglucosamine residue remains at the N-glycosylation site.

3. The method of claim 1 or claim 2, wherein the protein is a viral envelope protein.

4. The method of claim 3, wherein the viral envelope protein is selected from a group consisting of SARS-CoV2 spike protein, Pan-sarbecovirus spike protein, Pan-betacoronavirus spike protein, and Influenza hemagglutinin.

5. The method of claim 4, wherein the SARS-CoV2 spike protein is an alpha-SARS-CoV2 spike protein, beta-SARS-CoV2 spike protein, gamma-SARS-CoV2 spike protein, delta-SARS-CoV2 spike protein, or omicron-SARS-CoV2 spike protein.

6. The method of claim 5, wherein the SARS-CoV2 spike protein comprises SEQ ID NO: 16 or SEQ ID NO: 17.

7. The method of claim 1 or claim 2, wherein the protein is selected from a group consisting of alpha-fetoprotein, β-human chorionic gonadotropin, cancer antigens 15-3, 19-9, 27.29, 125, and 549, carcinoembryonic antigen, a carcinoembryonic antigen-related cell adhesion molecule, human epidermal growth factor receptor 2, oncofetal fibronectin, placental alkaline phosphatase, and prostate-specific antigen.

8. A method for producing a glycoprotein *in vitro*, the method comprising:

obtaining a mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase-deficient Chinese Hamster Ovary cell line, wherein the cell line comprises in its genome SEQ ID NO: 6;

expressing in the cell line a protein having an N-glycosylation site, whereby the protein is N-glycosylated at the N-glycosylation site with an oligomannose glycan; and

isolating the N-glycosylated protein from the cell line.

9. The method of claim 8, further comprising incubating the N-glycosylated protein with glycopeptide-D-mannosyl-N4-(N-acetyl-D-glucosaminyl)2-asparagine 1,4-N-acetyl-beta-glucosaminohydrolase (Endo H) to remove the oligomannose

glycan, whereby an N-acetylglucosamine residue remains at the N-glycosylation site.

10. The method of claim 8 or claim 9, wherein the protein is a viral envelope protein.

11. The method of claim 10, wherein the viral envelope protein is selected from a group consisting of SARS-CoV2 spike protein, Pan-sarbecovirus spike protein, Pan-betacoronavirus spike protein, and Influenza hemagglutinin.

12. The method of claim 11, wherein the SARS-CoV2 spike protein is an alpha-SARS-CoV2 spike protein, beta-SARS-CoV2 spike protein, gamma-SARS-CoV2 spike protein, delta-SARS-CoV2 spike protein, or omicron-SARS-CoV2 spike.

13. The method of claim 12, wherein the SARS-CoV2 spike protein comprises SEQ ID NO: 16 or SEQ ID NO: 17.

14. The method of claim 8 or claim 9, wherein the protein is selected from a group consisting of alpha-fetoprotein, β-human chorionic gonadotropin, cancer antigens 15-3, 19-9, 27.29, 125, and 549, carcinoembryonic antigen, a carcinoembryonic antigen-related cell adhesion molecule, human epidermal growth factor receptor 2, oncofetal fibronectin, placental alkaline phosphatase, and prostate-specific antigen.