[0007a] In one aspect, the present disclosure refers to a mammalian recombinant cell for producing lactose, wherein said recombinant cell comprises one or more expression constructs that encode an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), and a glucose transporter, wherein the glucose transporter is a glucose transporter 1 (GLUT1), wherein the one or more expression constructs are mammalian expression vectors.

[0007b] In another aspect, the present disclosure refers to one or more expression constructs, wherein the expression constructs comprise polynucleotides encoding an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), and a glucose transporter, wherein the glucose transporter is a glucose transporter 1 (GLUT1), wherein the one or more expression constructs are mammalian expression vectors

[0008] In another aspect, the present disclosure refers to a method of producing lactose using the recombinant cell as disclosed herein, wherein the method comprises the steps of: i) culturing the recombinant cell of any one of the preceding claims, and ii) detecting lactose from the recombinant cell in i).

[0009] In another aspect, the present disclosure refers to a cell culture comprising the cell as disclosed herein and a culture medium.

[00010] In yet another aspect, the present disclosure refers to a cryopreserved cell culture comprising the cell as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[00011] **Figure 1** shows the different subcellular localizations of GFP and Glucose Transporter 1 (GLUT1) fusion proteins in mammalian cells. Lactose synthesis takes place in the Golgi of the mammary gland epithelial cells with the functions of lactose synthase (LS) heterodimer α-Lactalbumin (LALBA) and β-1,4-galactosyltransferase (B4GalT1). One of starting materials for lactose synthesis, glucose, is transported by glucose transporters in cells. However, glucose transporter 1 (GLUT1) is normally expressed on the cell surface. Thus, GLUT1 variants were generated to identify a suitable variant that remains in the ER or Golgi apparatus, instead of being translocated to the cell membrane Figure 1A and Figure1C show localization of Golgi in Chinese Hamster Ovary (CHO) cells grown in suspension culture. Fluorescent microscopic image of GFP-GLUT1 (GFP fused at the N-terminus of huGLUT1) as shown in Figure 1B is mostly localized to the cell membrane whereas GLUT1-GFP (GFP fused at the C-terminus of huGLUT1) in Figure 1D is only partially localized to the cell membrane in CHO cells grown in suspension culture.

[00012] **Figure 2** provides fluorescent confocal microscopic images showing cellular localizations of additional variants of the GLUT1/GFP fusion proteins. The tested variants comprise: LGCT: a full-length human GLUT1 with a C-terminal fusion of GFP; LGNT: a full-length human GLUT1 with an N-terminal fusion of GFP; ST2: a full-length human GLUT1 with an N-terminal fusion of GFP and an ER retention signal (obtained from human Glucose Transporter 2, having the sequence of LLTKVKGS (SEQ ID NO: 30)); ST3: a full-length human GLUT1 with an N-terminal fusion of GFP and a Golgi

retention signal (obtained from the C-terminal 18 amino acids of human CMP-sialic acid transporter (CST), having the sequence of TTSIQQGETASKERVIGV (SEQ ID NO: 32); ST5: a truncated human GLUT1 with an N-terminal fusion of GFP; ST7: a full-length human GLUT1 with an N-terminal fusion of GFP and a C-terminal ER retention signal; ST4: a full-length human GLUT1 with an N-terminal fusion of GFP and a C-terminal Golgi retention signal; and ST6: a truncated human GLUT1 with an N-terminal fusion of GFP and a C-terminal Golgi retention signal. According to Figure 2, the exemplary variants show various degree of co-localisation between the GLUT1/GFP fusion proteins and the Golgi marker to carry out the desired function of transporting glucose into the ER and Golgi apparatus. In particular, ST7, ST5, and ST6 show high co-localisation between GLUT1/GFP fusion proteins and the Golgi marker, indicating retention of GLUT1/GFP fusion protein in the Golgi apparatus.

CLAIMS

What is claimed is:

1. A mammalian recombinant cell for producing lactose, wherein said recombinant cell comprises one or more expression constructs that encode an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), and a glucose transporter, wherein the glucose transporter is a glucose transporter 1 (GLUT1), wherein the one or more expression constructs are mammalian expression vectors.

2. One or more expression constructs, wherein the expression constructs comprise polynucleotides encoding an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), and a glucose transporter, wherein the glucose transporter is a glucose transporter 1 (GLUT1), wherein the one or more expression constructs are mammalian expression vectors.

3. The recombinant cell or expression constructs of claim 1 or 2, wherein the glucose transporter is from a mammal.

4. The recombinant cell or expression constructs of any one of claims 1 to 3, wherein the glucose transporter is a human glucose transporter 1 (huGLUT1).

5. The recombinant cell or expression constructs of any one of claims 1 to4, wherein the glucose transporter is a full-length GLUT1 or C-terminal truncated GLUT1.

6. The recombinant cell or expression constructs of any one of claims 1 to 5, wherein the alpha-lactalbumin (LALBA) and the beta-1,4-galactosyltransferase 1 (B4GalT1) are from the same mammalian species.

7. The recombinant cell or expression constructs of any one of claims 1 to 6, wherein both the alpha-lactalbumin (LALBA) and the beta-1,4-galactosyltransferase 1 (B4GalT1) are from human.

8. The recombinant cell or expression constructs of any one of claims 1 to 7, wherein both the alpha-lactalbumin (LALBA) and the beta-1,4-galactosyltransferase 1 (B4GalT1) are from hamster.

9. The recombinant cell of any one of claims 1 and 3 to 8, wherein the cell is a hamster cell or a human cell.

10. The recombinant cell of claim 9, wherein the hamster cell is a CHO cell or a Baby hamster kidney cell (BHK).

11. The recombinant cell of claim 10, wherein the CHO cell is a CHO-K1 or DUKX-X11 cell.

12. The recombinant cell of any one of claims 1 and 3 to 9, wherein the cell is a human cell selected from a group consisting of: HeLa, HEK293T, U2OS, A549, HT1080, MCF-7, Y79, SO-Rb50 and Hep G2 cells.

13. The recombinant cell or expression constructs of any one of claims 1 to 8 or the recombinant cell of any one of claims 9 to 12, wherein the sequences encoding alpha-lactalbumin (LALBA), beta-1,4-galactosyltransferase 1 (B4GalT1), glucose transporter, and combinations thereof, are engineered in at least one, at least two, at least three or at least four constructs.

14. The recombinant cell of any one of claims 1, and 3 to 13, wherein the recombinant cell comprises:

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a glucose transporter 1 (GLUT1), and a marker protein,
  wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of GLUT1, and C-terminus of GLUT1 is fused to N-terminus of the marker protein
  and an expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1) and an alpha-lactalbumin (LALBA),
  wherein C-terminus of B4GalT1 is linked to N-terminus of LALBA;

or

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a marker protein, a glucose transporter 1 (huGLUT1), and an ER localization signal,
  wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of the marker protein, C-terminus of the marker protein is linked to N-terminus of GLUT1, and C-terminus of GLUT1 is linked to N-terminus of the ER localization signal
  and an expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1) and an alpha-lactalbumin (LALBA),
  wherein C-terminus of B4GalT1 is linked to N-terminus of LALBA;

or

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a marker protein, and a glucose transporter 1 (GLUT1),
  wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of the marker protein, and C-terminus of the marker protein is linked to N-terminus of GLUT1

and an expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1) and an alpha-lactalbumin (LALBA),

wherein C-terminus of B4GalT1 is linked to N-terminus of LALBA;

or

- a first expression construct that encodes an alpha-lactalbumin (LALBA)

and a second expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1);

or

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a marker protein, and a C-terminal truncated glucose transporter 1 (GLUT1Δ),

wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of the marker protein, and C-terminus of the marker protein is linked to N-terminus of GLUT1Δ

and an expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1) and an alpha-lactalbumin (LALBA),

wherein C-terminus of B4GalT1 is linked to N-terminus of LALBA;

or

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a marker protein, a C-terminal truncated glucose transporter 1 (GLUT1Δ), and a Golgi localization sequence,

wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of the marker protein, C-terminus of the marker protein is linked to N-terminus of GLUT1Δ, and C-terminus of GLUT1Δ is linked to N-terminus of the Golgi localization sequence

and an expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1) and an alpha-lactalbumin (LALBA),

wherein C-terminus of B4GalT1 is linked to N-terminus of LALBA;

or

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a marker protein, a glucose transporter 1 (huGLUT1), and a Golgi localization sequence,

wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of the marker protein, C-terminus of the marker protein is linked to N-terminus of GLUT1, and C-terminus of GLUT1 is linked to N-terminus of C-terminal of the Golgi localization sequence

and an expression construct that encodes a beta-1,4-galactosyltransferase 1 (B4GalT1) linked to alpha-lactalbumin (LALBA)

wherein C-terminus of B4GalT1 is linked to N-terminus of LALBA;

or

- an expression construct that encodes an alpha-lactalbumin (LALBA), a beta-1,4-galactosyltransferase 1 (B4GalT1), a marker protein, and a glucose transporter 1 (GLUT1), wherein C-terminus of LALBA is linked to N-terminus of B4GalT1, C-terminus of B4GalT1 is linked to N-terminus of the marker protein, and C-terminus of the marker protein is linked to N-terminus of GLUT1.

15. The recombinant cell of any one of claims 1, and 3 to 14, wherein the expression constructs comprise, if present, the following:

- sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 1 (alpha-lactalbumin (LALBA));
- sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 2 (beta-1,4-galactosyltransferase 1 (B4GalT1)); and
- sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 3 (glucose transporter 1 (GLUT1)); or
- sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 4 (C-terminal truncated glucose transporter 1 (GLUT1Δ)).

16. The expression constructs of any one of claims 2 to 8 and 13, comprising the following:

- polynucleotide sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 1 (alpha-lactalbumin (LALBA));
- polynucleotide sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 2 (beta-1,4-galactosyltransferase 1 (B4GalT1)); and optionally
- polynucleotide sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 3 (glucose transporter 1 (GLUT1)); or
- polynucleotide sequences having at least 90% or 95% or 98% or 100% sequence identity with SEQ ID NO: 4 (C-terminal truncated glucose transporter 1 (GLUT1Δ)).

17. A method of producing lactose using the recombinant cell of any one of claims 1 and 3 to 17, wherein the method comprises the steps of:

i) culturing the recombinant cell of any one of the preceding claims, and

ii) detecting lactose from the recombinant cell culture media in i).

18. A cell culture comprising the recombinant cell of any one of claims 1 and 3 to 15, and a culture medium.

19. A cryopreserved cell culture comprising the recombinant cell of any one of claims 1 and 3 to 15.

20. The recombinant cell of any one of claims 1 and 3 to 15, wherein the lactose is further modified within said cell to produce human milk oligosaccharides (HMOs).

21. The recombinant cell of claim 20, wherein the human milk oligosaccharides (HMOs) produced include but not limited to sialyl-lactose, lacto-N-neotetraose (LNnT), sialyl-LNnT, para-lacto-N-hexaose (para-LNH), sialyl-para-LNH, para-lacto-N-octaose.

22. The recombinant cell of claim 21, wherein the sialyl-lactose is a 3' sialyl-lactose (3'SL).