[0005] In view of the above, in order to streamline the process of generating stable cell line, there is a need to provide an alternative expression vector comprising a polynucleotide encoding for an alternative glutamine synthetase.

SUMMARY

[0005a] In one aspect, there is provided an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest; wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, and K333A of a glutamine synthetase; and wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

[0006] In one example, there is provided an expression vector comprising a polynucleotide encoding for a glutamine synthetase with reduced activity compared to a wild type glutamine synthetase. In one example, the expression vector further comprising at least one polynucleotide encoding for a polypeptide of interest. In another example, the glutamine synthetase with reduced activity comprises one or more mutations as compared to a wild type glutamine synthetase. In yet another example, the glutamine synthetase with reduced activity comprises one or more mutations in the glutamate binding region, the ATP binding region and/or the ammonia binding region. In yet another example, (i) the mutation in the glutamate binding region is of at least one amino acid at the position selected from the group consisting of 134, 136, 196, 203, 248, 249, 253, 299, 319, 338, and 340 of a glutamine synthetase; or (ii)

the mutation in the ammonia binding region is of at least one amino acid at the position selected from the group consisting of 63, 66, 162, and 305 of a glutamine synthetase; or (iii) the mutation in the ATP binding region is of at least one amino acid at the position selected from the group consisting of 192, 255, 257, 262, 333, 336, and 324 of a glutamine synthetase; wherein the glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO:4. In yet another example, the mutation is of at least one amino acid at the position selected from the group consisting of 12, 19, 63, 66, 91, 116, 134, 136, 160, 162, 172, 176, 181, 192, 194, 196, 198, 199, 203, 230, 248, 249, 253, 255, 257, 260, 262, 271, 299, 305, 319, 336, 338, 333, 340, 324 and 341 of a glutamine synthetase, wherein the glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO:4. In yet another example, the mutation is a substitution. In yet another example, the mutation in the glutamine synthetase is selected from the group consisting of R324C, R341C, D63A, S66A, E134A, E136A, Y162A, G192A, E196A, E203A, N248A, G249A, H253A, N255A, S257A, R262A, R299A, E305A, R319A, R324A, Y336A, E338A, K333A, R340A, and R341A of a glutamine synthetase, wherein the glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO:4.

[0007] In yet another embodiment, the polynucleotide encoding for a glutamine synthetase with reduced activity is operatively linked to a promoter. In yet another embodiment, only one of the at least one polynucleotide encoding for a polypeptide of interest is operatively linked to a promoter. In yet another embodiment, the polynucleotide encoding for a glutamine synthetase with reduced activity compared to a wild type glutamine synthetase is not linked to a promoter. In yet another embodiment, the polypeptide of interest is selected from the group consisting of a recombinant protein and a part thereof, a fusion protein and a part thereof, an antibody and a part thereof. In yet another embodiment, the polypeptide of interest is the heavy chain of an antibody of interest and/or light chain of an antibody of interest. In yet another embodiment, the expression vector comprises two polynucleotides each encoding for a polypeptide of interest, wherein one of the polynucleotides encodes for the light chain of an antibody of interest, and one of the polynucleotides encodes for the heavy chain of the same antibody of interest. In yet another embodiment, the polynucleotide encoding for the light chain of an antibody of interest and the polynucleotide encoding for the heavy chain of the same antibody of interest are separated by polynucleotides encoding an internal ribosome entry site (IRES). In yet another embodiment, the polynucleotide encoding for the heavy chain of an antibody of interest is separated from the polynucleotide encoding for a glutamine synthetase with reduced activity by polynucleotide encoding an internal ribosome entry site (IRES). In yet another embodiment, the polynucleotide encoding the internal ribosome entry site separating the polynucleotide encoding for the light chain of an antibody of interest and the polynucleotide encoding for the heavy chain of the same antibody of interest is polynucleotide encoding a wild type internal ribosome entry site (IRES), and wherein the internal ribosome entry site separating the polynucleotide encoding for the heavy chain of an antibody of interest from the polynucleotide encoding for a glutamine synthetase with reduced activity is polynucleotide encoding an internal ribosome entry site with attenuated translation efficiency (IRESatt).

[0007a] In yet another aspect, there is provided a method for preparing stable cell line, comprising: (a) transforming a host cell having no glutamine synthetase activity with an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest, wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R341C, R324C, Y162A, G192A, G249A, S257A,

R262A, R299A, E305A, Y336A, K333A and R341A of a glutamine synthetase, wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4; and (b) culturing the transformed host cell in a medium that selectively allows the proliferation of the transformed host cells comprising an amplified number of copies of the vector to be selected.

[0008] In another aspect, there is provided a host cell comprising the expression vector as described herein. In yet another example, there is provided a method for preparing stable cell line, comprising: (a) transforming a host cell having no glutamine synthetase activity with the expression vector as described herein; and (b) culturing the transformed host cell in a medium that selectively allows the proliferation of the transformed host cells comprising an amplified number of copies of the vector to be selected.

[0008a] In yet another aspect, there is provided a method of producing polypeptide of interest, comprising: (a) transforming a host cell having no glutamine synthetase activity with an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest, wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R341C, R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, K333A and R341A of a glutamine synthetase, wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4; (b) culturing the transformed host cell in a medium that selectively allows the production of polypetide of interest; and (c) collecting the polypeptide of interest from the medium of (b).

[0009] In yet another example, there is provided a method of producing polypeptide of interest, comprising: (a) transforming a host cell having no glutamine synthetase activity with the expression vector as described herein; (b) culturing the transformed host cell in a medium that selectively allows the production of polypeptide of interest; and (c) collecting the polypeptide of interest from the medium of (b). In yet another embodiment, the medium is a glutamine free cell culture medium. In yet another embodiment, the method does not comprise the use of a glutamine synthetase inhibitor, optionally wherein the glutamine synthetase inhibitor is methionine sulphoximine (MSX).

[0009a] In yet another aspect, there is provided a kit comprising an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest, wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R341C, R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, K333A and R341A of a glutamine synthetase, wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

[0010] In yet another example, there is provided a kit comprising the expression vector as described herein. In yet another embodiment, the kit further comprises at least one of the following: (i) a host cell having no glutamine synthetase activity compared to a wild type

cell; and (ii) a transfection medium or means to carry out a transfection. In yet another embodiment, the kit further comprises a glutamine free cell culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0010] **Figure 1** shows a pair of line graphs depicting amount of antibodies produced using CHOK1 $GFT^{-/-}$ $GS^{-/-}$ cells that are transfected with either Tricistronic mAb-GS (SEQ ID NO: 14) or Tricistronic mAb-GSatt (R324C) (SEQ ID NO: 85) in minipools format. **Figure 1A** depicts the experimental result for Antibody A and **Figure 1B** depicts the experimental result for Antibody B. Thus, **Figure 1** illustrates that for production of both Antibody A and Antibody B, the amount of antibody produced using Tricistronic mAb-GSatt (R324C) is higher than the amount of antibody produced using Tricistronic mAb-GS.

Figure 2 shows a pair of bar graphs depicting amount of antibodies titer (mAb titer) produced using CHOK1 $GFT^{-/-}$ $GS^{-/-}$ cells that are transfected with either Tricistronic mAb-GS (SEQ ID NO: 14) or Tricistronic mAb-GSatt (R324C) (SEQ ID NO: 85) in stable suspension pool format. For preliminary analysis, the WT GS system (i.e. CHOK1 $GFT^{-/-}$ $GS^{-/-}$ cells that are transfected with Tricistronic mAb-GS) and attenuated GS system (i.e. CHOK1 $GFT^{-/-}$ $GS^{-/-}$ cells that are transfected with mAb-GSatt (R324C)) were compared directly in the absence of glutamine for a fair comparison. Thus, the WT GS pools were not

Claims

1. An expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest;
wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, and K333A of a glutamine synthetase; and
wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

2. The expression vector of claim 1, wherein (i) the polynucleotide encoding for a glutamine synthetase is operatively linked to a promoter; or (ii) wherein only one of the at least one polynucleotide encoding for a polypeptide of interest is operatively linked to a promoter; optionally wherein the polynucleotide encoding for a glutamine synthetase compared to a wild type glutamine synthetase is not linked to a promoter.

3. The expression vector of any one of claims 1 and 2, wherein (i) the polypeptide of interest is selected from the group consisting of a recombinant protein and a part thereof, a fusion protein and a part thereof, an antibody and a part thereof; or (ii) wherein the polypeptide of interest is the heavy chain of an antibody of interest and/or light chain of an antibody of interest; or (iii) wherein the expression vector comprises two polynucleotides each encoding for a polypeptide of interest, wherein one of the polynucleotides encodes for the light chain of an antibody of interest, and one of the polynucleotides encodes for the heavy chain of the same antibody of interest.

4. The expression vector of any one of claims 1 to 3, wherein (i) the polynucleotide encoding for the light chain of an antibody of interest and the polynucleotide encoding for the heavy chain of the same antibody of interest are separated by polynucleotides encoding an internal ribosome entry site (IRES); or (ii) wherein the polynucleotide encoding for the heavy chain of an antibody of interest is separated from the

polynucleotide encoding for a glutamine synthetase with reduced activity by polynucleotide encoding an internal ribosome entry site (IRES).

5. The expression vector of claim 4, wherein the polynucleotide encoding the internal ribosome entry site separating the polynucleotide encoding for the light chain of an antibody of interest and the polynucleotide encoding for the heavy chain of the same antibody of interest is polynucleotide encoding a wild type internal ribosome entry site (IRES), and wherein the internal ribosome entry site separating the polynucleotide encoding for the heavy chain of an antibody of interest from the polynucleotide encoding for a glutamine synthetase with reduced activity is polynucleotide encoding an internal ribosome entry site with attenuated translation efficiency (IRESatt).

6. A host cell comprising the expression vector of any one of claims 1 to 5.

7. A method for preparing stable cell line, comprising:

(a) transforming a host cell having no glutamine synthetase activity with an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest,
wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R341C, R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, K333A and R341A of a glutamine synthetase,
wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4; and

(b) culturing the transformed host cell in a medium that selectively allows the proliferation of the transformed host cells comprising an amplified number of copies of the vector to be selected.

8. A method of producing polypeptide of interest, comprising:

(a) transforming a host cell having no glutamine synthetase activity with an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest,

wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R341C, R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, K333A and R341A of a glutamine synthetase,

wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4;

(b) culturing the transformed host cell in a medium that selectively allows the production of polypetide of interest; and

(c) collecting the polypeptide of interest from the medium of (b).

9. The method of claim 7 or 8, wherein (i) the medium is a glutamine free cell culture medium; or (ii) wherein the method does not comprise the use of a glutamine synthetase inhibitor, optionally wherein the glutamine synthetase inhibitor is methionine sulphoximine (MSX).

10. A kit comprising an expression vector comprising a polynucleotide encoding for a selection marker and at least one polynucleotide encoding for a polypeptide of interest, wherein the selection marker is an attenuated glutamine synthetase comprising one or more mutations as compared to a wild type glutamine synthetase, and the one or more mutations in the glutamine synthetase is/are selected from the group consisting of R341C, R324C, Y162A, G192A, G249A, S257A, R262A, R299A, E305A, Y336A, K333A and R341A of a glutamine synthetase,

wherein the wildtype glutamine synthetase has the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

11. The kit of claim 10, wherein the kit further comprises at least one of the following:

(i) a host cell having no glutamine synthetase activity compared to a wild type cell; and

(ii) a transfection medium or means to carry out a transfection;

optionally wherein the kit further comprises a glutamine free cell culture medium.