[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

[0006] In one aspect, there is provided an expression vector comprising a polynucleotide encoding for a glutamine synthetase with reduced activity compared to a wild type glutamine synthetase; and wherein the expression vector further comprises at least one polynucleotide encoding for a polypeptide of interest; and wherein 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; and wherein (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, 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 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 example, the polynucleotide encoding for a glutamine synthetase with reduced activity is operatively linked to a promoter. In yet another example, only one of the at least one polynucleotide encoding for a polypeptide of interest is operatively linked to a promoter. In yet another example, 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 example, 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 example, 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 example, 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 example, 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 example, 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 example, 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).

[0008] In another aspect, there is provided a host cell comprising the expression vector as described herein. 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 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.

[0009] 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 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 example, the medium is a glutamine free cell culture medium. In yet another example, the method does not comprise the use of a glutamine synthetase inhibitor, optionally wherein the glutamine synthetase inhibitor is methionine sulphoximine (MSX).

[0010] In yet another aspect, there is provided a kit comprising the expression vector as described herein. In yet another example, 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 example, the kit further comprises a glutamine free cell culture medium.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0011] 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:

[0012] **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.

[0013] **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 glutamine synthetase with reduced activity compared to a wild type glutamine synthetase; and wherein the expression vector further comprises at least one polynucleotide encoding for a polypeptide of interest; and

wherein 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; and

wherein

(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, 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.

2. The expression vector of claim 1, wherein 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, 339, 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,

further wherein the mutation is a substitution.

3. The expression vector of claim 1 or claim 2, wherein 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.

4. The expression vector of any one of claims 1 to 3, wherein the polynucleotide encoding for a glutamine synthetase with reduced activity is operatively linked to a promoter.

5. The expression vector of any one of claims 1 to 4, wherein only one of the at least one polynucleotide encoding for a polypeptide of interest is operatively linked to a promoter.

6. The expression vector of claim 5, wherein the polynucleotide encoding for a glutamine synthetase with reduced activity compared to a wild type glutamine synthetase is not linked to a promoter.

7. The expression vector of any one of claims 1 to 6, wherein 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.

8. The expression vector of any one of claims 1 to 7, wherein the polypeptide of interest is the heavy chain of an antibody of interest and/or light chain of an antibody of interest.

9. The expression vector of any one of claims 1 to 8, 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.

10. The expression vector of any one of claims 1 to 9, wherein 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).

11. The expression vector of any one of claims 1 to 10, 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).

12. The expression vector of claim 10 or 11, 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).

13. A host cell comprising the expression vector of any one of claims 1 to 12.

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

(a) transforming a host cell having no glutamine synthetase activity with the expression vector of any one of claims 1 to 12; 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.

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

(a) transforming a host cell having no glutamine synthetase activity with the expression vector of any one of claims 1 to 12;

(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).

16. The method of claim 14 or 15, wherein the medium is a glutamine free cell culture medium.

17. The method of any one of claims 14 to 16, wherein the method does not comprise the use of a glutamine synthetase inhibitor, optionally wherein the glutamine synthetase inhibitor is methionine sulphoximine (MSX).

18. A kit comprising the expression vector of any of claims 1 to 12.

19. The kit of claim 18, 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.

20. The kit of claim 18 or 19, wherein the kit further comprises a glutamine free cell culture medium.