Claims

1. An expression vector comprising a polynucleotide encoding for a glutamine synthetase with reduced activity compared to a wild type glutamine synthetase.

2. The expression vector of claim 1, wherein the expression vector further comprising at least one polynucleotide encoding for a polypeptide of interest.

3. The expression vector of claim 1 or 2, wherein the glutamine synthetase with reduced activity comprises one or more mutations as compared to a wild type glutamine synthetase.

4. The expression vector of claim 3, 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.

5. The expression vector of claim 4, 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.

6. The expression vector of any one of claims 3 to 5, 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.

7. The expression vector of claim 6, wherein the mutation is a substitution.

8. The expression vector of any one of claims 4 to 7, 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.

9. The expression vector of any one of claims 1 to 8, wherein the polynucleotide encoding for a glutamine synthetase with reduced activity is operatively linked to a promoter.

10. The expression vector of any one of claims 1 to 9, wherein only one of the at least one polynucleotide encoding for a polypeptide of interest is operatively linked to a promoter.

11. The expression vector of claim 10, wherein the polynucleotide encoding for a glutamine synthetase with reduced activity compared to a wild type glutamine synthetase is not linked to a promoter.

12. The expression vector of any one of claims 2 to 11, 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.

13. The expression vector of any one of claims 2 to 12, wherein the polypeptide of interest is the heavy chain of an antibody of interest and/or light chain of an antibody of interest.

14. The expression vector of any one of claims 2 to 13, 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.

15. The expression vector of any one of claims 2 to 14, 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).

16. The expression vector of any one of claims 2 to 15, 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).

17. The expression vector of claim 15 or 16, 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).

18. A host cell comprising the expression vector of any one of claims 1 to 17.

19. 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 17; 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.

20. 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 2 to 17;

(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).

21. The method of claim 19 or 20, wherein the medium is a glutamine free cell culture medium.

22. The method of any one of claims 19 to 21, wherein the method does not comprise the use of a glutamine synthetase inhibitor, optionally wherein the glutamine synthetase inhibitor is methionine sulphoximine (MSX).

23. A kit comprising the expression vector of any of claims 1 to 17.

24. The kit of claim 23, 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.

25. The kit of claim 23 or 24, wherein the kit further comprises a glutamine free cell culture medium.