CLAIMS

What is claimed is:

1. A mammalian host cell for expressing an antigen binding protein having three or four different chains, comprising a first expression vector and a second expression vector, wherein:

(a) the first expression vector comprises nucleotide sequences encoding 1) a first chain and a second chain, wherein the first chain is operably linked to a first promoter, the second chain is operably linked to a second promoter or an IRES sequence or is linked to the first chain through a linker sequence; and 2) a first selectable marker operably linked to a third promoter; and

(b) the second expression vector comprises nucleotide sequences encoding 1) a third chain and a fourth chain, wherein the third chain is operably linked to a fourth promoter, the fourth chain is operably linked to a fifth promoter or an IRES sequence or is linked to the third chain through a linker sequence; and 2) a second selectable marker operably linked to a sixth promoter,

wherein the first, second, third and fourth chains are selected from the group consisting of a heavy chain, a light chain, an antibody heavy chain fusion, an antibody light chain fusion, an ScFv, and an ScFv-Fc, wherein two of the chains may be identical; and

wherein the sixth promoter is different from the third promoter.

2. The mammalian host cell of claim 1, wherein the second chain and the fourth chain are operably linked to a second promoter and fourth promoter, respectively.

3. The mammalian host cell of claim 1, wherein the first selectable marker and the second selectable marker are the same.

4. The mammalian host cell of claim 3, wherein the first selectable marker and the second selectable marker are selected from the group consisting of glutamine synthetase and dihydrofolate reductase.

5. The mammalian host cell of claim 4, wherein the first selectable marker and the second selectable marker are glutamine synthetase.

6. The mammalian host cell of claim 1, wherein the third promoter and the sixth promoter are selected from mPGK and Srα.

7. The mammalian host cell of claim 2, wherein the first, second, fourth and fifth promoters are different from the third and sixth promoters.

8. The mammalian host cell of claim 7, wherein the first, second, fourth and fifth promoters are selected from the group consisting of CMV/GAPDH, CMV/adL, and CMV/EF1α.

9. The mammalian host cell of claim 7, wherein the first and fourth promoters are identical and the second and fifth promoters are identical.

10. The mammalian host cell of claim 9, wherein the first, second, fourth and fifth promoters are identical.

11. The mammalian host cell of claim 9, wherein the first and fourth promoters are different from the second and fifth promoters.

12. The mammalian host cell of claim 1, wherein the stronger promoter of the third promoter and the sixth promoter is on the expression vector with the more difficult-to-express chains.

13. The mammalian host cell of claim 12, wherein Srα is on the expression vector with the more difficult-to-express chains and mPGK is on the expression vector with the easier-to-express chains.

14. The mammalian host cell of claim 5, wherein methionine sulfoximine (MSX) stringency is optimized to favor expression of the more difficult-to-express chain paired with the stronger promoter.

15. The mammalian host cell of claim 14, wherein the MSX stringency is less than 75μM.

16. The mammalian host cell of claim 1, wherein the stronger promoter of the third promoter and the sixth promoter is on the expression vector with the easier-to-express chains.

17. The mammalian host cell of claim 1, wherein the first and third chains are first and second antibody light chains, respectively, and the second and fourth chains are antibody heavy chains.

18. The mammalian host cell of claim 1, wherein a) the first and third chains are first and second antibody light chains, respectively, and b) one of the second or fourth chain is an antibody heavy chain and the other is an antibody heavy chain fusion.

19. The mammalian host cell of claim 17 or 18, wherein the first and second antibody light chains are antibody light chains that are identical.

20. The mammalian host cell of claim 17 or 18, wherein the first and second antibody light chains are antibody light chains that are different.

21. The mammalian host cell of claim 1, wherein the antigen binding protein having three or four chains is selected from a heteroIgG, and a C1mAb.

22. The mammalian host cell of claim 1, wherein the first expression vector comprises in 5’ to 3’ order a first promoter, a nucleotide sequence encoding a first antibody light chain, a second promoter or an IRES, a nucleotide sequence encoding a first heavy chain, a third promoter which is Srα, and a nucleotide sequence encoding a selectable marker which is glutamine synthetase; and the second expression vector comprises in 5’ to 3’ order a fourth promoter, a nucleotide sequence encoding a second antibody light chain, a fifth promoter or an IRES, a nucleotide sequence encoding a second heavy chain, a sixth promoter which is mPGK, and a nucleotide sequence encoding a selectable marker which is glutamine synthetase.

23. The mammalian host cell of claim 1, wherein the first expression vector comprises in 5’ to 3’ order a first promoter, a nucleotide sequence encoding a first antibody light chain, a second promoter, a nucleotide sequence encoding a first heavy chain, a third promoter which is Srα and a nucleotide sequence encoding a selectable marker which is glutamine synthetase; and the second expression vector comprises in 5’ to 3’ order a fourth promoter, a nucleotide sequence encoding a second antibody light chain, a fifth promoter, a nucleotide sequence encoding a second heavy chain, a sixth promoter which is mPGK, and a nucleotide sequence encoding a selectable marker which is glutamine synthetase.

24. The mammalian host cell of claim 22 or 23, wherein the first heavy chain is more difficult-to-express than the second heavy chain.

25. The mammalian host cell of claim 1, wherein the first expression vector comprises in 5’ to 3’ order a first promoter, a nucleotide sequence encoding an antibody light chain, a second promoter or an IRES, a nucleotide sequence encoding a heavy chain fusion, a third promoter which is Srα, and a nucleotide sequence encoding a selectable marker which is glutamine synthetase; and the second expression vector comprises in 5’ to 3’ order a fourth promoter, a nucleotide sequence encoding the antibody light chain, a fifth promoter or an IRES, a nucleotide sequence encoding a heavy chain, a sixth promoter which is mPGK, and a nucleotide sequence encoding a selectable marker which is glutamine synthetase.

26. The mammalian host cell of claim 1, wherein the first expression vector comprises in 5’ to 3’ order a first promoter, a nucleotide sequence encoding an antibody light chain, a second promoter, a nucleotide sequence encoding a heavy chain fusion, a third promoter which is Srα, and a selectable marker which is glutamine synthetase; and the second expression vector comprises in 5’ to 3’ order a fourth promoter, a nucleotide sequence encoding the antibody light chain, a fifth promoter, a nucleotide sequence encoding a heavy

chain, a sixth promoter which is mPGK, and a nucleotide sequence encoding a selectable marker which is glutamine synthetase.

27. The mammalian host cell of claim 22, 23, 25, or 26, wherein the first antibody light chain and the second antibody light chain are identical.

28. The mammalian host cell of claim 1, wherein the first and second expression vectors are integrated in the genome of the host cell.

29. The mammalian host cell of claim 1, wherein the host cell is a Chinese Hamster Ovary (CHO) cell.

30. The mammalian host cell of claim 29, wherein the CHO cell is deficient in dihydrofolate reductase ($DHFR^-$) or is a glutamine synthetase knock out (GSKO) cell.

31. A vector system for genetically engineering a mammalian host cell for expressing an antigen binding protein having three or four different chains, comprising a first expression vector and a second expression vector, wherein:

(a) the first expression vector comprises nucleotide sequences encoding 1) a first chain and a second chain, wherein the first chain is operably linked to a first promoter, the second chain is operably linked to a second promoter or an IRES sequence or is linked to the first chain through a linker sequence; and 2) a first selectable marker operably linked to a third promoter; and

(b) the second expression vector comprises nucleotide sequences encoding 1) a third chain and a fourth chain, wherein the third chain is operably linked to a fourth promoter, the fourth chain is operably linked to a fifth promoter or an IRES sequence or is linked to the third chain through a linker sequence; and 2) a second selectable marker operably linked to a sixth promoter,

wherein the first, second, third and fourth chains are selected from the group consisting of a heavy chain, a light chain, an antibody heavy chain fusion, an antibody light chain fusion, an ScFv, and an ScFv-Fc, wherein two of the chains may be identical; and

wherein the sixth promoter is different from the third promoter.

32. A method for producing an antigen binding protein having three or four chains, the method comprising:

a) culturing the mammalian host cell of claim 1 under conditions to express the antigen binding protein; and

b) recovering the antigen binding protein.

33. The method of claim 32, wherein the recovered antigen binding protein is purified and formulated in a pharmaceutically acceptable formulation.

34. The method of claim 32 or 33, wherein the mammalian host cell is:

a CHO DHFR- cell and is cultured under methotrexate stringency; or

a CHO GSKO cell and is cultured under methionine sulfoxamine stringency to favor expression of the difficult-to-express chain paired with the stronger GS promoter.