

WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising
 - a) at least one expression cassette comprising a DNA sequence encoding a protein of interest operably linked to a promoter and a transcription termination site;
 - 5 b) a selectable marker; and
 - c) an Epstein Barr virus (EBV) oriP or functional fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment.
2. The nucleic acid construct of claim 1, wherein the oriP functional fragment comprises a nucleic acid sequence at least 90% identity, at least 95% identity, at least 99% identity or
10 100% identity to the sequence as set forth in SEQ ID NO: 1.
3. The nucleic acid construct of claim 1, wherein the oriP or functional fragment comprises a nucleic acid sequence at least 90% identity, at least 95% identity, at least 99% identity or 100% identity to the sequence as set forth in SEQ ID NO: 2.
4. The nucleic acid construct of any one of claims 1 to 3, further comprising a Scaffold
15 Attachment Region (SAR).
5. The nucleic acid construct of any one of claims 1 to 4, wherein promoter is an inducible promoter.
6. The nucleic acid construct of claim 5, wherein the inducible promoter is a tetracycline response element (TRE), a ponA-inducible promoter, or a cumate-inducible promoter.
- 20 7. The nucleic acid construct of claim 6, wherein the inducible promoter is a cumate-inducible promoter.
8. The nucleic acid construct of any one of claims 1 to 4, wherein the promoter is a constitutive promoter.
9. The nucleic acid construct of claim 8, wherein the constitutive promoter is human
25 Ubiquitin C (UBC) promoter, human Elongation Factor 1 alpha (EF1A) promoter, human

phosphoglycerate kinase 1 (PGK) promoter, simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter (CMV), chicken b-Actin promoter coupled with CMV early enhancer (CAG), hybrid EF1-HTLV promoter or Chinese hamster EF1 (CHEF) promoter.

- 5 10. The nucleic acid construct of any one of claims 1 to 9, wherein the selectable marker is a neomycin resistance gene, a hygromycin resistance gene, a puromycin resistance gene, a blasticidin resistance gene, a zeocin resistance gene or a Glutamine Synthetase (GS) gene.
11. The nucleic acid construct of any one of claims 1 to 10, wherein the expression
10 cassette encodes an antibody fragment, an antibody heavy chain and/or an antibody light chain.
12. The nucleic acid construct of any one of claims 1 to 11, wherein the nucleic acid construct comprises two expression cassettes.
13. The nucleic acid construct of claim 12, wherein one expression cassette encodes an
15 antibody heavy chain and one expression cassette encodes an antibody light chain.
14. A method of production of a protein of interest in a mammalian cell, the method comprising:
- a) introducing into the mammalian cell one or more nucleic acid constructs according to any one of claims 1 to 13;
- 20 b) applying selective pressure to the cell to select for cells that carry the selectable marker; and
- c) culturing the cell under conditions for production of the protein of interest.
15. The method of claim 14, wherein two different nucleic acid constructs according to any one of claims 1 to 13 are introduced into the cell.
- 25 16. The method of claim 14 or 15, wherein the one or more nucleic acid constructs are introduced into the cell by transfection.

17. The method of claim 16, wherein the transfection is carried out using a transfection reagent, wherein the transfection reagent is a cationic lipid, a non-liposomal reagent, or a cationic polymer, optionally polyethylenimine (PEI).
18. The method of any one of claims 14 to 17, wherein the protein production is increased
5 by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, or at least 250% relative to protein production in a cell from a nucleic acid construct lacking an oriP when cultured under the same conditions.
19. The method of any one of claims 14 to 18, wherein the mammalian cell is a SP2/0
10 cell, NS/0 cell, HT-1080 cell, PER.C6 cell, HKB-11 cell, CAP cell, HuH-7 cell, Chinese Hamster Ovary (CHO) cell or a Human Embryonic Kidney 293 (HEK293) cell.
20. The method of claim 19, wherein the cell is a CHO cell.
21. The method of any one of claims 14 to 20, wherein the cell does not express EBNA1.
22. The method of any one of claims 14 to 21, wherein the selectable marker of the nucleic
15 acid construct is glutamine synthetase (GS) and the selective pressure applied is the withdrawal of glutamine from a growth medium.
23. The method of any one of claims 14 to 22, wherein the promoter is an inducible promoter and the conditions for the production of the protein of interest comprise the addition of an inducing agent.
- 20 24. The method of any one of claims 14 to 23, wherein the nucleic acid construct is integrated into the genome of the mammalian cell.
25. The method of any one of claims 14 to 24, wherein the method further comprises collecting the mammalian cell and/or a cell medium containing the protein of interest, and optionally purifying the protein of interest from the collected cell and/or cell medium.

26. The method of any one of claims 14 to 25, wherein the protein of interest is an antibody or antibody fragment.
27. The method of any one of claims 14 to 26, wherein the nucleic acid construct encodes an antibody heavy chain and/or an antibody light chain, and the protein being produced is an antibody, optionally the antibody is cetuximab.
28. A mammalian cell for increased production of a protein of interest, the cell comprising one or more nucleic acid constructs of any one of claims 1 to 13.
29. The mammalian cell of claim 28, wherein the cell comprises two nucleic acid constructs, each construct encoding a different protein of interest.
30. The mammalian cell of claim 28 or 29, wherein the cell is stably transfected with the nucleic acid construct or constructs.
31. The mammalian cell of claim 30, wherein the nucleic acid construct or constructs is or are integrated into the genome.
32. The mammalian cell of any one of claims 28 to 31, wherein the cell expresses an antibody or antibody fragment.
33. The mammalian cell of claim 32, wherein the antibody is cetuximab, palivizumab, rituximab, or trastuzumab.
34. The mammalian cell of any one of claims 28 to 33, wherein the cell is a SP2/0 cell, NS/0 cell, HT-1080 cell, PER.C6 cell, HKB-11 cell, CAP cell, HuH-7 cell, Chinese Hamster Ovary (CHO) cell or a Human Embryonic Kidney 293 (HEK293) cell.
35. The mammalian cell of any one of claims 28 to 34, wherein the cell does not express EBNA1.