

of the EBNA1 protein EBV oriP system for increasing productivity in stable cell lines. The presence of oriP *per se* was found to increase productivity of stable pools, and presence of EBNA1 in the cell line did not increase the productivity in this context.

5 [0006a] In one aspect, the present disclosure provides a method of production of a protein of interest in a mammalian cell, the method comprising: a) introducing into the mammalian cell a nucleic acid construct comprising a selectable marker; 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, wherein the mammalian cell does not express Epstein–Barr virus nuclear antigen 1 (EBNA1) and
10 wherein the nucleic acid construct comprises: i) at least one expression cassette comprising a DNA sequence encoding the protein of interest operably linked to a promoter and a transcription termination site; and ii) an Epstein Barr virus (EBV) oriP or a fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment, wherein the cell is a CHO cell.

15 [0006b] In another aspect, the present disclosure provides a mammalian cell for production of a protein of interest, the cell comprising a nucleic acid construct, wherein the cell does not express Epstein–Barr virus nuclear antigen 1 (EBNA1) and wherein the nucleic acid construct comprises: a) at least one expression cassette comprising a DNA sequence encoding the protein of interest operably linked to a promoter and a
20 transcription termination site; b) a selectable marker; and c) an Epstein Barr virus (EBV) oriP or a fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment, wherein the cell is a CHO cell.

[0007] Accordingly, one example of the disclosure is a nucleic acid construct for the expression of a protein of interest. The nucleic acid construct of the disclosure comprises
25 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; b) a selectable marker; and c) an Epstein Barr Virus (EBV) origin of replication (oriP) or functional fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment. In

WHAT IS CLAIMED IS:

1. A method of production of a protein of interest in a mammalian cell, the method comprising:
 - a) introducing into the mammalian cell a nucleic acid construct comprising a selectable marker;
 - 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,wherein the mammalian cell does not express Epstein–Barr virus nuclear antigen 1 (EBNA1) and
wherein the nucleic acid construct comprises:
 - i) at least one expression cassette comprising a DNA sequence encoding the protein of interest operably linked to a promoter and a transcription termination site; and
 - ii) an Epstein Barr virus (EBV) oriP or a fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segmentwherein the cell is a CHO cell.
2. The method of claim 1, wherein two different nucleic acid constructs are introduced into the cell and wherein each nucleic acid construct is as defined in claim 1.
3. The method of claim 1 or 2, wherein the nucleic acid construct comprises a Scaffold Attachment Region (SAR).
4. The method of any one of claims 1 to 3, wherein the oriP fragment comprises a sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

5. The method of any one of claims 1 to 4, wherein the protein production is increased by at least 20% relative to protein production in a cell from a control nucleic acid construct lacking the oriP or fragment thereof when cultured under the same conditions.
6. The method of any one of claims 1 to 5, wherein the nucleic acid construct is integrated into the genome of the mammalian cell.
7. The method of any one of claims 1 to 6, wherein the method further comprises purifying the protein of interest.
8. The method of any one of claims 1 to 7, wherein the protein of interest is an antibody or an antibody fragment.
9. The method of any one of claims 1 to 8, wherein the nucleic acid construct encodes an antibody heavy chain and/or an antibody light chain, and the protein being produced is an antibody.
10. A mammalian cell for production of a protein of interest, the cell comprising a nucleic acid construct,

wherein the cell does not express Epstein–Barr virus nuclear antigen 1 (EBNA1) and wherein the nucleic acid construct comprises:

- a) at least one expression cassette comprising a DNA sequence encoding the protein of interest operably linked to a promoter and a transcription termination site;
- b) a selectable marker; and
- c) an Epstein Barr virus (EBV) oriP or a fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment

wherein the cell is a CHO cell.

11. The mammalian cell of claim 10, wherein the cell comprises two nucleic acid constructs, wherein each nucleic acid construct is as defined in claim 12, and wherein each nucleic acid construct encodes a different protein of interest.
12. The mammalian cell of claim 10 or 11, wherein the oriP fragment comprises a sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
13. The mammalian cell of any one of claims 10 to 12, wherein the nucleic acid construct is integrated into the genome.
14. The mammalian cell of any one of claims 10 to 13, wherein the protein of interest is an antibody or antibody fragment.
15. The mammalian cell of any one of claims 10 to 14, wherein the nucleic acid construct encodes an antibody heavy chain and/or an antibody light chain, and the protein being produced is an antibody.
16. The mammalian cell of any one of claims 10 to 15, wherein the nucleic acid construct comprises a Scaffold Attachment Region (SAR).