

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.

[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 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 functional fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment.

[0006b] In another aspect, the present disclosure provides a mammalian cell for increased 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 functional fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment.

[0007] Accordingly, one ~~aspect~~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 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

an embodimentexample, the oriP or functional fragment thereof has at least 90% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[0008] In an embodimentexample, the nucleic acid construct further comprises a Scaffold Attachment Region (SAR).

5 [0009] In an embodimentexample, the promoter is an inducible promoter, optionally a tetracycline response element (TRE), a ponA-inducible promoter, or a cumate-inducible promoter.

[0010] In an embodimentexample, the promoter is a constitutive promoter, optionally a human Ubiquitin C (UBC) promoter, human Elongation Factor 1 alpha (EF1A) promoter,
10 human phosphoglycerate kinase 1 (PGK) promoter, simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter (CMV) promoter, chicken b-Actin promoter coupled with CMV early enhancer (CAG), the hybrid EF1-HTLV promoter, or the Chinese hamster EF1 promoter (CHEF).

[0011] In an embodimentexample, the selectable marker is a neomycin resistance
15 gene, a hygromycin resistance gene, a puromycin resistance gene, blasticidin resistance gene, zeocin resistance gene or optionally a Glutamine Synthetase (GS) gene.

[0012] In an embodimentexample, the expression cassette encodes an antibody or antibody fragment, or an antibody heavy chain and/or an antibody light chain.

[0013] In some embodimentexample, the nucleic acid construct comprises two expression cassettes. In an embodimentexample, one expression cassette encodes an antibody heavy chain and one expression cassette encodes an antibody light chain.

[0014] Another aspectexample of the disclosure is a method of production of a protein of interest, the method comprising: a) introducing into a mammalian cell the nucleic acid construct of the disclosure; 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. In some embodimentexample two different nucleic acid constructs of the disclosure are introduced into the mammalian cell.

[0015] In one embodimentexample, the nucleic acid construct or constructs are introduced into the cell by transfection. In some embodimentexamples, the transfection is carried out by a transfection reagent such as a cationic lipid, a non-liposomal reagent, or a cationic polymer. Optionally the cationic polymer is polyethylenimine (PEI). In other embodimentexamples, the transfection is calcium phosphate transfection or electroporation/nucleofection.

[0016] In one embodimentexample protein production is increased 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.

[0017] In one embodimentexample, the mammalian 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. In one embodimentexample, the cell does not express EBNA1.

[0018] In an embodimentexample, the selectable marker is glutamine synthetase (GS) and the selective pressure applied is the withdrawal of glutamine from a growth medium. In another embodimentexample, the selective agent is methionine sulfoximine (MSX) selection of glutamine synthetase overexpressing cells. In yet another embodimentexample, the selective marker is methotrexate selection of dihydrofolate reductase (DHFR) expressing cells.

[0019] In another embodimentexample, the promoter is an inducible promoter and the conditions for the production of the protein of interest comprise the addition of an inducing agent.

[0020] In a further embodimentexample, the nucleic acid is integrated into the genome of the mammalian cell.

[0021] In one embodimentexample, 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 the cell medium.

[0022] In some embodimentexamples, the nucleic acid encodes an antibody fragment, an antibody heavy chain and/or an antibody light chain. In some embodimentexamples, the protein of interest is an antibody or antibody fragment, optionally cetuximab or a fragment thereof.

[0023] A further aspectexample of the disclosure is a mammalian cell for increased production of a protein of interest comprising one or more nucleic acids constructs of the disclosure. In some embodimentexamples, the cell comprises two different nucleic acid constructs of the disclosure, each encoding a different protein of interest. In some embodimentexamples, the protein of interest is an antibody or an antibody fragment, optionally cetuximab or a fragment thereof.

[0024] In some embodimentexamples, the nucleic acid construct or constructs are stably transfected, optionally the construct or constructs are integrated into the genome of the mammalian cell.

[0025] In some embodimentexamples the mammalian cell is a Chinese Hamster Ovary (CHO) cell or a Human Embryonic Kidney 293 (HEK293) cell. In one embodimentexample, the cell does not express EBNA1.

[0026] The preceding section is provided by way of example only and is not intended to be limiting on the scope of the present disclosure and appended claims. Additional objects and advantages associated with the compositions and methods of the present disclosure will be appreciated by one of ordinary skill in the art in light of the instant claims, description, and examples. For example, the various aspectexamples and embodimentexamples of the disclosure may be utilized in numerous combinations, all of which are expressly contemplated by the present description. These additional advantages, objects and embodimentexamples are expressly included

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;~~
 - 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 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 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.~~
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 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.~~

~~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 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 antibody heavy chain and one expression cassette encodes an antibody light chain.~~

141. A method of production of a protein of interest in a mammalian cell, the method comprising:

a) introducing into the mammalian cell a one or more nucleic acid constructs comprising a selectable marker according to any one of claims 1 to 13;

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 functional fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment.

~~152.~~ 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 and wherein each nucleic acid construct is as defined in claim 1.

~~163.~~ The method of claim 1 or 2~~14 or 15~~, wherein the ~~one or more~~ nucleic acid constructs comprises a Scaffold Attachment Region (SAR)~~are introduced into the cell by transfection.~~

~~174.~~ The method of any one of claims 1 to 3~~16~~, wherein the oriP functional 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~~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).~~

~~185.~~ The method of any one of claims 1 to 4~~14 to 17~~, wherein the protein production is increased 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 control nucleic acid construct lacking ~~an the oriP or functional fragment thereof~~ when cultured under the same conditions.

~~196.~~ The method of any one of claims 1 to 5~~14 to 18~~, wherein the mammalian 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.

~~207.~~ The method of claim 6~~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 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.~~

248. The method of any one of claims 1 to 7~~14 to 23~~, wherein the nucleic acid construct is integrated into the genome of the mammalian cell.

259. The method of any one of claims 1 to 8~~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.~~

2610. The method of any one of claims 1 to 9~~14 to 25~~, wherein the protein of interest is an antibody or an antibody fragment.

2711. The method of any one of claims 1 to 10~~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.~~

2812. A mammalian cell for increased production of a protein of interest, the cell comprising ~~one or more~~a nucleic acid constructs ~~of any one of claims 1 to 13,~~

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 functional fragment thereof comprising a dyad symmetry (DS) region and a family of repeats (FR) segment.

~~29~~13. The mammalian cell of claim ~~12~~28, 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~~encoding a different protein of interest.

~~30~~14. The mammalian cell of claim ~~12 or 13~~28 or 29, wherein the oriP functional 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~~the cell is stably transfected with the nucleic acid construct or constructs.~~

~~31~~15. The mammalian cell of any one of claims 12 to 14~~claim 30~~, wherein the nucleic acid construct ~~or constructs is or are~~is integrated into the genome.

~~32~~16. The mammalian cell of any one of claims ~~12 to 15~~28 to 31, wherein the ~~cell expresses~~protein of interest is an antibody or antibody fragment.

~~33. The mammalian cell of claim 32, wherein the antibody is cetuximab, palivizumab, rituximab, or trastuzumab.~~

~~34~~17. The mammalian cell of any one of claims ~~12 to 16~~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~~18. The mammalian cell of any one of claims ~~12 to 17~~28 to 34, wherein the nucleic acid construct encodes an antibody heavy chain and/or an antibody light chain, and the protein being produced is an antibody~~cell does not express EBNA1.~~

19. The mammalian cell of any one of claims 12 to 18, wherein the nucleic acid construct comprises a Scaffold Attachment Region (SAR).

20. The mammalian cell of any one of claims 12 to 19, wherein the cell is a CHO cell.