

In one aspect, the present disclosure refers to a mammalian host cell comprising 10 to 200 copies of a nucleic acid construct for expression of a protein of interest, wherein the nucleic acid construct comprises the following elements in operable association in 5' to 3' order: (a) optionally, a first promoter sequence; (b) a selectable marker sequence; (c) a second promoter sequence; (d) a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; (e) and a poly A signal sequence; wherein the nucleic acid construct further comprises one or more insertion elements positioned at one or more locations selected from the group consisting of: (i) 5' to the first promoter, when present, or 5' to the selectable marker sequence when the first promoter sequence is absent, (ii) 3' to the poly A signal sequence, (iii) between the selectable marker sequence and the second promoter sequence, and (iv) both the location of (i) and the location of (ii), wherein the 10 to 200 copies of the nucleic acid construct are inserted into a plurality of exogenous dock sites integrated into the genome of the mammalian host cell; and wherein each of the plurality of exogenous dock sites is flanked on both its 5' side and its 3' side by exogenous retroviral vector sequences. In some examples,

examples, the IRES sequence is selected from the group consisting of foot and mouth disease virus (FMDV), encephalomyocarditis virus and poliovirus IRES sequences.

In some preferred examples, the nucleic acid construct further comprises a third promoter operably linked to a second nucleic acid sequence encoding a second protein of interest positioned 3' to the nucleic acid sequence encoding the first protein of interest. In some preferred examples, the third promoter sequence is selected from the group consisting of SV40, *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, human elongation factor 1 alpha (hEF1alpha), and mouse metallothionein-I promoter sequences. In some preferred examples, the constructs further comprise an RNA export element in operable association with the second nucleic acid sequence encoding a second protein of interest. In some preferred examples, the constructs further comprise a poly A signal sequence in operable association with the second nucleic acid sequence encoding a second protein of interest.

In some preferred examples, the first protein of interest is one of an antibody heavy and light chain and the second protein of interest is the other of an antibody heavy and light chain.

In some preferred examples, the nucleic acid construct further comprises an intron operably linked to a second nucleic acid sequence encoding a second protein of interest positioned 3' to the nucleic acid sequence encoding the first protein of interest. In some preferred examples, the constructs further comprise an RNA export element in operable association with the second nucleic acid sequence encoding a second protein of interest. In some preferred examples, the constructs further comprise a poly A signal sequence in operable association with the second nucleic acid sequence encoding a second protein of interest. In some preferred examples, the first protein of interest is one of an antibody heavy and light chain and the second protein of interest is the other of an antibody heavy and light chain.

In some preferred examples, the present disclosure describes a vector comprising a nucleic acid construct as described above. In some preferred examples, the vector is a plasmid.

In one example, the present disclosure refers to a host cell comprising the nucleic acid construct as disclosed herein. In some preferred examples, the present disclosure describes a host cell comprising a nucleic acid construct as described above or a vector as described above. In some preferred examples, the host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney

In some preferred examples, the present disclosure describes a host cell culture comprising a population of host cells as described above.

In another aspect, the present disclosure refers to a process for producing a protein of interest comprising culturing the mammalian host cells as disclosed herein and purifying the protein of interest from the culture of the mammalian host cell. In some preferred examples, the present disclosure describes processes for producing a protein of interest comprising culturing host cells as described above under conditions such that the protein(s) of interest is expressed and purifying the protein(s) of interest from the host cell culture. In some preferred examples, the host cells grown in a medium comprising an inhibitor of the selectable marker. In some preferred examples, the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulfoximine (Msx). In some preferred examples, the selectable marker is DHFR and the inhibitor is methotrexate.

In some preferred examples, the present disclosure describes a vector comprising the nucleic acid construct as described above. In some preferred examples, the vector is selected from the group consisting of a plasmid vector, a retroviral vector, a lentiviral vector, an AAV vector, and a transposon vector.

In one example, the present disclosure refers to a system comprising: a first nucleic acid construct as described herein; and a second nucleic acid construct encoding an enzyme. In some preferred examples, the constructs are provided on different vectors. In some preferred examples, the constructs are provided on the same vectors. In some preferred examples, the enzyme is selected from the group consisting of a transposase, an integrase, a recombinase, a nuclease and a nickase. In some preferred examples, the nuclease is a Cas nuclease. In some preferred examples, the nickase is a Cas nickase. In some preferred examples, the systems further comprise one or more RNA guide sequences. In some preferred examples, the enzyme facilitates insertion of the nucleic acid construct or a portion thereof into the genome of a host cell.

In some preferred examples, the systems further comprise at least a third nucleic acid construct as described above, the third nucleic acid construct encoding a protein of interest that is different from the protein of interest in the first nucleic acid construct. In some preferred examples, the third nucleic acid construct is provided in a separate vector. In some preferred examples, the third nucleic acid construct is provided in the same vector as the first and second nucleic acid constructs.

In some preferred examples, the present disclosure describes a system comprising at least first and second nucleic acid constructs as described above; wherein the first and second nucleic acid constructs each encode a different protein of interest. In some preferred examples, the first and second nucleic acid constructs are provided in separate vectors. In

CLAIMS

What is claimed is:

- 5 1. A mammalian host cell comprising 10 to 200 copies of a nucleic acid construct for expression of a protein of interest, wherein the nucleic acid construct comprises the following elements in operable association in 5' to 3' order:
- (a) optionally, a first promoter sequence;
 - (b) a selectable marker sequence;
 - 10 (c) a second promoter sequence;
 - (d) a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; and
 - (e) a poly A signal sequence;
- wherein the nucleic acid construct further comprises one or more insertion elements
- 15 positioned at one or more locations selected from the group consisting of:
- (i) 5' to the first promoter sequence, when present, or 5' to the selectable marker sequence when the first promoter sequence is absent;
 - (ii) 3' to the poly A signal sequence;
 - (iii) between the selectable marker sequence and the second promoter sequence;
 - 20 and
 - (iv) both the location of (i) and the location of (ii);
- wherein the 10 to 200 copies of the nucleic acid construct are inserted into a plurality of exogenous dock sites integrated into the genome of the mammalian host cell; and
- wherein each of the plurality of exogenous dock sites is flanked on both its 5' side and
- 25 its 3' side by exogenous retroviral vector sequences.
2. The mammalian host cell of claim 1, wherein the nucleic acid construct does not comprise a poly A signal sequence between the selectable marker sequence and the second promoter sequence.
- 30 3. The mammalian host cell of any one of claims 1 to 2, wherein the selectable marker sequence is adjacent to the second promoter sequence.

4. The mammalian host cell of any one of claims 1 to 3, wherein the second promoter sequence is adjacent to the nucleic acid sequence encoding the first protein of interest.
5. The mammalian host cell of any one of claims 1 to 4, wherein the nucleic acid construct comprises an extending packaging region (EPR) between the first promoter sequence and the selectable marker sequence.
6. The mammalian host cell of any one of claims 1 to 5, wherein the first promoter sequence is selected from the group consisting of SIN-LTR, SV40, *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, human elongation factor 1 alpha (hEF1alpha), and mouse metallothionein-I promoter sequences.
7. The mammalian host cell of any one of claims 1 to 6, wherein the selectable marker sequence is an amplifiable selectable marker sequence selected from the group consisting of the Glutamine Synthase (GS) sequence and the Dihydrofolate Reductase (DHFR) sequence.
8. The mammalian host cell of any one of claims 1 to 7, wherein the second promoter sequence is selected from the group consisting of SV40, *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, human elongation factor 1 alpha (hEF1alpha), and mouse metallothionein-I promoter sequences.
9. The mammalian host cell of any one of claims 1 to 8, wherein the nucleic acid sequence encoding the first protein of interest encodes a protein selected from the group consisting of a heavy-chain immunoglobulin sequence and a light-chain immunoglobulin sequence.
10. The mammalian host cell of any one of claims 1 to 9, wherein the insertion element is selected from the group consisting of a transposon insertion element, a recombinase insertion element, and an HDR insertion element.
11. The mammalian host cell of claim 10, wherein the transposon insertion element is an inverted terminal repeat

12. The mammalian host cell of claim 10, wherein the recombinase insertion element is an attachment site (att).

- 5 13. A process for producing a protein of interest comprising:
culturing the mammalian host cell of any one of claims 1 to 12; and
purifying the protein of interest from a culture of the mammalian host cell.