processes such as removal of virus particles by using filters or resins, as well as inactivation steps by using low pH or detergents, are implemented to prevent the serious safety issue of viral contamination of protein drug substances. Given the complexity of therapeutic proteins with respect to their large molecular size, post-translational modifications, and the variety of biological materials involved in their manufacturing process, the ability to enhance particular functional attributes while maintaining product safety and efficacy achieved through protein-engineering strategies is highly desirable.

While the integration of novel strategies and approaches to modify protein drug products is not a trivial matter, the potential therapeutic advantages have driven the increased use of such strategies during drug development. A number of protein-engineering platform technologies are currently in use to increase the circulating half-life, targeting, and functionality of novel therapeutic protein drugs as well as to increase production yield and product purity. For example, protein conjugation and derivatization approaches, including Fc-fusion, albumin-fusion, and PEGylation, are currently being used to extend a drug's circulating half-life.

The production of protein pharmaceutical (biologics) is expensive and time consuming. What is needed in the art are more efficient tools and processes for producing this important class of drugs.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention relates~~disclosure refers to nucleic acid constructs and their use to develop host cell lines for production of a protein of interest, and in particular to nucleic acid constructs which allow for improved selection to develop high-producing cell lines.

In some preferred ~~embodiments~~examples, the present ~~invention provides~~disclosure describes nucleic acid constructs for expression of a protein of interest comprising the following elements in operable association in 5' to 3' order: optionally, a first promoter sequence; a selectable marker sequence; a second promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; and a poly A signal sequence; the nucleic acid construct further comprising at least one insertion element at a position or positions selected from the group consisting of 5' to the optional first promoter or selectable marker sequence, 3' to the poly A signal sequence, between the optional first promoter and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the optional first promoter sequence or the selectable marker sequence and 3' to the poly A signal sequence.

In one aspect, the present disclosure refers to a nucleic acid construct for expression of a protein of interest comprising the following elements in operable association in 5' to 3' order: optionally, a first promoter sequence; a selectable marker sequence; a second promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; and a poly A signal sequence; the nucleic acid construct further comprising at least one insertion element at a position or positions selected from the group consisting of 5' to the optional first promoter or selectable marker sequence, 3' to the poly A signal sequence, between the optional first promoter and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the optional first promoter sequence or the selectable marker sequence and 3' to the poly A signal sequence. In some examples~~embodiments~~,

nucleic acid constructs comprise the first promoter sequence. In some preferred examplesembodiments, the construct does not comprise a poly A signal sequence between the selectable marker and the second promoter. In some preferred examplesembodiments, the selectable marker is adjacent to the second promoter. In some preferred examplesembodiments, the second promoter is adjacent to the nucleic acid sequence encoding the first protein of interest. In some preferred examplesembodiments, the nucleic acid construct comprises a non-coding region between the first promoter and the selectable marker. In some preferred examplesembodiments, the non-coding region comprises multiple potential Kozak sequences and/or ATG translation start sites. In some preferred examplesembodiments, the nucleic acid construct comprises an extending packaging region (EPR) between the first promoter and the selectable marker. In some preferred examplesembodiments, the EPR comprises multiple potential Kozak sequences and/or ATG translation start sites.

In some preferred examplesembodiments, 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. In some preferred examplesembodiments, the first promoter sequence is not a retroviral LTR promoter.

In some preferred examplesembodiments, 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. In some preferred examplesembodiments, the selectable marker sequence is an antibiotic resistance marker sequence selected from the group consisting of neomycin resistance gene (neo), hygromycin B phosphotransferase gene and puromycin N-acetyl transferase gene sequences.

In some preferred examplesembodiments, 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.

In some preferred examplesembodiments, the nucleic acid sequence encoding a protein of interest encodes a protein selected from the group consisting of heavy and light chain immunoglobulin sequences.

In some preferred examplesembodiments, the insertion element is selected from the group consisting of a transposon insertion element, a recombinase insertion element, and a HDR

insertion element. In some preferred examplesembodiments, the transposon insertion element is an inverted terminal repeat. In some preferred examplesembodiments, the construct comprises two inverted terminal repeats positioned 5' to the first promoter and 3' to the poly A signal sequence. In some preferred examplesembodiments, the recombinase insertion element is an attachment site (att). In some preferred examplesembodiments, the attachment site (att) is attB. In some preferred embodiments, the HDR insertion element comprises an AAVS1 safe harbor locus sequence. In some preferred examplesembodiments, the HDR insertion element is a nucleic acid sequence homologous to a target site in a chromosome. In some preferred examplesembodiments, the nucleic acid sequence homologous to a target site in a chromosome is from about 30 to 1000 bases in length. In some preferred examplesembodiments, the construct comprises two nucleic acid sequences homologous to a target site in a chromosome positioned 5' to the first promoter and 3' to the poly A signal sequence. In some preferred examplesembodiments, the recombinase insertion element is a Flp Recombination Target (FRT) site. In some preferred examplesembodiments, the recombinase insertion element is a LoxP sequence.

In some preferred examplesembodiments, the constructs further comprise an RNA export element. In some preferred examplesembodiments, the RNA export element is located 3' or 5' to the nucleic acid sequence encoding the protein of interest. In some preferred examplesembodiments, the RNA export element is a pre-mRNA processing enhancer (PPE). In some preferred examplesembodiments, the RNA export element is a posttranscriptional regulatory element (PRE). In some preferred examplesembodiments, the PRE RNA export element is a Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

In some preferred examplesembodiments, the constructs further comprise a signal peptide sequence operably linked to the first protein of interest. In some preferred examplesembodiments, the signal peptide sequence is selected from the group consisting of tissue plasminogen activator, human growth hormone, lactoferrin, alpha-casein and alpha-lactalbumin signal peptide sequences.

In some preferred examplesembodiments, the constructs further comprise a protein purification marker sequence. In some preferred examplesembodiments, the protein purification marker sequence is a hexahistidine tag or a hemagglutinin (HA) tag.

In some preferred examplesembodiments, the constructs further comprise an Internal Ribosome Entry Site (IRES) sequence and a second nucleic acid sequence encoding at least a second protein of interest (e.g., including third, fourth, fifth, etc. protein of interest) positioned 3' to the nucleic acid sequence encoding the first protein of interest. In some preferred

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

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

In one aspect, the present disclosure refers to a host cell comprising the nucleic acid construct as disclosed herein. In some preferred examplesembodiments, the present ~~invention provides~~disclosure describes a host cell comprising a nucleic acid construct as described above or a vector as described above. In some preferred examplesembodiments, 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

CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells and human hepatoma line cells. In some preferred examplesembodiments, the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cells, a HEK 293 cells and a CAP cells. In some preferred examplesembodiments, the host cell line is a GS knockout cell line. In some preferred examplesembodiments, the host cell line is a DHFR knockout cell line. In some preferred examplesembodiments, the host cell comprises from about 1 to 1000 copies of the nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from about 10 to 200 copies of the nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from about 10 to 100 copies of the nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from about 20 to 100 copies of the nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from 50 to 500 copies of the nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from 50 to 250 copies of the nucleic acid construct.

In some preferred examplesembodiments, the host cell further comprises at least a second nucleic acid construct that encodes and allows for expression of a second protein of interest. In some preferred examplesembodiments, the second nucleic acid construct does not include a selectable marker. In some preferred examplesembodiments, the second nucleic acid construct includes a selectable marker that is different from the selectable marker in the first nucleic acid construct. In some preferred examplesembodiments, the first protein of interest in the first nucleic acid construct is one of an immunoglobulin heavy or light chain and the second protein in the second nucleic acid construct is the other of an immunoglobulin heavy or light chain. In some preferred examplesembodiments, the first protein of interest is an immunoglobulin heavy chain and the second protein of interest is an immunoglobulin light chain. In some preferred examplesembodiments, the host cell comprises from about 1 to 1000 copies of the second nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from about 10 to 200 copies of the second nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from about 10 to 100 copies of the second nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from about 20 to 100 copies of the second nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from 50 to 500 copies of the second nucleic acid construct. In some preferred examplesembodiments, the host cell comprises from 50 to 250 copies of the second nucleic acid construct.

In some preferred examples~~embodiments~~, the present ~~invention provides~~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 host cells as disclosed herein and purifying the protein of interest from the host cell culture. In some preferred examples~~embodiments~~, the present ~~invention provides~~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~~embodiments~~, the host cells grown in a medium comprising an inhibitor of the selectable marker. In some preferred examples~~embodiments~~, the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulphoximine (Msx). In some preferred examples~~embodiments~~, the selectable marker is DHFR and the inhibitor is methotrexate.

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

In some preferred examples~~embodiments~~, 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~~embodiments~~, the third nucleic acid construct is provided in a separate vector. In some preferred examples~~embodiments~~, the third nucleic acid construct is provided in the same vector as the first and second nucleic acid constructs.

In some preferred examples~~embodiments~~, the present ~~invention provides~~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~~embodiments~~, the first and second nucleic acid constructs are provided in separate vectors. In

some preferred examplesembodiments, the first and second nucleic acid constructs are provided in the same vector. In some preferred examplesembodiments, the systems further comprise a third nucleic acid construct encoding an enzyme. In some preferred examplesembodiments, the enzyme is selected from the group consisting of a transposase, an integrase, a recombinase, a nuclease and a nickase. In some preferred examplesembodiments, the nuclease is a Cas nuclease. In some preferred examplesembodiments, the nickase is a Cas nickase. In some preferred examplesembodiments, the systems further comprise one or more RNA guide sequences. In some preferred examplesembodiments, the enzyme facilitates insertion of the nucleic acid construct or a portion thereof into the genome of a host cell. In some preferred examplesembodiments, the third nucleic acid construct is provided in a separate vector. In some preferred examplesembodiments, the third nucleic acid construct is provided in the same vector as the first and second nucleic acid constructs.

In some preferred examplesembodiments, the present invention providesdisclosure describes processes for producing a protein of interest comprising: introducing a nucleic acid construct, vector, or a system as described above into a host cell under conditions such that the nucleic acid construct is incorporated into the genome of the host cell; developing a host cell line that expresses the protein of interest; culturing host cells from the host cell line under conditions such that the protein of interested is produced by the host cells; and purifying the protein of interest from the host cell culture.

In some preferred examplesembodiments, 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 CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells and human hepatoma line cells. In some preferred examplesembodiments, the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cells, a HEK 293 cells and a CAP cells. In some preferred examplesembodiments, the host cell line is a GS knockout cell line. In some preferred examplesembodiments, the host cell line is a DHFR knockout cell line. In some preferred examplesembodiments, the host cells are grown in a medium comprising an inhibitor of the selectable marker. In some preferred examplesembodiments, the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulphoximine (Msx). In some preferred examplesembodiments, the selectable marker is DHFR and the inhibitor is methotrexate. In some preferred examplesembodiments, culturing host cells from the host cell line under conditions such that the protein of interested is produced by the host cells further comprises culturing in a system selected from the group

CLAIMS

What is claimed is:

1. A nucleic acid construct for expression of a protein of interest comprising the following elements in operable association in 5' to 3' order:

optionally, a first promoter sequence;

a selectable marker sequence;

a second promoter sequence;

a nucleic acid sequence encoding a first protein of interest that is operably linked to the second promoter sequence; and

a poly A signal sequence;

the nucleic acid construct further comprising at least one insertion element at a position or positions selected from the group consisting of 5' to the optional first promoter or selectable marker sequence, 3' to the poly A signal sequence, between the optional first promoter and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the optional first promoter sequence or the selectable marker sequence and 3' to the poly A signal sequence.

2. The nucleic acid construct of claim 1, wherein the nucleic acid construct does not comprise a poly A signal sequence between the selectable marker and the second promoter.

3. The nucleic acid construct of ~~any one of claims 1 to 2~~claim 1, wherein the selectable marker is adjacent to the second promoter.

4. The nucleic acid construct of ~~any one of claims 1 to 3~~claim 1, wherein the second promoter is adjacent to the nucleic acid sequence encoding the first protein of interest.

5. The nucleic acid construct of ~~any one of claims 1 to 4~~claim 1, wherein the nucleic acid construct comprises an extending packaging region between the first promoter and the selectable marker.

6. The nucleic acid construct of claim 5, wherein the EPR comprises multiple potential Kozak sequences and/or ATG translation start sites.

7. The nucleic acid construct of ~~any one of claims 1 to 6~~claim 1, wherein the first promoter sequence is a weak promoter sequence.

8. The nucleic acid construct of ~~any one of claims 1 to 7~~claim 1, 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.

~~9. The nucleic acid construct of any one of claims 1 to 8, wherein the first promoter sequence is not a retroviral LTR promoter.~~

~~10.~~9. The nucleic acid construct of ~~any one of claims 1 to 9~~claim 1, 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.

~~11.~~10. The nucleic acid construct of ~~any one of claims 1 to 10~~claim 1, wherein the selectable marker sequence is an antibiotic resistance marker sequence selected from the group consisting of neomycin resistance gene (neo), hygromycin B phosphotransferase gene and puromycin N-acetyl transferase gene sequences.

~~12.~~11. The nucleic acid construct of ~~any one of claims 1 to 11~~claim 1, 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.

~~13.~~12. The nucleic acid construct of ~~any one of claims 1 to 12~~claim 1, wherein the nucleic acid sequence encoding a protein of interest encodes a protein selected from the group consisting of heavy and light chain immunoglobulin sequences.

~~14.~~13. The nucleic acid construct of ~~any one of claims 1 to 13,~~claim 1 wherein the insertion element is selected from the group consisting of a transposon insertion element, a recombinase insertion element, and a HDR insertion element.

~~15.~~14. The nucleic acid construct of claim ~~14~~13, wherein the transposon insertion element is an inverted terminal repeat.

~~16.~~15. The nucleic acid construct of claim ~~15~~14, wherein the construct comprises two inverted terminal repeats positioned 5' to the first promoter and 3' to the poly A signal sequence.

~~17.~~16. The nucleic acid construct of claim ~~14~~13, wherein the recombinase insertion element is an attachment site (att).

~~18.~~17. The nucleic acid construct of claim ~~17~~16, wherein the attachment site (att) is attB.

~~19. The nucleic acid construct of claim 14, wherein the HDR insertion element comprises an AAVS1 safe harbor locus sequence.~~

~~20. The nucleic acid construct of claim 14, wherein the HDR insertion element is a nucleic acid sequence homologous to a target site in a chromosome.~~

~~21. The nucleic acid construct of claim 20, wherein the nucleic acid sequence homologous to a target site in a chromosome is from about 30 to 1000 bases in length.~~

~~22. The nucleic acid construct of claim 21, wherein the construct comprises two nucleic acid sequences homologous to a target site in a chromosome positioned 5' to the first promoter and 3' to the poly A signal sequence.~~

~~23. The nucleic acid construct of claim 14, wherein the recombinase insertion element is a Flp Recombination Target (FRT) site.~~

~~24. The nucleic acid construct of claim 14, wherein the recombinase insertion element is a LoxP sequence.~~

~~25. The nucleic acid construct of any one of claims 1 to 24, further comprising an RNA export element.~~

~~26. The nucleic acid construct of claim 25, wherein the RNA export element is located 3' or 5' to the nucleic acid sequence encoding the protein of interest.~~

~~27. The nucleic acid construct of claim 26, wherein the RNA export element is a pre-mRNA processing enhancer (PPE).~~

~~28. The nucleic acid construct of claim 26, wherein the RNA export element is a posttranscriptional regulatory element (PRE).~~

~~29. The nucleic acid construct of claim 28, wherein the PRE RNA export element is a Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).~~

~~30. The nucleic acid construct of any one of claims 1 to 29, further comprising a signal peptide sequence operably linked to the first protein of interest.~~

~~31. The nucleic acid construct of any one of claims 1 to 30, wherein the signal peptide sequence is selected from the group consisting of tissue plasminogen activator, human growth hormone, lactoferrin, alpha-casein and alpha-lactalbumin signal peptide sequences.~~

~~32. The nucleic acid construct of any one of claims 1 to 31, further comprising a protein purification marker sequence.~~

~~33. The nucleic acid construct of claim 32, wherein the protein purification marker sequence is a hexahistidine tag or a hemagglutinin (HA) tag.~~

~~34. The nucleic acid construct of any one of claims 1 to 33, further comprising an Internal Ribosome Entry Site (IRES) sequence and a second nucleic acid sequence encoding a second protein of interest positioned 3' to the nucleic acid sequence encoding the first protein of interest.~~

~~35. The nucleic acid construct of claim 34, wherein the IRES sequence is selected from the group consisting of foot and mouth disease virus (FDV), encephalomyocarditis virus and poliovirus IRES sequences.~~

~~36. The nucleic acid construct of any one of claims 1 to 33, wherein 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.~~

~~37. The nucleic acid construct of claim 36, further comprising an RNA export element in operable association with the second nucleic acid sequence encoding a second protein of interest.~~

~~38. The nucleic acid construct of claim 36, further comprising a poly A signal sequence in operable association with the second nucleic acid sequence encoding a second protein of interest.~~

~~39. The nucleic acid construct of any one of claims 36 to 38, wherein 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.~~

~~40. The nucleic acid construct of any one of claims 1 to 33, wherein 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.~~

~~41. The nucleic acid construct of claim 40, further comprising an RNA export element in operable association with the second nucleic acid sequence encoding a second protein of interest.~~

~~42. The nucleic acid construct of claim 40, further comprising a poly A signal sequence in operable association with the second nucleic acid sequence encoding a second protein of interest.~~

~~43. The nucleic acid construct of any one of claims 40 to 42, wherein 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.~~

~~44. A plasmid comprising the nucleic acid construct of any one of claims 1 to 43.~~

~~45.~~18. A host cell comprising the nucleic acid construct of ~~any one of claims 1 to 43~~claim 1.

~~46. The host cell of claim 45, wherein 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 CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells and human hepatoma line cells.~~

~~47. The host cell of any one of claims 45 to 46, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cells, a HEK 293 cells and a CAP cells.~~

~~48. The host cell of any one of claims 45 to 46, wherein the host cell line is a GS knockout cell line.~~

~~49. The host cell line of any one of claims 45 to 47, wherein the host cell line is a DHFR knockout cell line.~~

~~50.~~19. The host cell of ~~any one of claims 45 to 49~~claim 18, wherein the host cell comprises from about 1 to 1000 copies of the nucleic acid construct.

~~51. The host cell of any one of claims 45 to 49, wherein the host cell comprises from about 10 to 200 copies of the nucleic acid construct.~~

~~52. The host cell of any one of claims 45 to 49, wherein the host cell comprises from about 10 to 100 copies of the nucleic acid construct.~~

~~53. The host cell of any one of claims 45 to 49, wherein the host cell comprises from about 20 to 100 copies of the nucleic acid construct.~~

~~54. The host cell of any one of claims 45 to 53, wherein the host cell further comprises at least a second nucleic acid construct that encodes and allows for expression of a second protein of interest, and wherein said second nucleic acid construct does not include a selectable marker.~~

~~55. The host cell of any one of claims 45 to 53, wherein the host cell further comprises at least a second nucleic acid construct that encodes and allows for expression of a second protein of interest, and wherein said second nucleic acid construct includes a selectable marker that is different from the selectable marker in the first nucleic acid construct.~~

~~56. The host cell of any one of claims 54 to 55, wherein the first protein of interest in the first nucleic acid construct is one of an immunoglobulin heavy or light chain and the second protein in the second nucleic acid construct is the other of an immunoglobulin heavy or light chain.~~

~~57. The host cell of claim 56, wherein the first protein of interest is an immunoglobulin heavy chain and the second protein of interest is an immunoglobulin light chain.~~

~~58. The host cell of any one of claims 54 to 57, wherein the host cell comprises from about 1 to 1000 copies of the second nucleic acid construct.~~

~~59. The host cell of any one of claims 54 to 57, wherein the host cell comprises from about 10 to 200 copies of the second nucleic acid construct.~~

~~60. The host cell of any one of claims 54 to 57, wherein the host cell comprises from about 10 to 100 copies of the second nucleic acid construct.~~

~~61. The host cell of any one of claims 54 to 57, wherein the host cell comprises from about 20 to 100 copies of the second nucleic acid construct.~~

~~62. A host cell culture comprising host cells according to any one of claims 45 to 61.~~

~~63.~~20. A process for producing a protein of interest comprising culturing host cells according to ~~any one of claims 45 to 61~~claim 18 and purifying the protein of interest from the host cell culture.

~~64. The process of claim 63, wherein the host cells grown in a medium comprising an inhibitor of the selectable marker.~~

~~65. The process of claim 64, wherein the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulphoximine (Msx).~~

~~66. The process of claim 64, wherein the selectable marker is DHFR and the inhibitor is methotrexate.~~

~~67. A vector comprising the nucleic acid construct of any one of claims 1 to 43.~~

~~68. The vector of claim 67, wherein 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.~~

~~69.~~21. A system comprising:

a first nucleic acid construct according to ~~any one of claims 1 to 30~~claim 1; and

a second nucleic acid construct encoding an enzyme.

~~70.~~22. The system of claim ~~69~~21, wherein the enzyme is selected from the group consisting of a transposase, an integrase, a recombinase, a nuclease and a nickase.

~~71. The system of claim 70, wherein the nuclease is a Cas nuclease.~~

~~72. The system of claim 70, wherein the nickase is a Cas nickase.~~

~~73. The system of any one of claims 71 to 72, further comprising one or more RNA guide sequences.~~

~~74. The system of any one of claims 69 to 73, wherein the enzyme facilitates insertion of the nucleic acid construct or a portion thereof into the genome of a host cell.~~

~~75. The system of any one of claims 69 to 74, wherein the first and second nucleic acid constructs are provided in separate vectors.~~

~~76. The system of any one of claims 69 to 74, wherein the first and second nucleic acid constructs are provided in the same vector.~~

77. The system of any one of claims 69 to 76, further comprising at least a third nucleic acid construct according to any one of claims 1 to 43, the third nucleic acid construct encoding a protein of interest that is different from the protein of interest in the first nucleic acid construct.

78. The system of claim 77, wherein the third nucleic acid construct is provided in a separate vector.

79. The system of claim 77, wherein the third nucleic acid construct is provided in the same vector as the first and second nucleic acid constructs.

80. A system comprising:
first and second nucleic acid constructs according to any one of claims 1 to 35; wherein the first and second nucleic acid constructs each encode a different protein of interest.

81. The system of claim 80, wherein the first and second nucleic acid constructs are provided in separate vectors.

82. 82. The system of claim 80, wherein the first and second nucleic acid constructs are provided in the same vector.

83. The system of any one of claims 80 to 82, further comprising a third nucleic acid construct encoding an enzyme.

84. The system of claim 83, wherein the enzyme is selected from the group consisting of a transposase, an integrase, a recombinase, a nuclease and a nickase.

85. The system of claim 84, wherein the nuclease is a Cas nuclease.

86. The system of claim 84, wherein the nickase is a Cas nickase.

87. The system of any one of claims 85 to 86, further comprising one or more RNA guide sequences.

88. The system of any one of claims 83 to 87, wherein the enzyme facilitates insertion of the nucleic acid construct or a portion thereof into the genome of a host cell.

89. The system of any one of claims 83 to 87, wherein the third nucleic acid construct is provided in a separate vector.

90. The system of any one of claims 83 to 87, wherein the third nucleic acid construct is provided in the same vector as the first and second nucleic acid constructs.

91. A process for producing a protein of interest comprising:
introducing a nucleic acid construct of any one of claims 1 to 43, a vector of any one of claims 67 to 68, or a system of any of claims 69 to 90 into a host cell under conditions such that the nucleic acid construct is incorporated into the genome of the host cell;
developing a host cell line that expresses the protein of interest;
culturing host cells from the host cell line under conditions such that the protein of interested is produced by the host cells; and
purifying the protein of interest from the host cell culture.

92. The process of claim 91, wherein 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 CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells and human hepatoma line cells.

93. The process of claim 92, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cells, a HEK 293 cells and a CAP cells.

94. The process of any one of claims 91 to 93, wherein the host cell line is a GS knockout cell line.

~~95. The process of any one of claims 91 to 93, wherein the host cell line is a DHFR knockout cell line.~~

~~96. The process of any one of claims 91 to 95, wherein the host cells are grown in a medium comprising an inhibitor of the selectable marker.~~

~~97. The process of claim 96, wherein the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulphoximine (Msx).~~

~~98. The process of claim 96, wherein the selectable marker is DHFR and the inhibitor is methotrexate.~~

~~99. The process of any one of claims 91 to 98, wherein culturing host cells from the host cell line under conditions such that the protein of interested is produced by the host cells further comprises culturing in a system selected from the group consisting of petri dishes, well plates, roller bottles, bioreactors, perfusion systems and fed batch cultures.~~