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 to~~disclosure describes host cell lines containing multiple dock sites for insertion of a nucleic acid construct, and in particular nucleic acid constructs that express an exogenous gene of interest.

In one aspect, the present disclosure refers to a host cell comprising a genome, the genome comprising from 1 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred ~~embodiments~~examples, the present ~~invention provides~~disclosure describes a host cell (or population of host cells) comprising a genome, the genome comprising from 1 to 1000 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred ~~embodiments~~examples, the genome comprises from 1 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred examples~~embodiments~~, the genome comprises from 5 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred examples~~embodiments~~, the genome comprises from 5 to 250 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred examples~~embodiments~~, the genome comprises from 5 to 100 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred examples~~embodiments~~, the genome comprises

from 5 to 50 integrated docking sites, each docking site comprising at least one dock site insertion element. In some preferred examples~~embodiments~~, the integrated docking sites are independently positioned throughout the genome.

In some preferred examples~~embodiments~~, the dock site insertion element is targeted by enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase. In some preferred examples~~embodiments~~, the dock site insertion element is selected from the group consisting of a recombinase dock site insertion element and a HDR dock site insertion element. In some preferred examples~~embodiments~~, the dock site insertion element is a recombinase dock site insertion element. In some preferred examples~~embodiments~~, the recombinase dock site insertion element comprises an attachment site (att). In some preferred examples~~embodiments~~, the attachment site (att) is selected from the group consisting of attB and attP. In some preferred examples~~embodiments~~, the attachment site (att) is selected from the group consisting of attR and attL. In some preferred examples~~embodiments~~, the recombinase dock site insertion element comprises a LoxP sequence. In some preferred examples~~embodiments~~, the recombinase dock site insertion element is a Flp Recombination Target (FRT) site. In some preferred examples~~embodiments~~, the dock site insertion element is a HDR dock site insertion element. In some preferred examples~~embodiments~~, the HDR dock site insertion element comprises one or two dock site homology arms. In some preferred examples~~embodiments~~, the HDR dock site insertion element further comprises one or more sequences homologous to a guide RNA sequence. In some preferred examples~~embodiments~~, the dock site homology arms are from about 30 to 1000 bases in length. In some preferred examples~~embodiments~~, the integrase dock site insertion element comprises an AAVS1 safe harbor locus sequence.

In some preferred examples~~embodiments~~, each docking site is flanked by exogenous integrating vector sequences. In some preferred examples~~embodiments~~, the exogenous integrating vector sequences are selected from the group consisting of viral vector sequences and transposon vector sequences. In some preferred examples~~embodiments~~, the docking sites each further comprise a sequence encoding a selectable maker operably linked to a promoter.

In some preferred examples~~embodiments~~, the host cell line further comprises an exogenous sequence encoding an enzyme operably linked to a promoter. In some preferred examples~~embodiments~~, the exogenous sequence encoding an enzyme operably linked to a promoter is inserted into the genome of the host cell. In some preferred examples~~embodiments~~, the exogenous sequence encoding an enzyme operably linked to a promoter is inserted at the dock site. In some preferred examples~~embodiments~~, the exogenous sequence encoding an enzyme operably linked to a promoter is provided in an episomal expression vector. In some preferred examples~~embodiments~~,

the episomal expression vector is a plasmid. In some preferred examplesembodiments, the enzyme is selected from the group consisting of 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 dock site insertion element is positioned to facilitate cassette exchange. In some preferred examplesembodiments, each docking site comprises two dock site insertion elements. In some preferred examplesembodiments, the two dock site insertion elements are positioned to facilitate cassette exchange. In some preferred examplesembodiments, the two dock site insertion elements flank sequences encoding a selectable marker, an enzyme, or a combination thereof.

In some preferred examplesembodiments, the host cells further comprise nucleic acid expression constructs inserted at 1 or more of the docking sites. In some preferred examplesembodiments, the nucleic acid expression construct comprises a second (i.e., internal) promoter operably linked to a sequence encoding a protein of interest. In some preferred examplesembodiments, the nucleic acid expression construct further comprises a 5' promoter (or first promoter) operably linked and 5' to a selectable marker.

In some preferred examplesembodiments, the nucleic acid expression construct further comprises the following elements in operable association in 5' to 3' order: a first (or 5') promoter sequence; a selectable marker sequence; a second (or internal) promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the internal promoter; and a poly A signal sequence. In some preferred examplesembodiments, 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 first promoter, 3' to the poly A signal sequence, between the first promoter and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the first promoter and 3' to the poly A signal sequence. In some preferred examplesembodiments, the nucleic acid expression 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 (i.e., the 5' promoter sequence) is selected from the group consisting of SIN-LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences. In some preferred examplesembodiments, the first promoter sequence is a weak promoter sequence. In some preferred examplesembodiments, the first promoter sequence is not a retroviral LTR promoter.

In some preferred examplesembodiments, the integrated docking sites further comprise an integrated exogenous promoter. In some preferred examplesembodiments, the promoter is a weak promoter. In some preferred examplesembodiments, the integrated exogenous promoter is selected from the group consisting of SIN-LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences. In some preferred examplesembodiments, the integrated promoter is a retroviral LTR. In some preferred examplesembodiments, the retroviral LTR is a SIN LTR. In some preferred examplesembodiments, each docking site comprises a SIN LTR EPR 5' of the docking site and a SIN LTR 3' of the docking site. In some preferred examplesembodiments, the host cell further comprise nucleic acid expression constructs inserted at 1 or more of the docking sites with an integrated promoter.

In some preferred examplesembodiments, nucleic acid expression constructs for use in cell lines with an integrated exogenous promoter in the docking site comprise an internal (or second) promoter sequence operably linked to a sequence encoding a protein of interest. In some preferred examplesembodiments, the nucleic acid expression construct comprises the following elements in operable association in 5' to 3' order: a selectable marker sequence; an internal promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the internal promoter; and a poly A signal sequence. In some preferred examplesembodiments, the nucleic acid construct further comprises at least one insertion element at a position or positions selected from the group consisting of 5' to the selectable marker sequence, 3' to the poly A signal sequence, between the selectable marker sequence and the poly A signal sequence, between the selectable marker and the internal promoter sequence, and both 5' to the selectable marker sequence and 3' to the poly A signal sequence. In some preferred examplesembodiments, the nucleic acid expression construct does not comprise a poly A

signal sequence between the selectable marker and the second promoter. In some preferred examples~~embodiments~~, the selectable marker is adjacent to the internal promoter sequence. In some preferred examples~~embodiments~~, the internal promoter sequence is adjacent to the nucleic acid sequence encoding the first protein of interest. In some preferred examples~~embodiments~~, the internal 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, and mouse metallothionein-I promoter sequences.

In some preferred examples~~embodiments~~, the selectable marker sequence in any of the foregoing expression constructs 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 examples~~embodiments~~, 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 examples~~embodiments~~, the second promoter sequence in any of the foregoing expression constructs 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, and mouse metallothionein-I promoter sequences.

In some preferred examples~~embodiments~~, 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 examples~~embodiments~~, the insertion element in any of the foregoing expression constructs is selected from the group consisting of a recombinase insertion element and a HDR insertion element. In some preferred examples~~embodiments~~, the expression construct insertion element is a recombinase expression construct insertion element compatible with the recombinase dock site insertion element. In some preferred examples~~embodiments~~, the recombinase expression construct insertion element is an attachment site (att). In some preferred examples~~embodiments~~, the attachment site (att) is selected from the group consisting of attP and attB. In some preferred examples~~embodiments~~, the recombinase expression construct insertion element is a Flp Recombination Target (FRT) site. In some preferred examples~~embodiments~~, the recombinase expression construct insertion element is a LoxP sequence. In some preferred examples~~embodiments~~, the expression construct insertion element is a HDR expression

construct insertion element compatible with the HDR dock site insertion element. In some preferred examplesembodiments, the HDR expression construct insertion element comprises one or two expression construct homology arms compatible with or homologous to the one or two dock site homology arms. In some preferred examplesembodiments, the homology arms are from about 30 to 1000 bases in length. In some preferred examplesembodiments, the recombinase expression construct comprises two homology arms positioned 5' to the first promoter and 3' to the poly A signal sequence. In some preferred examplesembodiments, the HDR expression construct insertion element comprises an AAVS1 safe harbor locus sequence. In some preferred embodiments,

In some preferred examplesembodiments, the nucleic acid expression constructs in any of the preceding embodiments further comprise a first RNA export element. In some preferred examplesembodiments, the first RNA export element is located 3' or 5' to the nucleic acid sequence encoding the protein of interest. In some preferred examplesembodiments, the first RNA export element is a pre-mRNA processing enhancer (PPE). In some preferred examplesembodiments, the first 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 nucleic acid expression constructs described in the preceding examplesembodiments further comprise a nucleic acid sequence encoding at least a second protein of interest (e.g., including third, fourth, fifth, etc. proteins of interest). In some preferred examplesembodiments, the nucleic acid sequence encoding the second protein of interest is operably associated with a construct element selected from the group consisting of a third promoter, an intron, a second RNA export element and an IRES sequence and combinations thereof. In some preferred examplesembodiments, the second RNA export element is a pre-mRNA processing enhancer (PPE). In some preferred examplesembodiments, suitable third promoters include, but are not limited to, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences. In some preferred examplesembodiments, the second 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 second nucleic acid sequence encoding a second protein of interest is positioned 3' to the nucleic acid sequence encoding the first protein of interest. In some preferred examplesembodiments, the second nucleic acid sequence encoding a second protein of

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

In some preferred examples~~embodiments~~, the nucleic acid expression constructs of any of the preceding examples ~~embodiments~~ further comprise a signal peptide sequence operably linked to the first protein of interest. In some preferred examples~~embodiments~~, 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 examples~~embodiments~~, the nucleic acid expression constructs further comprise a protein purification marker sequence. In some preferred examples~~embodiments~~, the protein purification marker sequence is a hexahistidine tag or a hemagglutinin (HA) tag.

In some preferred examples~~embodiments~~, the nucleic acid constructs encode a viral vector (i.e., instead of a protein of interest). In some preferred examples~~embodiments~~, the viral vector is selected from the group consisting of a retroviral vector, adenoviral vector and a adenovirus-associated viral vector. In some preferred examples~~embodiments~~, the retroviral vector is a lentiviral vector.

In some preferred examples~~embodiments~~, 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 examples~~embodiments~~, 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 examples~~embodiments~~, the host cell line is a GS knockout cell line. In some preferred examples~~embodiments~~, the host cell line is a DHFR knockout cell line.

In some preferred examples~~embodiments~~, the host cell further comprises at least a second nucleic acid construct that encodes and allows for expression of at least a second protein of interest, and wherein said second nucleic acid construct does not include a selectable marker. In some preferred examples~~embodiments~~, 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. In some preferred examples~~embodiments~~, the first protein of interest in the first vector is one of an immunoglobulin heavy or light chain and the

second protein in the second vector 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 second nucleic acid construct is inserted at one or more dock sites.

In some preferred embodiments, the present invention providesIn one aspect, the present disclosure refers to a cell culture comprising host cells as described aboveherein. In some preferred examplesembodiments, the cell culture is a master cell culture.

In one aspect, the present disclosure refers to a process for producing a protein of interest comprising culturing host cells as disclosed herein under conditions that the protein of interest is expressed and purifying the protein of interest from the host cell culture. In some preferred examplesembodiments, the present invention providesdisclosure describes processes for producing a protein of interest comprising culturing host cells as described above that comprise a nucleic acid expression construct encoding a protein of interest under conditions such that the protein of interest is expressed and purifying the protein of interest from the host cell culture. In some preferred examplesembodiments, the host cells 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, the present invention providesdisclosure describes processes for producing a viral vector comprising culturing host cells comprising an expression construct for a viral vector as described above under conditions that the viral vector is expressed (or synthesized) and purifying the viral vectors from the host cell culture.

In some embodiments, the present invention providesone aspect, the present disclosure refers to a system comprising a host cell or population of host cells as described above herein and one or more nucleic acid expression constructs, wherein the nucleic acid expression construct further comprises the following elements in operable association in 5' to 3' order: a selectable marker sequence; an internal promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the internal promoter; and a poly A signal sequence, wherein the nucleic acid construct further comprises at least one insertion element, and wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site. In some preferred examplesembodiments, the one or more nucleic acid expression constructs further comprise a 5' promotor sequence 5' to the selectable marker sequence. In some preferred examplesembodiments, the 5' promoter is a weak promoter. In some preferred examplesembodiments, suitable 5' promoters include, but are not limited to, SIN-LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter

sequences. In some preferred examplesembodiments, the nucleic acid expression constructs are provided in a vector. In some preferred examplesembodiments, the vector is a plasmid vector. Other suitable vectors include cosmids, YACs and small circular constructs.

In some preferred examplesembodiments, the systems further comprise a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site. In some preferred examplesembodiments, the enzyme is selected from the group consisting of 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 nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector. In some preferred examplesembodiments, the vector is a different vector from the vector comprising the nucleic acid expression construct. In some preferred examplesembodiments, the vector is the same vector as the vector comprising the nucleic acid expression construct.

In some preferred examplesembodiments, the systems further comprise at least a second nucleic acid expression construct encoding a different protein of interest. In some preferred examplesembodiments, the second nucleic acid expression construct further comprises the following elements in operable association in 5' to 3' order: a selectable marker sequence; an internal promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the internal promoter; and a poly A signal sequence, wherein the nucleic acid construct further comprises at least one insertion element, and wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site. In some preferred examplesembodiments, the at least a second nucleic acid expression construct encoding a different protein of interest is provided on a separate vector. In some preferred examplesembodiments, suitable internal promoters include, but are not limited to, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

In some preferred examplesembodiments, the nucleic acid expression construct included in the system further comprises a nucleic acid sequence encoding at least a second protein of interest (e.g., including third, fourth, fifth, etc. proteins of interest).. In some preferred examplesembodiments, the nucleic acid sequence encoding the second protein of interest is operably

associated with a construct element selected from the group consisting of a third promoter, an intron, a second RNA export element and an IRES sequence and combinations thereof. In some preferred examplesembodiments, suitable third promoters include, but are not limited to, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences. In some preferred examplesembodiments, the second RNA export element is a pre-mRNA processing enhancer (PPE). In some preferred examplesembodiments, the second nucleic acid sequence encoding a second protein of interest is 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 embodiments, the present invention providesone aspect, the present disclosure refers to a methods comprising: providing a host cell(s) as described above herein and introducing into the host cell(s) one or more nucleic acid expression constructs encoding a first protein of interest under conditions such that the nucleic acid expression constructs are inserted at the dock sites, wherein the nucleic acid expression construct further comprises at least the following elements in operable association in 5' to 3' order: a selectable marker sequence; an internal promoter sequence; a nucleic acid sequence encoding the first protein of interest that is operably linked to the internal promoter; and a poly A signal sequence, wherein the nucleic acid construct further comprises at least one insertion element, and wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site. In some preferred examplesembodiments, suitable internal promoters include, but are not limited to, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

In some preferred examplesembodiments, the one or more nucleic acid expression constructs further comprise a 5' promotor sequence 5' to the selectable marker sequence. In some preferred examplesembodiments, the nucleic acid expression constructs are provided in a vector. In some preferred examplesembodiments, the vector is a plasmid vector. Other suitable vectors include cosmids, YACs and small circular constructs. In some preferred examplesembodiments, the vector is transiently introduced into the host cell. In some preferred examplesembodiments, the 5' promoter is a weak promoter. In some preferred examplesembodiments, suitable 5' promoters include, but are not limited to, SIN-LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

In some preferred examples~~embodiments~~, the host cell line used in the method comprises a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site. In some preferred examples~~embodiments~~, the methods further comprise transiently introducing into the host cell a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site. In some preferred examples~~embodiments~~, the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector. In some preferred examples~~embodiments~~, the vector is a plasmid vector. In some preferred examples~~embodiments~~, the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell line is from 1:1000 to 1:10. In some preferred examples~~embodiments~~, the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell line is from 1:100 to 1:750. In some preferred examples~~embodiments~~, the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell line is from 1:400 to 1:600.

In some preferred examples~~embodiments~~, the enzyme is selected from the group consisting of 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 methods further comprise introducing into the host cell one or more RNA guide sequences.

In some preferred examples~~embodiments~~, the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector. In some preferred examples~~embodiments~~, the vector is a different vector from the vector comprising the nucleic acid expression construct. In some preferred examples~~embodiments~~, the vector is the same vector as the vector comprising the nucleic acid expression construct.

In some preferred examples~~embodiments~~, the methods further comprise introducing into the host cell at least a second nucleic acid expression construct encoding a different protein of interest. In some preferred examples~~embodiments~~, the at least a second nucleic acid expression

construct encoding a different protein of interest is provided on a separate vector. In some preferred examples~~embodiments~~, the second nucleic acid expression construct comprises the following elements in operable association in 5' to 3' order: a selectable marker sequence; an internal promoter sequence; a nucleic acid sequence encoding a first protein of interest that is operably linked to the internal promoter; and a poly A signal sequence, wherein the nucleic acid construct further comprises at least one insertion element, and wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site. In some preferred examples~~embodiments~~, the nucleic acid expression construct further comprises a nucleic acid sequence encoding at least a second protein of interest. In some preferred examples~~embodiments~~, suitable internal promoters include, but are not limited to, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

In some preferred examples~~embodiments~~, the nucleic acid sequence encoding the second protein of interest is operably associated with a construct element selected from the group consisting of a third promoter, an intron, a second RNA export element and an IRES sequence and combinations thereof. In some preferred examples~~embodiments~~, suitable third promoters include, but are not limited to, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences. In some preferred examples~~embodiments~~, the second RNA export element is a pre-mRNA processing enhancer (PPE). In some preferred examples~~embodiments~~, the second nucleic acid sequence encoding a second protein of interest is positioned 3' to the nucleic acid sequence encoding the first protein of interest. In some preferred ~~embodiments~~examples, 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 ~~embodiments~~examples, the present ~~invention provides~~disclosure describes methods for making a host cell line comprising inserting into the genome of the host cell multiple docking sites, each docking site comprising at least one dock site insertion element. In some preferred ~~embodiments~~examples, the inserting comprises insertion of the docking sites via a vector selected from the group consisting of a viral vector and a transposon vector. In some preferred ~~embodiments~~examples, the viral vector is a retroviral vector. In some preferred ~~embodiments~~examples, from 1 to 500 integrated docking sites are inserted, each docking site comprising at least one dock

site insertion element. In some preferred ~~embodiments~~examples, from 5 to 500 integrated docking sites are inserted, each docking site comprising at least one dock site insertion element. In some preferred ~~embodiments~~examples, the integrated docking sites are independently inserted throughout the genome.

In some preferred ~~embodiments~~examples, the dock site insertion element is targeted by enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase. In some preferred ~~embodiments~~examples, the dock site insertion element is selected from the group consisting of a recombinase dock site insertion element and a HDR dock site insertion element. In some preferred ~~embodiments~~examples, the dock site insertion element is a recombinase dock site insertion element. In some preferred ~~embodiments~~examples, the recombinase dock site insertion element comprises an attachment site (att). In some preferred ~~embodiments~~examples, the attachment site (att) is selected from the group consisting of attB and attP. In some preferred ~~embodiments~~examples, the attachment site (att) is selected from the group consisting of attR and attL. In some preferred ~~embodiments~~examples, the recombinase dock site insertion element comprises a LoxP sequence. In some preferred ~~embodiments~~examples, the recombinase dock site insertion element is a Flp Recombination Target (FRT) site. In some preferred ~~embodiments~~examples, the dock site insertion element is a HDR dock site insertion element. In some preferred ~~embodiments~~examples, the HDR dock site insertion element comprises one or two dock site homology arms. In some preferred ~~embodiments~~examples, the HDR dock site insertion element further comprises one or more sequences homologous to a guide RNA sequence. In some preferred ~~embodiments~~examples, the dock site homology arms are from about 30 to 1000 bases in length. In some preferred ~~embodiments~~examples, the integrase dock site insertion element comprises an AAVS1 safe harbor locus sequence.

In some preferred ~~embodiments~~examples, each docking site is flanked by exogenous integrating vector sequences following insertion. In some preferred ~~embodiments~~examples, the exogenous integrating vector sequences are selected from the group consisting of viral vector sequences and transposon vector sequences.

In some preferred ~~embodiments~~examples, the docking sites each further comprise a sequence encoding a selectable maker operably linked to a promoter.

In some preferred ~~embodiments~~examples, the docking site comprises a promoter. In some preferred ~~embodiments~~examples, the promoter is a weak promoter. In some preferred ~~embodiments~~examples, suitable promoters included in the docking site include, but are not limited to, SIN-LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

In some preferred ~~embodiments~~examples, each docking site comprises two dock site insertion elements. In some preferred ~~embodiments~~examples, the two dock site insertion elements are positioned to facilitate cassette exchange. In some preferred ~~embodiments~~examples, the two dock site insertion elements flank sequences encoding a selectable marker, an enzyme, or a combination thereof.

DESCRIPTION OF THE FIGURES

Abbreviations used in figures:

AmpR= bacterial ampicillin resistance gene

attB= Bacterial Attachment Site

attP= Phage Attachment Site

attR= Recombined Upstream Attachmend Site

Backbone=Plasmid Backbone

CDS= Coding Sequence

EPR=MMLV Extended Packaging Region

GCI = Gene Copy Index

GS= Glutamine Synthetase

H or HC= Heavy Chain

hCMV= Human Cytomegalovirus immediate-early Promoter

I= intron

L or LC= Light Chain

MoMuSV 5'LTR= Moloney Murine Sarcoma Virus 5' Long Terminal Repeat

Neo= Neomycin resistance genePA or PolyA= Polyadenylation signal

ProV SIN-LTR= Proviral Self- Inactivating Long Terminal Repeat

sCMV= Simian Cytomegalovirus immediate-early Promoter

SDS-PAGE= Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis

SIN-3'LTR= Self-Inactivation 3' Long Terminal Repeat

SV40= Simian Virus 40

TK= Thymidine Kinase

UTR= Untranslated Region

W or WPRE= Woodchuck Post-transcriptional Regulatory Element

FIG. 1. Nucleic acid construct design for certain embodiments of the invention

CLAIMS

What is claimed is:

1. A host cell comprising a genome, the genome comprising from 1 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element.

2. The host cell of claim 1, wherein the genome comprises from 5 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element.

~~3. The host cell of claim 1, wherein the genome comprises from 5 to 250 integrated docking sites, each docking site comprising at least one dock site insertion element.~~

~~4. The host cell of claim 1, wherein the genome comprises from 5 to 100 integrated docking sites, each docking site comprising at least one dock site insertion element.~~

~~5. The host cell of claim 1, wherein the genome comprises from 5 to 50 integrated docking sites, each docking site comprising at least one dock site insertion element.~~

~~6.~~3. The host cell of ~~any one of claims 1 to 5~~claim 1, wherein the integrated docking sites are independently positioned throughout the genome.

~~7.~~4. The host cell of ~~any one of claims 1 to 6~~claim 1, wherein the dock site insertion element is targeted by enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.

~~8.~~5. The host cell of ~~any one of claims 1 to 7~~claim 1, wherein the dock site insertion element is selected from the group consisting of a recombinase dock site insertion element and a HDR dock site insertion element.

~~9.~~6. The host cell of claim ~~8~~5, wherein the dock site insertion element is a recombinase dock site insertion element.

~~10.~~7. The host cell of claim ~~9~~6, wherein the recombinase dock site insertion element comprises an attachment site (att).

~~11.~~8. The host cell of claim ~~10~~7, wherein the attachment site (att) is selected from the group consisting of attB and attP and attR and attL.

~~12.~~9. The host cell of claim ~~9~~6, wherein the recombinase dock site insertion element comprises a LoxP sequence.

~~13.~~10. The host cell line of claim ~~9~~6, wherein the recombinase dock site insertion element is a Flp Recombination Target (FRT) site.

~~14. The host cell of claim 8, wherein the dock site insertion element is a HDR dock site insertion element.~~

~~15. The host cell line of claim 14, wherein the HDR dock site insertion element comprises one or two dock site homology arms.~~

~~16. The host cell line of claim 15, wherein the HDR dock site insertion element further comprises one or more sequences homologous to a guide RNA sequence.~~

~~17. The host cell line of any of claims 15 to 16, wherein the dock site homology arms are from about 30 to 1000 bases in length.~~

~~18. The host cell of claim 14, wherein the integrase dock site insertion element comprises an AAVS1 safe harbor locus sequence.~~

~~19.~~11. The host cell line of ~~any one of claims 1 to 18~~claim 1, wherein each docking site is flanked by exogenous integrating vector sequences.

~~20.~~12. The host cell line of claim ~~18~~11, wherein the exogenous integrating vector sequences are selected from the group consisting of viral vector sequences and transposon vector sequences.

~~21.~~13. The host cell line of ~~any one of claims 1 to 20~~claim 1, wherein the docking sites each further comprise a sequence encoding a selectable maker operably linked to a promoter.

~~22.~~14. The host cell line of ~~any one of claims 1 to 21~~claim 1, wherein the host cell line further comprises an exogenous sequence encoding an enzyme operably linked to a promoter.

~~23.~~15. The host cell line of ~~any one of claims 1 to 22~~claim 1, wherein the exogenous sequence encoding an enzyme operably linked to a promoter is inserted into the genome of the host cell.

~~24.~~16. The host cell line of claim ~~23~~15, wherein the exogenous sequence encoding an enzyme operably linked to a promoter is inserted at the dock site.

~~25.~~17. The host cell line of ~~any one of claims 1 to 22~~claim 1, wherein the exogenous sequence encoding an enzyme operably linked to a promoter is provided in an episomal expression vector.

~~26. The host cell line of claim 25, wherein the episomal expression vector is a plasmid.~~

~~27.~~18. The host cell line of ~~any one of claims 22 to 26~~claim 17, wherein the exogenous enzyme is selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.

~~28. The host cell line of claim 27, wherein the nuclease is a Cas nuclease.~~

~~29. The host cell of claim 27, wherein the nickase is a Cas nickase.~~

~~30.~~19. The host cell line of ~~any one of claims 1 to 29~~claim 1, wherein the dock site insertion element is positioned to facilitate cassette exchange.

~~31.~~20. The host cell line of ~~any one of claims 1 to 30~~claim 1, wherein each docking site comprises two dock site insertion elements.

~~32.~~21. The host cell line of claim ~~31~~20, wherein the two dock site insertion elements are positioned to facilitate cassette exchange.

~~33. The host cell line of any one of claims 31 to 32, wherein the two dock site insertion elements flank sequences encoding a selectable marker, an enzyme, or a combination thereof.~~

~~34.~~22. The host cell line of ~~any one of claims 1 to 33~~claim 1, further comprising nucleic acid expression constructs inserted at 1 or more of the docking sites.

~~35. The host cell line of claim 34, wherein the nucleic acid expression construct further comprises a first promoter operably linked to a selectable marker.~~

~~36. The host cell line of claim 34, wherein the nucleic acid expression construct comprises a second promoter operably linked to a sequence encoding a protein of interest.~~

~~37.~~23. The host cell line of ~~any one of claims 34 to 36~~claim 22, wherein the nucleic acid expression construct further comprises the following elements in operable association in 5’ to 3’ order:

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 internal promoter; and

a poly A signal sequence.

~~38.~~24. The host cell line of ~~any one of claims 34 to 37~~claim 23, wherein 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 first promoter, 3’ to the poly A signal sequence, between the first promoter and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5’ to the first promoter and 3’ to the poly A signal sequence.

~~39. The host cell line of any one of claims 34 to 38, wherein the nucleic acid expression construct does not comprise a poly-A signal sequence between the selectable marker and the second promoter.~~

~~40. The host cell line of any one of claims 34 to 39, wherein the selectable marker is adjacent to the second promoter.~~

~~41. The host cell line of any one of claims 34 to 40, wherein the second promoter is adjacent to the nucleic acid sequence encoding the first protein of interest.~~

~~42. The host cell line of any one of claims 34 to 41, wherein the nucleic acid construct comprises an extending packaging region (EPR) between the first promoter and the selectable marker.~~

~~43. The host cell line of claim 42, wherein the EPR comprises multiple potential Kozak sequences and/or ATG translation start sites.~~

~~44. The host cell line of any one of claims 34 to 43, wherein the first promoter sequence is selected from the group consisting of SIN LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.~~

~~45. The host cell line of any one of claims 34 to 44, wherein the first promoter sequence is a weak promoter sequence.~~

~~46. The host cell line of any one of claims 34 to 45, wherein the first promoter sequence is not a retroviral LTR promoter.~~

~~47. The host cell line of any one of claims 1 to 34, wherein the integrated docking sites further comprise an exogenous promoter.~~

~~48. The host cell line of claim 47, wherein the exogenous promoter is selected from the group consisting of SIN LTR, SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

49. The host cell line of claim 48, wherein the promoter is a retroviral LTR.

50. The host cell line of claim 49, wherein the retroviral LTR is a SIN LTR.

51. The host cell line of claim 50, wherein each docking site comprising a SIN LTR EPR 5' of the docking site and a SIN LTR 3' of the docking site.

52. The host cell line of any one of claims 47 to 51, further comprising nucleic acid expression constructs inserted at 1 or more of the docking sites.

53. The host cell line of claim 52, wherein the nucleic acid expression construct comprises a second promoter operably linked to a sequence encoding a protein of interest.

54. The host cell line of any one of claims 1 to 34 and 47 to 52, wherein the nucleic acid expression construct further comprises the following elements in operable association in 5' to 3' order:

a selectable marker sequence;

an internal promoter sequence;

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

a poly A signal sequence.

55. The host cell line of any one of claims 52 to 54, wherein the nucleic acid construct further comprises at least one insertion element at a position or positions selected from the group consisting of 5' to the selectable marker sequence, 3' to the poly A signal sequence, between the selectable marker sequence and the poly A signal sequence, between the selectable marker and the internal promoter sequence, and both 5' to the selectable marker sequence and 3' to the poly A signal sequence.

56. The host cell line of any one of claims 52 to 55, wherein the nucleic acid expression construct does not comprise a poly-A signal sequence between the selectable marker and the second promoter.

57. The host cell line of any one of claims 54 to 56, wherein the selectable marker is adjacent to the internal promoter sequence.

58. The host cell line of any one of claims 54 to 56, wherein the internal promoter sequence is adjacent to the nucleic acid sequence encoding the first protein of interest.

59. The host cell line of any one of claims 54 to 58, wherein the internal promoter sequence is selected from the group consisting of SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

60. The host cell line of any one of claims 34 to 59, 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.

61. The host cell line of any one of claims 34 to 59, 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.

62. The host cell line of any one of claims 34 to 61, wherein the second promoter sequence is selected from the group consisting of SV40, EF1α, *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, and mouse metallothionein-I promoter sequences.

63. The host cell line of any one of claims 34 to 62, 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.

64. The host cell line of any one of claims 34 to 63, wherein the insertion element is selected from the group consisting of a recombinase insertion element and a HDR insertion element.

65. The host cell line of claim 64, wherein the expression construct insertion element is a recombinase expression construct insertion element compatible with the recombinase dock site insertion element.

66. The host cell line of claim 65, wherein the recombinase expression construct insertion element is an attachment site (att).

67. The host cell line of claim 66, wherein the attachment site (att) is selected from the group consisting of attP and attB.

68. The host cell line of claim 64, wherein the recombinase expression construct insertion element is a Flp Recombination Target (FRT) site.

69. The host cell line of claim 64, wherein the recombinase expression construct insertion element is a LoxP sequence.

70. The host cell line of claim 64, wherein the expression construct insertion element is a HDR expression construct insertion element compatible with the HDR dock site insertion element.

71. The host cell line of claim 70, wherein the HDR expression construct insertion element comprises one or two expression construct homology arms compatible with or homologous to the one or two dock site homology arms.

72. The host cell line of claim 71, wherein the homology arms are from about 30 to 1000 bases in length.

~~73. The host cell line of claim 72, wherein the recombinase expression construct comprises two homology arms positioned 5' to the first promoter and 3' to the poly-A signal sequence.~~

~~74. The host cell line of claim 70, wherein the HDR expression construct insertion element comprises an AAVS1 safe harbor locus sequence.~~

~~75. The host cell line of any one of claims 34 to 74, wherein the nucleic acid expression constructs further comprise a first RNA export element.~~

~~76. The host cell line of claim 75, wherein the first RNA export element is located 3' or 5' to the nucleic acid sequence encoding the protein of interest.~~

~~77. The host cell line of claim 76 wherein the first RNA export element is a pre-mRNA processing enhancer (PPE).~~

~~78. The host cell line of claim 76, wherein the first RNA export element is a posttranscriptional regulatory element (PRE).~~

~~79. The host cell line of claim 78, wherein the PRE RNA export element is a Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).~~

~~80. The host cell line of any one of claims 34 to 79 wherein the nucleic acid expression construct further comprises a nucleic acid sequence encoding a second protein of interest.~~

~~81. The host cell line of claim 80 wherein the nucleic acid sequence encoding the second protein of interest is operably associated with a construct element selected from the group consisting of a third promoter, an intron, a second RNA export element and an IRES sequence and combinations thereof.~~

~~82. The host cell line of claim 81 wherein the second RNA export element is a pre-mRNA processing enhancer (PPE).~~

~~83. The host cell line of claim 82, wherein the second RNA export element is a posttranscriptional regulatory element (PRE).~~

84. The host cell line of claim 83, wherein the PRE RNA export element is a Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

85. The host cell line of any one of claims 80 to 84, wherein the second nucleic acid sequence encoding a second protein of interest is positioned 3' to the nucleic acid sequence encoding the first protein of interest.

86. The host cell line of claim 85, wherein the second nucleic acid sequence encoding a second protein of interest is operably associated with IRES sequence is selected from the group consisting of foot and mouth disease virus (FDV), encephalomyocarditis virus and poliovirus IRES sequences.

87. The host cell line of any one of claims 34 to 86, wherein the nucleic acid expression constructs further comprise a signal peptide sequence operably linked to the first protein of interest.

88. The host cell of claim 87, 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.

89. The host cell line of any one of claims 34 to 88, wherein the nucleic acid expression constructs further comprise a protein purification marker sequence.

90. The host cell line of claim 89, wherein the protein purification marker sequence is a hexahistidine tag or a hemagglutinin (HA) tag.

91. The host cell line of any one of claims 1 to 34 and 47 to 52, wherein the nucleic acid construct encodes a viral vector.

92. The host cell line of claim 91, wherein the viral vector is selected from the group consisting of a retroviral vector, adenoviral vector and an adenovirus-associated viral vector.

93. The host cell line of claim 92, wherein the retroviral vector is a lentiviral vector.

94. The host cell of any one of claims 1 to 93, 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.~~

~~95. The host cell of claim 94, 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.~~

~~96. The host cell of any one of claims 94 to 95, wherein the host cell line is a GS knockout cell line.~~

~~97. The host cell line of any one of claims 94 to 95, wherein the host cell line is a DHFR knockout cell line.~~

~~98. The host cell of any one of claims 34 to 90 and 94 to 97, wherein the host cell further comprises at least a second nucleic acid construct that encodes and allows for expression of at least a second protein of interest, and wherein said second nucleic acid construct does not include a selectable marker.~~

~~99. The host cell of any one of claims 34 to 90 and 94 to 97, 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.~~

~~100. The host cell of any one of claims 98 to 99, wherein the first protein of interest in the first vector is one of an immunoglobulin heavy or light chain and the second protein in the second vector is the other of an immunoglobulin heavy or light chain.~~

~~101. The host cell of claim 100, wherein the first protein of interest is an immunoglobulin heavy chain and the second protein of interest is an immunoglobulin light chain.~~

~~102. The host cell line of claim 99, wherein the second nucleic acid construct is inserted at one or more dock sites.~~

~~103. The host cell line of claim 25, wherein the ratio of the episomal expression vector to inserted nucleic acid expression constructs is from 1:1000 to 1:10.~~

~~104. The host cell line of claim 25, wherein the ratio of the episomal expression vector to inserted nucleic acid expression constructs is from 1:100 to 1:750.~~

~~105. The host cell line of claim 25, wherein the ratio of the episomal expression vector to inserted nucleic acid expression constructs is from 1:400 to 1:600.~~

~~106.~~25. A cell culture comprising host cells of ~~any one of claims 1 to 105~~claim 1.

~~107. The cell culture of claim 106, wherein the cell culture is a master cell culture.~~

~~108.~~26. A process for producing a protein of interest comprising culturing host cells according to ~~any one of claims 34 to 90 and 94 to 107~~claim 22 under conditions that the protein of interest is expressed and purifying the protein of interest from the host cell culture.

~~109. The process of claim 108, wherein the host cells are grown in a medium comprising an inhibitor of the selectable marker.~~

~~110. The process of claim 109, wherein the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulphoximine (Msx).~~

~~111. The process of claim 109, wherein the selectable marker is DHFR and the inhibitor is methotrexate.~~

~~112. A process for producing a viral vector comprising culturing host cells according to any one of claims 91 to 93 under conditions such that the viral vector is expressed and purifying the viral vectors from the host cell culture.~~

~~113.~~27. A system comprising:

a host cell of ~~any one of claims 1 to 37, 47 to 51 and 94 to 97~~claim 1;

one or more nucleic acid expression constructs, wherein the nucleic acid expression construct further comprises the following elements in operable association in 5’ to 3’ order:

a selectable marker sequence;

an internal promoter sequence;

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

a poly A signal sequence,

wherein the nucleic acid construct further comprises at least one insertion element, and

wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site.

~~114. The system of claim 113, wherein the one or more nucleic acid expression constructs further comprise a 5' promotor sequence 5' to the selectable marker sequence.~~

~~115. The system of claim 113, wherein the nucleic acid expression constructs are provided in a vector.~~

~~116. The system of claim 113, wherein the vector is a plasmid vector.~~

~~117. The system of claim 113, further comprising a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site.~~

~~118. The system of claim 117, wherein the enzyme is selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.~~

~~119. The system of claim 118, wherein the nuclease is a Cas nuclease.~~

~~120. The system of claim 118, wherein the nickase is a Cas nickase.~~

~~121. The system of any one of claims 119 to 120, further comprising one or more RNA guide sequences.~~

~~122. The system of any one of claims 117 to 121, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.~~

~~123. The system of claim 122, wherein the vector is a different vector from the vector comprising the nucleic acid expression construct.~~

~~124. The system of claim 122, wherein the vector is the same vector as the vector comprising the nucleic acid expression construct.~~

~~125. The system of any one of claims 113 to 124, further comprising at least a second nucleic acid expression construct encoding a different protein of interest.~~

~~126. The system of claim 125, wherein the second nucleic acid expression construct further comprises the following elements in operable association in 5' to 3' order:~~

~~a selectable marker sequence;~~

~~an internal promoter sequence;~~

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

~~a poly A signal sequence,~~

~~wherein the nucleic acid construct further comprises at least one insertion element, and~~

~~wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site.~~

~~127. The system of any one of claims 125 to 126, wherein the at least a second nucleic acid expression construct encoding a different protein of interest is provided on a separate vector.~~

~~128. The system of any one of claims 113 to 124, wherein the nucleic acid expression construct further comprises a nucleic acid sequence encoding at least a second protein of interest.~~

~~129. The system of claim 128 wherein the nucleic acid sequence encoding the second protein of interest is operably associated with a construct element selected from the group consisting of a third promoter, an intron, a second RNA export element and an IRES sequence and combinations thereof.~~

~~130. The system of claim 129 wherein the second RNA export element is a pre-mRNA processing enhancer (PPE).~~

~~131. The system of any one of claims 128 to 130, wherein the second nucleic acid sequence encoding a second protein of interest is positioned 3' to the nucleic acid sequence encoding the first protein of interest.~~

~~132. The system of claim 129, wherein the IRES sequence is selected from the group consisting of foot and mouth disease virus (FDV), encephalomyocarditis virus and poliovirus IRES sequences.~~

~~133.~~28. A method comprising:

providing a host cell of ~~any one of claims 1 to 37, 47 to 51 and 94 to 97~~claim 1;

introducing into the host cell one or more nucleic acid expression constructs encoding a first protein of interest under conditions such that the nucleic acid expression constructs are inserted at the dock sites, wherein the nucleic acid expression construct further comprises at least the following elements in operable association in 5' to 3' order:

a selectable marker sequence;

an internal promoter sequence;

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

a poly A signal sequence,

wherein the nucleic acid construct further comprises at least one insertion element, and

wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site.

~~134. The method of claim 133, wherein the one or more nucleic acid expression constructs further comprise a 5' promotor sequence 5' to the selectable marker sequence.~~

~~135. The method of claim 133, wherein the nucleic acid expression constructs are provided in a vector.~~

~~136. The method of claim 127, wherein the vector is a plasmid vector.~~

~~137. The method of any one of claims 135 to 136, wherein the vector is transiently introduced into the host cell.~~

~~138. The method of any one of claims 133 to 137, wherein the host cell line comprises a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site.~~

~~139. The method of claim 133 to 137, further comprising transiently introducing into the host cell a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site.~~

~~140. The method of claim 139, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.~~

~~141. The method of claim 139, wherein the vector is a plasmid vector.~~

~~142. The method of any one of claims 139 to 141, wherein the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell line is from 1:1000 to 1:10.~~

~~143. The method of any one of claims 139 to 141, wherein the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell line is from 1:100 to 1:750.~~

144. The method of any one of claims 139 to 141, wherein the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell line is from 1:400 to 1:600.

145. The method of any one of claims 138 to 144, wherein the enzyme is selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.

146. The method of claim 145, wherein the nuclease is a Cas nuclease.

147. The method of claim 145, wherein the nickase is a Cas nickase.

148. The method of any one of claims 146 to 147, further comprising introducing into the host cell one or more RNA guide sequences.

149. The method of any one of claims 138 to 148, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.

150. The method of claim 149, wherein the vector is a different vector from the vector comprising the nucleic acid expression construct.

151. The method of claim 149, wherein the vector is the same vector as the vector comprising the nucleic acid expression construct.

152. The method of any one of claims 133 to 151, further comprising introducing into the host cell at least a second nucleic acid expression construct encoding a different protein of interest.

153. The method of claim 152, wherein the at least a second nucleic acid expression construct encoding a different protein of interest is provided on a separate vector.

~~154. The method of any one of claims 152 to 153, wherein the second nucleic acid expression construct further comprises the following elements in operable association in 5' to 3' order:~~

~~a selectable marker sequence;~~

~~an internal promoter sequence;~~

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

~~a poly A signal sequence,~~

~~wherein the nucleic acid construct further comprises at least one insertion element, and~~

~~wherein the expression construct insertion elements are compatible with the dock site insertion elements to facilitate insertion of the nucleic acid expression construct at the dock site.~~

~~155. The method of any one of claims 133 to 151, wherein the nucleic acid expression construct further comprises a nucleic acid sequence encoding at least a second protein of interest.~~

~~156. The method of claim 155 wherein the nucleic acid sequence encoding the second protein of interest is operably associated with a construct element selected from the group consisting of a third promoter, an intron, a second RNA export element and an IRES sequence and combinations thereof.~~

~~157. The method of claim 156 wherein the second RNA export element is a pre-mRNA processing enhancer (PPE).~~

~~158. The method of any one of claims 155 to 156, wherein the second nucleic acid sequence encoding a second protein of interest is positioned 3' to the nucleic acid sequence encoding the first protein of interest.~~

~~159. The method of claim 156, wherein the IRES sequence is selected from the group consisting of foot and mouth disease virus (FDV), encephalomyocarditis virus and poliovirus IRES sequences.~~

~~160. A method for making a host cell line comprising inserting into the genome of the host cell multiple docking sites, each docking site comprising at least one dock site insertion element.~~

~~161. The method of claim 160, wherein the inserting comprises insertion of the docking sites via a vector selected from the group consisting of a viral vector and a transposon vector.~~

~~162. The method of claim 161, wherein the viral vector is a retroviral vector.~~

~~163. The method of claim 160, wherein from 1 to 500 integrated docking sites are inserted, each docking site comprising at least one dock site insertion element.~~

~~164. The method of claim 160, wherein from 5 to 500 integrated docking sites are inserted, each docking site comprising at least one dock site insertion element.~~

~~165. The method of claim 160, wherein the integrated docking sites are independently inserted throughout the genome.~~

~~166. The method of any one of claims 160 to 165, wherein the dock site insertion element is targeted by enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.~~

~~167. The method of any one of claims 160 to 166, wherein the dock site insertion element is selected from the group consisting of a recombinase dock site insertion element and a HDR dock site insertion element.~~

~~168. The method of claim 167, wherein the dock site insertion element is a recombinase dock site insertion element.~~

~~169. The method of claim 168, wherein the recombinase dock site insertion element comprises an attachment site (att).~~

~~170. The method of claim 169, wherein the attachment site (att) is selected from the group consisting of attB and attP.~~

~~171. The method of claim 169, wherein the recombinase dock site insertion element comprises a LoxP sequence.~~

~~172. The method of claim 169, wherein the recombinase dock site insertion element is a Flp Recombination Target (FRT) site.~~

~~173. The method of claim 167, wherein the dock site insertion element is a HDR dock site insertion element.~~

~~174. The method of claim 172, wherein the HDR dock site insertion element comprises one or two dock site homology arms.~~

~~175. The method of claim 174, wherein the HDR dock site insertion element further comprises one or more sequences homologous to a guide RNA sequence.~~

~~176. The method of any of claims 174 to 175, wherein the dock site homology arms are from about 30 to 1000 bases in length.~~

~~177. The method of claim 172, wherein the integrase dock site insertion element comprises an AAVS1 safe harbor locus sequence.~~

~~178. The method of any one of claims 160 to 176, wherein each docking site is flanked by exogenous integrating vector sequences following insertion.~~

~~179. The method of claim 178, wherein the exogenous integrating vector sequences are selected from the group consisting of viral vector sequences and transposon vector sequences.~~

~~180. The method of any one of claims 160 to 179, wherein the docking sites each further comprise a sequence encoding a selectable maker operably linked to a promoter.~~

~~181. The method of any one of claim 160 to 180, wherein the docking site comprises a promoter.~~

~~182. The method of any one of claims 180 to 181, wherein each docking site comprises two dock site insertion elements.~~

~~183. The method of claim 182, wherein the two dock site insertion elements are positioned to facilitate cassette exchange.~~

~~184. The method of any one of claims 182 to 183, wherein the two dock site insertion elements flank sequences encoding a selectable marker, an enzyme, or a combination thereof.~~