event but, given the randomness as to where the nucleotide sequence integrates, these rare events can result in a variety of gene expression and cell growth phenotypes. Such variation, known as "position effect variation," originates, at least in part, from the complex gene regulatory networks present in eukaryotic cell genomes and the accessibility of certain genomic loci for integration and gene expression. Second, random integration strategies generally do not offer control over the number of gene copies integrated into a host cell genome. In fact, gene amplification methods are often used to achieve high-producing cells. Such gene amplification, however, can lead to unwanted cell phenotypes such as unstable cell growth and/or product expression. Third, because of the integration loci heterogeneity inherent in the random integration process, it is time-consuming and labor-intensive to screen thousands of clones after transfection to isolate cell lines demonstrating a desirable level of expression of the polypeptides of interest. Even after isolating such cell lines, stable expression of the polypeptide of interest is not guaranteed and further screening may be required to obtain a stable commercial cell line. Finally, polypeptides produced from randomly integrated cell lines exhibit a high degree of sequence variance, which may be, in part, due to the mutagenicity of the selective agents used to select for a high level of expression of polypeptides of interest.

SUMMARY

In one aspect, the present disclosure refers to a host cell capable of expressing a polypeptide of interest comprising:

a) a targeted integrated exogenous nucleic acid sequence of interest (SOI) encoding a first polypeptide of interest and a first selection marker flanked by two recombination recognition sequences (RRSs), wherein the targeted integrated exogenous SOI is integrated within a targeted locus of the genome of the host cell; and

b) a randomly integrated exogenous nucleic acid SOI encoding a second polypeptide of interest and a second selection marker, wherein the randomly integrated SOI is integrated at least once in the genome of the host cell.

In another aspect, the present disclosure refers to a method of expressing a polypeptide of interest comprising:

a) providing a host cell comprising an exogenous nucleotide sequence integrated at a targeted locus of the genome of the host cell, wherein the exogenous nucleotide sequence comprises two RRSs flanking a first selection marker;

b) introducing into the cell provided in (a) a nucleic acid comprising two RRSs matching the two RRSs of the integrated exogenous nucleotide sequence and flanking a first exogenous SOI encoding a first polypeptide of interest and a second selection marker;

c) introducing a recombinase or a nucleic acid encoding a recombinase, wherein the recombinase recognizes the RRSs;

d) selecting for cells expressing the second selection marker;

e) introducing, via random integration, a second exogenous SOI encoding a second polypeptide of interest and a third selection marker into the genome of the host cell;

f) selecting for cells expressing the third selection marker; and

g) culturing the host cell under conditions sufficient to express the first and second polypeptides of interest.

The presently disclosed subject matter relates, in part, to targeted integration (TI) host cells suitable for the expression of recombinant proteins where the TI host cell is subjected to supertransfection resulting in the random integration (RI) of exogenous nucleic acids encodes into their genome, as well as methods of producing and using said supertransfected TI host cells. The presently disclosed subject matter not only provides host cell TI sites that have high productivity, it also provides a novel method of introducing multiple sequences of interest into a single TI locus in a host cell by recombinase-mediated cassette exchange (RMCE) and, as outlined herein, achieving increased expression of the sequences of interest by subjecting the cells to supertransfection resulting in the random integration (RI) of exogenous nucleic acids encodes into the TI host cell genome.

In certain examples, the supertransfected TI host cell will initially (prior to being subjected to RI) or subsequently (after beign subjected to RI) comprise an exogenous nucleotide sequence integrated at an integration site within a locus of the

genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7. In certain examples, the nucleotide sequence immediately 5' of the integrated exogenous nucleotide sequence is selected from the group consisting of sequences at least about 90% homologous to nucleotides 41190-45269 of NW_006874047.1, nucleotides 63590-207911 of NW_006884592.1, nucleotides 253831-491909 of NW_006881296.1, nucleotides 69303-79768 of NW_003616412.1, nucleotides 293481-315265 of NW_003615063.1, nucleotides 2650443-2662054 of NW_006882936.1, and nucleotides 82214-97705 of NW_003615411.1. In certain examples, the nucleotide sequence immediately 3' of the integrated exogenous nucleotide sequence is selected from the group consisting of sequences at least about 90% homologous to nucleotides 45270-45490 of NW_006874047.1, nucleotides 207912-792374 of NW_006884592.1, nucleotides 491910-667813 of NW_006881296.1, nucleotides 79769-100059 of NW_003616412.1, nucleotides 315266-362442 of NW_003615063.1, nucleotides 2662055-2701768 of NW_006882936.1, or nucleotides 97706-105117 of NW_003615411.1. In certain examples, the integrated exogenous nucleotide sequence is operably linked to a nucleotide sequence selected from the group consisting of sequences at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7.

In certain examples, the supertransfected TI host cell will initially (prior to being subjected to RI) or subsequently (after beign subjected to RI) comprise an exogenous nucleotide sequence integrated at an integration site within an endogenous gene selected from the group consisting of LOC107977062 (SEQ ID NO. 1), LOC100768845 (SEQ ID NO. 2), ITPR2 (SEQ ID NO. 3), ERE67000.1 (SEQ ID NO. 4), UBAP2 (SEQ ID NO. 5), MTMR2 (SEQ ID NO. 6), XP_003512331.2 (SEQ ID NO. 7), and sequences at least about 90% homologous thereto. In certain examples, the exogenous nucleotide sequence is integrated at an integration site operably linked to an endogenous gene selected from the group consisting of LOC107977062 (SEQ ID NO. 1), LOC100768845 (SEQ ID NO. 2), ITPR2 (SEQ ID NO. 3), ERE67000.1 (SEQ ID NO. 4), UBAP2 (SEQ ID NO. 5), MTMR2 (SEQ ID NO. 6), XP_003512331.2 (SEQ ID NO. 7), and sequences at least about 90% homologous thereto. In certain examples, the integrated exogenous nucleotide is flanked by a nucleotide sequence selected from the group consisting of sequences at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7. In certain

examples, the exogenous nucleotide sequence is integrated at an integration site immediately adjacent to all or a portion of a sequence selected from the group consisting of sequences at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7.

In certain examples, a TI host cell is a mammalian host cell. In certain examples, a TI host cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell. In certain examples, a TI host cell is a Chinese hamster ovary (CHO) host cell, a CHO K1 host cell, a CHO K1SV host cell, a DG44 host cell, a DUKXB-11 host cell, a CHOK1S host cell, or a CHO K1M host cell.

In certain examples, a host cell comprises an exogenous nucleotide sequence wherein the integration of that seqeunce was targeted, and wherein the exogenous nucleotide sequence comprises one or more recombination recognition sequence (RRS). In certain examples, the exogenous nucleotide sequence comprises at least two RRSs. The RRS can be recognized by a recombinase, for example, a Cre recombinase, an FLP recombinase, a Bxb1 integrase, or a φC31 integrase. The RRS can be selected from the group consisting of a LoxP sequence, a LoxP L3 sequence, a LoxP 2L sequence, a LoxFas sequence, a Lox511 sequence, a Lox2272 sequence, a Lox2372 sequence, a Lox5171 sequence, a Loxm2 sequence, a Lox71 sequence, a Lox66 sequence, a FRT sequence, a Bxb1 attP sequence, a Bxb1 attB sequence, a φC31 attP sequence, and a φC31 attB sequence.

In certain examples, the exogenous nucleotide sequence integrated by targeted integration comprises a first and a second RRS, and at least one selection marker located between the first and the second RRS. In certain examples, the exogenous nucleotide sequence integrated by targeted integration further comprises a third RRS, wherein the third RRS is located between the first and the second RRS, and the third RRS is different from the first or the second RRS. In certain examples, the exogenous nucleotide sequence integrated by targeted integration comprises a first and a second RRS, and a first selection marker located between the first and the second RRS. In certain examples, the exogenous nucleotide sequence integrated by targeted integration further comprises a second selection marker, and the first and the second selection markers are different. In certain examples, the exogenous nucleotide sequence integrated by targeted integration can further comprise a third selection marker and an internal ribosome

entry site (IRES), wherein the IRES is operably linked to the third selection marker. The third selection marker can be different from the first or the second selection marker. The selection markers can be selected from the group consisting of an aminoglycoside phosphotransferase (APH) (e.g., hygromycin phosphotransferase (HYG), neomycin and G418 APH), dihydrofolate reductase (DHFR), thymidine kinase (TK), glutamine synthetase (GS), asparagine synthetase, tryptophan synthetase (indole), histidinol dehydrogenase (histidinol D), and genes encoding resistance to puromycin, blasticidin, bleomycin, phleomycin, chloramphenicol, Zeocin, and mycophenolic acid. The selection markers can also be selected from the group consisting of a green fluorescent protein (GFP) marker, an enhanced GFP (eGFP) marker, a synthetic GFP marker, a yellow fluorescent protein (YFP) marker, an enhanced YFP (eYFP) marker, a cyan fluorescent protein (CFP) marker, an mPlum marker, an mCherry marker, a tdTomato marker, an mStrawberry marker, a J-red marker, a DsRed-monomer marker, an mOrange marker, an mKO marker, an mCitrine marker, a Venus marker, a YPet marker, an Emerald6 marker, a CyPet marker, an mCFPm marker, a Cerulean marker, and a T-Sapphire marker. In some examples, the selection marker is selected from the group consisting of a green fluorescent protein (GFP) marker, an enhanced GFP (eGFP) marker, a synthetic GFP marker.

In certain examples, the exogenous nucleotide sequence integrated by targeted integration comprises at least one selection marker and at least one exogenous sequence of interest (SOI). In certain examples, the exogenous nucleotide sequence integrated by targeted integration comprises a first and a second RRS, and at least one selection marker and at least one exogenous SOI located between the first and the second RRS. In certain examples, the exogenous nucleotide sequence integrated by targeted integration comprises a first, second, and third RRS, at least one selection marker and at least one exogenous SOI located between the first and the third RRS, and at least one exogenous SOI located between the third and the second RRS. The sequence of interest can encode any polypeptide of interest, including but not limited to the examples listed herein. The SOIs can be the same or they can be different, for example the SOIs can include coding sequences to both chains of an antibody. In the case where two or more different polypeptides are expressed, the sequence of interest can include the two polypeptides at various ratios, e.g., when eight coding sequences encode two different

polypeptides including, but not limited to, 1:7, 2:6, etc. The SOIs can encode a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.

The presently disclosed subject matter also provides methods for the targeted integration of an exogenous nucleic acid into a host cell to facilitate the expression of a polypeptide of interest wherein the targted integration is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest. In certain examples, such methods comprise the targeted integration of an exogenous nucleic acid into a host cell via recombinase-mediated recombination. In certain examples, such methods relate to a cell comprising an exogenous nucleotide sequence integrated at a site within an endogenous gene selected from the group consisting of LOC107977062, LOC100768845, ITPR2, ERE67000.1, UBAP2, MTMR2, XP_003512331.2, and sequences least about 90% homologous thereto. In certain examples, such methods relate to a cell comprising an exogenous nucleotide sequence integrated within a locus of the genome of the host cell, wherein the locus comprises a nucleotide sequence that is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7.

In certain examples, the present disclosure provides a method of preparing a TI host cell expressing a polypeptide of interest, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest, and where the method also comprises: a) providing a TI host cell comprising an exogenous nucleotide sequence integrated at a site within a locus of the genome of the TI host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7, wherein the exogenous nucleotide sequence comprises two RRSs, flanking at least one first selection marker; b) introducing into the cell provided in a) a vector comprising two RRSs matching the two RRSs on the integrated exogenous nucleotide sequence and flanking at least one exogenous SOI and at least one second selection marker; c) introducing a recombinase, wherein the recombinase recognizes the RRSs; and d) selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the polypeptide of interest.

In certain examples, the present disclosure provides a method of preparing

a TI host cell expressing a first and second polypeptide of interest (where the first and second polypeptides can be the same or different), wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid(s) encoding the same or different polypeptide(s) of interest, and where the method also comprises: a) providing a TI host cell comprising an exogenous nucleotide sequence integrated at a site within a locus of the genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7, wherein the exogenous nucleotide sequence comprises a first and a second RRS flanking at least one first selection marker, and a third RRS located between the first and the second RRS, wherein the first, second, and third RRSs are heterospecific, i.e., the first, second, and third RRSs are not matched RRSs and therefore any two of the first, second, and third RRSs would not be amenable to recombinase-mediated recombination,; b) introducing into the cell provided in a) a first vector comprising two RRSs matching the first and the third RRS on the integrated exogenous nucleotide sequence and flanking at least one first exogenous SOI and at least one second selection marker; c) introducing into the cell provided in a) a second vector comprising two RRSs matching the second and the third RRS on the integrated exogenous nucleotide sequence and flanking at least one second exogenous SOI; d) introducing one or more recombinases, wherein the one or more recombinases recognize the RRSs; and e) selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the first and second polypeptides of interest.

In certain examples, the present disclosure provides a method for expressing a polypeptide of interest, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest, and where the method also comprises: a) providing a host cell comprising at least one exogenous SOI and at least one selection marker flanked by two RRSs integrated within a locus of the genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7; and b) culturing the cell in a) under conditions suitable for expressing the SOI and recovering a polypeptide of interest therefrom.

In certain examples, the present disclosure provides a method for

expressing a polypeptide of interest, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest, where the method also comprises: a) providing a host cell comprising at least two exogenous SOIs and at least one selection marker integrated within a locus of the genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7, wherein at least one exogenous SOI and one selection marker is flanked by a first and a third RRS and at least one exogenous SOI is flanked by a second and the third RRS; and b) culturing the cell in a) under conditions suitable for expressing the SOI and recovering a polypeptide of interest therefrom.

The presently disclosed subject matter also provides compositions and methods to produce polypeptides of interest via the use of TI host cells, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest. Such compositions and methods include, but are not limited to, polynucleotides and vectors, facilitating the integration of an exogenous nucleotide sequence into a TI host cell as well as TI host cells and compositions comprising exogenous nucleotide sequences and methods of using the same. In certain examples, a vector can comprise nucleotide sequences at least 50% homologous to a sequence selected from SEQ ID Nos. 1-7 flanking a DNA cassette, wherein the DNA cassette comprises at least one selection marker and at least one exogenous SOI flanked by two RRSs. The vector can be selected from the group consisting of an adenovirus vector, an adeno-associated virus vector, a lentivirus vector, a retrovirus vector, an integrating phage vector, a non-viral vector, a transposon and/or transposase vector, an integrase substrate, and a plasmid.

In certain examples, the methods of the present disclosure comprise the targeted integration of an exogenous nucleic acid encoding a polypeptide of interest into a host cell via homologous recombination, homology-directed repair (HDR), and/or non-homologous end joining (NHEJ). In certain examples, such methods involve the integration of an exogenous nucleic acid encoding a polypeptide of interest into a cell at a site within an endogenous gene selected from the group consisting of LOC107977062 (SEQ ID NO. 1), LOC100768845 (SEQ ID NO. 2), ITPR2 (SEQ ID NO. 3), ERE67000.1

(SEQ ID NO. 4), UBAP2 (SEQ ID NO. 5), MTMR2 (SEQ ID NO. 6), XP_003512331.2 (SEQ ID NO. 7), and sequences at least about 90% homologous thereto. In certain examples, such methods involve the integration of an exogenous nucleic acid encoding a polypeptide of interest into a cell at a site within a locus of the genome of the host cell, wherein the locus comprises a nucleotide sequence that is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7.

In certain examples, the present disclosure provides a method for preparing a TI host cell expressing a polypeptide of interest, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest, and where the method also comprises: a) providing a host cell comprising a locus of the genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7; b) introducing a vector into the host cell, wherein the vector comprises nucleotide sequences at least 50% homologous to a sequence selected from SEQ ID Nos. 1-7 flanking a DNA cassette, wherein the DNA cassette comprises at least one selection marker and at least one exogenous SOI flanked by two RRSs; and c) selecting for the selection marker to isolate a TI host cell with the SOI integrated in the locus of the genome, and expressing the polypeptide of interest. In certain examples, such methods involve the use of an exogenous nuclease to promote the targeted integration. The exogenous nuclease can be selected from the group consisting of a zinc finger nuclease (ZFN), a ZFN dimer, a transcription activator-like effector nuclease (TALEN), a TAL effector domain fusion protein, an RNA-guided DNA endonuclease, an engineered meganuclease, and a clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) endonuclease.

In certain examples, the present disclosure provides a method of preparing a TI host cell expressing a polypeptide of interest, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest, and where the method also comprises: a) providing a TI host cell comprising at least one exogenous nucleotide sequence integrated at a site within one or more loci of the genome of the TI host cell, wherein the one or more loci are at least about 90% homologous to a sequence selected from SEQ ID No. 1-7, wherein the

exogenous nucleotide sequence comprises one or more RRSs; b) introducing into the cell provided in a) a vector comprising one or more RRSs matching the one or more RRSs on the integrated exogenous nucleotide sequence and flanking at least one exogenous SOI operably linked to a regulatable promoter; c) introducing a recombinase or a nucleic acid encoding a recombinase, wherein the recombinase recognizes the RRSs; and d) selecting for TI cells expressing the exogenous SOI in the presence of an inducer to thereby isolate a TI host cell expressing the polypeptide of interest.

In certain examples, the present disclosure provides a method for expressing a polypeptide of interest, wherein the targted integration of the sequence encoding the polypeptide of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide of interest, and where the method also comprises: a) providing a host cell comprising at least one exogenous SOI flanked by two RRSs and operably linked to a regulatable promoter integrated within a locus of the genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7; and b) culturing the cell under conditions suitable for expressing the SOI and recovering a polypeptide of interest therefrom.

In certain examples, the present disclosure provides a method for preparing a TI host cell expressing a first and second polypeptide of interest (where the first and second polypeptides can be the same or different), wherein the targted integration of the sequence(s) encoding the polypeptide(s) of interest is preceded by, followed by, or introduced concurrently with a randomly integrated exogenous nucleic acid encoding the same or different polypeptide(s) of interest, and where the method also comprises: a) providing a TI host cell comprising an exogenous nucleotide sequence integrated at a site within a locus of the genome of the host cell, wherein the locus is at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7, wherein the exogenous nucleotide sequence comprises a first, second RRS and a third RRS located between the first and the second RRS, wherein the first, second, and third RRSs are heterospecific, i.e. the first, second, and third RRSs are not matched RRSs and therefore any two of the first, second, and third RRSs would not be amenable to recombinase-mediated recombination; b) introducing into the cell provided in a) a first vector comprising two RRSs matching the first and the third RRS on the integrated exogenous nucleotide sequence and flanking at

least one first exogenous SOI operably linked to a regulatable promoter; c) introducing into the cell provided in a) a second vector comprising two RRSs matching the second and the third RRS on the integrated exogenous nucleotide sequence and flanking at least one second exogenous SOI operably linked to a regulatable promoter; d) introducing one or more recombinases, or one or more nucleic acids encoding one or more recombinases, wherein the one or more recombinases recognize the RRSs; and e) selecting for TI cells expressing the exogenous SOI in the presence of an inducer to thereby isolate a TI host cell expressing the first and second polypeptides of interest.

In certain examples, the present disclosure provides a host cell capable of expressing a polypeptide of interest comprising: a) a targeted integrated exogenous nucleic acid sequence of interest (SOI) encoding a first polypeptide of interest and a first selection marker flanked by two recombination recognition sequences (RRSs), wherein the targeted integrated exogenous SOI is integrated within a targeted locus of the genome of the host cell; and b)a randomly integrated exogenous nucleic acid SOI encoding a second polypeptide of interest and a second selection marker, wherein the randomly integrated SOI is integrated at least once in the genome of the host cell. In certain examples, the wherein the first and the second polypeptide of interest can be the same. In certain examples, the first and the second selection marker can be the same. In certain examples, the host cell may comprise one to ten randomly integrated exogenous nucleic acid SOIs. In certain examples, the targeted locus can be at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7. In certain examples, the host cell of the present disclosure may further comprise a second targeted integrated exogenous nucleic acid SOI encoding a second polypeptide of interest and a second selection marker integrated within a targeted locus of the genome of the host cell, wherein the first targeted integrated exogenous nucleic acid SOI and the first selection marker can be flanked by a first and a third RRS and the second targeted exogenous SOI and second selection marker can be flanked by a second and the third RRS. In certain examples, the polypeptides of interest can be selected from the group consisting of: a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), and an Fc fusion protein. In certain examples, the host cell can be a mammalian host cell. In certain examples, the host cell can be a hamster host cell, a human host cell, a rat host cell, or a mouse host cell. In certain

examples, the host cell can be a CHO host cell, a CHO K1 host cell, a CHO K1SV host cell, a DG44 host cell, a DUKXB-11 host cell, a CHOK1S host cell, or a CHO K1M host cell. In certain examples, the targeted integration of the SOIs and selection markers can be promoted by an exogenous nuclease. In certain examples, the exogenous nuclease can be selected from the group consisting of a zinc finger nuclease (ZFN), a ZFN dimer, a transcription activator-like effector nuclease (TALEN), a TAL effector domain fusion protein, an RNA-guided DNA endonuclease, an engineered meganuclease, and a clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) endonuclease.

The presently disclosed subject matter also provides methods of expressing a polypeptide of interest. In certain examples, the present disclosure provides a method of expressing a polypeptide of interest comprising: a) providing a host cell comprising an exogenous nucleotide sequence integrated at a targeted locus of the genome of the host cell, wherein the exogenous nucleotide sequence comprises two RRSs flanking a first selection marker; b) introducing into the cell provided in (a) a nucleic acid comprising two RRSs matching the two RRSs of the integrated exogenous nucleotide sequence and flanking a first exogenous SOI encoding a first polypeptide of interest and a second selection marker; c) introducing a recombinase or a nucleic acid encoding a recombinase, wherein the recombinase recognizes the RRSs; d) selecting for cells expressing the second selection marker; e) introducing, via random integration, a second exogenous SOI encoding a second polypeptide of interest and a third selection marker into the genome of the host cell; f) selecting for cells expressing the third selection marker; and g) culturing the host cell under conditions sufficient to express the first and second polypeptides of interest. In certain examples, such methods may further comprise recovering the first and second polypeptides of interest from the host cell culture. In certain examples, the first and the second polypeptides of interest can be the same. In certain examples, the targeted locus can be at least about 90% homologous to a sequence selected from SEQ ID Nos. 1-7. In certain examples, the first and second polypeptides of interest can be selected from the group consisting of: a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), and an Fc fusion protein. In certain examples, the host cell can be a mammalian host cell. In certain examplets, the host cell can be a hamster host cell, a human host cell, a rat host cell, or a mouse host

cell. In certain examples, the host cell can be a CHO host cell, a CHO K1 host cell, a CHO K1SV host cell, a DG44 host cell, a DUKXB-11 host cell, a CHOK1S host cell, or a CHO K1M host cell. In certain examples, the targeted integration of any of the SOIs can promoted by an exogenous nuclease. In certain examples, the exogenous nuclease can be selected from the group consisting of a zinc finger nuclease (ZFN), a ZFN dimer, a transcription activator-like effector nuclease (TALEN), a TAL effector domain fusion protein, an RNA-guided DNA endonuclease, an engineered meganuclease, and a clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) endonuclease. In certain examples, the expression of the SOIs can be controlled by a regulatable promoter. In certain examples, the regulatable promoter can be selected from the group consisting of SV40 and CMV promoters.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing an outline of the genome-wide screening steps for identifying CHO TI loci that allow for stable and high expression of antibodies.

Figures 2A and 2B depict two double selection schemes which were utilized to generate the targeted antibody expressing population. Transposase generated TI hosts are depicted in Figure 2A and random integration generated TI hosts are depicted in Figure 2B.

Figure 3 depicts the productivity of the top clones generated by two particular TI hosts.

Figure 4 depicts a two-plasmid RMCE strategy involving the use of three RRS sites to carry out two independent RMCEs simultaneously. One vector (front) comprises the RRS1, a first SOI and a promoter (P) followed by a start codon (ATG) and RRS3. The other vector (back) comprises the RRS3 fused to the coding sequence of a marker without the start codon (ATG), an SOI 2 and the RRS2. Only when the two plasmids are correctly targeted will the full expression cassette of the selection marker be assembled thus rendering cells resistance to selection.

Figure 5 depicts the results of using either single-vector or two-vector RMCE in connection with the expression of Antibodies D, E, F, G, and J. FACS, genomic PCR, and gene copy analysis were assessed to confirm both plasmid cassettes were targeted correctly to the TI site. In two-plasmid RMCEs, more total HC and LC copies were