

What is claimed is:

1. A method of preparing a TI host cell expressing sequences of interest (SOIs) comprising:

a) providing a TI host cell comprising an exogenous nucleotide sequence integrated at an integration site that is within a sequence:

5 a. at least about 90% homologous to all or part of nucleotides 69303-79768 of
NW_003616412.1, all or part of nucleotides 41190-45269 of
NW_006874047.1, all or part of nucleotides 63590-207911 of
NW_006884592.1, all or part of nucleotides 253831-491909 of
NW_006881296.1, all or part of nucleotides 293481-315265 of
10 NW_003615063.1, all or part of nucleotides 2650443-2662054 of
NW_006882936.1, or all or part of nucleotides 82214-97705 of
NW_003615411.1; or

 b. at least about 90% homologous to all or part of nucleotides 45270-45490 of
NW_006874047.1, all or part of nucleotides 207912-792374 of
15 NW_006884592.1, all or part of nucleotides 491910-667813 of
NW_006881296.1, all or part of nucleotides 79769-100059 of
NW_003616412.1, all or part of nucleotides 315266-362442 of
NW_003615063.1, all or part of nucleotides 2662055-2701768 of
NW_006882936.1, or all or part of nucleotides 97706-105117 of
20 NW_003615411.1; and

wherein the exogenous nucleotide sequence comprises four or more incompatible
recombination recognition sequences (RRSs);

b) introducing into the cell provided in a) at least three vectors, each vector comprising:

25 a. a pair of two RRSs matching two sequentially oriented RRSs on the integrated
exogenous nucleotide sequence; and
 b. each pair of RRSs flanking at least one exogenous SOI and at least one
selection marker;

c) introducing recombinases or nucleic acids encoding recombinases, wherein the
recombinases recognize the RRSs; and

d) selecting for TI host cells expressing the selection markers to thereby isolate a TI host cell expressing the SOIs.

2. A method for expressing SOIs comprising:

a) providing a TI host cell comprising an exogenous nucleotide sequence integrated at an integration site that is within a sequence:

a. at least about 90% homologous to all or part of nucleotides 69303-79768 of NW_003616412.1, all or part of nucleotides 41190-45269 of NW_006874047.1, all or part of nucleotides 63590-207911 of NW_006884592.1, all or part of nucleotides 253831-491909 of NW_006881296.1, all or part of nucleotides 293481-315265 of NW_003615063.1, all or part of nucleotides 2650443-2662054 of NW_006882936.1, or all or part of nucleotides 82214-97705 of NW_003615411.1; or

b. at least about 90% homologous to all or part of nucleotides 45270-45490 of NW_006874047.1, all or part of nucleotides 207912-792374 of NW_006884592.1, all or part of nucleotides 491910-667813 of NW_006881296.1, all or part of nucleotides 79769-100059 of NW_003616412.1, all or part of nucleotides 315266-362442 of NW_003615063.1, all or part of nucleotides 2662055-2701768 of NW_006882936.1, or all or part of nucleotides 97706-105117 of NW_003615411.1; and

wherein the exogenous nucleotide sequence comprises four or more incompatible RRSs;

b) introducing into the cell provided in a) at least three vectors, each vector comprising:

a. a pair of two RRSs matching two sequentially oriented RRSs on the integrated exogenous nucleotide sequence; and
b. each pair of RRSs flanking at least one exogenous SOI and at least one selection marker;

c) introducing recombinases or nucleic acids encoding recombinases, wherein the recombinases recognize the RRSs;

d) selecting for TI host cells expressing the selection markers to thereby isolate a TI host cell expressing the SOIs; and

e) culturing the cell in d) under conditions suitable for expressing the SOIs and recovering the expressed protein therefrom.

5 3. The method of claim 1 or 2, wherein the recombinases are selected from Cre recombinase, FLP recombinase, Bxb1 integrase, and a ϕ C31 integrase.

4. The method of any one of claims 1-3, wherein the SOIs encode a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.

10 5. The method of any one of claims 1-4, wherein the TI host cell is a mammalian host cell; particularly wherein the TI host cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell; more particularly wherein the TI host cell is a CHO host cell, a CHO K1 host cell, a CHO K1SV host cell, a DG44 host cell, a DUKXB-l 1 host cell, a CHOK1S host cell, or a CHO K1M host cell.

15 6. The method of any of claims 1-5, wherein the vector is 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.

20 7. A method for producing a recombinant mammalian cell comprising a nucleic acid encoding an antibody, comprising:

a) providing a recombinant mammalian cell comprising at least a single exogenous nucleic acid incorporated at a predetermined locus of the genome of the mammalian cell comprising four or more incompatible RRSs;

25 b) introducing into the recombinant mammalian cell of a) at least three vectors, each vector comprising a pair of incompatible RRSs matching two of the incompatible RRSs comprised in the exogenous nucleic acid incorporated at a predetermined locus of the genome of the mammalian cell and each pair of incompatible RRSs flank one

or more SOIs, where the SOIs encode an antibody, and/or one or more selection markers;

c) introducing one or more recombinases, simultaneously or sequentially, with the introduction of the at least three vectors comprising the SOIs and/or selection markers; and

d) selecting for cells expressing one or more of the SOIs and/or selection markers,

thereby producing a recombinant mammalian cell comprising nucleic acid SOIs encoding the antibody.

8. The method of claim 7, wherein the exogenous nucleic acid is incorporated at a locus:

a. at least about 90% homologous to all or part of nucleotides 69303-79768 of NW_003616412.1, all or part of nucleotides 41190-45269 of NW_006874047.1, all or part of nucleotides 63590-207911 of NW_006884592.1, all or part of nucleotides 253831-491909 of NW_006881296.1, all or part of nucleotides 293481-315265 of NW_003615063.1, all or part of nucleotides 2650443-2662054 of NW_006882936.1, or all or part of nucleotides 82214-97705 of NW_003615411.1; or

b. at least about 90% homologous to all or part of nucleotides 45270-45490 of NW_006874047.1, all or part of nucleotides 207912-792374 of NW_006884592.1, all or part of nucleotides 491910-667813 of NW_006881296.1, all or part of nucleotides 79769-100059 of NW_003616412.1, all or part of nucleotides 315266-362442 of NW_003615063.1, all or part of nucleotides 2662055-2701768 of NW_006882936.1, or all or part of nucleotides 97706-105117 of NW_003615411.1.

9. A method for producing a recombinant mammalian cell comprising nucleic acids encoding one or more antibodies, comprising:

a) providing a recombinant mammalian cell comprising at least two exogenous nucleic acid incorporated at predetermined loci of the genome of the mammalian cell

comprising, each exogenous nucleic acid comprising four or more incompatible RRSs, where the exogenous nucleic acids can comprise the same or different RRSs;

b) introducing into the recombinant mammalian cell of a) at least three vectors, each vector comprising a pair of incompatible RRSs matching two of the incompatible RRSs comprised in one or both of the exogenous nucleic acids incorporated at predetermined loci of the genome of the mammalian cell and each pair of incompatible RRSs flank one or more SOIs, where the SOIs encode an antibody, and/or one or more selection markers;

c) introducing one or more recombinases, simultaneously or sequentially, with the introduction of the at least three vectors comprising the SOIs and/or selection markers; and

d) selecting for cells expressing one or more of the SOIs and/or selection markers,

thereby producing a recombinant mammalian cell comprising nucleic acid SOIs encoding the antibody.

10. The method of claim 9, wherein the exogenous nucleic acids are incorporated at distinct loci selected from loci:

a. at least about 90% homologous to all or part of nucleotides 69303-79768 of NW_003616412.1, all or part of nucleotides 41190-45269 of NW_006874047.1, all or part of nucleotides 63590-207911 of NW_006884592.1, all or part of nucleotides 253831-491909 of NW_006881296.1, all or part of nucleotides 293481-315265 of NW_003615063.1, all or part of nucleotides 2650443-2662054 of NW_006882936.1, or all or part of nucleotides 82214-97705 of NW_003615411.1; or

b. at least about 90% homologous to all or part of nucleotides 45270-45490 of NW_006874047.1, all or part of nucleotides 207912-792374 of NW_006884592.1, all or part of nucleotides 491910-667813 of NW_006881296.1, all or part of nucleotides 79769-100059 of NW_003616412.1, all or part of nucleotides 315266-362442 of NW_003615063.1, all or part of nucleotides 2662055-2701768 of

NW_006882936.1, or all or part of nucleotides 97706-105117 of
NW_003615411.1.

11. The method of claim 9 or 10, wherein the recombinases are selected from Cre recombinase, FLP recombinase, Bxb1 integrase, and a ϕ C31 integrase.

5 12. The method of any one of claims 9-11, wherein the SOIs encode a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.

13. The method of any one of claims 9-12, wherein the mammalian cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell; particularly wherein the mammalian
10 cell is a CHO host cell, a CHO Kl host cell, a CHO K1SV host cell, a DG44 host cell, a DUKXB-l 1 host cell, a CHOK1S host cell, or a CHO K1M host cell.

14. The method of any of claims 9-13, wherein the vectors are selected from the group consisting of adenovirus vectors, adeno-associated virus vectors, lentivirus vectors, retrovirus vectors, integrating phage vectors, non-viral vectors, transposon and/or transposase vectors,
15 integrase substrates, and plasmids.