

What is claimed is:

1. A method for generating and high throughput screening a library of targeted integration (TI) host cells expressing at least one sequence of interest (SOI) comprising:
 - a) generating a library of TI host cells, wherein said library comprises a plurality of TI host cells expressing one or more SOIs, by:
 - i) providing a plurality of TI host cells;
 - ii) contacting the plurality of TI host cells with a plurality of vectors, wherein the vectors comprise one or more SOIs;
 - iii) introducing the one or more SOIs into one or more of the plurality of TI host cells;
 - b) separating said library into single clones; and
 - c) screening the clones for a specific cellular or product attribute.
2. The method of claim 1, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells by recombinase-mediated integration.
3. The method of claim 1, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells by gene editing-mediated integration.
4. The method of claim 1, wherein the one or more SOIs are operably linked to one or more regulatable promoters.
5. The method of claim 4, wherein the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.
6. The method of claim 1, wherein the TI host cells are mammalian host cells.
7. The method of claim 6, wherein the TI host cells are hamster host cells, human host cells, rat host cells, or mouse host cells.

8. The method of claim 7, wherein the TI host cells are CHO host cells, CHO K1 host cells, CHO K1SV host cells, DG44 host cells, DUKXB-11 host cells, CHOK1S host cells, or CHO K1M host cells.
9. The method of claim 1, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.
10. The method of claim 1, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.
11. The method of claim 1, wherein the specific cellular attribute is selected from: cell growth, cell titer, specific productivity, volumetric productivity, clone stability.
12. The method of claim 1, wherein the specific product attribute is selected from: level of glycosylation, level of charge variance, reduced mismatch, reduced protein/peptide aggregation, protein sequence heterogeneity.
13. The method of claim 1, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells at one or more loci at least about 90% homologous to a sequence selected from: SEQ ID Nos. 1-12; NW_006874047.1; NW_006884592.1; NW_006881296.1; NW_003616412.1; NW_003615063.1; NW_006882936.1; and NW_003615411.1.
14. A method of generating a library of TI host cells comprising a plurality of exogenous nucleotide SOIs comprising:
 - a) providing a plurality of TI host cells;
 - b) contacting the plurality of TI host cells with a plurality of vectors, wherein the vectors comprise one or more SOIs;
 - c) introducing the one or more SOIs into one or more of the plurality of TI host cells.
15. The method of claim 14, wherein one or more of the plurality of TI host cells comprises one or more exogenous nucleotide sequences integrated at one or more loci of the

genome of the TI host cell, and wherein the exogenous nucleotide sequence comprises at least two RRSs, flanking at least one first selection marker.

16. The method of claim 14, wherein the vectors comprise:

- a) at least two RRSs matching the at least two RRSs on the integrated exogenous nucleotide sequence; and
- b) one or more exogenous SOI and at least one second selection marker flanked by said RRSs

17. The method of claim 16, wherein the one or more SOIs are introduced into the one or more of the plurality of TI host cells by recombinase-mediated integration.

18. The method of claim 14, wherein the one or more SOIs are introduced into the one or more of the plurality of TI host cells by gene editing-mediated integration.

19. The method of claim 14, wherein the one or more SOIs are operably linked to one or more regulatable promoters.

20. The method of claim 19, wherein the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.

21. The method of claim 14, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.

22. The method of claim 14, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.

23. The method of claim 14, wherein the one or more exogenous nucleotide sequences of (a) are integrated at one or more loci at least about 90% homologous to a sequence selected from: SEQ ID Nos. 1-12; NW_006874047.1; NW_006884592.1; NW_006881296.1; NW_003616412.1; NW_003615063.1; NW_006882936.1; and NW_003615411.1.

24. The method of claim 14, wherein at least one sequence of interest comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 sequences of interest.
25. The method of claim 14, wherein the TI host cells are mammalian host cells.
26. The method of claim 25, wherein the TI host cells are hamster host cells, human host cells, rat host cells, or mouse host cells.
27. The method of claim 26, wherein the TI host cells are CHO host cells, CHO K1 host cells, CHO K1SV host cells, DG44 host cells, DUKXB-11 host cells, CHOK1S host cells, or CHO K1M host cells.
28. A method of preparing a TI host cell expressing one or more SOIs comprising:
- a) providing a plurality of TI host cells;
 - b) contacting the plurality of TI host cells with a plurality of vectors, wherein the vectors comprise one or more SOIs;
 - c) introducing the one or more SOIs into one or more of the plurality of TI host cells; and
 - d) selecting the TI host cells expressing the one or more SOIs.
29. The method of claim 28, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells by recombinase-mediated integration.
30. The method of claim 28, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells by gene editing-mediated integration.
31. The method of claim 28, wherein the one or more SOIs are operably linked to one or more regulatable promoters.
32. The method of claim 31, wherein the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.
33. The method of claim 28, wherein the TI host cell is a mammalian host cell.

34. The method of claim 33, wherein the TI host cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell.
35. The method of claim 34, 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-11 host cell, a CHOK1S host cell, or a CHO K1M host cell.
36. The method of claim 28, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.
37. The method of claim 28, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.
38. The method of claim 28, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells at one or more loci at least about 90% homologous to a sequence selected from: SEQ ID Nos. 1-12; NW_006874047.1; NW_006884592.1; NW_006881296.1; NW_003616412.1; NW_003615063.1; NW_006882936.1; and NW_003615411.1.
39. The method of claim 28, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.
40. The method of claim 28, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein, and fragments thereof.
41. The method of claim 28, wherein:
 - a) one or more of the plurality of TI host cells comprises one or more exogenous nucleotide sequences integrated at one or more loci of the genome of the TI host cell, and wherein the exogenous nucleotide sequence comprises at least two RRSs, flanking at least one first selection marker;
 - b) the vectors comprise:

- a. at least two RRSs matching the at least two RRSs on the integrated exogenous nucleotide sequence; and
 - b. one or more exogenous SOI and at least one second selection marker flanked by said RRSs;
 - c) introducing into one or more of the plurality of TI host cells one or more recombinase or a nucleic acid encoding a recombinase, wherein the one or more recombinase recognizes the RRSs; and
 - d) selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the sequence of interest.
42. The method of claim 41, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.
43. The method of claim 41, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein, and fragments thereof.
44. The method of claim 41, wherein the one or more exogenous nucleotide sequences of (a) are integrated at one or more loci at least about 90% homologous to a sequence selected from: SEQ ID Nos. 1-12; NW_006874047.1; NW_006884592.1; NW_006881296.1; NW_003616412.1; NW_003615063.1; NW_006882936.1; and NW_003615411.1.
45. The method of claim 41, wherein:
- a. 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, and all the RRSs are heterospecific; and
 - b. the plurality of vectors comprise:
 - i. 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;
 - ii. 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; and

- c. selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the first and second sequences of interest.

46. The method of any one of claims 28, 41, and 45, wherein the vector comprising at least one sequence of interest comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 sequences of interest.

47. The method of any one of claims 28, 41, and 45, wherein the TI host cell is a mammalian host cell.

48. The method of claim 47, wherein the TI host cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell.

49. The method of claim 48, 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-11 host cell, a CHOK1S host cell, or a CHO K1M host cell.

50. A method of expressing a SOI comprising:

- a) providing a plurality of TI host cells;
- b) contacting the plurality of TI host cells with a plurality of vectors, wherein the vectors comprise one or more SOIs;
- c) introducing the one or more SOIs into one or more of the plurality of TI host cells; and
- d) selecting for a TI host cell expressing the sequence of interest;
- e) culturing the cell in d) under conditions suitable for expressing the sequence of interest therefrom.

51. The method of claim 50, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells by recombinase-mediated integration.

52. The method of claim 50, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells by gene editing-mediated integration.

53. The method of claim 50, wherein the one or more SOIs are operably linked to one or more regulatable promoters.
54. The method of claim 53, wherein the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.
55. The method of claim 50, wherein the TI host cell is a mammalian host cell.
56. The method of claim 55, wherein the TI host cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell.
57. The method of claim 56, 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-11 host cell, a CHOK1S host cell, or a CHO K1M host cell.
58. The method of claim 50, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.
59. The method of claim 50, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein.
60. The method of claim 50, wherein the one or more SOIs are introduced into one or more of the plurality of TI host cells at one or more loci at least about 90% homologous to a sequence selected from: SEQ ID Nos. 1-12; NW_006874047.1; NW_006884592.1; NW_006881296.1; NW_003616412.1; NW_003615063.1; NW_006882936.1; and NW_003615411.1.
61. The method of claim 50, wherein at least one sequence of interest comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 sequences of interest.
62. A method of expressing a SOI comprising:
- a) providing a plurality of TI host cells wherein each TI host cell comprises one or more exogenous nucleotide sequences integrated at one or more loci of the genome of the TI host cell, and wherein the exogenous nucleotide sequence comprises at least two RRSs, flanking at least one first selection marker;

- b) contacting the plurality of TI host cells with a plurality of vectors, wherein the vectors comprise:
 - a. at least two RRSs matching the at least two RRSs on the integrated exogenous nucleotide sequence; and
 - b. one or more exogenous SOI and at least one second selection marker flanked by said RRSs;
 - c) introducing one or more recombinase or a nucleic acid encoding a recombinase, wherein the one or more recombinase recognizes the RRSs;
 - d) selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the sequence of interest; and
 - e) culturing the cell in d) under conditions suitable for expressing the sequence of interest and recovering the product of the sequence of interest therefrom.
63. The method of claim 62, wherein the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein.
64. The method of claim 62, wherein the one or more SOIs are operably linked to one or more regulatable promoters.
65. The method of claim 64, wherein the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.
66. The method of claim 62, wherein the SOI encodes a single chain antibody, an antibody light chain, an antibody heavy chain, a single-chain Fv fragment (scFv), or an Fc fusion protein, and fragments thereof.
67. The method of claim 62, wherein the one or more exogenous nucleotide sequences of (a) are integrated at one or more loci at least about 90% homologous to a sequence selected from: SEQ ID Nos. 1-12; NW_006874047.1; NW_006884592.1; NW_006881296.1; NW_003616412.1; NW_003615063.1; NW_006882936.1; and NW_003615411.1.

68. The method of claim 62, wherein:
- a. 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, and all the RRSs are heterospecific; and
 - b. the plurality of vectors comprises:
 - i. 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;
 - ii. 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; and
 - c. selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the first and second sequences of interest.
69. The method of claim 62 or 68, wherein the at least one sequence of interest comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 sequences of interest.
70. The method of claim 62, wherein the at least one first and the at least one second SOI are operably linked to one or more regulatable promoters.
71. The method of claim 70, wherein the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.
72. The method of claim 62 or 68, wherein the TI host cell is a mammalian host cell.
73. The method of claim 72, wherein the TI host cell is a hamster host cell, a human host cell, a rat host cell, or a mouse host cell.
74. The method of claim 73, 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-11 host cell, a CHOK1S host cell, or a CHO K1M host cell