SUMMARY

The presently disclosed subject matter relates to "Randomized Configuration Targeted Integration" (also referred to herein as "Randomized Chain Targeted Integration") (RCTI) strategies for the generation and identification of host cells capable of expressing recombinant proteins, e.g., monoclonal antibodies, as well as compositions derived from the same, e.g., bispecific antibodies, and other complex format proteins, e.g., membrane protein complexes and other difficult to express molecules. The RCTI methods described in the present disclosure can be used to screen the same number, if not more, vector configurations than the standard cell line development methods while using fewer resources. Fewer individual clones can be screened as well by using the RCTI methods described in the present disclosure.

In one aspect, the present disclosure provides 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. In certain examples, the present disclosure provides a method for generating and high throughput screening a library of targeted integration (TI) host cells expressing at least one sequence of interest (SOI) that comprises: 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.

In another aspect, the present disclosure provides 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. In certain examples, the 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 the exogenous nucleotide sequence comprises at least two recombinase recognition sequences (RRSs), flanking at least one first selection marker. In certain examples, 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.

The presently disclosed subject matter also provides methods for RCTI of one

or more exogenous nucleic acid into a host cell to facilitate the expression of a sequence of interest. In certain examples, such methods comprise the targeted integration of one or more exogenous nucleic acids into a host cell via recombinase-mediated integration or via gene editing-mediated integration. In certain examples, such methods relate to a cell comprising one or more exogenous nucleotide sequences 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. 1-12.

In yet another aspect, the present disclosure provides 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. In certain examples, the 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. In certain examples, 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. In certain examples, the method comprises 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. In certain examples, 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 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 selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the first and second sequences of interest.

In one aspect, the present disclosure provides 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.

In another aspect, the present disclosure provides 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. In certain examples, 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 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 selecting for TI cells expressing the second selection marker to thereby isolate a TI host cell expressing the first and second sequences of interest.

In certain of the above described examples, the one or more SOIs are introduced into one or more of the plurality of TI host cells by recombinase-mediated integration. In certain of the above described examples, the one or more SOIs are introduced into one or more of the plurality of TI host cells by gene editing-mediated

integration.

In certain of the above described examples, the one or more SOIs are operably linked to one or more regulatable promoters. In certain of the above described examples, the one or more regulatable promoters are selected from the group consisting of SV40 and CMV promoters.

In certain of the above described examples, the TI host cells are mammalian host cells. In certain of the above described examples, the TI host cells are hamster host cells, human host cells, rat host cells, or mouse host cells. In certain of the above described examples, the TI host cells 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.

In certain of the above described examples, the SOI encodes a polypeptide subunit, or fragment thereof, of a multisubunit protein. In certain of the above described examples, 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.

In certain of the above described examples, the specific cellular attribute is selected from: cell growth, cell titer, specific productivity, volumetric productivity, clone stability. In certain of the above described examples, the specific product attribute is selected from: level of glycosylation, level of charge variance, reduced mismatch, reduced protein/peptide aggregation, protein sequence heterogeneity.

In certain of the above described examples, the one or more SOIs are introduced into one or more of the plurality of TI host cells at one or more loci that are 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.

In certain of the above described examples, the at least one sequence of interest comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 sequences of interest.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B are schematics illustrating exemplary targeted integration (TI) Cell Line Development (CLD) workflows.

Figures 2A-2B are schematics illustrating ex the comparison of standard TI and “Randomized Configuration Targeted Integration” (also referred to herein as