COMPOSITIONS AND METHODS FOR PRODUCING STABLE VIRAL VECTOR PRODUCER CELLS FOR CELL AND GENE THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority from U.S. Provisional Patent Application No. 63/025,812, filed May 15, 2020, which is herein incorporated by reference in its entirety.

FIELD

[002] The present disclosure relates to the field of the production of viral vectors for cell and gene therapy.

BACKGROUND

[003] The growing number of gene therapy candidates combined with rapid progression through the clinical development has created a world-wide shortage of gene therapy vectors. More than 500 gene therapy and 100 cell therapy candidates are in different stages of development. Greater than 2200 clinical studies are ongoing across the globe. The strong and proven safety profile of viral vectors (*e.g.*, lentiviral vectors) has underpinned a robust clinical development pipeline. However, the clinical manufacture and use of viral vectors, especially lentiviral vectors, also comes with several limitations. For example, conventional manufacturing methods and associated technologies are outdated and not scalable, provide low downstream process yields (~20%), and furthermore require significant upfront capital and ongoing operational costs to establish. Furthermore, traditionally, viral vector manufacturing is seen as unpredictable and highly risky, resulting in demand greatly exceeding supply, which in turn drives up prices. There is a need to identify new methods and improvement for manufacturing viral vectors by generating stable producer lines with high titer at high volumes.

SUMMARY

[004] In an ~~aspect~~example, the present disclosure provides a method of making a stable viral vector producer cell line, the method comprising:

a. introducing into a population of cells a viral vector genome construct encoding a gene of interest (GOI) and one or more viral accessory constructs encoding one or more viral accessory proteins;

b. producing a population of transgenic cells comprising integrated or episomal sequences encoding the GOI and the one or more viral accessory proteins;

c. selecting from the population of transgenic cells a cell clone producing a desired viral titer; and

d. generating from the cell clone a stable viral vector producer cell line,

wherein the introduction of the one or more accessory constructs occurs concurrently.

[004a] In one aspect, the present disclosure provides a method of making a stable viral vector producer cell line, said method comprising:

a. introducing into a population of cells a viral vector genome construct encoding a gene of interest (GOI) and one or more viral accessory constructs encoding one or more viral accessory proteins;

b. producing a population of transgenic cells comprising integrated or episomal sequences encoding said GOI and said one or more viral accessory proteins;

c. selecting from said population of transgenic cells a cell clone producing a desired viral titer; and

d. generating from said cell clone a stable viral vector producer cell line,

wherein the introduction of said one or more accessory constructs occurs concurrently.

[005] In another ~~aspect~~example, the present disclosure provides a method of making a stable viral vector producer cell line, the method comprising:

a. introducing into a population of cells a viral vector genome construct encoding a gene of interest (GOI) and one or more viral accessory constructs encoding one or more viral accessory proteins;

b. producing a population of transgenic cells comprising integrated or episomal sequences encoding the GOI and the one or more viral accessory proteins;

c. selecting from the population of transgenic cells a cell clone producing a desired viral titer; and

d. generating from the cell clone a stable viral vector producer cell line,

wherein the introduction of the one or more accessory constructs occurs via one or more sequential steps with no intervening cell culturing.

[005a] In one aspect, the present disclosure provides a method of making a stable viral vector producer cell line, said method comprising:

a. introducing into a population of cells a viral vector genome construct encoding a gene of interest (GOI) and one or more viral accessory constructs encoding one or more viral accessory proteins;

b. producing a population of transgenic cells comprising integrated or episomal sequences encoding said GOI and said one or more viral accessory proteins;

c. selecting from said population of transgenic cells a cell clone producing a desired viral titer; and

d. generating from said cell clone a stable viral vector producer cell line,

wherein the introduction of said one or more accessory constructs occurs via one or more sequential steps with no intervening cell culturing.

BRIEF DESCRIPTION OF THE DRAWINGS

[006] **Figure 1** provides an illustration of the genome organization of the HIV-1 Virus. The HIV-1 genome contains 9,749 bp. In addition to the *gag*, *pol*, and *env* genes common to all retroviruses, HIV-1 contains a regulatory gene - *rev* - that is indispensable for virus replication, and five accessory genes - *vif*, *vpr*, *vpu*, *tat*, and *nef* - that, while dispensable for *in vitro* virus growth, are key for *in vivo* replication and pathogenesis. Further information about the biological functions of each of the HIV-encoded proteins is provided in Table 1.

[007] **Figure 2** provides an exemplary work flow of generating a cell clone with stable introduction of various construct elements.

[008] **Figure 3** provides exemplary vector constructs used in Example 2.

[009] **Figure 4** provides results of the Example 2 experiments showing optimal conditions for GFP (combinations 16 and 4) and optimal conditions for Globin-LCR-GFP (combinations 12 and 6).