

SUMMARY

[005] In an example, the present disclosure provides a viral vector construct comprising a barcoded concatemer molecule. In one aspect, the present disclosure provides a viral vector construct, wherein the construct comprises a barcoded concatemer molecule comprising one or more copies of an expression cassette encoding a gene of interest (GOI).

[006] In another aspect, the present disclosure provides a cell line comprising a viral vector construct comprising a barcoded concatemer molecule.

[007] In another example, the present disclosure provides a method of making a viral vector construct comprising a barcoded concatemer molecule, wherein the method comprises ligating a unique molecular identifier to a viral vector genome construct to generate a barcoded viral vector genome construct, and ligating a barcoded viral vector genome construct to generate a barcoded concatemer molecule. In one aspect, the present disclosure provides a method of making a viral vector construct comprising a barcoded concatemer molecule, the method comprising: a. ligating a unique molecular identifier to a viral vector genome construct to generate a barcoded viral vector genome construct; and b. ligating a concatemer to the barcoded viral vector genome construct to generate the barcoded concatemer molecule.

[008] In another example, the present disclosure provides a method of making a stable viral vector producer cell line comprising a barcoded concatemer, wherein the method comprises introducing into a population of cells a viral vector genome construct, wherein the viral vector genome construct comprises a barcoded concatemer, comprising an expression cassette encoding one or more copies of a gene of interest (GOI) and one or more unique molecular identifiers, producing a population of transgenic cells comprising integrated sequences encoding a GOI and one or more unique molecular identifiers, selecting from a population of transgenic cells a cell clone producing a desired viral titer, and generating from a selected cell clone a stable viral vector producer cell line. In one aspect, the present disclosure provides a method of making a stable viral vector producer cell line comprising a barcoded concatemer, the method comprising: a) introducing into a population of cells a viral vector genome construct, wherein the viral vector genome construct comprises a barcoded concatemer, comprising an expression cassette encoding one or more copies of a gene of interest (GOI) and one or more unique molecular identifiers; b) producing a population of transgenic cells comprising integrated sequences encoding the GOI and the unique molecular identifiers; 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.

[009] In a further example, the present disclosure provides a method of characterizing a viral vector comprising a barcoded concatemer molecule, wherein the viral vector is integrated, and wherein the barcoded concatemer comprises one or more unique molecular identifiers, and wherein the method comprises sequencing the barcoded concatemer molecule to generate sequence reads, assembling the generated sequence reads, and mapping the assembled sequence reads. In one aspect, the disclosure provides a method of characterizing a viral vector comprising a barcoded concatemer molecule, wherein the viral vector is integrated, and wherein the barcoded concatemer comprises one or more unique molecular identifiers, the method comprising: a. sequencing the barcoded concatemer molecule to generate sequence reads; b. assembling the generated sequence reads; and c. mapping the assembled sequence reads.

BRIEF DESCRIPTION OF THE DRAWINGS

[010] **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 - *tat*, *vif*, *vpr*, *vpu* and *nef* - that, while dispensable for