

**[0004]** In the most common approach, the above four plasmids are transiently transfected into cells to produce lentiviral vectors, which is labor-intensive and costly. In addition, transient transfection has a limitation in scalability as it requires large amounts of plasmid DNAs, which also raises a concern for safety.

**[0005]** What are needed to overcome difficulties associated with transient transfection, are methods for preparing a producer cell line (PCL), in which all or most of the genes or sequences required for lentiviral vector production are stably integrated into the chromosome of a mammalian cell allowing for the production of lentiviral vectors by a simple induction method. The present invention fulfills these needs.

## **SUMMARY**

**[0006]** In some embodiments, provided herein is a method of producing a lentiviral packaging vector-containing mammalian cell, comprising: transfecting a mammalian cell with: a packaging vector including an expression cassette, encoding: a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter; a lentiviral envelope gene under control of a second promoter; and a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter, wherein the expression cassette is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR); and culturing the transfected mammalian cell; and isolating the lentiviral packaging vector-containing mammalian cell. In one aspect, the present disclosure refers to a method of producing a lentiviral packaging vector-containing mammalian cell, comprising:

- a. transfecting a mammalian cell with:
  - i. a packaging vector including an expression cassette, encoding:
    - 1. a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter;
    - 2. a lentiviral envelope gene under control of a second promoter; and
    - 3. a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter,
 wherein the expression cassette is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR); and
  - b. culturing the transfected mammalian cell; and
  - c. isolating the lentiviral packaging vector-containing mammalian cell.

**[0007]** Also provided herein is a method of producing a lentiviral vector-producing mammalian cell, comprising: transfecting a mammalian cell with: a packaging vector including an expression cassette, encoding: a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter; a lentiviral envelope gene under control of a second promoter; and a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter, wherein the expression cassette is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR); and a transfer vector, comprising: a nucleic acid sequence encoding a gene of interest under control of a fourth promoter, wherein the nucleic acid sequence is flanked on both the 5' and 3' ends by transposon-specific inverted terminal

repeats (ITR); culturing the transfected mammalian cell; and isolating the lentiviral vector-producing mammalian cell. In one aspect, the present disclosure refers to a method of producing a lentiviral packaging vector-containing mammalian cell, comprising:

- a. transfecting a mammalian cell with:
  - i. a packaging vector including an expression cassette, encoding:
    1. a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter;
    2. a lentiviral envelope gene under control of a second promoter; and
    3. a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter,
 wherein the expression cassette is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR); and
  - ii. a transfer vector, comprising:
    1. a nucleic acid sequence encoding a gene of interest under control of a fourth promoter,
 wherein the nucleic acid sequence is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR);
- b. culturing the transfected mammalian cell; and
- c. isolating the lentiviral vector-producing mammalian cell.

**[0008]** In further embodiments, provided herein is a method of producing a lentiviral vector, comprising: producing a lentiviral packaging vector-containing mammalian cell according to methods described herein; transfecting the mammalian cell with a transfer vector, comprising: a nucleic acid sequence encoding a gene of interest under control of a fourth promoter; inducing production of the expression cassette and the nucleic acid; culturing the transfected mammalian cell; and harvesting the lentiviral vector.

**[0009]** Also provided herein is a method of producing a lentiviral vector, comprising: producing a lentiviral vector-producing mammalian cell according to the methods described herein, inducing production of the expression cassette and the nucleic acid; culturing the mammalian cell; and harvesting the lentiviral vector.

**[0010]** In additional embodiments, provided herein is a method of treatment with a lentiviral vector, comprising: administering the lentiviral vector produced according to the methods described herein to a mammalian subject.

**[0011]** In further embodiments, provided herein is a mammalian cell for producing a lentiviral vector, comprising: a nucleic acid molecule chromosomally integrated into the mammalian cell, the nucleic acid molecule comprising a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter; a lentiviral envelope gene under control of a second promoter; and a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter, wherein the nucleic acid sequence is flanked on both the 5' and 3' ends by sequences resulting from the recombination of transposon-specific inverted terminal repeats (ITRs). In one aspect, the present disclosure refers to a mammalian cell for producing a lentiviral vector, comprising:

- a. a nucleic acid molecule chromosomally integrated into the mammalian cell, the nucleic acid molecule comprising
  - i. a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter;

- ii. a lentiviral envelope gene under control of a second promoter; and
- iii. a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter,

wherein the nucleic acid sequence is flanked on both the 5' and 3' ends by sequences resulting from the recombination of transposon-specific inverted terminal repeats (ITRs).

**[0012]** Also provided herein is a method of producing a lentiviral vector, comprising: transfecting the mammalian cells described herein with a transfer vector, comprising: an nucleic acid sequence encoding a gene of interest under control of a fourth promoter; inducing production of the expression cassette and the nucleic acid; culturing the transfected mammalian cell; and harvesting the lentiviral vector. In one aspect, the present disclosure refers to a method of producing a lentiviral vector, comprising:

- a. transfecting the mammalian cell described herein with a transfer vector, comprising:
  - i. a nucleic acid sequence encoding a gene of interest under control of a fourth promoter;
- b. inducing production of the expression cassette and the nucleic acid;
- c. culturing the transfected mammalian cell; and
- d. harvesting the lentiviral vector.

**[0013]** In further embodiments, provided herein is a method of producing a lentiviral vector, comprising: inducing production of the chromosomally integrated nucleic acid sequence and the chromosomally integrated nucleic acid sequence encoding a gene of interest of the mammalian cells described herein; culturing the mammalian cell; and harvesting the lentiviral vector. In one aspect, the present disclosure refers to a method of producing a lentiviral vector, comprising:

- a. inducing production of the chromosomally integrated nucleic acid sequence and the chromosomally integrated nucleic acid sequence encoding a gene of interest of the mammalian described herein;
- b. culturing the mammalian cell; and
- c. harvesting the lentiviral vector.

## **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0014]** FIG. 1 shows a packaging plasmid in accordance with embodiments hereof.

**[0015]** FIG. 2 shows a transfer vector in accordance with embodiments hereof.

**[0016]** FIGS. 3A-3D show HEK293T cells transfected with packaging and transfer vectors, in accordance with embodiments hereof.

**[0017]** FIG. 4 shows the production of lentiviral vectors using exemplary cell lines described herein.

**[0018]** FIG. 5 shows lentivirus titer for PEI and lipofectamine transfection with and without transposase.

**[0019]** FIG. 6 shows lentivirus productivity and stability of a single cell clone DH4.

**[0020]** FIG. 7 shows lentivirus productivity and stability of a single cell clone ED8.

**[0021]** FIG. 8 shows the results of a copy number analysis.

**[0022]** FIG. 9 shows potency comparison for lentivirus produced using transient transfection and producer cell lines.

**[0023]** FIG. 10 shows an exemplary manufacturing flow for lentivirus PCL without antibiotics during parts of a seed train culture.

## **DETAILED DESCRIPTION OF THE INVENTION**

**CLAIMS**

What is claimed is:

1. A method of producing a lentiviral packaging vector-containing mammalian cell, comprising:
  - a. transfecting a mammalian cell with:
    - i. a packaging vector including an expression cassette, encoding:
      1. a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter;
      2. a lentiviral envelope gene under control of a second promoter; and
      3. a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter,wherein the expression cassette is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR); and
  - b. culturing the transfected mammalian cell; and
  - c. isolating the lentiviral packaging vector-containing mammalian cell.
2. The method of claim 1, wherein the mammalian cell is a mammalian cell culture.
3. The method of claim 2, wherein the mammalian cell culture is a suspension culture.
4. The method of claim 3, wherein the mammalian cell is an HEK293T cell.

5. The method of any one of claims 1-4, wherein the GAG gene is an HIV GAG gene and the POL gene is an HIV POL gene.
6. The method of any one of claims 1-5, wherein the lentiviral envelope gene is a Vesicular Stomatitis Virus Glycoprotein (VSV-G) gene.
7. The method of any one of claim 1-6, wherein the first, second and third promoters are derepressible promoters.
8. The method of claim 7, wherein the expression cassette further encodes a repressor element of the first, second and third derepressible promoters
9. The method of claim 8, wherein each of the derepressible promoters comprises a functional promoter and a tetracycline operator sequence (TetO), and the repressor element is a tetracycline repressor protein.
10. The method of claim 9, wherein the expression cassette further comprises a Kruppel-associated box sequence following a sequence encoding the tetracycline repressor protein.
11. The method of any one of claims 1-10, wherein the transposon-specific ITRs are *Lepidopteran* transposon (PIGGYBAC®) ITRs.
12. The method of any one of claims 1-11, wherein the transfecting is in the presence of a transposase that recognizes the transposon-specific ITRs.
13. The method of claim 12, wherein the transposase is *Lepidopteran* (PIGGYBAC®) transposase mRNA or *Lepidopteran* (PIGGYBAC®) transposase cDNA.

14. A method of producing a lentiviral vector-producing mammalian cell, comprising:
  - a. transfecting a mammalian cell with:
    - i. a packaging vector including an expression cassette, encoding:
      1. a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter;
      2. a lentiviral envelope gene under control of a second promoter; and
      3. a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter,wherein the expression cassette is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR); and
    - ii. a transfer vector, comprising:
      1. a nucleic acid sequence encoding a gene of interest under control of a fourth promoter,wherein the nucleic acid sequence is flanked on both the 5' and 3' ends by transposon-specific inverted terminal repeats (ITR);
  - b. culturing the transfected mammalian cell; and
  - c. isolating the lentiviral vector-producing mammalian cell.
15. A mammalian cell for producing a lentiviral vector, comprising:

- a. a nucleic acid molecule chromosomally integrated into the mammalian cell, the nucleic acid molecule comprising
    - i. a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter;
    - ii. a lentiviral envelope gene under control of a second promoter; and
    - iii. a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a third promoter,
 wherein the nucleic acid sequence is flanked on both the 5' and 3' ends by sequences resulting from the recombination of transposon-specific inverted terminal repeats (ITRs).
16. The mammalian cell of claim 15, further comprising a chromosomally integrated nucleic acid sequence encoding a gene of interest under control of a fourth promoter.
17. A method of producing a lentiviral vector, comprising:
  - a. transfecting the mammalian cell of claim 15 with a transfer vector, comprising:
    - i. a nucleic acid sequence encoding a gene of interest under control of a fourth promoter;
  - b. inducing production of the expression cassette and the nucleic acid;
  - c. culturing the transfected mammalian cell; and
  - d. harvesting the lentiviral vector.
18. A method of producing a lentiviral vector, comprising:
  - a. inducing production of the chromosomally integrated nucleic acid sequence and the chromosomally integrated nucleic acid sequence encoding a gene of interest of the mammalian of claim 16;

- b. culturing the mammalian cell; and
- c. harvesting the lentiviral vector.