[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 mamalian cell allowing for the production of lentiviral vectors by a simple induction method. The present invention fulfills these needs.

SUMMARY ~~OF THE INVENTION~~

[0006] In some embodiments, provided herein is 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®) transpose 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. The method of claim 14, wherein the mammalian cell is a mammalian cell culture.~~

16. The method of claim 15, wherein the mammalian cell culture is a suspension culture.

17. The method of claim 16, wherein the mammalian cell is an HEK293T cell.

18. The method of any one of claims 14-17, wherein the GAG gene is an HIV GAG gene and the POL gene is an HIV POL gene.

19. The method of any one of claims 14-18, wherein the lentiviral envelope gene is a Vesicular Stomatitis Virus Glycoprotein (VSV-G) gene.

20. The method of any one of claims 14-19, wherein the first, second and third promoters are derepressible promoters.

21. The method of claim 20, wherein the expression cassette further encodes a repressor element of the first, second and third derepressible promoters

22. The method of claim 21, 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.

23. The method of claim 22, wherein the expression cassette further comprises a Kruppel-associated box sequence following a sequence encoding the tetracycline repressor protein.

24. The method of any one of claims 14-23, wherein the transposon-specific ITRs are *Lepidopteran* transposon (PIGGYBAC®) ITRs.

25. The method of any one of claims 14-24, wherein the transfecting is in the presence of a transposase that recognizes the transposon-specific ITRs.

26. The method of claim 25, wherein the transposase is *Lepidopteran* (PIGGYBAC®) transposase mRNA or *Lepidopteran* (PIGGYBAC®) transpose cDNA.

27. The method of any one of claims 14-26, wherein the gene of interest is a gene of therapeutic interest.

28. A method of producing a lentiviral vector, comprising:

a. producing a lentiviral packaging vector-containing mammalian cell according to claim 1;

b. transfecting the mammalian cell with a transfer vector, comprising:

i. a nucleic acid sequence encoding a gene of interest under control of a fourth promoter;

c. inducing production of the expression cassette and the nucleic acid;

d. culturing the transfected mammalian cell; and

e. harvesting the lentiviral vector.

29. A method of producing a lentiviral vector, comprising:

a. producing a lentiviral vector-producing mammalian cell according to claim 14;

b. inducing production of the expression cassette and the nucleic acid;

c. culturing the mammalian cell; and

d. harvesting the lentiviral vector.

~~30. The method of claim 28 or claim 29, wherein each of the promoters in the expression cassette is a derepressible promoter comprising a functional promoter and a tetracycline operator sequence (TetO), and the repressor element is a tetracycline repressor protein, and the inducing includes adding doxycycline to the mammalian cell.~~

~~31. The method of claim 28 or claim 29, wherein an amount of the lentiviral vector produced is at least about 10^6 transduction units/mL two days after the inducing.~~

~~32. A method of treatment with a lentiviral vector, comprising:~~

~~a. administering the lentiviral vector produced according to claim 28 or claim 29 to a mammalian subject.~~

~~33. The method of claim 32, wherein the administering comprises inhalation, injection or intravenous administration.~~

~~34.~~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).

~~35.~~16. The mammalian cell of claim ~~34~~15, further comprising a chromosomally integrated nucleic acid sequence encoding a gene of interest under control of a fourth promoter.

~~36. The mammalian cell of claim 34 or claim 35, wherein the mammalian cell is a mammalian cell culture.~~

~~37. The mammalian cell of claim 36, wherein the mammalian cell culture is a suspension culture.~~

~~38. The mammalian cell of claim 37, wherein the mammalian cell is an HEK293T cell.~~

~~39. The mammalian cell of any one of claims 34-38, wherein the GAG gene is an HIV GAG gene and the POL gene is an HIV POL gene.~~

~~40. The mammalian cell of any one of claims 34-39, wherein the lentiviral envelope gene is a Vesicular Stomatitis Virus Glycoprotein (VSV-G) gene.~~

~~41. The mammalian cell of any one of claims 34-40, wherein the first, second and third promoters are derepressible promoters.~~

~~42. The mammalian cell claim 41, wherein the expression cassette further encodes a repressor element of the first, second and third derepressible promoters~~

~~43. The mammalian cell of claim 42, 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.~~

~~44. The mammalian cell of claim 43, wherein the expression cassette further comprises a Kruppel-associated box sequence following a sequence encoding the tetracycline repressor protein.~~

~~45. The mammalian cell of any one of claims 34-44, wherein the transposon-specific ITRs are *Lepidopteran* transposon (PIGGYBAC®) ITRs.~~

~~46. The mammalian cell of claim 35, wherein the gene of interest is a gene of therapeutic interest.~~

~~47.~~17. A method of producing a lentiviral vector, comprising:

a. transfecting the mammalian cell of claim ~~34~~ 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.

~~48.~~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 ~~35~~16;

b. culturing the mammalian cell; and

c. harvesting the lentiviral vector.

~~49. The method of claim 47 or claim 48, wherein each of the promoters in the expression cassette is a derepressible promoter comprising a functional promoter and a tetracycline~~

~~operator sequence (TetO), and the repressor element is a tetracycline repressor protein, and the inducing includes adding doxycycline to the mammalian cell.~~

~~50. The method of claim 47 or claim 48, wherein an amount of the lentiviral vector produced is at least about 10^6 transduction units/mL two days after the inducing.~~

~~51. A method of treatment with a lentiviral vector, comprising:~~

~~a. administering the lentiviral vector produced according to claim 47 or claim 48 to a mammalian subject.~~

~~52. The method of claim 51, wherein the administering comprises inhalation, injection or intravenous administration.~~

~~53. The method of any one of claims 28-31 and 47-50, wherein at least a portion of the culturing the mammalian cell occurs in the absence of an antibiotic.~~

~~54. The method of claim 53, wherein the portion of the culturing is from passage 3 up to an induction phase.~~