

[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 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.

[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 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.

[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).

[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.

[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.

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 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.
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. The method of claim 1, wherein the gene of interest is a gene of therapeutic interest.