[0004] What are needed to overcome difficulties associated with transient transfection of four plasmids, are methods for producing scalable amounts of lentiviral vector using transfection of fewer than four plasmids including a plasmid encoding a gene of interest. The present invention fulfills these needs.

SUMMARY ~~OF THE INVENTION~~

[0005] In some ~~embodiments~~examples, provided herein is a method of producing a lentiviral vector, comprising: transfecting a mammalian cell with a first nucleic acid encoding a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter and an envelope glycoprotein gene under control of a second promoter, a second nucleic acid encoding a gene of interest under control of a third promoter, and a third nucleic acid encoding a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene under control of a fourth promoter; culturing the transfected mammalian cell; and isolating the lentiviral vector. In one aspect, the present disclosure refers to a method of producing a lentiviral vector, comprising:

a. transfecting a mammalian cell with:
   i. a first nucleic acid encoding a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter and an envelope glycoprotein gene under control of a second promoter;
   ii. a second nucleic acid encoding a gene of interest under control of a third promoter; and
   iii. a third nucleic acid encoding a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a fourth promoter,
b. culturing the transfected mammalian cell; and
c. isolating the lentiviral vector.

[0005a] In another aspect, the present disclosure refers to a lentiviral vector produced by the method disclosed herein.

[0005b] In another aspect, the present disclosure refers to use of a lentiviral vector disclosed herein in the manufacture of a medicament for treating inflammatory diseases, autoimmune, chronic and infectious diseases, including such disorders as AIDS, cancer, neurological diseases, cardiovascular disease, hypercholestemia; various blood disorders including various anemias, thalassemias and hemophilia; genetic defects such as cystic fibrosis, Gaucher's Disease, adenosine deaminase (ADA) deficiency, emphysema, wherein the lentiviral vector ~~of claim 11 to~~is to be administered to a mammalian subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIGS. 1A-1B are schematic representations of plasmids for lentivirus production in accordance with embodiments hereof.

[0007] FIG. 2 shows the lentiviral titer achieved using the methods described herein (3-plasmid system) using GFP as the gene of interest.

[0008] FIG. 3 shows the lentiviral titer achieved using the methods described herein (3-plasmid system) compared to commercially available products (4 plasmid system) using CD-19 CAR-T as the gene of interest.

DETAILED DESCRIPTION OF THE INVENTION

[0009] The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0010] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the method/device being employed to determine the value.

CLAIMS

What is claimed is:

1. A method of producing a lentiviral vector, comprising:
    a. transfecting a mammalian cell with:
        i. a first nucleic acid encoding a lentiviral regulator of expression of virion proteins (REV) gene under control of a first promoter and an envelope glycoprotein gene under control of a second promoter;
        ii. a second nucleic acid encoding a gene of interest under control of a third promoter; and
        iii. a third nucleic acid encoding a lentiviral group specific antigen (GAG) gene and a lentiviral polymerase (POL) gene both under control of a fourth promoter,
    b. culturing the transfected mammalian cell; and
    c. isolating the lentiviral vector.
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 1, wherein the mammalian cell is an HEK293T cell.
5. The method of any one of claims 1-4, wherein the envelope glycoprotein gene is a Vesicular Somatitis Virus Glycoprotein (VSV-G) gene.
6. The method of any one of claims 1-5, wherein the GAG gene is an HIV GAG gene and the POL gene is an HIV POL gene.
7. The method of any one of claims 5-6, wherein the nucleic acids encoding VSV-G and REV are codon-optimized.

8. The method of any one of claims 1-7, wherein the VSV-G gene expression is driven by a Rous sarcoma virus (RSV) promoter.

9. The method of any one of claims 1-8, wherein the REV gene expression is driven by a cytomegalovirus (CMV) promoter.

10. The method of any one of claims 1-9, wherein the gene of interest is a gene of therapeutic interest.

11. A lentiviral vector produced by the method of any one of claims 1-10.

12. ~~A method of treatment with~~Use of a lentiviral vector of claim 11 in the manufacture of a medicament for treating inflammatory diseases, autoimmune, chronic and infectious diseases, including such disorders as AIDS, cancer, neurological diseases, cardiovascular disease, hypercholestemia; various blood disorders including various anemias, thalassemias and hemophilia; genetic defects such as cystic fibrosis, Gaucher's Disease, adenosine deaminase (ADA) deficiency, emphysema, ~~comprising:~~

~~a.~~ ~~administering~~ wherein the lentiviral vector ~~of claim 11 to~~is to be administered to a mammalian subject.