SUMMARY

[0005] In some examples, provided herein is a method for automated production of an adeno-associated virus (AAV) viral vector, comprising: introducing an engineered mammalian AAV viral producer cell into a fully enclosed cell engineering system, the engineering mammalian AAV viral producer cell including integrated into its genome: an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter; an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; a viral-associated, non-coding RNA under control of a third derepressible promoter; and a repressor element of the first, second and third derepressible promoters; transducing the mammalian AAV viral producer cell with a vector encoding a gene of interest to produce a transduced viral producer cell; treating the mammalian AAV viral producer cell with a binding partner of the repressor element; activating the first, second and third derepressible promoters; expanding the transduced viral producer cell and producing the AAV viral vector within the transduced viral producer cell; and isolating the viral vector, wherein the steps are performed in a closed and automated process.

[0006] Also provided herein is a method for automated production of an adeno-associated virus (AAV) viral vector, comprising: transducing a mammalian cell stably expressing one or more nucleic acids encoding TetR and/or TetR-KRAB with: a first nucleic acid encoding an adenovirus helper gene comprising an E2A gene, a E4Orf gene and a viral-associated non-coding RNA, under control of a first derepressible promoter; a second nucleic acid encoding an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; and optionally, a third nucleic acid encoding a gene of interest under control of a third derepressible promoter; treating the mammalian cell with a binding partner of the TetR; activating the first, second and third derepressible promoters; expanding the transduced cell and producing the AAV viral vector within the transduced cell; and isolating the viral vector, wherein the steps are performed in a closed and automated process.

[0007] ~~In one aspect, the present disclosure refers to~~In further embodiments, provided herein is a method for automated production of a lentiviral vector, comprising: ~~(a)~~ introducing an engineered mammalian lentiviral vector producer cell into a fully enclosed cell engineering system, the engineering mammalian lentiviral vector producer cell including integrated into its genome: ~~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); ~~(b)~~ transducing the mammalian lentiviral vector producer cell with a vector encoding a gene of interest to produce a transduced viral producer cell; ~~(c)~~ activating the first, second and third promoters; ~~(d)~~ expanding the transduced viral producer cell and producing the lentiviral vector within the transduced viral producer cell; and ~~(e)~~ isolating the viral vector, wherein (a) through (e) are performed in a closed and automated process.

[0008] In further examples, provided herein is a method for automated production of a lentiviral vector, comprising: introducing a mammalian cell into a fully enclosed cell engineering system; transducing 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 both under control of a fourth promoter, expanding the transduced cell and producing the lentiviral vector within the transduced cell; and isolating the viral vector, wherein the steps are performed in a closed and automated process.

[0008a] In one aspect, the present disclosure refers to a method for automated production of a lentiviral vector, comprising:

(a) introducing an engineered mammalian lentiviral vector producer cell into a fully enclosed cell engineering system, the engineering mammalian lentiviral vector producer cell including integrated into its genome a nucleic acid 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);
(b) transducing the mammalian lentiviral vector producer cell with a vector encoding a gene of interest to produce a transduced viral producer cell;
(c) activating the first, second and third promoters;

(d) expanding the transduced viral producer cell and producing the lentiviral vector within the transduced viral producer cell; and

(e) isolating the viral vector,

wherein (a) through (e) are performed in a closed and automated process, wherein the closed and automated process comprises:

monitoring with one or more of a temperature sensor, a pH sensor, a glucose sensor, a lactose sensor, an oxygen sensor, a carbon dioxide sensor, and an optical density sensor, and

automatically adjusting one or more of a temperature, a pH level, a glucose level, a lactose level, an oxygen level, a carbon dioxide level, and an optical density.

[0008b] In one aspect, the present disclosure refers to a method for automated production of a lentiviral vector comprising:

(a) introducing a mammalian cell into a fully enclosed cell engineering system;

(b) transducing the 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, and

(c) expanding the transduced cell and producing the engineered mammalian lentiviral vector producer cell within the transduced cell, and

(e) isolating the viral vector,

wherein (a) through (e) are performed in a closed and automated process, wherein the closed and automated process comprises:

monitoring with one or more of a temperature sensor, a pH sensor, a glucose sensor, a lactose sensor, an oxygen sensor, a carbon dioxide sensor, and an optical density sensor, and

automatically adjusting one or more of a temperature, a pH level, a glucose level, a lactose level, an oxygen level, a carbon dioxide level, and an optical density.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 shows a closed and automated system useful for producing viral vectors as described in embodiments herein.

[0010] FIG. 2 shows a lab space containing exemplary closed and automated systems useful for producing viral vectors as described in embodiments herein.

[0011] FIG. 3 shows flow diagram of a viral vector production process that can be performed in a closed and automated system as described in embodiments herein.

CLAIMS

What is claimed is:

1. A method for automated production of a lentiviral vector, comprising:

(a) introducing an engineered mammalian lentiviral vector producer cell into a fully enclosed cell engineering system, the engineering mammalian lentiviral vector producer cell including integrated into its genome a nucleic acid 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);

(b) transducing the mammalian lentiviral vector producer cell with a vector encoding a gene of interest to produce a transduced viral producer cell;

(c) activating the first, second and third promoters;

(d) expanding the transduced viral producer cell and producing the lentiviral vector within the transduced viral producer cell; and

(e) isolating the viral vector,

wherein (a) through (e) are performed in a closed and automated process, wherein the closed and automated process comprises:

monitoring with one or more of a temperature sensor, a pH sensor, a glucose sensor, a lactose sensor, an oxygen sensor, a carbon dioxide sensor, and an optical density sensor, and

automatically adjusting one or more of a temperature, a pH level, a glucose level, a lactose level, an oxygen level, a carbon dioxide level, and an optical density.

2. The method of claim 1, wherein the engineered mammalian lentiviral vector producer cell is a mammalian cell culture, and wherein the mammalian cell culture is preferably a suspension culture.
3. The method of claim 1, wherein the engineered mammalian lentiviral vector producer cell is a human cell, and wherein the human cell is preferably a human embryonic kidney (HEK) cell.
4. The method of claim 1, wherein the GAG gene is an HIV GAG gene and the POL gene is an HIV POL gene.
5. The method of claim 1, wherein the lentiviral envelope gene is a Vesicular Somatitis Virus Glycoprotein (VSV-G) gene.
6. The method of claim 1, wherein the first, second and third promoters are derepressible promoters.
7. The method of claim 6, wherein the engineered mammalian lentiviral vector producer cell further includes integrated into its genome a repressor element of the first, second and third derepressible promoters.
8. The method of claim 7, 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.

9. The method of claim 8, further comprising a Kruppel-associated box sequence following a sequence encoding the tetracycline repressor protein.

10. The method of claim 1, wherein the transposon-specific ITRs are Lepidopteran transposon (PIGGYBAC®) ITRs.

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

12. The method of claim 1, wherein an amount of lentiviral vectors produced is at least about 10^{10} viral vectors.

13. The method of claim 1, wherein the closed and automated process comprises:

    (a) monitoring with one or more of a temperature sensor, a pH sensor, a glucose sensor, a lactose sensor, an oxygen sensor, a carbon dioxide sensor, and an optical density sensor, and

    (b) automatically adjusting one or more of a temperature, a pH level, a glucose level, a lactose level, an oxygen level, a carbon dioxide level, and an optical density.

14. The method of claim 1, wherein the transducing comprises viral infection, electroporation, liposome transfection, or membrane disruption.

15. ~~The~~ A method for automated production of a lentiviral vector comprising~~of claim 1, wherein the method further comprises the steps prior to step (a) of~~:

    (a) introducing a mammalian cell into ~~the~~ a fully enclosed cell engineering system;

    (b) transducing the 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, and

(c) expanding the transduced cell and producing the engineered mammalian lentiviral vector producer cell within the transduced cell, and

(e) isolating the viral vector,

wherein (a) through (e) are performed in a closed and automated process, wherein the closed and automated process comprises:

monitoring with one or more of a temperature sensor, a pH sensor, a glucose sensor, a lactose sensor, an oxygen sensor, a carbon dioxide sensor, and an optical density sensor, and

automatically adjusting one or more of a temperature, a pH level, a glucose level, a lactose level, an oxygen level, a carbon dioxide level, and an optical density.

16. The method of claim 15, wherein the mammalian cell is a human cell, preferably wherein the human cell is a human embryonic kidney (HEK) cell.

17. The method of claim 15, wherein the GAG gene is an HIV GAG gene and the POL gene is an HIV POL gene.

18. The method of claim 15, wherein the envelope glycoprotein gene is a Vesicular Somatitis Virus Glycoprotein (VSV-G) gene, and wherein the VSV-G gene and the REV gene are codon optimized.

19. The method of claim 15, wherein the gene of interest is a gene of therapeutic interest.

20. The method of claim 15, wherein an amount of lentiviral vectors produced is at least about 10^{10} viral vectors.