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. 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. The mammalian cell of claim 34, 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. A method of producing a lentiviral vector, comprising:
    a. transfecting the mammalian cell of claim 34 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. 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;
    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.