et al., "Insect Cells as a Factory to Produce Adeno-Associated Virus Type 2 Vectors," *Human Gene Therapy 13*:1935-1943 (2002)). HeLa packaging cells with stably integrated Rep-Cap and GOI have also been developed. These systems, nevertheless, still require the wildtype Adenovirus as a helper virus, which poses risks of contamination of replicative adenovirus in AAV products (*see, e.g*., Robert *et al*., "Manufacturing of recombinant adeno-associated viruses using mammalian expression platforms," *Biotechnology Journal 12*:1600193 (1-16) (2017).

[0005] What are needed, are cell lines and related methods for production of AAV that are easily scalable to large volume production, to provide reproducible and stable results, while limiting contamination and reducing cost.

SUMMARY

[0006] In some examples, provided herein is a mammalian cell for producing an adeno-associated virus (AAV), comprising a nucleic acid molecule encoding a viral helper gene under control of a first derepressible promoter, a nucleic acid molecule encoding an AAV gene under control of a second derepressible promoter, and a nucleic acid molecule encoding a repressor element of the first and the second derepressible promoters.

[0006a] In one aspect, the present disclosure refers to a mammalian cell for producing an adeno-associated virus (AAV), comprising: a. a nucleic acid molecule encoding a viral helper gene under control of a first derepressible promoter; b. a nucleic acid molecule encoding an AAV gene under control of a second derepressible promoter; and c. a nucleic acid molecule encoding a repressor element of the first and the second derepressible promoters.

[0007] In additional examples, provided herein is a mammalian cell for producing an adeno-associated virus (AAV), comprising a nucleic acid molecule encoding 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 depressible promoter, two inverted terminal repeat (ITR) sequences, and a repressor element of the first, second and third derepressible promoters.

[0007a] In one aspect, the present disclosure refers to a mammalian cell for producing an adeno-associated virus (AAV), comprising: a. a nucleic acid molecule encoding: i. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter; ii. an AAV gene comprising *Rep* and *Cap* genes under control of a second derepressible promoter; iii. a viral-associated, non-coding RNA under control of a third depressible promoter; iv. two inverted terminal repeat (ITR) sequences; and v. a repressor element of the first, second and third derepressible promoters.

[0008] In further examples, provided herein is an isolated nucleic acid molecule encoding 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 depressible promoter, two inverted terminal repeat (ITR) sequences, and a repressor element of the first, second and third derepressible promoters.

[0008a] In one aspect, the present disclosure refers to an isolated nucleic acid molecule encoding: a. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter; b. an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; c. a viral-associated, non-coding RNA under control of a third depressible promoter; d. two inverted terminal repeat (ITR) sequences; and e. a repressor element of the first, second and third derepressible promoters.

[0009] In still further examples, provided herein is a method of producing an adeno-associated virus (AAV) in a mammalian cell comprising transfecting the mammalian cell with an isolated nucleic acid molecule encoding 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 depressible promoter, two inverted terminal repeat (ITR) sequences, and a repressor element of the first, second and third derepressible promoters, treating the mammalian cell with a binding partner of the repressor element, activating the first, second and third derepressible promoters, producing the AAV, and harvesting the AAV.

[0009a] In one aspect, the present disclosure refers to a method of producing an adeno-associated virus (AAV) in a mammalian cell comprising: a. transfecting the mammalian cell with an isolated nucleic acid molecule encoding: i. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter; ii. an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; iii. a viral-associated, non-coding RNA under control of a third depressible promoter; iv. two inverted terminal repeat (ITR) sequences; and v. a repressor element of the first, second and third derepressible promoters; b. treating the mammalian cell with a binding partner of the repressor element; c. activating the first, second and third derepressible promoters; d. producing the AAV; and e. harvesting the AAV.

[0010] In further examples, provided herein is a method of treatment with an adeno-associated virus (AAV) comprising: transfecting the mammalian cell with an isolated nucleic acid molecule encoding 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 depressible promoter, two inverted terminal repeat (ITR) sequences, and a repressor element of the first, second and third derepressible promoters, treating the mammalian cell with a binding partner of the repressor element, activating the first, second and third derepressible promoters, producing the AAV, harvesting the AAV and administering the AAV to a mammalian patient.

[0010a] In one aspect, the present disclosure refers to the use of an adeno-associated virus (AAV) in the manufacture of a medicament for treatment, wherein: a. a mammalian cell is to be transfected with an isolated nucleic acid molecule encoding: i. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter; ii. an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; iii. a viral-associated, non-coding RNA under control of a third depressible promoter; iv. two inverted terminal repeat (ITR) sequences; and v. a repressor element of the first, second and third derepressible promoters; b. the mammalian cell is to be treated with a binding partner of the repressor element; c. the first, second and third derepressible promoters are to be activated; d. the AAV is to be produced; e. the AAV is to be harvested; and f. the AAV is to be administered to a mammalian patient.

[0011] In still further examples, provided herein is a method of producing an adeno-associated virus (AAV) comprising: transfecting 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 and/or TetR-KRAB, activating the first, second, and third derepressible promoters, producing the AAV, and harvesting the AAV.

[0011a] In one aspect, the present disclosure refers to a method of producing an adeno-associated virus (AAV), comprising: a. transfecting a mammalian cell stably expressing one or more nucleic acids encoding TetR and/or TetR-KRAB with: i. 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; ii. a second nucleic acid encoding an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; and iii. optionally, a third nucleic acid encoding a gene of interest under control of a third derepressible promoter; b. treating the mammalian cell with a binding partner of the TetR; c. activating the first, second and third derepressible promoters; d. producing the AAV; and e. harvesting the AAV.

[0012] In still further examples, provided herein is a method for producing an adeno-associated virus (AAV) , comprising: stably transfecting a mammalian cell with a nucleic acid

encoding a TetR and/or TetR-KRAB repressor, chicken hypersensitive site-4 (cHS4) sequences flanking the TetR and/or TetR-KRAB repressor, and a selection gene, transfecting the stably transfected mammalian cell 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; producing the AAV; and harvesting the AAV.

[0012a] In one aspect, the present disclosure refers to a method for producing an adeno-associated virus (AAV), comprising: a. stably transfecting a mammalian cell with: a nucleic acid encoding a TetR and/or TetR-KRAB repressor; chicken hypersensitive site-4 (cHS4) sequences flanking the TetR and/or TetR-KRAB repressor; and a selection gene; b. transfecting the stably transfected mammalian cell with: i. 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; ii. a second nucleic acid encoding an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; and iii. optionally, a third nucleic acid encoding a gene of interest under control of a third derepressible promoter; c. treating the stably transfected mammalian cell with a binding partner of the TetR; d. activating the first, second and third derepressible promoters; e. producing the AAV; and f. harvesting the AAV.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 shows a schematic of the use of derepressible promoters to control the expression of helper and VA genes in accordance with embodiments hereof.

[0014] FIGS. 2A and 2B show exemplary nucleic acid molecules for production of helper and VA genes in accordance with embodiments hereof.

[0015] FIG. 3 shows the results of induction of helper and AAV genes in accordance with embodiments hereof.

[0016] FIGS. 4A-4C shows schematics of derepressible constructs for expression of AAV genes in accordance with embodiments hereof.

[0017] FIG. 5 shows exemplary derepressible p5 promoters in accordance with embodiments hereof.

[0018] FIG. 6 shows exemplary derepressible p19 promoters in accordance with embodiments hereof.

[0019] FIG. 7A-7B shows exemplary derepressible p19 promoters, including an artificial intron, in accordance with embodiments hereof.

[0020] FIGS. 8A-8B show the results of Rep-Cap expression and AAV titers using the Rep-Cap vectors, in accordance with embodiments hereof.

CLAIMS

What is claimed is:

1. A mammalian cell for producing an adeno-associated virus (AAV), comprising:

    a. a nucleic acid molecule encoding a viral helper gene under control of a first derepressible promoter;

    b. a nucleic acid molecule encoding an AAV gene under control of a second derepressible promoter; and

    c. a nucleic acid molecule encoding a repressor element of the first and the second derepressible promoters.

2. The mammalian cell of claim 1, wherein the mammalian cell is a mammalian cell culture.

3. The mammalian cell of claim 2, wherein the mammalian cell culture is a suspension culture.

4. The mammalian cell of any one of claims 1-3, wherein the viral helper gene is an adenovirus helper gene.

5. The mammalian cell of claim 4, wherein the adenovirus helper gene comprises E2A and E4Orf6 genes.

6. The mammalian cell of claim 5, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

7. The mammalian cell of any one of claims 1-6, wherein the AAV gene comprises *Rep* and *Cap* genes.

8. The mammalian cell of claim 7, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a third derepressible promoter.

9. The mammalian cell of claim 7, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a third derepressible promoter contained within an artificial intron.

10. The mammalian cell of claim 8 or claim 9, wherein the *Cap* gene is under control of a native promoter.

11. The mammalian cell of any one of claims 1-10, wherein each of the derepressible promoters comprise a functional promoter and two tetracycline operator sequences ($TetO_2$).

12. The mammalian cell of claim 11, wherein the functional promoter of the first derepressible promoter is a cytomegalovirus (CMV) promoter.

13. The mammalian cell of any one of claims 1-12, wherein the repressor element is under control of a constitutive promoter.

14. The mammalian cell of any one of claims 1-13, wherein the repressor element is a tetracycline repressor protein.

15. The mammalian cell of claim 14, further comprising a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

16. The mammalian cell of any one of claims 1-15, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

17. The mammalian cell of any one of claims 1-15, wherein the mammalian cell is a human cell.

18. The mammalian cell of claim 17, wherein the human cell is a human embryonic kidney (HEK) cell.

19. The mammalian cell of any one of claims 1-18, further comprising a nucleic acid molecule including two inverted terminal repeat (ITR) sequences.

20. The mammalian cell of any one of claims 1-19, further comprising a nucleic acid molecule encoding a gene of interest.

21. The mammalian cell of any one of claims 1-20, further comprising a nucleic acid encoding a viral-associated, non-coding RNA under control of a fourth derepressible promoter.

22. A mammalian cell for producing an adeno-associated virus (AAV), comprising:

a. a nucleic acid molecule encoding:

i. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter;

ii. an AAV gene comprising *Rep* and *Cap* genes under control of a second derepressible promoter;

iii. a viral-associated, non-coding RNA under control of a third depressible promoter;

iv. two inverted terminal repeat (ITR) sequences; and

v. a repressor element of the first, second and third derepressible promoters.

23. The mammalian cell of claim 22, wherein the mammalian cell is a mammalian cell culture.

24. The mammalian cell of claim 23, wherein the mammalian cell culture is a suspension culture.

25. The mammalian cell of any one of claims 22-24, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

26. The mammalian cell of any one of claims 22-25, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter.

27. The mammalian cell of any one of claims 22-25, wherein a Rep78 gene is under control of the second derepressible promoter and a Rep52 gene is under control of a fourth derepressible promoter contained within an artificial intron.

28. The mammalian cell of claim 26 or claim 27, wherein the *Cap* gene is under control of a native promoter.

29. The mammalian cell of any one of claims 22-28, wherein each of the derepressible promoters comprise a functional promoter and two tetracycline operator sequences ($TetO_2$).

30. The mammalian cell of claim 29, wherein the functional promoter of the first derepressible promoter is a cytomegalovirus (CMV) promoter.

31. The mammalian cell of any one of claims 22-30, wherein the repressor element is under control of a constitutive promoter.

32. The mammalian cell of any one of claims 22-31, wherein the repressor element is a tetracycline repressor protein.

33. The mammalian cell of claim 25, further comprising a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

34. The mammalian cell of any one of claims 22-33, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

35. The mammalian cell of any one of claims 22-33, wherein the mammalian cell is a human cell.

36. The mammalian cell of claim 35, wherein the human cell is a human embryonic kidney (HEK) cell.

37. The mammalian cell of any one of claims 22-36, further comprising a nucleic acid molecule encoding a gene of interest.

38. An isolated nucleic acid molecule encoding:

    a. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter;

    b. an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter;

    c. a viral-associated, non-coding RNA under control of a third depressible promoter;

    d. two inverted terminal repeat (ITR) sequences; and

    e. a repressor element of the first, second and third derepressible promoters.

39. The isolated nucleic acid of claim 38, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

40. The isolated nucleic acid of any one of claims 38-39, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter.

41. The isolated nucleic acid of any one of claims 38-39 wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter contained within an artificial intron.

42. The isolated nucleic acid of claim 40 or claim 41, wherein the Cap gene is under control of a native promoter.

43. The isolated nucleic acid of any one of claims 38-42, wherein each of the derepressible promoters comprise a functional promoter and two tetracycline operator sequences (TetO2).

44. The isolated nucleic acid of claim 43, wherein the functional promoter of the first derepressible promoter is a cytomegalovirus (CMV) promoter.

45. The isolated nucleic acid of any one of claims 38-44, wherein the repressor element is under control of a constitutive promoter.

46. The isolated nucleic acid of any one of claims 38-45, wherein the repressor element is a tetracycline repressor protein.

47. The isolated nucleic acid of claim 46, further comprising a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

48. The isolated nucleic acid of any one of claims 38-47, further comprising a gene of interest.

49. A method of producing an adeno-associated virus (AAV) in a mammalian cell comprising:

a. transfecting the mammalian cell with an isolated nucleic acid molecule encoding:

i. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter;

ii. an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter;

iii. a viral-associated, non-coding RNA under control of a third depressible promoter;

iv. two inverted terminal repeat (ITR) sequences; and

v. a repressor element of the first, second and third derepressible promoters;

b. treating the mammalian cell with a binding partner of the repressor element;

c. activating the first, second and third derepressible promoters;

d. producing the AAV; and

e. harvesting the AAV.

50. The method of claim 49, wherein the mammalian cell is a mammalian cell culture.

51. The method of claim 50, wherein the mammalian cell culture is a suspension culture.

52. The method of any one of claims 49-51, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

53. The method of any one of claims 49-52, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter.

54. The method of any one of claims 49-52, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter contained within an artificial intron.

55. The method of claim 54, wherein the fourth derepressible promoter is removed following the activating the derepressible promoters and prior to the producing the AAV.

56. The method of claim 53 or claim 54, wherein the *Cap* gene is under control of a native promoter.

57. The method of any one of claims 49-56, wherein each of the derepressible promoters comprise a functional promoter and two tetracycline operator sequences ($TetO_2$).

58. The method of claim 57, wherein the functional promoter of the first derepressible promoter is a cytomegalovirus (CMV) promoter.

59. The method of any one of claims 49-58, wherein the repressor element is under control of a constitutive promoter.

60. The method of any one of claims 49-59, wherein the repressor element is a tetracycline repressor protein.

61. The method of claim 60, wherein the nucleic acid further comprises a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

62. The method of claim 60 or 61, wherein the treating comprises treating with doxycycline.

63. The method of any one of claims 49-62, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

64. The method of any one of claims 49-63, wherein the mammalian cell is a human cell.

65. The method of claim 64, wherein the human cell is a human embryonic kidney (HEK) cell.

66. The method of any one of claims 49-65, wherein the AAV comprises a nucleic acid molecule encoding a gene of interest.

67. The method of claim 66, wherein the AAV comprises a gene of therapeutic interest.

68. Use of an adeno-associated virus (AAV) in the manufacture of a medicament for treatment, wherein:

a. a mammalian cell is to be transfected with an isolated nucleic acid molecule encoding:

i. an adenovirus helper gene comprising E2A and E4Orf6 genes under control of a first derepressible promoter;

ii. an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter;

iii. a viral-associated, non-coding RNA under control of a third depressible promoter;

iv. two inverted terminal repeat (ITR) sequences; and

v. a repressor element of the first, second and third derepressible promoters;

b. the mammalian cell is to be treated with a binding partner of the repressor element;

c. the first, second and third derepressible promoters are to be activated;

d. the AAV is to be produced;

e. the AAV is to be harvested; and

f. the AAV is to be administered to a mammalian patient.

69. The use of claim 68, wherein the mammalian cell is a mammalian cell culture.

70. The use of claim 69, wherein the mammalian cell culture is a suspension culture.

71. The use of any one of claims 68-70, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

72. The use of any one of claims 68-71, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter.

73. The use of any one of claims 68-72, wherein a *Rep78* gene is under control of the second derepressible promoter and a *Rep52* gene is under control of a fourth derepressible promoter contained within an artificial intron.

74. The use of claim 73, wherein the fourth derepressible promoter is removed following the activating the derepressible promoters and prior to the producing the AAV.

75. The use of claim 73 or claim 74, wherein the *Cap* gene is under control of a native promoter.

76. The use of any one of claims 68-75, wherein each of the derepressible promoters comprise a functional promoter and two tetracycline operator sequences ($TetO_2$).

77. The use of claim 76, wherein the functional promoter of the first derepressible promoter is a cytomegalovirus (CMV) promoter.

78. The use of any one of claims 68-77, wherein the repressor element is under control of a constitutive promoter.

79. The use of any one of claims 68-78, wherein the repressor element that is a tetracycline repressor protein.

80. The use of claim 79, wherein the nucleic acid further comprises a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

81. The use of claim 79 or 80, wherein the treatment comprises treatment with doxycycline.

82. The use of any one of claims 68-81, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

83. The use of any one of claims 68-82, wherein the mammalian cell is a human cell.

84. The use of claim 83, wherein the human cell is a human embryonic kidney (HEK) cell.

85. The use of any one of claims 68-84, wherein the AAV comprises a nucleic acid molecule encoding a gene of interest.

86. The use of claim 85, wherein the AAV comprises a gene of therapeutic interest.

87. The use of any one of claims 68-86, wherein the administration comprises inhalation, injection or intravenous administration.

88. A method of producing an adeno-associated virus (AAV), comprising:

a. transfecting a mammalian cell stably expressing one or more nucleic acids encoding TetR and/or TetR-KRAB with:

i. 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;

ii. a second nucleic acid encoding an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; and

iii. optionally, a third nucleic acid encoding a gene of interest under control of a third derepressible promoter;

b. treating the mammalian cell with a binding partner of the TetR;

c. activating the first, second and third derepressible promoters;

d. producing the AAV; and

e. harvesting the AAV.

89. The method of claim 88, wherein the mammalian cell is a mammalian cell culture.

90. The method of any one of claims 88-89, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

91. The method of any one of claims 88-90, wherein the mammalian cell is a human cell.

92. The method of claim 91, wherein the human cell is a human embryonic kidney (HEK) cell.

93. The method of claim 89, wherein the mammalian cell culture is a suspension culture.

94. The method of any one of claims 88-90, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

95. The method of any one of claims 88-91, wherein each of the derepressible promoters comprise a functional promoter and two tetracycline operator sequences ($TetO_2$).

96. The method of claim 95, wherein the functional promoter of the first derepressible promoter is a cytomegalovirus (CMV) promoter.

97. The method of any one of claims 88-96, wherein the TetR and/or TetR-KRAB is under control of a constitutive promoter.

98. The method of claim 97, wherein the nucleic acid encoding the TetR-KRAB comprises KRAB fused in frame with the TetR.

99. The method of any one of claims 88-98, wherein the treating comprises treating with doxycycline.

100. The method of any one of claims 88-99, wherein each of the first, second and third nucleic acids are flanked by transposon-specific inverted terminal repeats (ITRs).

101. The method of any one of claims 88-100, wherein the mammalian cell expresses sufficient amounts of TetR and/or TetR-KRAB.

102. The method of any one of claims 88-101, wherein the AAV comprises a gene of therapeutic interest.

103. The method of any one of claims 88-102, wherein the mammalian cell further comprises stable expression of one or more nucleic acids encoding chicken hypersensitive site-4 (cHS4) flanking the TetR and/or TetR-KRAB repressor sequences.

104. The method of any one of claims 88-103, wherein the mammalian cell further comprises a selection gene.

105. A method for producing an adeno-associated virus (AAV), comprising:

a. stably transfecting a mammalian cell with:

a nucleic acid encoding a TetR and/or TetR-KRAB repressor;

chicken hypersensitive site-4 (cHS4) sequences flanking the TetR and/or TetR-KRAB repressor; and

a selection gene;

b. transfecting the stably transfected mammalian cell with:

i. 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;

ii. a second nucleic acid encoding an AAV gene comprising Rep and Cap genes under control of a second derepressible promoter; and

iii. optionally, a third nucleic acid encoding a gene of interest under control of a third derepressible promoter;

c. treating the stably transfected mammalian cell with a binding partner of the TetR;

d. activating the first, second and third derepressible promoters;

e. producing the AAV; and

f. harvesting the AAV.

106. The method of claim 105, wherein the stably transfected mammalian cell produces a sufficient amounts of TetR.

107. The method of any one of claims 105 and 106, wherein the KRAB repressor domain is fused in frame with the TetR.