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.

In one aspect, provided herein is a human embryonic kidney (HEK) 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 comprising a cytomegalovirus (CMV) promoter and two tetracycline operator sequences ($TetO_2$); ii. an AAV gene comprising a *Rep78* gene under control of a second derepressible promoter comprising a p5 promoter and a $TetO_2$, a Cap gene under control of a native promoter and a Rep52 gene under control of a fourth derepressible promoter comprising a p19 promoter and a $TetO_2$; iii. a viral-associated (VA 1), non-coding RNA under control of a third derepressible promoter comprising an H1 promoter and a $TetO_2$ sequence; iv. two inverted terminal repeat (ITR) sequences; and v. a repressor element of the first, second, third, and fourth derepressible promoters under the control of a constitutive promoter.

In another aspect, provided herein is a method of producing an adeno-associated virus (AAV) in a human embryonic kidney (HEK) cell comprising a. transfecting the HEK 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 comprising a cytomegalovirus (CMV) promoter and two tetracycline operator sequences (TetO2); ii. an AAV gene comprising a Rep78 gene under control of a second derepressible promoter comprising a p5 promoter and a TetO2, a Cap gene under control of a native promoter and a Rep52 gene under control of a fourth derepressible promoter comprising a p19 promoter and a TetO2; iii. a viral-associated (VA 1), non-coding RNA under control of a third derepressible promoter comprising an H1 promoter and a TetO2 sequence; iv. two inverted terminal repeat (ITR) sequences; and v. a repressor element of the first, second, third, and fourth derepressible promoters under the control of a constitutive promoter, b. treating the HEK cell with a binding partner of the repressor element; c. activating the first, second, third, and fourth derepressible promoters; d. producing the AAV; and e. harvesting the AAV.

[0006a] In one example, provided herein is a human embryonic kidney (HEK) 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 comprising a cytomegalovirus (CMV) promoter and two tetracycline operator sequences ($TetO_2$);
   ii. an AAV gene comprising a *Rep78* gene under control of a second derepressible promoter comprising a p19 promoter and a $TetO_2$, a Cap gene under control of a native promoter and a Rep52 gene under control of a fourth derepressible promoter comprising a p19 promoter and a $TetO_2$;
   iii. a viral-associated (VA 1), non-coding RNA under control of a third derepressible promoter comprising an H1 promoter and a $TetO_2$ sequence;
   iv. two inverted terminal repeat (ITR) sequences; and
   v. a repressor element of the first, second, and third derepressible promoters under the control of a constitutive promoter,

wherein the nucleic acid molecule is integrated into the HEK cell's genomic DNA and/or is maintained by the HEK cell extra-chromosomally.

[0006b] In another example, provided herein is a method of producing an adeno-associated virus (AAV) in a human embryonic kidney (HEK) cell comprising:

a. transfecting the HEK 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 comprising a cytomegalovirus (CMV) promoter and two tetracycline operator sequences ($TetO_2$);
   ii. an AAV gene comprising a *Rep78* gene under control of a second derepressible promoter comprising a p19 promoter and a $TetO_2$, a Cap gene under control of a native promoter and a Rep52 gene under control of a fourth derepressible promoter comprising a p19 promoter and a $TetO_2$;
   iii. a viral-associated (VA 1), non-coding RNA under control of a third derepressible promoter comprising an H1 promoter and a $TetO_2$ sequence;
   iv. two inverted terminal repeat (ITR) sequences; and
   v. a repressor element of the first, second, and third derepressible promoters under the control of a constitutive promoter,
b. treating the HEK 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.

[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.

[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.

CLAIMS

What is claimed is:

1. A human embryonic kidney (HEK) 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 comprising a cytomegalovirus (CMV) promoter and two tetracycline operator sequences ($TetO_2$);

        ii. an AAV gene comprising a *Rep78* gene under control of a second derepressible promoter comprising a p5 promoter and a $TetO_2$, a Cap gene under control of a native promoter and a Rep52 gene under control of a fourth derepressible promoter comprising a p19 promoter and a $TetO_2$;

        iii. a viral-associated (VA 1), non-coding RNA under control of a third derepressible promoter comprising an H1 promoter and a $TetO_2$ sequence;

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

        v. a repressor element of the first, second, third, and fourth derepressible promoters under the control of a constitutive promoter,

    wherein the nucleic acid molecule is integrated into the HEK cell's genomic DNA and/or is maintained by the HEK cell extra-chromosomally.

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

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

4. The HEK cell of any one of claims 1-3, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

5. The HEK cell of any one of claims 1-4, wherein the repressor element is a tetracycline repressor protein.

6. The HEK cell of claim 5, further comprising a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

7. The HEK cell of any one of claims 1-6, further comprising a nucleic acid molecule encoding a gene of interest.

8. A method of producing an adeno-associated virus (AAV) in a human embryonic kidney (HEK) cell comprising:

   a. transfecting the HEK 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 comprising a cytomegalovirus (CMV) promoter and two tetracycline operator sequences ($TetO_2$);

      ii. an AAV gene comprising a *Rep78* gene under control of a second derepressible promoter comprising a p5 promoter and a $TetO_2$, a Cap gene under control of a native promoter and a Rep52 gene under control of a fourth derepressible promoter comprising a p19 promoter and a $TetO_2$;

      iii. a viral-associated (VA 1), non-coding RNA under control of a third derepressible promoter comprising an H1 promoter and a $TetO_2$ sequence;

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

      v. a repressor element of the first, second, third, and fourth derepressible promoters under the control of a constitutive promoter;

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

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

d. producing the AAV; and

e. harvesting the AAV.

9. The method of claim 8, wherein the HEK cell is a HEK cell culture.

10. The method of claim 9, wherein the HEK cell culture is a suspension culture.

11. The method of any one of claims 8-10, further comprising an internal ribosome entry site (IRES) element between the E2A and E4Orf6 genes.

12. The method of claim 8-11, wherein the fourth derepressible promoter is removed following the activating the derepressible promoters and prior to the producing the AAV.

13. The method of any one of claims 8-12, wherein the repressor element is a tetracycline repressor protein.

14. The method of claim 13, wherein the nucleic acid molecule further comprises a nucleic acid encoding a transcriptional repression domain in frame with the nucleic acid encoding the tetracycline repressor protein.

15. The method of claim 13 or 14, wherein the treating comprises treating with doxycycline.

16. The method of any one of claims 8-15, wherein the AAV comprises a nucleic acid molecule encoding a gene of interest.

17. The method of claim 16, wherein the AAV comprises a gene of therapeutic interest.