

WHAT IS CLAIMED IS:

1. A method of producing an adeno-associated virus (AAV) in an E1 complementary producer cell, comprising:
 - a. transfecting the E1 complementary producer cell with one or more vectors comprising:
 - i. an E1A adenovirus helper gene;
 - ii. an adenovirus helper gene selected from E2A, E4, or both;
 - iii. a viral-associated, non-coding RNA (VA RNA); and
 - iv. an AAV gene selected from Rep, Cap, or both;
 - b. culturing the transfected E1 complementary producer cell under conditions suitable for producing the AAV; and
 - c. purifying the AAV from the cultured E1 complementary producer cell, thereby obtaining the AAV.
2. The method of claim 1, wherein (i), (ii), (iii), and (iv) are on a single vector, or wherein (i), (ii), (iii), and (iv) are on more than one vector.
- ~~3. The method of claim 1, wherein (i), (ii), (iii), and (iv) are on more than one vector.~~
- ~~4.3.~~ The method of claim ~~3~~2, wherein (i), (ii), and (iii) are on a first vector, and (iv) is a second vector;
or wherein (i) is on a first vector, (ii) and (iii) are on a second vector, and (iv) is on a third vector;
or wherein each of (i), (ii), (iii), and (iv) is on a separate vector.
- ~~5. The method of claim 3, wherein (i) is on a first vector, (ii) and (iii) are on a second vector, and (iv) is on a third vector.~~
- ~~6. The method of claim 3, wherein each of (i), (ii), (iii), and (iv) is on a separate vector.~~
- ~~7.4.~~ The method of claim ~~5 or 6~~3, wherein (i) is on a separate vector from (ii), (iii), and (iv), and wherein the vector comprising (i) is transfected into the E1 complementary producer

cell at a ratio of about 1:1 to about 5:1 to each of the vectors comprising (ii), (iii), and (iv).

~~8.5.~~ The method of claim ~~74~~, wherein the ratio of the vector comprising (i) to each of the vectors comprising (ii), (iii), and (iv) transfected into the E1 complementary producer cell is 1:1, 2:1, or 3:1.

~~9.~~ ~~The method of claim 7, wherein the ratio of the vector comprising (i) to each of the vectors comprising (ii), (iii), and (iv) transfected into the E1 complementary producer cell is 2:1.~~

~~10.~~ ~~The method of claim 7, wherein the ratio of the vector comprising (i) to each of the vectors comprising (ii), (iii), and (iv) transfected into the E1 complementary producer cell is 3:1.~~

~~11.6.~~ The method of any one of claims 1 to ~~105~~, wherein (i), (ii), (iii), and (iv) are operably linked to one or more promoters.

~~12.~~ ~~The method of claim 11, wherein each of (i), (ii), (iii), and (iv) is operably linked to a separate promoter.~~

~~13.7.~~ The method of claim ~~126~~, wherein each of (i), (ii), (iii), and (iv) is operably linked to a separate promoter, and wherein the promoter operably linked to (i) provides substantially a same an expression level that is substantially the same, or at least 2-fold higher, or at least 3-fold higher as compared to the promoters operably linked to (ii), (iii), and (iv).

~~14.~~ ~~The method of claim 12, wherein the promoter operably linked to (i) provides at least 2-fold higher expression as compared to the promoters operably linked to (ii), (iii), and (iv).~~

~~15.~~ ~~The method of claim 12, wherein the promoter operably linked to (i) provides at least 3-fold higher expression as compared to the promoters operably linked to (ii), (iii), and (iv).~~

~~16.8.~~ The method of any one of claims 11 to 15claim 6 or 7, wherein (i) is operably linked to a promoter and further operably linked to an enhancer, and wherein (ii), (iii), and (iv) are not operably linked to an enhancer.

~~17.9.~~ The method of any one of claims 1 to ~~168~~, wherein a copy number ratio of (i) to each of (ii), (iii), and (iv) on the one or more vectors is about 1:1 to about 5:1.

~~18.~~ ~~The method of claim 17, wherein the copy number ratio of (i) to each of (ii), (iii), and (iv) on the one or more vectors is 1:1.~~

~~19.~~ ~~The method of claim 17, wherein the copy number ratio of (i) to each of (ii), (iii), and (iv) on the one or more vectors is 2:1.~~

~~20.~~ ~~The method of claim 17, wherein the copy number ratio of (i) to each of (ii), (iii), and (iv) on the one or more vectors is 3:1.~~

~~21.10.~~ The method of any one of claims 1 to ~~209~~, wherein (ii) comprises E2A and E4; and/or wherein (iv) comprises Rep and Cap.

~~22.~~ ~~The method of any one of claims 1 to 21, wherein (iv) comprises Rep and Cap.~~

~~23.11.~~ The method of any one of claims 1 to ~~2210~~, wherein the culturing comprises expressing E1A at a ratio of about 1:1 to about 5:1 to each of (ii), (iii), and (iv).

~~24.~~ ~~The method of claim 23, wherein the ratio of the expression of E1A to the expression of each of (ii), (iii), and (iv) is 1:1, 2:1, or 3:1.~~

~~25.12.~~ The method of any one of claims 1 to ~~2411~~, wherein the one or more vectors further comprises one or both of:
(v) a helper gene selected from E1B, NP1, NS2, or a combination thereof, optionally wherein the E1B is E1B-19k or E1B-55k; and
a gene of interest.

~~26.~~ ~~The method of claim 25, wherein the E1B is E1B-19k or E1B-55k.~~

~~27.~~ ~~The method of claim 25, wherein (v) comprises NP1 and NS2.~~

~~28.13.~~ The method of ~~any one of claims 25 to 27~~claim 12, wherein (i) and (v) are on a single vector; or wherein (v) is on a separate vector from (i), (ii), (iii), and (iv).

~~29. The method of any one of claims 25 to 27, wherein (v) is on a separate vector from (i), (ii), (iii), and (iv).~~

~~30. The method of any one of claims 1 to 29, wherein the one or more vectors further comprises a gene of interest.~~

~~31. The method of any one of claims 1 to 29, further comprising transfecting the E1 complementary producer cell with a vector comprising a gene of interest, wherein the gene of interest is on a separate vector from (i), (ii), (iii), and (iv).~~

~~32.14.~~ The method of any one of claims 1 to ~~34~~13, wherein the method produces at least 1.5-fold or higher titer of the AAV as compared to a method that does not comprise transfecting a E1 complementary producer cell with an E1A adenovirus helper gene.

~~33.15.~~ A method of producing an adeno-associated virus (AAV) in an E1 complementary producer cell, comprising:

a. transfecting the E1 complementary producer cell with:

- i. a first vector comprising E1A operably linked to a first promoter;
- ii. a second vector comprising E2A, E4, and a viral-associated, non-coding RNA (VA RNA), all operably linked to a second promoter; ~~and~~
- iii. a third vector comprising Rep and Cap, both operably linked to a third promoter; and

iv. optionally, one or both of:

(A) a helper gene selected from E1B, NP1, NS2, or a combination thereof, wherein the helper gene is on the first, second, or third vector, or wherein the helper gene is on a fourth vector; and
(B) a gene of interest, wherein the gene of interest is on the first, second, third, or fourth vector, or wherein the gene of interest is on a fifth vector,

wherein a transfection ratio of (i) to each of (ii) and (iii) is about 1:1 to about 3:1;

- b. culturing the transfected E1 complementary producer cell under conditions suitable for producing the AAV; and

- c. purifying the AAV from the cultured E1 complementary producer cell, thereby obtaining the AAV.

34.16. A method of producing an adeno-associated virus (AAV) in an E1 complementary producer cell, comprising:

- a. transfecting the E1 complementary producer cell with:
 - i. a first vector comprising E1A operably linked to a first promoter and an enhancer;
 - ii. a second vector comprising E2A, E4, and a viral-associated, non-coding RNA (VA RNA), all operably linked to a second promoter; ~~and~~
 - iii. a third vector comprising Rep and Cap, each operably linked to a third promoter; and
 - iv. optionally, one or both of:
 - (A) a helper gene selected from E1B, NP1, NS2, or a combination thereof, wherein the helper gene is on the first, second, or third vector, or wherein the helper gene is on a fourth vector; and
 - (B) a gene of interest, wherein the gene of interest is on the first, second, third, or fourth vector, or wherein the gene of interest is on a fifth vector,
- b. culturing the transfected E1 complementary producer cell under conditions suitable for producing the AAV, wherein E1A is expressed at a ratio of about 1:1 to about 3:1 to each of E2A, E4, VA RNA, Rep, and Cap; and
- c. purifying the AAV from the cultured E1 complementary producer cell, thereby obtaining the AAV.

35.17. A method of producing an adeno-associated virus (AAV) in an E1 complementary producer cell, comprising:

- a. transfecting the E1 complementary producer cell with:
 - i. a first vector comprising E1A, E2A, E4 and VA RNA, all operably linked to a first promoter; ~~and~~
 - ii. a second vector comprising Rep and Cap, operably linked to a second promoter, and

iii. optionally, one or both of:

(A) a helper gene selected from E1B, NP1, NS2, or a combination thereof, wherein the helper gene is on the first or second vector, or wherein the helper gene is on a third vector; and

(B) a gene of interest, wherein the gene of interest is on the first, second, or third vector, or wherein the gene of interest is on a fourth vector,

wherein a copy number ratio of E1A to each of E2A, E4, VA RNA, Rep, and Cap is about 1:1 to about 3:1;

- b. culturing the transfected E1 complementary producer cell under conditions suitable for producing the AAV; and
- c. purifying the AAV from the cultured E1 complementary producer cell, thereby obtaining the AAV.

~~36. The method of claim 33 or 34, wherein one or more of the first, second, or third vectors further comprises a helper gene selected from E1B, NP1, NS2, or a combination thereof.~~

~~37. The method of claim 35, wherein one or both of the first or second vectors further comprises a helper gene selected from E1B, NP1, NS2, or a combination thereof.~~

~~38. The method of any one of claims 33 to 35, further comprising transfecting the E1 complementary producer cell with a vector comprising helper gene selected from E1B, NP1, NS2, or a combination thereof.~~

~~39. The method of any one of claims 36 to 38, wherein the E1B is E1B-19k or E1B-55k.~~

~~40. The method of any one of claims 36 to 38, wherein the helper gene comprises a combination of NP1 and NS2.~~

~~41. The method of any one of claims 33 to 40, further comprising transfecting the E1 complementary producer cell with a vector comprising a gene of interest.~~

~~42.18.~~ The method of any one of claims 1 to ~~41.17~~, wherein the E1 complementary producer cell is a HEK293 cell, a 911 cell, a pTG6559 cell, a PER.C6 cell, a GH329 cell, an N52.E6

(CAP®) cell, a HeLa-E1 cell, an UR cell, a VLI-293 cell, an Ac51 cell, an Ac139 cell, or a variant or derivative thereof.

~~43.19.~~ The method of claim ~~42~~18, wherein the E1 complementary producer cell is a HEK293 cell, a PER.C6 cell, or a N52.E6 (CAP®) cell.

~~44.~~ ~~The method of claim 43, wherein the E1 complementary producer cell is a HEK293 cell.~~

~~45.20.~~ The method of claim ~~44~~19, wherein the E1 complementary producer cell is a HEK293 cell, and wherein the HEK293 cell is a HEK293S cell, a HEK293T cell, a HEK293F cell, a HEK293FT cell, a HEK293FTM cell, a HEK293SG cell, a HEK293SGGD cell, a HEK293H cell, a HEK293E cell, a HEK293MSR cell, a HEK293A cell, or a combination thereof.

~~46.~~ ~~The method of claim 45, wherein the HEK293 cell is a HEK293T cell.~~

~~47.~~ ~~The method of claim 43, wherein the E1 complementary producer cell is a PER.C6 cell.~~

~~48.~~ ~~The method of claim 43, wherein the E1 complementary producer cell is a N52.E6 (CAP®) cell.~~