

What is claimed is:

1. A non-naturally occurring nucleic acid molecule comprising a modified adeno-associated virus (AAV) *rep* gene having an AAV *rep* gene encoding four Rep proteins Rep78, Rep68, Rep52 and Rep40 and an artificial intron inserted into a coding sequence of the *rep* gene shared by the four Rep proteins, wherein the artificial intron comprises a stop cassette inserted downstream of the 5' splice site and upstream of the branch site of the artificial intron, and the stop cassette comprises, in 5' to 3' order:
 - (a) an *attP* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO:7, preferably, an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (b) a splice acceptor;
 - (c) a terminator; and
 - (d) an *attB* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical of SEQ ID NO:8 or SEQ ID NO:9, preferably, an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9.
2. The non-naturally occurring nucleic acid molecule of claim 1, wherein the splice acceptor comprises the nucleotide sequence of SEQ ID NO:17.
3. The non-naturally occurring nucleic acid molecule of claim 1 or 2, wherein the terminator comprises a polyadenylation signal.
4. The non-naturally occurring nucleic acid molecule of claim 3, wherein the terminator further comprises the nucleotide sequence of SEQ ID NO:19.
5. The non-naturally occurring nucleic acid molecule of any one of claims 1 to 4, wherein the stop cassette comprises a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18.
6. The non-naturally occurring nucleic acid molecule of any one of claims 1 to 5, wherein the artificial intron comprises, in 5' to 3' order, the nucleotide sequence of SEQ ID NO:14, the stop cassette, and the nucleotide sequence of SEQ ID NO:15.

7. The non-naturally occurring nucleic acid molecule of any one of claims 1 to 6, wherein the AAV *rep* gene comprises a *rep* gene of one of AAV1 to AAV8, or a hybrid thereof.
8. The non-naturally occurring nucleic acid molecule of claim 7, wherein the AAV *rep* gene comprises the *rep* gene of human AAV2 having nucleotide numbers 190 to 2202 of the nucleotide sequence of GenBank accession number NC_001401.2.
9. The non-naturally occurring nucleic acid molecule of claim 8, wherein the artificial intron is inserted between nucleotide numbers 996 to 1905 of the nucleotide sequence of GenBank accession number NC_001401.2.
10. The non-naturally occurring nucleic acid molecule of claim 9, wherein the artificial intron is inserted immediately downstream of nucleotide number 1052, 1061, 1712, 1906, 1022, 1112, 1475, 1514, 1700, 1742, 1784 or 1340, preferably nucleotide number 1052, of the nucleotide sequence of GenBank accession number NC_001401.2.
11. A non-naturally occurring nucleic acid molecule comprising a modified AAV *rep* gene comprising, in 5' to 3' order:
 - (a) a 5' portion of an AAV *rep* gene having the nucleotide sequence of SEQ ID NO:55;
 - (b) an artificial intron comprising, in 5' to 3' order:
 - (i) a 5' intron fragment having the nucleotide sequence of SEQ ID NO:14;
 - (ii) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor having the nucleotide sequence of SEQ ID NO:17;
 - (3) a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator having the nucleotide sequence of SEQ ID NO:19;and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and

- (iii) a 3' intron fragment having the nucleotide sequence of SEQ ID NO:15;
and
 - (c) a 3' portion of the AAV *rep* gene having the nucleotide sequence of SEQ ID NO:56.
- 12. A non-naturally occurring nucleic acid molecule comprising a modified AAV *rep* gene comprising, in 5' to 3' order:
 - (a) a 5' portion of an AAV *rep* gene having the nucleotide sequence of SEQ ID NO:73;
 - (b) an artificial intron comprising, in 5' to 3' order:
 - (i) a 5' intron fragment having the nucleotide sequence of SEQ ID NO:14;
 - (ii) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor having the nucleotide sequence of SEQ ID NO:17;
 - (3) a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator having the nucleotide sequence of SEQ ID NO:19;
and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (iii) a 3' intron fragment having the nucleotide sequence of SEQ ID NO:66;
and
 - (c) a 3' portion of the AAV *rep* gene having the nucleotide sequence of SEQ ID NO:56.
- 13. The non-naturally occurring nucleic acid molecule of claim 11 or 12, wherein the stop cassette comprises the nucleotide sequence of SEQ ID NO:16.
- 14. The non-naturally occurring nucleic acid molecule of any one of claims 1 to 13, further comprising an AAV *cap* gene encoding three capsid proteins VP1, VP2 and VP3.

15. The non-naturally occurring nucleic acid molecule of claim 14, wherein the AAV *cap* gene comprises a *cap* gene of one of AAV1 to AAV9 and AAVDJ, or a hybrid thereof.
16. The non-naturally occurring nucleic acid molecule of claim 15, wherein the AAV *cap* gene comprises the *cap* gene of human AAV9 having the nucleotide sequence of GenBank accession number AY530579.1.
17. The non-naturally occurring nucleic acid molecule of any one of claims 14 to 16, wherein the AAV *cap* gene further comprises a polyadenylation signal, preferably a polyadenylation signal of AAV2 having nucleotide numbers 4411 to 4466 of the nucleotide sequence of GenBank accession number NC_001401.2, and an enhancer, preferably an AAV2 *rep* P5 promoter having nucleotide numbers 190 to 313 of the nucleotide sequence of GenBank accession number NC_001401.2, wherein the polyadenylation signal and the enhancer are both downstream of the coding sequence of the *cap* gene.
18. The non-naturally occurring nucleic acid molecule of any one of claims 14 to 17, further comprising a transgene flanked by a pair of AAV inverted terminal repeats (ITRs) downstream of the AAV *cap* gene.
19. The non-naturally occurring nucleic acid molecule of claim 18, further comprising a first insulator upstream of the modified AAV *rep* gene and optionally a second insulator downstream of the transgene flanked by the ITRs, preferably, the first insulator and the second insulator are independently selected from the group consisting of:
 - (a) a human anti-repressor element 40 having the nucleotide sequence of SEQ ID NO:24;
 - (b) a mouse anti-repressor element 40 having the nucleotide sequence of SEQ ID NO:25;
 - (c) an anti-repressor element 04 having the nucleotide sequence of GenBank accession number AY190749.1;
 - (d) an anti-repressor element 06 having the nucleotide sequence of GenBank accession number AY190750.1;

- (e) an anti-repressor element 07 having the nucleotide sequence of GenBank accession number AY190751.1;
 - (f) an anti-repressor element 12 having the nucleotide sequence of GenBank accession number AY190752.1;
 - (g) an anti-repressor element 13 having the nucleotide sequence of GenBank accession number AY190753.1;
 - (h) an anti-repressor element 35 having the nucleotide sequence of GenBank accession number AY190754.1;
 - (i) an anti-repressor element 36 having the nucleotide sequence of GenBank accession number AY190755.1;
 - (j) an anti-repressor element 52 having the nucleotide sequence of GenBank accession number AY190757.1;
 - (k) an anti-repressor element 53 having the nucleotide sequence of GenBank accession number AY190758.1; and
 - (l) a Chicken HS4 insulator from the globin locus having the nucleotide sequence of AY040835.1 in two or more copies,
more preferably, the first insulator and the second insulator have the nucleotide sequences of SEQ ID NO:24 and SEQ ID NO:25, respectively.
20. The non-naturally occurring nucleic acid molecule of claim 19, wherein the non-naturally occurring nucleic acid molecule comprises the first insulator upstream of the modified AAV *rep* gene, and further comprises a first spacer sequence and a second spacer sequence upstream and downstream of the transgene, respectively, wherein the first spacer sequence and the second spacer sequence are independently selected from the group consisting of:
- (a) a nucleotide sequence of SEQ ID NO:67; and
 - (b) a nucleotide sequence of SEQ ID NO:68.
21. The non-naturally occurring nucleic acid molecule of any one of claims 18 to 20, wherein the ITR has the nucleotide sequence of SEQ ID NO:20, the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; preferably, the promoter has the nucleotide sequence of

SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23.

22. A non-naturally occurring nucleic acid molecule comprising, in 5' to 3' order:
- (A) a first insulator, preferably the first insulator has the nucleotide sequence of SEQ ID NO:24;
 - (B) a modified AAV *rep* gene comprising, in 5' to 3' order:
 - (i) a 5' portion of an AAV *rep* gene, preferably the 5' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:55;
 - (ii) an artificial intron comprising, in 5' to 3' order:
 - (a) a 5' intron fragment, preferably the 5' intron fragment has the nucleotide sequence of SEQ ID NO:14;
 - (b) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor, preferably the splice acceptor has the nucleotide sequence of SEQ ID NO:17;
 - (3) a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator, preferably the terminator has the nucleotide sequence of SEQ ID NO:19; and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (c) a 3' intron fragment, preferably the 3' intron fragment has the nucleotide sequence of SEQ ID NO:15;
 - (iii) a 3' portion of the AAV *rep* gene, preferably the 3' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:56;
 - (C) an AAV *cap* gene, preferably the AAV *cap* gene comprises the nucleotide sequence of SEQ ID NO:57;
 - (D) a transgene flanked by a pair of AAV ITRs, preferably, the AAV ITR has the nucleotide sequence of SEQ ID NO:20, and the transgene comprises a promoter

- operably linked to a coding sequence, and the coding sequence is operably linked to a polyadenylation signal; more preferably, the promoter has the nucleotide sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23; and
- (E) a second insulator, preferably the second insulator has the nucleotide sequence of SEQ ID NO:25.
23. A non-naturally occurring nucleic acid molecule comprising, in 5' to 3' order:
- (A) a first insulator, preferably the first insulator has the nucleotide sequence of SEQ ID NO:24;
- (B) a modified AAV *rep* gene comprising, in 5' to 3' order:
- (i) a 5' portion of an AAV *rep* gene, preferably the 5' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:73;
- (ii) an artificial intron comprising, in 5' to 3' order:
- (a) a 5' intron fragment, preferably the 5' intron fragment has the nucleotide sequence of SEQ ID NO:14;
- (b) a stop cassette comprising, in 5' to 3' order:
- (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
- (2) a splice acceptor, preferably the splice acceptor has the nucleotide sequence of SEQ ID NO:17;
- (3) a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
- (4) a terminator, preferably the terminator has the nucleotide sequence of SEQ ID NO:19; and
- (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
- (c) a 3' intron fragment, preferably the 3' intron fragment has the nucleotide sequence of SEQ ID NO:66;
- (iii) a 3' portion of the AAV *rep* gene, preferably the 3' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:56;

- (C) an AAV *cap* gene;
 - (D) a transgene flanked by
 - (1) a pair of AAV ITRs, preferably, the AAV ITR has the nucleotide sequence of SEQ ID NO:20, and the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; more preferably, the promoter has the nucleotide sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23; and
 - (2) a pair of spacer sequences, preferably, the spacer sequences have a nucleotide sequence of SEQ ID NO:67 and SEQ ID NO:68.
24. A vector comprising the non-naturally occurring nucleic acid molecule of any one of claims 1 to 22; preferably, the vector is a plasmid; more preferably, the plasmid comprises the nucleotide sequence of SEQ ID NO:12.
25. A vector comprising the non-naturally occurring nucleic acid molecule of any one of claims 1 to 21 or 23; preferably, the vector is a plasmid; more preferably, the plasmid comprises the nucleotide sequence of SEQ ID NO:70.
26. A method of making the non-naturally occurring nucleic acid molecule of any one of claims 1 to 23.
27. A method of making the vector of claim 24 or 25.
28. A cell comprising a non-naturally occurring nucleic acid molecule comprising a modified adeno-associated virus (AAV) *rep* gene having an AAV *rep* gene encoding four Rep proteins Rep78, Rep68, Rep52 and Rep40 and an artificial intron inserted into a coding sequence of the *rep* gene shared by the four Rep proteins, wherein the artificial intron comprises a stop cassette inserted downstream of the 5' splice site and upstream of the branch site of the artificial intron, and the stop cassette comprises, in 5' to 3' order:
- (a) an *attP* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO:7, preferably, an *attP* site having the nucleotide sequence of SEQ ID NO:7;

- (b) a splice acceptor;
 - (c) a terminator; and
 - (d) an *attB* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical of SEQ ID NO:8 or SEQ ID NO:9, preferably, an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9.
29. The cell of claim 28, wherein the splice acceptor comprises the nucleotide sequence of SEQ ID NO:17.
30. The cell of claim 28 or 29, wherein the terminator comprises a polyadenylation signal.
31. The cell of claim 30, wherein the terminator further comprises the nucleotide sequence of SEQ ID NO:19.
32. The cell of any one of claims 28 to 31, wherein the stop cassette comprises a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18.
33. The cell of any one of claims 28 to 32, wherein the artificial intron comprises, in 5' to 3' order, the nucleotide sequence of SEQ ID NO:14, the stop cassette, and the nucleotide sequence of SEQ ID NO:15.
34. The cell of any one of claims 28 to 32, wherein the artificial intron comprises, in 5' to 3' order, the nucleotide sequence of SEQ ID NO:14, the stop cassette, and the nucleotide sequence of SEQ ID NO:66.
35. The cell of any one of claims 28 to 33, wherein the AAV *rep* gene comprises a *rep* gene of one of AAV1 to AAV8, or a hybrid thereof.
36. The cell of claim 35, wherein the AAV *rep* gene comprises the *rep* gene of human AAV2 having nucleotide numbers 190 to 2202 of the nucleotide sequence of GenBank accession number NC_001401.2.

37. The cell of claim 36, wherein the artificial intron is inserted between nucleotide numbers 996 to 1905 of the nucleotide sequence of GenBank accession number NC_001401.2.
38. The cell of claim 37, wherein the artificial intron is inserted immediately downstream of nucleotide number 1052, 1061, 1712, 1906, 1022, 1112, 1475, 1514, 1700, 1742, 1784 or 1340, preferably nucleotide number 1052, of the nucleotide sequence of GenBank accession number NC_001401.2.
39. A cell comprising a non-naturally occurring nucleic acid molecule comprising a modified AAV *rep* gene comprising, in 5' to 3' order:
- (a) a 5' portion of an AAV *rep* gene having the nucleotide sequence of SEQ ID NO:55;
 - (b) an artificial intron comprising, in 5' to 3' order:
 - (i) a 5' intron fragment having the nucleotide sequence of SEQ ID NO:14;
 - (ii) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor having the nucleotide sequence of SEQ ID NO:17;
 - (3) a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator having the nucleotide sequence of SEQ ID NO:19;and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (iii) a 3' intron fragment having the nucleotide sequence of SEQ ID NO:15;and
 - (c) a 3' portion of the AAV *rep* gene having the nucleotide sequence of SEQ ID NO:56.
40. A cell comprising a non-naturally occurring nucleic acid molecule comprising a modified AAV *rep* gene comprising, in 5' to 3' order:

- (a) a 5' portion of an AAV *rep* gene having the nucleotide sequence of SEQ ID NO:73;
 - (b) an artificial intron comprising, in 5' to 3' order:
 - (i) a 5' intron fragment having the nucleotide sequence of SEQ ID NO:14;
 - (ii) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor having the nucleotide sequence of SEQ ID NO:17;
 - (3) a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator having the nucleotide sequence of SEQ ID NO:19; and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (iii) a 3' intron fragment having the nucleotide sequence of SEQ ID NO:66; and
 - (c) a 3' portion of the AAV *rep* gene having the nucleotide sequence of SEQ ID NO:56.
41. The cell of claim 39 or 40, wherein the stop cassette comprises the nucleotide sequence of SEQ ID NO:16.
42. The cell of any one of claims 28 to 41, further comprising an AAV *cap* gene encoding three capsid proteins VP1, VP2 and VP3.
43. The cell of claim 42, wherein the AAV *cap* gene comprises a *cap* gene of one of AAV1 to AAV9 and AAVDJ, or a hybrid thereof.
44. The cell of claim 43, wherein the AAV *cap* gene comprises the *cap* gene of human AAV9 having the nucleotide sequence of GenBank accession number AY530579.1.
45. The cell of claim 43, wherein the AAV *cap* gene comprises the *cap* gene of a hybrid of AAV9.

46. The cell of claims 42 to 45, wherein the AAV *cap* gene further comprises a polyadenylation signal, preferably a polyadenylation signal of AAV2 having nucleotide numbers 4411 to 4466 of the nucleotide sequence of GenBank accession number NC_001401.2, and an enhancer, preferably an AAV2 *rep* P5 promoter having nucleotide numbers 190 to 313 of the nucleotide sequence of GenBank accession number NC_001401.2, wherein the polyadenylation signal and the enhancer are both downstream of the coding sequence of the *cap* gene.
47. The cell of any one of claims 42 to 46, further comprising a transgene flanked by a pair of AAV inverted terminal repeats (ITRs) downstream of the AAV *cap* gene.
48. The cell of claim 47, further comprising a first insulator upstream of the modified AAV *rep* gene and optionally a second insulator downstream of the transgene flanked by the ITRs, preferably, the first insulator and the second insulator are independently selected from the group consisting of:
- (a) a human anti-repressor element 40 having the nucleotide sequence of SEQ ID NO:24;
 - (b) a mouse anti-repressor element 40 having the nucleotide sequence of SEQ ID NO:25;
 - (c) an anti-repressor element 04 having the nucleotide sequence of GenBank accession number AY190749.1;
 - (d) an anti-repressor element 06 having the nucleotide sequence of GenBank accession number AY190750.1;
 - (e) an anti-repressor element 07 having the nucleotide sequence of GenBank accession number AY190751.1;
 - (f) an anti-repressor element 12 having the nucleotide sequence of GenBank accession number AY190752.1;
 - (g) an anti-repressor element 13 having the nucleotide sequence of GenBank accession number AY190753.1;
 - (h) an anti-repressor element 35 having the nucleotide sequence of GenBank accession number AY190754.1;

- (i) an anti-repressor element 36 having the nucleotide sequence of GenBank accession number AY190755.1;
 - (j) an anti-repressor element 52 having the nucleotide sequence of GenBank accession number AY190757.1;
 - (k) an anti-repressor element 53 having the nucleotide sequence of GenBank accession number AY190758.1; and
 - (l) a Chicken HS4 insulator from the globin locus having the nucleotide sequence of AY040835.1 in two or more copies,
more preferably, the first insulator and the second insulator have the nucleotide sequences of SEQ ID NO:24 and SEQ ID NO:25, respectively.
49. The cell of claim 48, wherein the cell comprises the first insulator upstream of the modified AAV *rep* gene, and further comprises a first spacer sequence and a second spacer sequence upstream and downstream of the transgene, respectively, wherein the first spacer sequence and the second spacer sequence are independently selected from the group consisting of:
- (a) a nucleotide sequence of SEQ ID NO:67; and
 - (b) a nucleotide sequence of SEQ ID NO:68.
50. The cell of any one of claims 47 to 49, wherein the ITR has the nucleotide sequence of SEQ ID NO:20, the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; preferably, the promoter has the nucleotide sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23.
51. A cell comprising a non-naturally occurring nucleic acid molecule comprising, in 5' to 3' order:
- (A) a first insulator, preferably the first insulator has the nucleotide sequence of SEQ ID NO:24;
 - (B) a modified AAV *rep* gene comprising, in 5' to 3' order:
 - (i) a 5' portion of an AAV *rep* gene, preferably the 5' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:55;

- (ii) an artificial intron comprising, in 5' to 3' order:
 - (a) a 5' intron fragment, preferably the 5' intron fragment has the nucleotide sequence of SEQ ID NO:14;
 - (b) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor, preferably the splice acceptor has the nucleotide sequence of SEQ ID NO:17;
 - (3) a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator, preferably the terminator has the nucleotide sequence of SEQ ID NO:19; and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (c) a 3' intron fragment, preferably the 3' intron fragment has the nucleotide sequence of SEQ ID NO:15;
 - (iii) a 3' portion of the AAV *rep* gene, preferably the 3' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:56;
 - (C) an AAV *cap* gene, preferably the AAV *cap* gene comprises the nucleotide sequence of SEQ ID NO:57;
 - (D) a transgene flanked by a pair of AAV ITRs, preferably, the AAV ITR has the nucleotide sequence of SEQ ID NO:20, and the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; more preferably, the promoter has the nucleotide sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23; and
 - (E) a second insulator, preferably the second insulator has the nucleotide sequence of SEQ ID NO:25.
52. A cell comprising a non-naturally occurring nucleic acid molecule comprising, in 5' to 3' order:

- (A) a first insulator, preferably the first insulator has the nucleotide sequence of SEQ ID NO:24;
- (B) a modified AAV *rep* gene comprising, in 5' to 3' order:
 - (i) a 5' portion of an AAV *rep* gene, preferably the 5' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:73;
 - (ii) an artificial intron comprising, in 5' to 3' order:
 - (a) a 5' intron fragment, preferably the 5' intron fragment has the nucleotide sequence of SEQ ID NO:14;
 - (b) a stop cassette comprising, in 5' to 3' order:
 - (1) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (2) a splice acceptor, preferably the splice acceptor has the nucleotide sequence of SEQ ID NO:17;
 - (3) a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (4) a terminator, preferably the terminator has the nucleotide sequence of SEQ ID NO:19; and
 - (5) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (c) a 3' intron fragment, preferably the 3' intron fragment has the nucleotide sequence of SEQ ID NO:66;
 - (iii) a 3' portion of the AAV *rep* gene, preferably the 3' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:56;
- (C) an AAV *cap* gene; and
- (D) a transgene flanked by
 - (i) a pair of AAV ITRs, preferably, the AAV ITR has the nucleotide sequence of SEQ ID NO:20, and the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; more preferably, the promoter has the nucleotide

- sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23; and
- (ii) a pair of spacer sequences, preferably, the spacer sequences have a nucleotide sequence of SEQ ID NO:67 and SEQ ID NO:68.
53. The cell of any one of claims 28 to 51, wherein the non-naturally occurring nucleic acid molecule is episomal, having the nucleotide sequence of SEQ ID NO:12.
54. The cell of any one of claims 28 to 50 or 52, wherein the non-naturally occurring nucleic acid molecule is episomal, having the nucleotide sequence of SEQ ID NO:70.
55. The cell of any one of claims 52 to 54, further comprising a nucleic acid molecule encoding a recombinase having the amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2; preferably, the nucleic acid comprises the nucleotide sequence at least 85%, at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleotide sequence of SEQ ID NO:3; more preferably, the cell comprises a recombinant Δ E1/ Δ E3 adenovirus serotype 5 (Ad5) virus encoding the recombinase having the amino acid sequence of SEQ ID NO:2.
56. The cell of any one of claims 52 to 55, further comprising adenovirus E1A and E1B genes, preferably the cell is a 911 cell, a pTG6559 cell, a GH329 cell, a N52.E6 cell, a HeLa-E1 cell, an UR cell, a VLI-293 cell, a HEK293 cell, or a PER.C6 cell.
57. A method of producing a recombinant AAV comprising a transgene, comprising:
- (A) obtaining a first host cell comprising:
- (i) a modified AAV *rep* gene comprising, in 5' to 3' order:
- (a) a 5' portion of an AAV *rep* gene, preferably the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:55;
- (b) an artificial intron comprising, in 5' to 3' order:
- (1) a 5' intron fragment, preferably the 5' intron fragment has the nucleotide sequence of SEQ ID NO:14;

- (2) a stop cassette comprising, in 5' to 3' order:
 - (aa) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (bb) a splice acceptor, preferably the splice acceptor has the nucleotide sequence of SEQ ID NO:17;
 - (cc) a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (dd) a terminator, preferably the terminator has the nucleotide sequence of SEQ ID NO:19; and
 - (ee) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
- (3) a 3' intron fragment, preferably the 3' intron fragment has the nucleotide sequence of SEQ ID NO:15;
- (c) a 3' portion of the AAV *rep* gene, preferably the 3' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:56;
- (ii) an AAV *cap* gene, preferably the AAV *cap* gene comprises the nucleotide sequence of SEQ ID NO:57; and
- (iii) the transgene flanked by a pair of AAV ITRs, preferably, the ITR has the nucleotide sequence of SEQ ID NO:20, the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; more preferably, the promoter has the nucleotide sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23;
- (B) infecting the first host cell with a recombinant adenovirus comprising a recombinase gene encoding a recombinase having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:2 to obtain a second host cell further containing the recombinase gene;
- (C) growing the second host cell under conditions in which the recombinant AAV comprising the transgene is produced; and

- (D) optionally collecting the recombinant AAV.
58. A method of producing a recombinant AAV comprising a transgene, comprising:
- (A) obtaining a first host cell comprising:
- (i) a modified AAV *rep* gene comprising, in 5' to 3' order:
 - (a) a 5' portion of an AAV *rep* gene, preferably the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:73;
 - (b) an artificial intron comprising, in 5' to 3' order:
 - (1) a 5' intron fragment, preferably the 5' intron fragment has the nucleotide sequence of SEQ ID NO:14;
 - (2) a stop cassette comprising, in 5' to 3' order:
 - (aa) an *attP* site having the nucleotide sequence of SEQ ID NO:7;
 - (bb) a splice acceptor, preferably the splice acceptor has the nucleotide sequence of SEQ ID NO:17;
 - (cc) a gene encoding a selectable marker, preferably a neomycin phosphotransferase expression cassette having the nucleotide sequence of SEQ ID NO:18;
 - (dd) a terminator, preferably the terminator has the nucleotide sequence of SEQ ID NO:19; and
 - (ee) an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9; and
 - (3) a 3' intron fragment, preferably the 3' intron fragment has the nucleotide sequence of SEQ ID NO:66;
 - (c) a 3' portion of the AAV *rep* gene, preferably the 3' portion of the AAV *rep* gene has the nucleotide sequence of SEQ ID NO:66;
 - (ii) an AAV *cap* gene; and
 - (iii) the transgene flanked by:
 - (a) a pair of AAV ITRs, preferably, the ITR has the nucleotide sequence of SEQ ID NO:20, the transgene comprises a promoter operably linked to a coding sequence, and the coding sequence is operably linked a polyadenylation signal; more preferably, the

promoter has the nucleotide sequence of SEQ ID NO:21 and the polyadenylation signal has the nucleotide sequence SEQ ID NO:23; and

- (b) a pair of spacer sequences, preferably, the spacer sequences have a nucleotide sequence of SEQ ID NO:67 and SEQ ID NO:68;
 - (B) infecting the first host cell with a recombinant adenovirus comprising a recombinase gene encoding a recombinase having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:2 to obtain a second host cell further containing the recombinase gene;
 - (C) growing the second host cell under conditions in which the recombinant AAV comprising the transgene is produced; and
 - (D) optionally collecting the recombinant AAV.
59. The method of claim 57 or 58, wherein the first host cell further comprises a first insulator upstream of the modified AAV *rep* gene and optionally a second insulator downstream of the transgene flanked by the ITRs, preferably, the first insulator and the second insulator are independently selected from the group consisting of:
- (a) a human anti-repressor element 40 having the nucleotide sequence of SEQ ID NO:24;
 - (b) a mouse anti-repressor element 40 having the nucleotide sequence of SEQ ID NO:25;
 - (c) an anti-repressor element 04 having the nucleotide sequence of GenBank accession number AY190749.1;
 - (d) an anti-repressor element 06 having the nucleotide sequence of GenBank accession number AY190750.1;
 - (e) an anti-repressor element 07 having the nucleotide sequence of GenBank accession number AY190751.1;
 - (f) an anti-repressor element 12 having the nucleotide sequence of GenBank accession number AY190752.1;
 - (g) an anti-repressor element 13 having the nucleotide sequence of GenBank accession number AY190753.1;

- (h) an anti-repressor element 35 having the nucleotide sequence of GenBank accession number AY190754.1;
 - (i) an anti-repressor element 36 having the nucleotide sequence of GenBank accession number AY190755.1;
 - (j) an anti-repressor element 52 having the nucleotide sequence of GenBank accession number AY190757.1;
 - (k) an anti-repressor element 53 having the nucleotide sequence of GenBank accession number AY190758.1; and
 - (l) a Chicken HS4 insulator from the globin locus having the nucleotide sequence of AY040835.1 in two or more copies,
more preferably, the first insulator and the second insulator have the nucleotide sequences of SEQ ID NO:24 and SEQ ID NO:25, respectively.
60. The method of claim 59, wherein the first host cell comprises the first insulator upstream of the modified AAV *rep* gene, and further comprises a first spacer sequence and a second spacer sequence upstream and downstream of the transgene, respectively, wherein the first spacer sequence and the second spacer sequence are independently selected from the group consisting of:
- (a) a nucleotide sequence of SEQ ID NO:67; and
 - (b) a nucleotide sequence of SEQ ID NO:68.
61. The method of any one of claims 57 to 59, wherein the first host cell is obtained by introducing into a cell one or more nucleic acid molecules comprising the modified AAV *rep* gene, the AAV *cap* gene, the transgene flanked by the ITRs, the first insulator and the second insulator.
62. The method of claim 61, wherein the first host cell is obtained by introducing into the cell a nucleic acid molecule comprising, in 5' to 3' order, the first insulator, the modified AAV *rep* gene, the AAV *cap* gene, the transgene flanked by the ITRs, the first insulator and the second insulator, preferably, a plasmid comprising the nucleotide sequence of SEQ ID NO:12.

63. The method of claim 57, 58, or 60, wherein the first host cell is obtained by introducing into a cell one or more nucleic acid molecules comprising the modified AAV *rep* gene, the AAV *cap* gene, the transgene flanked by the ITRs, the first insulator, the first spacer sequence, and the second spacer sequence.
64. The method of claim 63, wherein the first host cell is obtained by introducing into a cell one or more nucleic acid molecules comprising the modified AAV *rep* gene, the AAV *cap* gene, the transgene flanked by the ITRs, the first insulator, the first spacer sequence, and the second spacer sequence, preferably, a plasmid comprising the nucleotide sequence of SEQ ID NO:70.
65. The method of any one of claims 57 to 62, wherein the recombinant adenovirus is a recombinant $\Delta E1/\Delta E3$ adenovirus serotype 5 (Ad5) virus comprising a nucleotide sequence of SEQ ID NO:3.
66. The method of any one of claims 57 to 65, wherein the host cell comprises adenovirus E1A and E1B genes, preferably the host cell is a 911 cell, pTG6559 cell, GH329 cell, N52.E6 cell, HeLa-E1 cell, UR cell, VLI-293 cell, HEK293 cell, or a PER.C6 cell.
67. The method of any one of claims 57 to 66, wherein said conditions for growing the second host cell comprise culturing the second cell with 2-aminopurine.
68. The method of claim 67, wherein the 2-aminopurine concentration is less than about 1.25 mM.
69. The method of claim 67 or 68, wherein the 2-aminopurine concentration is about 1 μ M to about 1.25 mM.
70. The method of claim 67 or 68, wherein the 2-aminopurine concentration is about 10 μ M to about 1.25 mM.
71. The method of claim 67 or 68, wherein the 2-aminopurine concentration is about 100 μ M to about 1.25 mM.

72. The method of claim 67 or 68, wherein the 2-aminopurine concentration is about 1.25 mM.
73. The method of any one of claims 67 to 72, wherein culturing the second cell with 2-aminopurine is initiated about 24 hours post-infection with the first host cell with a recombinant adenovirus.
74. A composition comprising the cell of claim 55, and 2-aminopurine.
75. The composition of claim 74, wherein the 2-aminopurine concentration is less than about 1.25 mM.
76. The composition of claim 74, wherein the 2-aminopurine concentration is about 1 μ M to about 1.25 mM.
77. The composition of claim 74, wherein the 2-aminopurine concentration is about 10 μ M to about 1.25 mM.
78. The composition of claim 74, wherein the 2-aminopurine concentration is about 100 μ M to about 1.25 mM.
79. The composition of claim 74, wherein the 2-aminopurine concentration is about 1.25 mM.
80. A non-naturally occurring nucleic acid molecule comprising a nucleotide sequence encoding a serine recombinase having an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identity to the amino acid sequence of SEQ ID NO:2.
81. The non-naturally occurring nucleic acid molecule of claim 74, comprising a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identity to the nucleotide sequence of SEQ ID NO:3.
82. A vector comprising the non-naturally occurring nucleic acid of claim 80 or 81.

83. The vector of claim 82, further comprising a promoter, preferably a cytomegalovirus (CMV) promoter operably linked to the nucleotide sequence encoding the serine recombinase.
84. The vector of claim 82 or 83, further comprising a polyadenylation signal, such as a simian virus 40 (SV40) polyadenylation signal, operably linked to the nucleotide sequence encoding the serine recombinase.
85. The vector of any one of claims 82 to 84, being a DNA plasmid.
86. The vector of any one of claims 82 to 85, being a recombinant adenoviral vector.
87. The vector of claim 86, being a recombinant $\Delta E1/\Delta E3$ adenovirus serotype 5 (Ad5) virus comprising a nucleotide sequence encoding a serine recombinase having the amino acid sequence of SEQ ID NO:2 under the control of a CMV promoter, wherein the nucleotide sequence is further operably linked to a SV40 polyadenylation signal (NC_001669.1, nt 2550 to 2774).
88. A cell comprising a non-naturally occurring nucleic acid molecule comprising a nucleotide sequence encoding a serine recombinase having an amino acid sequence having at least 85%, such as at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identity to the amino acid sequence of SEQ ID NO:2.
89. The cell of claim 88, comprising a nucleotide sequence having at least 85%, such as at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identity to the nucleotide sequence of SEQ ID NO:3.
90. A cell comprising a vector comprising the non-naturally occurring nucleic acid of claim 88 or 89.
91. The cell of claim 90, further comprising a promoter, preferably a cytomegalovirus (CMV) promoter operably linked to the nucleotide sequence encoding the serine recombinase.

92. The cell of claim 90 or 91, further comprising a polyadenylation signal, such as a simian virus 40 (SV40) polyadenylation signal, operably linked to the nucleotide sequence encoding the serine recombinase.
93. The cell of claims 90 to 92, wherein the vector is a DNA plasmid.
94. The cell of any one of claims 90 to 93, wherein the vector is a recombinant adenoviral vector.
95. The cell of claim 94, wherein the recombinant adenoviral vector is a recombinant $\Delta E1/\Delta E3$ adenovirus serotype 5 (Ad5) virus comprising a nucleotide sequence encoding a serine recombinase having the amino acid sequence of SEQ ID NO:2 under the control of a CMV promoter, wherein the nucleotide sequence is further operably linked to a SV40 polyadenylation signal (NC_001669.1, nt 2550 to 2774).
96. The cell of claims 88 to 95, comprising adenovirus E1A and E1B genes, preferably the cell is a 911 cell, pTG6559 cell, GH329 cell, N52.E6 cell, HeLa-E1 cell, UR cell, VLI-293 cell, HEK293 cell, or a PER.C6 cell.
97. A method of conducting a site-specific recombination in a cell, comprising:
- (a) obtaining a cell comprising a nucleic acid molecule having an *attP* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO:7, preferably, an *attP* site having the nucleotide sequence of SEQ ID NO:7, and an *attB* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical of SEQ ID NO:8 or SEQ ID NO:9, preferably, an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9;
 - (b) introducing to the cell a non-naturally occurring nucleic acid molecule encoding a serine recombinase having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to SEQ ID NO:2; and
 - (c) growing the cell under conditions to allow the serine recombinase to catalyze the site-specific recombination between the *attP* and *attB* sites.

98. A product produced by the process of conducting a site-specific recombination in a cell, comprising:
- (a) obtaining a cell comprising a nucleic acid molecule having an *attP* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO:7, preferably, an *attP* site having the nucleotide sequence of SEQ ID NO:7, and an *attB* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical of SEQ ID NO:8 or SEQ ID NO:9, preferably, an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9;
 - (b) introducing to the cell a non-naturally occurring nucleic acid molecule encoding a serine recombinase having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to SEQ ID NO:2; and
 - (c) growing the cell under conditions to allow the serine recombinase to catalyze the site-specific recombination between the *attP* and *attB* sites.
99. A process for obtaining a product from a cell, comprising:
- (a) obtaining a cell comprising a nucleic acid molecule having an *attP* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO:7, preferably, an *attP* site having the nucleotide sequence of SEQ ID NO:7, and an *attB* site having a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical of SEQ ID NO:8 or SEQ ID NO:9, preferably, an *attB* site having the nucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9;
 - (b) introducing to the cell a non-naturally occurring nucleic acid molecule encoding a serine recombinase having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to SEQ ID NO:2;
 - (c) growing the cell under conditions to allow the serine recombinase to catalyze the site-specific recombination between the *attP* and *attB* sites; and
 - (d) producing and recovering from the cell a product.
100. A non-naturally occurring system, comprising:
a means for AAV mediated recombination, wherein the means optionally

comprises a transgenic element.

101. A means for transferring the non-naturally occurring system of claim 100.
102. A non-naturally occurring system, comprising:
 - a recombination means for recombining the system of claim 100, wherein the recombination means includes using at least one serine residue during catalysis.
103. A means for transferring the non-naturally occurring system of claim 102.
104. A means for manufacturing a molecule, wherein the means for manufacturing a molecule comprises the means of any one of claims 100 to 103 and is capable of replication.
105. A process for AAV mediated site-specific recombination, comprising:
 - (a) a step for performing a function of obtaining a cell comprising the means of claim 100;
 - (b) a step for performing a function of growing the cell under conditions to allow site-specific recombination using at least one serine residue during catalysis.
106. The process for AAV mediated site-specific recombination of claim 105, comprising obtaining a product, wherein, optionally the product is a therapeutic product.