

CLAIMS

1. An adeno-associated virus genome that has a mutation that reduces expression of full-length wild-type membrane associated accessory protein (MAAP) yet maintains expression of VP1.
2. An adeno-associated virus genome that transcribes to MAAP mRNA, the genome
5 having a mutation whereby the MAAP mRNA is altered from wild-type MAAP mRNA, the alteration selected from: changing the MAAP translation initiation codon to a sequence that is not an initiation codon, and creating at least one stop codon in the MAAP mRNA; wherein said mutation does not prevent expression of VP1 from said genome.
- 10 3. An adeno-associated virus genome that has a mutation that inactivates the MAAP mRNA translation initiation codon and/or that introduces at least one stop codon to stop translation of full-length wild-type MAAP.
4. The adeno-associated virus genome of claim 3, that has a mutation that inactivates the MAAP mRNA translation initiation codon and further comprises at least
15 one mutation that introduces at least one stop codon to stop translation of full-length wild-type MAAP.
5. The adeno-associated virus genome of claim 1 or 2, wherein said mutation inactivates the MAAP translation initiation codon and/or introduces at least one stop codon to stop translation of full-length wild-type MAAP.
- 20 6. The adeno-associated virus genome of any one of claims 1 to 5, wherein said mutation introduces at least one stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 from residue numbers 9 to 110, preferably from residues numbers 39 to 103.
7. The adeno-associated virus genome of claim 6, wherein said mutation introduces
25 at least one stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 9, 33, 39, 47, 65, 90, 100, 103, 105, 106 or 110.
8. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning
30 with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 9.
9. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 33.
10. The adeno-associated virus genome of claim 6, wherein said mutation introduces
35 a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 39.

11. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 47.
- 5 12. The adeno-associated virus genome of claim 6, wherein said mutation introduces stop codons to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue numbers 33, 39 and 47.
- 10 13. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 65.
14. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 90.
- 15 15. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 100.
16. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 103.
- 20 17. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 105.
18. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 106.
- 25 19. The adeno-associated virus genome of claim 6, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 110.
20. The adeno-associated virus genome of any one of claims 1-19, where the genome is selected from the group consisting of: a naturally-occurring serotype and a non-naturally-occurring serotype.
- 30 21. The adeno-associated virus genome of claim 20, where the genome is a non-naturally-occurring serotype.
22. The adeno-associated virus genome of claim 20, where the genome is a naturally-occurring serotype.
- 35 23. The adeno-associated virus genome of claim 22, where the genome comprises a serotype 2 genome.
24. The adeno-associated virus genome of claim 22, where the genome comprises a serotype 5 genome.

25. The adeno-associated virus genome of claim 22, where the genome is selected from a serotype 6 genome and a serotype 8 genome.
26. The adeno-associated virus genome of claim 22, where the genome is selected from a serotype 1 genome and a serotype 9 genome.
- 5 27. The adeno-associated virus genome of claim 22, where the genome is selected from a serotype 1 genome, serotype 2 genome, serotype 5 genome, serotype 6 genome, serotype 8 genome and a serotype 9 genome.
28. The adeno-associated virus genome of claim 21, where the genome comprises a non-naturally-occurring serotype.
- 10 29. The adeno-associated virus genome of any one of claims 2-28, wherein the VP1 peptide sequence is unaltered from wild type.
30. The adeno-associated virus genome of any one of claims 2-28, wherein the VP1 peptide sequence contains a mutation.
31. The adeno-associated virus genome of any one of claims 2-28, wherein the MAAP and VP1 peptide sequences each have at least 80% homology to wild type.
- 15 32. The adeno-associated virus genome of claim 31, wherein the MAAP and VP1 peptide sequences each have at least 90% homology to wild type.
33. An adeno-associated virus genome that does not express a polypeptide having a primary amino acid sequence having at least 50% homology to any 33 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
- 20 34. The adeno-associated virus genome of claim 33, where the 33 contiguous residues comprise MAAP consensus polypeptide sequence SEQ. ID NO. 11 residues 93 to 97.
35. The adeno-associated virus genome of claim 33, where the 33 contiguous residues comprise MAAP consensus polypeptide sequence SEQ. ID NO. 11 residues 107 to 119.
- 25 36. The adeno-associated virus genome of claim 33 where the 33 contiguous residues comprise MAAP consensus polypeptide sequence SEQ. ID NO. 11 residues 1 to 30.
37. The adeno-associated virus genome of claim 33, free of any sequence having at least 60% homology to MAAP consensus polypeptide sequence SEQ. ID NO. 11 residues 1 to 33.
- 30 38. The adeno-associated virus genome of claim 33, free of any sequence having at least 60% homology to MAAP consensus polypeptide sequence SEQ. ID NO. 11 residues 1 to 39.
39. The adeno-associated virus genome of claim 33, free of any sequence having at least 60% homology to MAAP consensus polypeptide sequence SEQ. ID NO. 11 residues 1 to 47.
- 35 40. The adeno-associated virus genome of claim 33, free of any sequence having at least 60% homology to any 30 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.

41. The adeno-associated virus genome of claim 40, free of any sequence having at least 70% homology to any 30 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
- 5 42. The adeno-associated virus genome of claim 40, free of any sequence having at least 80% homology to any 30 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
- 10 43. The adeno-associated virus genome of claim 33, wherein the genome does not express a polypeptide having a primary amino acid sequence having at least 95% homology to any 15 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
44. The adeno-associated virus genome of claim 43, where the genome does not express a polypeptide having a primary amino acid sequence having at least 90% homology to any 17 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
- 15 45. The adeno-associated virus genome of claim 43, where the genome does not express a polypeptide having a primary amino acid sequence having at least 90% homology to any 19 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
- 20 46. The adeno-associated virus genome of claim 43, where the genome does not express a polypeptide having a primary amino acid sequence having at least 90% homology to any 21 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11.
- 25 47. The adeno-associated virus genome of claim 33, wherein the genome does not express a polypeptide having a primary amino acid sequence having at least 50% homology to any 10 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11 residue numbers 94 to 120.
48. A producer cell that produces adeno-associated virus, the producer cell comprising the adeno-associated virus genome of any one of claims 1-47.
- 30 49. The producer cell of claim 48, wherein the producer cell is eukaryotic.
50. The producer cell of claim 49, wherein the producer cell is mammalian.
51. The producer cell of claim 50, wherein the producer cell is a human cell.
52. The producer cell of claim 49, wherein the producer cell is selected from of yeast cells and insect cells.
- 35 53. A method for producing adeno-associated virus, the method comprising: obtaining an adeno-associated virus genome, and then introducing said genome into a cell to create the producer cell of any of claims 48-52, and then culturing said producer cell whereby said producer cell produces adeno-associated virus.
54. The method of claim 53, further comprising harvesting said adeno-associated virus, where said harvested adeno-associated virus comprises a transgene.

55. The method of claim 53, where the producer cell produces virus preparation wherein the ratio of the number of capsids containing the gene or genome of interest to the number of total physical capsids is at least as high as the ratio of the number of capsids containing the gene or genome of interest to the number of total physical capsids produced by a similar cell containing a wild-type adeno-associated virus genome.
56. The method of claim 53, where the producer cell produces virus having a ratio of full : empty virus capsids least as high as does a similar cell infected with a wild-type adeno-associated virus genome.
57. The method of claim 56, where the producer cell produces virus having a ratio of full : empty virus capsids 30% higher than does a similar cell infected with wild-type adeno-associated virus.
58. The method of claim 53, where the producer cell produces virus having at least as many viral genomes / mL as does a similar cell infected with wild-type adeno-associated virus.
59. The method of claim 58, where the producer cell produces virus having at least four times as many viral genomes / mL as does a similar cell infected with wild-type adeno-associated virus.
60. Adeno-associated virus produced by the process of claim 53.
61. A producer cell that produces adeno-associated virus, the producer cell substantially free of polypeptide having:
- (a) at least 50% homology to any 30 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11;
 - (b) at least 95% homology to any 15 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11;
 - (c) at least 50% homology to any 10 contiguous residues of MAAP consensus polypeptide sequence SEQ. ID NO. 11 residue numbers 94 to 120.
62. A method for producing adeno-associated virus, the method comprising culturing the producer cell of claim 61, whereby the producer cell produces adeno-associated virus, and then harvesting said adeno-associated virus.
63. Adeno-associated virus produced by the method of claim 62.
64. A producer cell comprising an adeno-associated virus genome, the producer cell able to express adeno-associated virus, the producer cell substantially free of full-length functional MAAP.
65. A method for producing adeno-associated virus, the method comprising culturing the producer cell of claim 64 whereby the producer cell produces adeno-associated virus, and then harvesting said adeno-associated virus.
66. Adeno-associated virus produced by the method of claim 65.
67. The producer cell of claim 64, where said adeno-associated virus genome has a mutation that interferes with the expression of full-length, wild-type functional MAAP.

68. A method for producing adeno-associated virus, the method comprising culturing the producer cell of claim 67 whereby the producer cell produces adeno-associated virus, and then harvesting said adeno-associated virus.

69. Adeno-associated virus produced by the method of claim 68.

5 70. The producer cell of claim 64, said producer cell comprising interfering RNA that interferes with the expression of full-length, wild-type functional MAAP.

71. A method for producing adeno-associated virus, the method comprising culturing the producer cell of claim 70 whereby the producer cell produces adeno-associated virus, and then harvesting said adeno-associated virus.

10 72. Adeno-associated virus produced by the method of claim 71.

73. The producer cell of claim 64, said producer cell comprising a protein, such as a monoclonal antibody or affibody, directed against MAAP that binds to MAAP and impairs the function of MAAP.

15 74. A method for producing adeno-associated virus, the method comprising culturing the producer cell of claim 73 whereby the producer cell produces adeno-associated virus, and then harvesting said adeno-associated virus.

75. Adeno-associated virus produced by the method of claim 71.

20 76. A method of increasing stability, increasing capsid integrity, or reducing capsid degradation of an adeno-associated virus (AAV), comprising including in the AAV the adeno-associated virus genome of any one of claims 1-47.

77. A method of increasing the proportion of AAV capsids containing a gene or genome of interest, comprising including in the AAV the adeno-associated virus genome of any one of claims 1-47 and the gene or genome of interest.

25 78. A method of the increasing the viral titre (viral genomes / mL) of a producer cell producing an AAV, comprising including in the AAV the adeno-associated virus genome of any one of claims 1-47 and introducing the AAV in the producer cell.

79. The method of claim 53, wherein the producer cell is cultured for at least 30 hours.

30 80. The method of claim 79, wherein the producer cell is cultured for at least 36 hours, 48 hours, 72 hours or 96 hours.

81. A method for increasing the retention of viral genomes or viral particles in a producer cell producing an AAV, comprising including in the AAV the adeno-associated virus genome of any one of claims 1-47 and introducing the AAV in the producer cell.

35 82. The method of claim 81, further comprising harvesting and/or purifying the viral genomes or viral particles from the producer cells, preferably substantially free of media.

CLAIMS

1. An adeno-associated virus genome that has a mutation that reduces expression of full-length wild-type membrane associated accessory protein (MAAP) yet maintains expression of VP1, wherein said mutation introduces at least one stop codon in the MAAP mRNA.
2. The adeno-associated virus genome of claim 1, that has a mutation that inactivates the MAAP mRNA translation-initiation codon and further comprises at least one mutation that introduces at least one stop codon to stop translation of full-length wild-type MAAP.
3. The adeno-associated virus genome of any one of claims 1 to 2, wherein said mutation introduces at least one stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 from residue numbers 9 to 110, preferably from residues numbers 39 to 103.
4. The adeno-associated virus genome of claim 3, wherein said mutation introduces at least one stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 9, 33, 39, 47, 65, 90, 100, 103, 105, 106 or 110.
5. The adeno-associated virus genome of claim 3, wherein said mutation introduces a stop codon to stop translation of MAAP polypeptide at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 11 residue number 100.
6. The adeno-associated virus genome of any one of claims 1-5, where the genome is a naturally-occurring serotype.
7. The adeno-associated virus genome of claim 6, where the genome is selected from a serotype 1 genome, serotype 2 genome, serotype 5 genome, serotype 6 genome, serotype 8 genome and a serotype 9 genome.
8. The adeno-associated virus genome of any one of claims 1-7, wherein the VP1 peptide sequence (a) is unaltered from wild type, or (b) contains a mutation.
9. The adeno-associated virus genome of any one of claims 1-7, wherein the MAAP and VP1 peptide sequences each have at least 80% homology to wild type.
10. A producer cell that produces adeno-associated virus, the producer cell comprising the adeno-associated virus genome of any one of claims 1-9.
11. The producer cell of claim 10, wherein the producer cell is eukaryotic, mammalian or human.
12. The producer cell of claim 11, wherein the producer cell is selected from of yeast cells and insect cells.
13. A method for producing adeno-associated virus, the method comprising: obtaining an adeno-associated virus genome, and then introducing said genome into a

cell to create the producer cell of any of claims 10-12, and then culturing said producer cell whereby said producer cell produces adeno-associated virus.

14. The method of claim 13, further comprising harvesting said adeno-associated virus, where said harvested adeno-associated virus comprises a transgene.

15. The method of claim 13, where the producer cell produces virus having a ratio of full : empty virus capsids 30% higher than does a similar cell infected with wild-type adeno-associated virus.

16. The method of claim 13, where the producer cell produces virus having at least as many viral genomes / mL as does a similar cell infected with wild-type adeno-associated virus.

17. Adeno-associated virus produced by the process of claim 13.

18. A method of increasing stability, increasing capsid integrity, or reducing capsid degradation of an adeno-associated virus (AAV), comprising including in the AAV the adeno-associated virus genome of any one of claims 1-9.

19. A method of increasing the proportion of AAV capsids containing a gene or genome of interest, comprising including in the AAV the adeno-associated virus genome of any one of claims 1-9 and the gene or genome of interest.

20. A method of the increasing the viral titre (viral genomes / mL) of a producer cell producing an AAV, comprising including in the AAV the adeno-associated virus genome of any one of claims 1-9 and introducing the AAV in the producer cell.

21. The method of claim 20, wherein the producer cell is cultured for at least 30 hours, at least 36 hours, at least 48 hours, at least 72 hours, at least 96 hours.

22. A method for increasing the retention of viral genomes or viral particles in a producer cell producing an AAV, comprising including in the AAV the adeno-associated virus genome of any one of claims 1-9 and introducing the AAV in the producer cell.

23. The method of claim 22, further comprising harvesting and/or purifying the viral genomes or viral particles from the producer cells, preferably substantially free of media.