CLAIMS

1. A two-plasmid system comprising a helper plasmid and a vector plasmid, wherein the helper plasmid comprises at least one rep gene encoding at least one functional Rep protein and does not comprise a cap gene encoding a functional set of Cap proteins.

2. The two-plasmid system of claim 1, wherein the two-plasmid system comprises a molar excess of vector plasmid compared to helper plasmid.

3. The two-plasmid system of claim 1 or 2, wherein the ratio of helper plasmid to vector plasmid is between 3:1 to 1:10, between 1.5:1 and 1:9, between 1.4:1 and 1:8, between 1.3:1 and 1:7; between 1.2:1 and 1:6; between 1.1:1 and 1:5; between 1:1 and 1:4; or between 1:1.5 and 1:3.

4. The two-plasmid system of any one of claims 1-3, wherein the ratio of helper plasmid to vector plasmid is between 1:2 and 1:4; or around 1:3.

5. The two-plasmid system of any one of claims 1-4, wherein the vector plasmid comprises:

(a) a cap gene encoding at least one functional Cap protein; or

(b) at least one cap gene promoter, a cloning site operably linked to the cap gene promoter, and an expression cassette flanked on at least one side by an ITR; wherein the vector plasmid does not comprise a rep gene encoding a functional Rep protein and the expression cassette comprises a transgene operably linked to at least one regulatory control element.

6. A helper plasmid comprising at least one rep gene encoding at least one functional Rep protein and at least one helper virus gene, and which does not comprise a cap gene encoding a functional set of Cap proteins.

7. The two-plasmid system of any one of claims 1-5 or the helper plasmid of claim 6, wherein the at least one rep gene comprises a gene encoding a functional Rep 52 protein, at least one gene encoding a functional Rep 40 protein, and a gene encoding a functional Rep 68 protein.

8. The two-plasmid system of any one of claims 1-5 or 7 or the helper plasmid of claim 6 or 7, wherein the at least one rep gene does not comprise a functional internal p40 promoter.

9. The two-plasmid system of any one of claims 1-5 or 7-8 or the helper plasmid of any one of claims 6-8, wherein:

(i) the at least one rep gene comprises a C nucleotide at a position corresponding to position 1823 of SEQ ID NO: 1;

(ii) the helper plasmid does not comprise a contiguous stretch of exclusively cap gene sequence of more than 250 nucleotides, more than 100 nucleotides, or more than 60 nucleotides; and/or

(iii) the helper plasmid comprises a portion of cap gene sequence, and the portion of cap gene sequence does not encode a functional set of Cap proteins.

10. The two-plasmid system of any one of claims 1-5 or 7-9 or the helper plasmid of any one of claims 6-9, wherein the helper plasmid comprises at least one helper virus gene, optionally wherein:

(i) the at least one helper virus gene is an adenovirus gene, optionally an Adenovirus 5 or Adenovirus 2 gene;

(ii) the at least one helper virus gene comprises a VA nucleic acid encoding functional VA RNA I and II, an E2A gene encoding a functional E2A protein and an E4 gene encoding a functional E4 protein;

wherein:

(iii) the at least one helper virus gene comprises a VA nucleic acid encoding functional VA RNA I and II, an E2A gene encoding a functional E2A protein and

an E4 gene encoding a functional E4 protein, and wherein the E4 gene is not located between the VA nucleic acid and the E2A gene;
(iv) the helper plasmid is less than 25000 bp, less than 20000 bp, less than 15000 bp, less than 14500 bp, between 10000 bp and 25000 bp, between 10000 bp and 20000 bp, between 12000 bp and 15000 bp, or around 14021 bp in length;
(v) the helper plasmid and/or the vector plasmid does not comprise an artificial Rep binding site; and/or
(vi) the helper plasmid and/or the vector plasmid comprises a plasmid backbone, and the plasmid backbone does not comprise an artificial Rep binding site.

11. A vector plasmid comprising:
(a) a cap gene encoding at least one functional Cap protein; or
(b) at least one cap gene promoter, a cloning site operably linked to the cap gene promoter, and an expression cassette flanked on at least one side by an ITR;
wherein the vector plasmid does not comprise a rep gene encoding a functional Rep protein and the expression cassette comprises a transgene operably linked to at least one regulatory control element.

12. The two-plasmid system of any one of claims 1-5 or 7-10 or the vector plasmid of claim 11, wherein the vector plasmid comprises a cap gene and further comprises an expression cassette flanked on at least one side by an ITR.

13. The two-plasmid system of any one of claims 1-5, 7-10 or 12 or the vector plasmid of claim 11 or 12, wherein the vector plasmid comprises a cap gene and the cap gene encodes a Cap protein selected from the group of AAV serotypes consisting of serotypes 2, 5, 8, 9, and Mut C (SEQ ID NO: 3 from WO 2016/181123).

14. The two-plasmid system of any one of claims 1-5, 7-10 or 12-14 or the vector plasmid of any one of claims 11-13, wherein:

(i) the vector plasmid does not comprise any dispensable translation initiation codons; and/or

(ii) the vector plasmid does not comprise any dispensable translation initiation codons, wherein the vector plasmid comprises a promoter region comprising one or more promoters, and the promoter region does not comprise ATG or GTG codons, optionally wherein the promoter region comprises p5, p19 and p40 promoters, and wherein ATG or GTG codons at one or more positions corresponding to positions (a) 321-323, (b) 766-768, (c) 955-957, (d) 993-995 and (e) 1014-1016 of SEQ ID NO: 1 are absent or mutated.

15. Use of the two-plasmid system as defined in any one of claims 1-5, 7-10 or 12-14, the helper plasmid as defined in any one of claims 6-10 or the vector plasmid as defined in any one of claims 11-14 for producing a recombinant AAV preparation:

(a) having a desired ratio of full to total particles; and/or

(b) at a high or desired yield.

16. Use of the two-plasmid system, the helper plasmid or the vector plasmid as defined in any one of claims 1-15 for:

(a) controlling or maximising the ratio of full to total particles produced during recombinant AAV production; and/or

(b) increasing, optimising or maximising the yield of recombinant AAV produced during recombinant AAV production.

17. The use of claim 15 or 16, wherein the use comprises transfecting a host cell with the two-plasmid system as defined in any one of claims 1-5, 7-10 or 12-14, the helper plasmid as defined in any one of claims 6-10 or the vector plasmid of any one of claims 11-14 and culturing the host cell under conditions suitable for recombinant AAV production.

18. A method for controlling or maximising the ratio of full to total particles produced during recombinant AAV production comprising:

(a) obtaining the two-plasmid system as defined in any one of claims 1-5, 7-10 or 12-14, the helper plasmid as defined in any one of claims 6-10 or the vector plasmid as defined in any one of claims 11-14;

(b) transfecting a host cell with the two-plasmid system as defined in any one of claims 1-5, 7-10 or 12-14, the helper plasmid as defined in any one of claims 6-10 or the vector plasmid as defined in any one of claims 11-14; and

(c) culturing the host cell under conditions suitable for recombinant AAV production.

19. The method of claim 18, further comprising a step of harvesting the recombinant AAV to provide a recombinant AAV preparation comprising a desired ratio of full to total particles.

20. The method of claim 18 or 19, wherein the method is a method for producing a recombinant AAV preparation at a high or desired yield.

21. A method for increasing, optimising or maximising the yield of recombinant AAV produced during recombinant AAV production comprising:

(a) obtaining the two-plasmid system as defined in any one of claims 1-5, 7-10 or 12-14, the helper plasmid as defined in any one of claims 6-10 or the vector plasmid as defined in any one of claims 11-14;

(b) transfecting a host cell with the two-plasmid system as defined in any one of claims 1-5, 7-10 or 12-14, the helper plasmid as defined in any one of claims 6-10 or the vector plasmid as defined in any one of claims 11-14; and

(c) culturing the host cell under conditions suitable for recombinant AAV production.

22. The method of claim 21, further comprising a step of harvesting the recombinant AAV to provide a recombinant AAV preparation comprising a high or desired yield of recombinant AAV.

23. The use or method of any one of claims 15, 17 or 20, wherein the high or desired yield is a yield that is at least 2-fold, at least 4-fold, at least 5-fold, or at least 6-fold higher than the yield achieved using an equivalent method with a ratio of helper plasmid to vector plasmid of 1.8:1.

24. The use or method of any one of claims 15, 17, 19, 20 or 23, wherein the desired ratio of full to total particles is a ratio of full to total particles that is at least 20% or at least 30% of the ratio of full to total particles achieved using an equivalent method with a ratio of helper plasmid to vector plasmid of 1.8:1.

25. The use or method of any one of claims 15-20, 23 or 24, wherein the ratio of helper plasmid to vector plasmid is between 3:1 to 1:10, between 1.5:1 and 1:9, between 1.4:1 and 1:8, between 1.3:1 and 1:7; between 1.2:1 and 1:6; between 1.1:1 and 1:5; between 1:1 and 1:4; between 1:1.5 and 1:3; between 1:2 and 1:4; or around 1:3.