Summary

The present disclosure relates to a two-plasmid system for manufacturing of rAAV, wherein at least some of the adenovirus helper gene functions are combined with AAV rep on one plasmid, and AAV cap is combined with the rAAV vector genome on a second plasmid. The 'trans-split Rep-Cap' two-plasmid system is superior over known systems in achieving a combination of improved economics resulting from the simpler and more flexible format, with enhanced safety and quality attributes in addition to other surprising advantages as disclosed herein.

Accordingly, in a first example of the disclosure, there is provided 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 Cap protein. In a first aspect of the disclosure, there is provided a helper plasmid comprising at least one adeno-associated virus (AAV) rep gene encoding at least one functional AAV Rep protein and at least one helper virus gene, and which does not comprise a cap gene encoding a functional set of Cap proteins.

In a second example of the disclosure, there is provided a two-plasmid system comprising a helper plasmid and a vector plasmid, wherein the helper plasmid comprises at least one helper virus gene and does not comprise a cap gene encoding a functional Cap protein, and the at least one helper virus gene is comprised in a contiguous stretch of the plasmid having (on one strand) at least 95%, at least 98%, at least 99%, or 100% identity to the full length or to a fragment at least 6000, at least 7000, or at least 8000 nucleotides in length of SEQ ID NO: 4.

Furthermore, the present disclosure relates to helper plasmids and vector plasmids that are designed for use within a two-plasmid system of the disclosure.

Accordingly, in a third example of the disclosure, there is provided 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 Cap protein.

Similarly, in a fourth example of the disclosure, there is provided a helper plasmid comprising at least one helper virus gene and which does not comprise a cap gene encoding a functional Cap protein, wherein the at least one helper virus gene is comprised in a contiguous stretch of the plasmid having at least 95%, at least 98%, at least 99%, or 100% identity to the full length or to a fragment at least 6000, at least 7000, or at least 8000 nucleotides in length of SEQ ID NO: 4.

Furthermore, in a fifth example of the disclosure, there is provided 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.

In a sixth example of the disclosure, there is provided a host cell comprising the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure.

The present disclosure also relates to the use of such two-plasmid systems, helper plasmids and vector plasmids in the manufacture of a rAAV preparation. The plasmids of the disclosure may be used to obtain rAAV preparations having a low level of rcAAV, having a desired ratio of full to total particles, and/or at a high or desired yield.

In a seventh example of the disclosure, there is provided a use of the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure for producing a rAAV preparation:

(a) having a low level of replication competent (rcAAV);

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

(c) at a high or desired yield.

In an eighth example of the disclosure, there is provided a use of the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure for:

(a) reducing or minimising the level of replication competent (rcAAV) produced during rAAV production;
(b) reducing or minimising the level of pseudo-wild type replication competent AAV (rcAAV) produced during recombinant AAV production;
(c) controlling or maximising the ratio of full to total particles produced during rAAV production; and/or
(d) increasing, optimising or maximising the yield of rAAV produced during rAAV production.

In a ninth example of the disclosure, there is provided a method for producing a rAAV preparation comprising:

(a) obtaining the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure;
(b) transfecting a host cell with the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure; and
(c) culturing the host cell under conditions suitable for rAAV production.

In a tenth example of the disclosure, there is provided a method for reducing or minimising the level of replication competent AAV (rcAAV) produced during rAAV production comprising:

(a) obtaining the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure;
(b) transfecting a host cell with the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure; and
(c) culturing the host cell under conditions suitable for rAAV production.

In an eleventh example of the disclosure, there is provided a method for controlling or maximising the ratio of full to total particles produced during rAAV production comprising:

(a) obtaining the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure;
(b) transfecting a host cell with the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure; and
(c) culturing the host cell under conditions suitable for rAAV production.

In a twelfth example of the disclosure, there is provided a method for increasing, optimising or maximising the yield of rAAV produced during rAAV production comprising:

(a) obtaining the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure;
(b) transfecting a host cell with the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure; and
(c) culturing the host cell under conditions suitable for rAAV production.

In a thirteenth example of the disclosure, there is provided a rAAV preparation obtainable by the methods of the disclosure.

In a fourteenth example of the disclosure, there is provided a rAAV preparation obtained by the methods of the disclosure.

In a fifteenth example of the disclosure, there is provided a method for reducing or minimising the level of pseudo-wild type replication competent AAV (rcAAV) produced during rAAV production comprising:

(a) obtaining the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure;
(b) transfecting a host cell with the two-plasmid system, the helper plasmid or the vector plasmid of the disclosure; and
(c) culturing the host cell under conditions suitable for rAAV production.

CLAIMS

1. A helper plasmid comprising at least one adeno-associated virus (AAV) rep gene encoding at least one functional AAV Rep protein and at least one helper virus gene, and which does not comprise a cap gene encoding a functional set of Cap proteins.

2. A two-plasmid system comprising the helper plasmid of claim 1 and a vector plasmid.

3. The two-plasmid system of claim 2, wherein:
(i) the vector plasmid comprises:
(a) an AAV cap gene encoding at least one functional AAV Cap protein; or
(b) at least one AAV cap gene promoter, a cloning site operably linked to the AAV cap gene promoter, and an expression cassette flanked on at least one side by an inverted terminal repeat (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; and/or
(ii) the ratio of helper plasmid to vector plasmid is between 1:1 and 1:4.

4. The two-plasmid system or helper plasmid of any one of the preceding claims , wherein the at least one AAV 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.

5. The two-plasmid system or helper plasmid of any one of the preceding claims, wherein:
(i) the at least one AAV rep gene comprises a gene encoding a functional Rep 52 protein and the gene encoding a functional Rep 52 protein comprises a nucleic acid sequence having at least 98% identity to the full length or a fragment of at least 1000 nucleotides in

length of nucleotides 993-2186 of SEQ ID NO: 1, or to a corresponding stretch of nucleotides in a different serotype of AAV; and/or

(ii) (a) the at least one AAV rep gene comprises a gene encoding a functional Rep 40 protein and the gene encoding a functional Rep 40 protein comprises a nucleic acid sequence having at least 98% identity to the full length or to a fragment of at least 800 nucleotides in length of a stretch of nucleotides corresponding to nucleotides 993-2252 minus nucleotides 1907-2227 of SEQ ID NO: 1, or to corresponding stretches of nucleotides in a different serotype of AAV; and/or

(b) the at least one AAV rep gene comprises a gene encoding a functional Rep 40 protein and the gene encoding a functional Rep 40 protein comprises a nucleic acid sequence having at least 98% identity to the full length or to a fragment of at least 1100 nucleotides in length of nucleotides 993-2252 of SEQ ID NO: 1, or to a corresponding stretch of nucleotides in a different serotype of AAV; and/or

(iii) the at least one AAV rep gene comprises a gene encoding a functional Rep 68 protein and the gene encoding a functional Rep 68 protein comprises a nucleic acid sequence having at least 98% identity to the full length or to a fragment of at least 1500 nucleotides in length of a stretch of nucleotides corresponding to nucleotides 321-2252 minus nucleotides 1907-2227 of SEQ ID NO: 1, or to corresponding stretches of nucleotides in a different serotype of AAV.

6. The two-plasmid system or helper plasmid of any one of the preceding claims, wherein:

(i) the helper plasmid does not comprise a gene encoding a functional Rep 78 protein; and/or

(ii) the helper plasmid does not comprise a contiguous sequence of at least 1800 nucleotides corresponding to a contiguous stretch of nucleotides of equivalent length comprised within nucleotides 321-2186 of SEQ ID NO: 1 or within a corresponding stretch of nucleotides in a different serotype of AAV; and/or

(iii) the at least one AAV rep gene does not comprise a functional internal p40 promoter; and/or

(iv) the helper plasmid does not comprise a contiguous stretch of exclusively cap gene sequence of more than 60 nucleotides.

7. The two-plasmid system or helper plasmid of any one of the preceding claims, wherein:

(i) the helper plasmid comprises at least one helper virus gene and the at least one helper virus gene comprises a viral associated (VA) nucleic acid, an E2A gene and an E4 gene;

and/or:

(ii) the helper plasmid comprises at least one helper virus gene and the at least one helper virus gene comprises a VA nucleic acid, an E2A gene and an E4 gene and the E4 gene is not located between the VA nucleic acid and the E2A gene; and/or

(iii) the helper plasmid comprises at least one helper virus gene and the helper plasmid between 12000 bp and 15000 bp in length; and/or

(iv) the helper plasmid does not comprise a contiguous sequence of at least 3000 nucleotides of a contiguous stretch of nucleotides of equivalent length comprised within nucleotides 194-3620 of SEQ ID NO: 2, or a corresponding stretch of nucleotides in a different serotype of adenovirus ; and/or(v) the helper plasmid does not comprise a contiguous sequence of at least 60 nucleotides of a contiguous stretch of nucleotides of equivalent length comprised within nucleotides 4032-4100 of SEQ ID NO: 2, or a corresponding stretch of nucleotides in a different serotype of adenovirus; and/or

(vi) the helper plasmid does not comprise a contiguous sequence of at least 350 nucleotides of a contiguous stretch of nucleotides of equivalent length comprised within nucleotides 4051-4413 of SEQ ID NO: 1, or a corresponding stretch of nucleotides in a different serotype of AAV.

8. The two-plasmid system or helper plasmid of any one of the preceding claims, wherein:

(i) the helper plasmid does not comprise a contiguous sequence of at least 600 nucleotides of a contiguous stretch of nucleotides of equivalent length comprised within nucleotides 2301-2947 of SEQ ID NO: 1, or a corresponding stretch of nucleotides in a different serotype of AAV; and/or

(ii) the helper plasmid comprises at least one helper virus gene and the at least one helper virus gene comprises a VA nucleic acid, an E2A gene and an E4 gene, and the VA nucleic acid, the E2A gene and the E4 gene are comprised within a contiguous portion of fewer than 10000 nucleotides; and/or

(iii) the helper plasmid does not comprise a contiguous sequence of at least 22000 nucleotides of a contiguous stretch of nucleotides of equivalent length comprised within nucleotides 10619-32756 of SEQ ID NO: 2, or a corresponding stretch of nucleotides in a different serotype of adenovirus; and/or

(iv) the helper plasmid and/or the vector plasmid does not comprise an artificial Rep binding site.

9. The two-plasmid system or helper plasmid of any one of the preceding claims, wherein the helper plasmid comprises at least one helper virus gene and the at least one helper virus gene is comprised in a contiguous stretch of the plasmid having at least 98% identity to the full length or to a fragment of at least 8000 nucleotides in length of SEQ ID NO: 4.

10. The two-plasmid system of any one of claims 2-9, wherein:

(i) the vector plasmid comprises an at least one AAV cap gene promoter, which comprises an AAV p40 promoter, a p5 promoter, and a p19 promoter; and/or

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

(iii) the vector plasmid comprises a promoter region comprising one or more promoters, and in the promoter region:

(a) nucleotides corresponding to nucleotides 321-323 of SEQ ID NO: 1 are absent,

(b) nucleotides corresponding to nucleotides 766-768 of SEQ ID NO: 1 are not ATG,

(c) nucleotides corresponding to nucleotides 955-957 of SEQ ID NO: 1 are absent,

(d) nucleotides corresponding to nucleotides 993-995 of SEQ ID NO: 1 are absent and

(e) nucleotides corresponding to nucleotides 1014-1016 of SEQ ID NO: 1 are absent; and/or

(iv) the vector plasmid comprises a backbone less than 3000 nucleotides in length..

11. The two plasmid system of claim 10(iii)(b), wherein nucleotides corresponding to nucleotides 766-768 of SEQ ID NO: 1 are ATT.

12. A method for producing a recombinant AAV preparation comprising:

(a) obtaining the two-plasmid system, the helper plasmid or the vector plasmid as defined in any one of claims 1-11;

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

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

13. The method of claim 12:

(i) further comprising a step of harvesting the recombinant AAV to provide a recombinant AAV preparation; and/or

(ii) wherein the recombinant AAV preparation comprises a low level of replication competent AAV (rcAAV); and/or

(iii) wherein the level of rcAAV is measured to be less than 1 rcAAV in 10^7 recombinant AAV, less than 1 rcAAV in 10^9 recombinant AAV, or less than 1 rcAAV in 10^{10} recombinant AAV; and/or

(iv) wherein the recombinant AAV preparation comprises a low level of rcAAV and the level of rcAAV is less than the level of rcAAV produced using an equivalent method

except that the vector plasmid comprises both at least one AAV rep gene and at least one AAV cap gene; and/or

(v) comprising a step of selecting a ratio of helper plasmid to vector plasmid wherein the ratio of helper plasmid to vector plasmid is selected or adjusted to a ratio that allows the user to obtain the desired ratio of full to total particles or the high or desired yield of recombinant AAV vector; and/or

(vi) wherein the ratio of helper plasmid to vector plasmid is selected or adjusted to a ratio that achieves a maximum yield of recombinant AAV with the minimum yield of empty particles achievable at such maximum yield of recombinant AAV; and/or

(vii) 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; and/or

(viii) further comprising a step of purifying the recombinant AAV.