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

(ii) the at least one AAV rep gene does not comprise a functional internal p40 promoter; and/or
(iii) the helper plasmid does not comprise a contiguous stretch of exclusively cap gene sequence of more than 60 nucleotides.

6. 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.

7. 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.

8. The two-plasmid system of any one of claims 2-7, 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 backbone less than 3000 nucleotides in length.

9. A method for producing a recombinant AAV preparation comprising:
(a) obtaining the two-plasmid system as defined in any one of claims 2-8, or the helper plasmid as defined in any one of claims 1-8 and the vector plasmid as defined in any one of claims 2-8;
(b) transfecting a host cell with the two-plasmid system as defined in any one of claims 2-8, or the helper plasmid as defined in any one of claims 1-8 and the vector plasmid as defined in any one of claims 2-8; and

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