

CLAIMS

1. A process for producing recombinant adeno-associated virus (AAV) particles,
the process comprising the steps:

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(a) culturing a population of first host cells, wherein each first host cell comprises:

(i) a nucleic acid molecule encoding a recombinant AAV genome;

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(ii) a nucleic acid molecule encoding a first AAV *cap* gene which encodes first AAV capsid polypeptides, wherein the first AAV capsid polypeptides confer a tropism on AAV particles which comprise such polypeptides towards second host cells;

(iii) a nucleic acid molecule encoding an AAV *rep* gene;

(iv) nucleic acid molecules encoding viral helper genes;

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such that the expression of each of (i)-(iv) in the first host cells is sufficient to produce first recombinant AAV particles comprising the AAV genome in the first host cells, wherein the first AAV particles are encapsidated by first AAV capsid polypeptides;

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(b) infecting a population of second host cells with the first recombinant AAV particles which are produced from Step (a);

(c) expressing in the population of second host cells:

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(i) a nucleic acid molecule encoding a second AAV *cap* gene which encodes second AAV capsid polypeptides, wherein the second AAV capsid polypeptides confer a tropism on AAV particles which comprise such polypeptides towards third host cells;

(ii) a nucleic acid molecule encoding an AAV *rep* gene;

(iii) nucleic acid molecules encoding viral helper genes;

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wherein all of (i)-(iii) are independently either present in the second host cells or are subsequently introduced into the second host cells,

such that the expression of each of (i)-(iii) in the second cells is sufficient to produce second recombinant AAV particles comprising the recombinant AAV genome in the second host cells,

wherein the second recombinant AAV particles are encapsidated by the second AAV capsid polypeptides;

(d) culturing the second host cells in a culture medium under conditions such that second recombinant AAV particles comprising the recombinant AAV genome are produced, the second recombinant AAV particles each being encapsidated by a capsid comprising the second capsid polypeptides;

and optionally,

(e) purifying and/or isolating second recombinant AAV particles from the second host cells or from the culture medium.

2. The process as claimed in claim 1, wherein Step (a) additionally comprises the prior step of introducing the nucleic acid molecule encoding a recombinant AAV genome into the first host cells.

3. The process as claimed in claim 1 or claim 2, wherein the first host cells and/or second host cells are HEK293, HEK293T, HEK293A, PerC6 or 911 cells.

4. The process as claimed in any one of the preceding claims, wherein the nucleic acid molecule encoding the recombinant AAV genome introduced into the first host cells is in the form of a plasmid, recombinant adenovirus, or is integrated into the genome of a cell or a recombinant AAV particle.

5. The process as claimed in any one of the preceding claims, wherein the nucleic acid molecule encoding the first AAV *cap* gene and/or the nucleic acid encoding the *rep* gene is introduced into the first host cells in the form of a plasmid or in an adenovirus recombinant genome.

6. The process as claimed in any one of the preceding claims, wherein the nucleic acid molecule encoding the second AAV *cap* gene and/or the nucleic acid encoding the *rep* gene is introduced into the second host cells in the form of a plasmid or in an adenovirus recombinant genome.

7. The process as claimed in any one of the preceding claims, wherein the amino acid sequence identity between the first and second AAV capsid polypeptides is less than 99.5%, preferably less than 99%, 98%, 97%, 96%, 95% or less than 90%; and at least 50%.

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8. The process as claimed in any one of the preceding claims, wherein the first recombinant AAV particles are of serotype 2 (AAV2).

9. The process as claimed in any one of the preceding claims, wherein the second recombinant AAV particles are of serotype 9 (AAV9), or modified or mutated derivative of serotype 9.

10. The process as claimed in any one of the preceding claims, wherein the third host cells are selected from the group consisting of neurons (e.g. retinal neurons), hepatocytes, muscle cells, stem cells (preferably mesenchymal stem cells, haematopoietic stem cells, embryonic stem cells, adipose stem cells and induced pluripotent stem cells and their derivatives), immune cells (including B and T lymphocytes, natural killer cells, monocytes and macrophages and granulocytes), endothelial cells, cardiovascular cells, epithelial cells, mesenchymal cells, pancreatic a cells or b cells, cardiomyocytes, spleen cells, fat cells, glial cells, fibroblasts, Kupffer cells and cancer cells (preferably leukaemia, lymphoma, myeloma, carcinoma, sarcoma or melanoma cells).

11. The process as claimed in any one of the preceding claims, wherein the nucleic acid molecules encoding the first and/or second *cap* genes and/or the *rep* genes are present in a recombinant adenovirus (AV), wherein the recombinant adenovirus comprises a repressible Major Late Promoter (MLP) and a plurality of adenoviral late genes, wherein the MLP comprises one or more repressor elements which are capable of regulating or controlling transcription of the adenoviral late genes, and wherein one or more of the repressor elements are inserted downstream of the MLP TATA box.

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