

SUMMARY

The inventors have found that the production of a single AAV vector comprising a first nucleic acid encoding RdCVF and a second nucleic acid encoding RdCVFL could be subject to problems of incomplete packaging of the AAV single strand DNA within the viral capsid. This incomplete encapsidation leads to the production of an incomplete AAV genome, so a DNA of a smaller size.

The inventors have surprisingly discovered that this incomplete encapsidation was due to the presence of direct repeated sequences within the AAV genome and that limiting the length of nucleotides that are identical between the first and second expression cassettes to at most 200 contiguous identical nucleotides, AAV can be fully packaged.

Thus, the present disclosure provides a solution to this problem, by providing AAV vectors comprising a first expression cassette comprising a first nucleic acid encoding RdCVF and a second expression cassette comprising a second nucleic acid encoding RdCVFL, in which production of AAV particles is optimized.

AAV according to the disclosure comprise a first and a second expression cassettes which display at most 200 contiguous identical nucleotides, preferably at most 190, even more preferably at most 180, 170, 167, 165, 164, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 55, 54, 50, 40, 30, 20, 15, 10, 9 or 8 contiguous identical nucleotides.

The inventors have developed several alternative and/or cumulative solutions to the problem of direct repeated sequences between the first and second expression cassettes.

Thus, in one aspect, the present disclosure refers to an adeno-associated vector (AAV) comprising:

- a first expression cassette comprising a first nucleic acid encoding RdCVF
- and
- a second expression cassette comprising a second nucleic acid encoding

RdCVFL,

wherein said first and second expression cassettes display less than 200 contiguous identical nucleotides.

In another example, the present disclosure relates to an adeno-associated vector (AAV) comprising:

- a first expression cassette comprising a first nucleic acid encoding RdCVF
- and
- a second expression cassette comprising a second nucleic acid encoding RdCVFL,

for use in a method of treatment of a retinal neurodegenerative disorder, wherein said first and second expression cassettes display less than 200 contiguous identical nucleotides.

In another aspect, the present disclosure refers to an AAV as disclosed herein, wherein said AAV is for use in a method of treatment of a retinal neurodegenerative disorder. In yet another aspect, the present disclosure refers to use of an AAV as disclosed herein in the manufacture of a medicament for the treatment of a retinal neurodegenerative disease.

The present disclosure also relates to a method for treating a patient suffering from a retinal degenerative disease comprising the step consisting of administering to said patient a therapeutically effective amount of an adeno-associated vector (AAV) comprising:

- a first expression cassette comprising a first nucleic acid encoding RdCVF
- and
- a second expression cassette comprising a second nucleic acid encoding RdCVFL,

wherein said first and second expression cassettes display less than 200 contiguous identical nucleotides.

The disclosure also relates to the use of an inert human DNA sequence in an AAV construct. In one example, the disclosure relates to an AAV comprising a nucleic acid having the sequence set forth in SEQ ID NO: 10, wherein said nucleic acid having the sequence as set forth in SEQ ID NO: 10 is not present in an expression cassette.

CLAIMS

1. An adeno-associated vector (AAV) comprising:

- a first expression cassette comprising a first nucleic acid encoding RdCVF

and

- a second expression cassette comprising a second nucleic acid encoding RdCVFL,

wherein said first and second expression cassettes display less than 200 contiguous identical nucleotides.

2. An AAV according to claim 1, wherein the AAV is a serotype AAV2/8.

3. An AAV according to any one of claims 1 or 2, wherein the first nucleic acid encoding RdCVF is under the control of an ubiquitous promoter, preferably the CMV/CBA promoter.

4. An AAV according to any one of claims 1 to 3, wherein the second nucleic acid encoding RdCVFL is under the control of the cone-opsin promoter.

5. An AAV according to any one of claims 1 to 4, wherein the AAV has a nucleic acid sequence as set forth in the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9, preferably SEQ ID NO: 6.

6. An AAV according to any one of claims 1 to 4, wherein the AAV further comprises a stuffer sequence of SEQ ID NO:10.

7. An AAV according to any of the above claims, wherein said AAV is for use in a method of treatment of a retinal neurodegenerative disorder.

8. Use of an AAV according to any of claims 1 to 6 in the manufacture of a medicament for the treatment of a retinal neurodegenerative disease.