

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 aspectexample, 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 one aspect, the present disclosure refers to an 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 at most 70 contiguous identical nucleotides. In another aspect, the present disclosure refers to a pharmaceutical composition comprising an AAV as disclosed herein and a pharmaceutically acceptable carrier.

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 at most 70 ~~less than 200~~ contiguous identical nucleotides.

2. An AAV according to claim 1, wherein said first and second expression cassettes display at most 54 contiguous identical nucleotides.

3. An AAV according to claim 1, wherein said first and second expression cassettes display at most 9 contiguous identical nucleotides.

4. An AAV according to any one of claims 1 to 3 ~~claim 1~~, wherein the AAV is a serotype AAV2/8.

5. An AAV according to any one of claims 1 ~~or 2~~ to 4, wherein the first nucleic acid encoding RdCVF is under the control of an ubiquitous promoter, ~~preferably the CMV/CBA promoter.~~

6. An AAV according to claim 5 wherein said ubiquitous promoter is the CMV/CBA promoter.

7. An AAV according to any one of claims 1 to 6, wherein the second nucleic acid encoding RdCVFL is under the control of the cone-opsin promoter.

8. An AAV according to any one of claims 1 to 7, 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 and SEQ ID NO: 14, ~~preferably SEQ ID NO: 6~~.

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

106. An AAV according to any one of claims 1 to 74, wherein the AAV further comprises a stuffer sequence of SEQ ID NO:10.

11. An AAV according to any one of claims 1 to 7, wherein the first nucleic acid comprises a codon-optimized cDNA encoding RdCVF as set forth in SEQ ID NO:12, and/or wherein the second nucleic acid comprises a codon-optimized cDNA encoding RdCVFL as set forth in SEQ ID NO:13.

12. A pharmaceutical composition comprising an AAV according to any one of claims 1 to 11 and a pharmaceutically acceptable carrier.

137. An AAV according to any ~~of the above claims~~ one of claims 1 to 11, wherein said AAV is for use in a method of treatment of a retinal neurodegenerative disorder.

14. An AAV for use according to claim 13, wherein said retinal neurodegenerative disorder is chosen in the group consisting of: retinitis pigmentosa, age-related macular degeneration, Bardet-Biedel syndrome, Bassen- Komzweig syndrome, Best disease, choroidema, gyrate atrophy, Leber congenital amaurosis, Refsum disease, Stargardt disease or Usher syndrome.

15. An AAV for use according to claim 14, wherein said retinal neurodegenerative disorder is retinitis pigmentosa.

16. An AAV for use according to any one of claims 13 to 15, wherein said AAV is to be administered by sub-retinal injection.

178. Use of an AAV according to any of claims 1 to 116 in the manufacture of a medicament for the treatment of a retinal neurodegenerative disease.

18. Use of an AAV according to claim 17, wherein said retinal neurodegenerative disease is chosen in the group consisting of: retinitis pigmentosa, age-related macular degeneration, Bardet-Biedel

syndrome, Bassen- Komzweig syndrome, Best disease, choroidema, gyrate atrophy, Leber congenital amaurosis, Refsum disease, Stargardt disease or Usher syndrome.

19. Use of an AAV according to claim 18, wherein said retinal neurodegenerative disease is retinitis pigmentosa.

20. Use of an AAV according to any one of claims 17 to 19, wherein said AAV is to be administered by sub-retinal injection.