

CLAIMS

1. A recombinant nucleic acid comprising (sequentially from the 5' end to the 3' end): a mitochondrial targeting sequence, a mitochondrial protein coding sequence, a 3' UTR sequence, and a polyA signal sequence, wherein the polyA signal sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 173;

wherein the mitochondrial targeting sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 1; and

the mitochondrial protein coding sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 6.

2. The recombinant nucleic acid according to claim 1, comprising a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of sequences as set forth in SEQ ID NOs: 176, and 180.

3. The recombinant nucleic acid according to any one of claims 1 to 2, comprising a Kozak sequence positioned before the 5' end of the mitochondrial targeting sequence, wherein optionally, the Kozak sequence is SEQ ID NO: 171; and optionally, no redundant nucleotides are present between the Kozak sequence and the mitochondrial targeting sequence.

4. The recombinant nucleic acid according to any one of claims 1 to 3, comprising an intron sequence, wherein optionally, the intron sequence is positioned before the 5' end of the mitochondrial targeting sequence; optionally, the intron sequence is positioned before the 5' end of the Kozak sequence; and optionally, the intron sequence comprises a sequence having at least

90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 170.

5. The recombinant nucleic acid according to any one of claims 1 to 4, comprising a promoter sequence, wherein optionally, the promoter sequence is positioned before the 5' end of the mitochondrial targeting sequence, the Kozak sequence, and/or the intron sequence; and optionally, the promoter sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 169.

6. The recombinant nucleic acid according to any one of claims 1 to 5, wherein the 3' UTR sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 13.

7. The recombinant nucleic acid according to any one of claims 1 to 6, wherein the mitochondrial protein coding sequence encodes an ND4 protein; wherein optionally, the ND4 protein comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 160.

8. The recombinant nucleic acid according to any one of claims 1 to 7, comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% identity to a spacer sequence as set forth in SEQ ID NO: 185 between the 3' UTR sequence and the polyA signal sequence.

9. The recombinant nucleic acid according to any one of claims 1 to 8, further comprising a first inverted terminal repeat (ITR) sequence and a second ITR sequence, wherein optionally, the first ITR sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%,

at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 178, and the second ITR sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 179.

10. The recombinant nucleic acid according to any one of claims 1 to 9, wherein no redundant nucleotides are present between the mitochondrial protein coding sequence and the 3' UTR sequence.

11. The recombinant nucleic acid according to any one of claims 1 to 10, wherein no redundant nucleotides are present between the mitochondrial targeting sequence and the mitochondrial protein coding sequence.

12. The recombinant nucleic acid according to any one of claims 1 to 11, comprising (sequentially from the 5' end to the 3' end): a first ITR sequence, a promoter sequence, an intron sequence, a Kozak sequence, a mitochondrial targeting sequence, a mitochondrial protein coding sequence, a 3' UTR sequence, a polyA signal sequence, and a second ITR sequence, wherein preferably, the first ITR sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 178; the promoter sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 169; the intron sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 170; the Kozak sequence is SEQ ID NO: 171; the mitochondrial targeting sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 1; the mitochondrial protein coding sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at

least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 6; the 3' UTR sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 13; the polyA signal sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 173; and the second ITR sequence comprises a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence as set forth in SEQ ID NO: 179.

13. The recombinant nucleic acid according to any one of claims 1 to 11, comprising (sequentially from the 5' end to the 3' end): a first ITR sequence, a promoter sequence, an intron sequence, a Kozak sequence, a mitochondrial targeting sequence, a mitochondrial protein coding sequence, a 3' UTR sequence, a polyA signal sequence, and a second ITR sequence, wherein preferably, the first ITR sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 178; the promoter sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 169; the intron sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 170; the Kozak sequence is SEQ ID NO: 171; the mitochondrial targeting sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 1; the mitochondrial protein coding sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 6; the 3' UTR sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 13; the polyA signal sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO: 173; and the second ITR sequence comprises a sequence having at least 99%, at least 99.5%, or 100% identity to a sequence as set forth in SEQ ID NO:

179.

14. The recombinant nucleic acid according to any one of claims 1 to 11, comprising (sequentially from the 5' end to the 3' end): a first ITR sequence, a promoter sequence, an intron sequence, a Kozak sequence, a mitochondrial targeting sequence, a mitochondrial protein coding sequence, a 3' UTR sequence, a polyA signal sequence, and a second ITR sequence, wherein preferably, the first ITR sequence comprises a sequence as set forth in SEQ ID NO: 178; the promoter sequence comprises a sequence as set forth in SEQ ID NO: 169; the intron sequence comprises a sequence as set forth in SEQ ID NO: 170; the Kozak sequence is SEQ ID NO: 171; the mitochondrial targeting sequence comprises a sequence as set forth in SEQ ID NO: 1; the mitochondrial protein coding sequence comprises a sequence as set forth in SEQ ID NO: 6; the 3' UTR sequence comprises a sequence as set forth in SEQ ID NO: 13; the polyA signal sequence comprises a sequence as set forth in SEQ ID NO: 173; and the second ITR sequence comprises a sequence as set forth in SEQ ID NO: 179.

15. A viral vector comprising the recombinant nucleic acid according to any one of claims 1 to 14.

16. The viral vector according to claim 15, wherein the viral vector is a recombinant adeno-associated virus (rAAV) vector.

17. The viral vector according to claim 16, wherein the rAAV vector is an rAAV2 vector.

18. A pharmaceutical composition comprising an adeno-associated virus (AAV), wherein the AAV comprises the recombinant nucleic acid according to any one of claims 1 to 14.

19. The pharmaceutical composition according to claim 18, wherein the pharmaceutically

acceptable excipient comprises 0.0001%–0.01% poloxamer 188.

20. The pharmaceutical composition according to any one of claims 18 to 19, wherein the pharmaceutically acceptable excipient further comprises one or more salts, wherein the one or more salts comprise NaCl, Na₂HPO₄, and KH₂PO₄.

21. The pharmaceutical composition according to any one of claims 18 to 20, wherein the pharmaceutical composition has a pH of 7.2–7.4.

22. The pharmaceutical composition according to any one of claims 18 to 21, wherein the pharmaceutical composition has a viral titer of at least 1.0×10^{10} vg/mL, at least 3.0×10^{10} vg/mL, at least 6.0×10^{10} vg/mL, or at least 9.0×10^{10} vg/mL.

23. Use of the pharmaceutical composition according to any one of claims 18 to 22 in the preparation of a medicament for treating an ocular disease.

24. The use according to claim 23, wherein the ocular disease is Leber's hereditary optic neuropathy (LHON).

25. The use according to claim 23 or 24, wherein the pharmaceutical composition is to be administered by intraocular or intravitreal injection.

26. The use according to any one of claims 23-25, wherein the adeno-associated virus is to be administered at a dose of at least 1.5×10^9 vg/eye, at least 3.0×10^9 vg/eye, or at least 4.5×10^9 vg/eye.

27. The pharmaceutical composition according to any one of claims 18 to 22 for use as a medicament.