

mutations: only very recently a promising research programme was delayed due to a mutation discovered in the ORF15 region of the transgene cassette. In another programme, alternative RPGR splice variants were detected by Western blot (Wu, Z. et al. (2015) Hum. Mol. Genet. 24: 3956-3970).

- 5 There is currently no approved therapy to prevent the development of RP or to improve vision following the onset of the disease. Accordingly, there remains a significant need for treatments of RP, in particular to prevent deterioration in visual function or to enable improvement in the vision of affected individuals.

SUMMARY

- 10 The present inventors have surprisingly found that the sequence of the retinitis pigmentosa GTPase regulator ORF15 isoform (RPGR^{ORF15}) gene can be engineered to increase sequence stability, such as by reducing or preventing the occurrence of mutations during replication (i.e. increasing the fidelity of replication). The present inventors have also
15 unexpectedly discovered that risks of the engineering affecting the function of the gene can be mitigated. Moreover, the engineered genes have been demonstrated by the present inventors to successfully treat animal models of retinitis pigmentosa.

Accordingly, in one example, the disclosure provides a polynucleotide comprising a nucleotide sequence encoding the retinitis pigmentosa GTPase regulator ORF15 isoform (RPGR^{ORF15}), wherein the RPGR^{ORF15}-encoding nucleotide sequence has been codon
20 optimised to increase fidelity of replication of the sequence.

In one aspect, the present disclosure refers to a composition comprising a nucleic acid comprising

- (a) a sequence encoding a rhodopsin kinase promoter, and
(b) a sequence encoding a retinitis pigmentosa GTPase regulator ORF15 isoform
25 (RPGR^{ORF15}) and comprising the nucleic acid sequence of SEQ ID NO: 3.

In another aspect, the present disclosure refers to a composition comprising a nucleic acid comprising

- (a) a sequence encoding a rhodopsin kinase promoter, and
(b) a sequence encoding a retinitis pigmentosa GTPase regulator ORF15 isoform
30 (RPGR^{ORF15}) and comprising a nucleotide sequence that has at least 97% identity to the nucleic acid sequence of SEQ ID NO: 3, wherein the sequence encoding the RPGR^{ORF15} exhibits increased fidelity of replication as compared to a wild type RPGR^{ORF15} sequence.

Preferably, the RPGR^{ORF15}-encoding nucleotide sequence of the disclosure has been codon optimised to reduce the generation of alternative splice variants.

- 35 Preferably, the RPGR^{ORF15}-encoding nucleotide sequence of the disclosure has been codon optimised to minimise or avoid the creation of new CpG sites in comparison to the wild type sequence. In one example, the RPGR^{ORF15}-encoding nucleotide sequence of the disclosure comprises less than 220, 210, 200, 190, 170, 165, 160, 150, 140, 130, 120, 110 or 100 CpG sites.

- 40 Preferably, the RPGR^{ORF15}-encoding nucleotide sequence of the disclosure has been codon optimised by replacing some or all GGN codons with GGC codons (when the GGN codons were not originally GGC codons).

Preferably, the RPGR^{ORF15}-encoding nucleotide sequence of the disclosure has been codon optimised to minimise or avoid the introduction of new thymine nucleotides in the purine-rich

CLAIMS

1. A composition comprising a nucleic acid comprising
(a) a sequence encoding a rhodopsin kinase promoter, and
5 (b) a sequence encoding a retinitis pigmentosa GTPase regulator ORF15 isoform (RPGR^{ORF15}) and comprising the nucleic acid sequence of SEQ ID NO: 3.
2. The composition of claim 1, wherein the sequence encoding the promoter has at least 90% identity to the nucleic acid sequence of SEQ ID NO: 7.
3. The composition of claim 1, wherein the sequence encoding the promoter comprises
10 the nucleic acid sequence of SEQ ID NO: 7.
4. The composition of claim 1, wherein the sequence encoding the promoter consists of the nucleic acid sequence of SEQ ID NO: 7.
5. The composition of claim 1, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:
15 1.
6. The composition of claim 1, wherein the nucleic acid further comprises a regulatory sequence.
7. The composition of claim 6, wherein the regulatory sequence comprises a polyadenylation site, and wherein the polyadenylation site comprises a Bovine
20 Growth Hormone (BGH) polyadenylation (poly-A) signal.
8. The composition of claim 7, wherein the BGH poly-A signal comprises the sequence of SEQ ID NO: 5.
9. A composition comprising a nucleic acid comprising
(a) a sequence encoding a rhodopsin kinase promoter, and
25 (b) a sequence encoding a retinitis pigmentosa GTPase regulator ORF15 isoform (RPGR^{ORF15}) and comprising a nucleotide sequence that has at least 97% identity to the nucleic acid sequence of SEQ ID NO: 3, wherein the sequence encoding the RPGR^{ORF15} exhibits increased fidelity of replication as compared to a wild type RPGR^{ORF15} sequence.

10. The composition of claim 9, wherein the sequence encoding the promoter has at least 90% identity to the nucleic acid sequence of SEQ ID NO: 7.
11. The composition of claim 9, wherein the sequence encoding the promoter comprises the nucleic acid sequence of SEQ ID NO: 7.
- 5 12. The composition of claim 9, wherein the sequence encoding the promoter consists of the nucleic acid sequence of SEQ ID NO: 7.
13. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 80% identity to the amino acid sequence of SEQ ID NO: 1.
- 10 14. The composition of claim 10, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 80% identity to the amino acid sequence of SEQ ID NO: 1.
- 15 15. The composition of claim 11, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 80% identity to the amino acid sequence of SEQ ID NO: 1.
16. The composition of claim 12, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 80% identity to the amino acid sequence of SEQ ID NO: 1.
- 20 17. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence that has at least 98% identity to the nucleic acid sequence of SEQ ID NO: 3.
18. The composition of claim 10, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence that has the nucleic acid sequence of SEQ ID NO: 3.
- 25 19. The composition of claim 11, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence that has the nucleic acid sequence of SEQ ID NO: 3.
- 30 20. The composition of claim 12, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence that has the nucleic acid sequence of SEQ ID NO: 3.

21. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence that has at least 99% identity to the nucleic acid sequence of SEQ ID NO: 3.
- 5 22. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence that has the nucleic acid sequence of SEQ ID NO: 3.
23. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 85% identity to the amino acid sequence of SEQ ID NO: 1.
- 10 24. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 90% identity to the amino acid sequence of SEQ ID NO: 1.
- 15 25. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO: 1.
26. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding an amino acid sequence that has at least 99% identity to the amino acid sequence of SEQ ID NO: 1.
- 20 27. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1.
- 25 28. The composition of claim 9, wherein the sequence encoding the RPGR^{ORF15} does not comprise a purine nucleotide at a position corresponding to nucleotide 33, 58, 59, 114, 123, 129, 181, 182, 213, 219, 226, 227, 237, 267, 285, 306, 309, 315, 324, 330, 339, 400, 401 , 444, 456, 478, 480, 510, 594, 606, 618, 639, 697, 726, 744, 777, 807, 852, 877, 879, 888, 891 , 921 , 930, 960, 1042, 1050, 1116, 1140, 1183, 1184, 1194, 1197, 1221, 1249, 1251, 1257, 1273, 1276, 1281, 1290, 1293, 1357, 1372, 1373, 1413, 1446, 1452, 1464, 1474, 1475, 1482, 1519, 1520, 1542, 1584, 1590, 1599, 1608, 1653, 1668, 1674, 1689, 1692, 1734, 1761 , 1776, 1788, 1803, 1824, 1845, 1854, 1881, 1893, 1902, 1909, 1910, 1929, 1932, 1960, 1987, 1988, 1992, 1998, 2020, 2031, 2047, 2049, 2062, 2067, 2076, 2121, 2167, 2169, 2190, 2193, 2208, 2229, 2259, 2283, 2298, 2323, 2343, 2346, 2361, 2373, 2382, 2388, 2403, 2409, 2410, 2412, 2448, 2457, 2472, 2476, 2478, 2505, 2520, 2547,
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2574, 2622, 2634, 2661, 2673, 2706, 2712, 2763, 2775, 2796, 2811, 2817, 2832, 2838, 2871, 2886, 2892, 2898, 2910, 2922, 2931, 2937, 2958, 2970, 2997, 3033, 3039, 3069, 3075, 3117, 3129, 3156, 3166, 3235, 3246, 3273, 3285, 3306, 3321, 3369, 3399, 3405 or 3438 of SEQ ID NO: 2.

5 29. The composition of claim 28, wherein the sequence encoding the RPGR^{ORF15} does not comprise purine nucleotides at at least 2, of the nucleotide positions provided in claim 28.

30. The composition of claim 9, wherein the nucleic acid further comprises a regulatory sequence.

10 31. The composition of claim 30, wherein the regulatory sequence comprises a polyadenylation site, and wherein the polyadenylation site comprises a Bovine Growth Hormone (BGH) polyadenylation (poly-A) signal.

32. The composition of claim 31, wherein the BGH poly-A signal comprises the sequence of SEQ ID NO: 5.