(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 September 2016 (22.09.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/149664 A1

(51) International Patent Classification:
A61K 39/12 (2006.01) *C12N 15/00* (2006.01)
A61K 48/00 (2006.01) *C07H 21/04* (2006.01)

(21) International Application Number:
PCT/US2016/023252

(22) International Filing Date:
18 March 2016 (18.03.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/134,822 18 March 2015 (18.03.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: METHODS AND COMPOSITIONS FOR RESTORATION OF CONE FUNCTION IN BCM

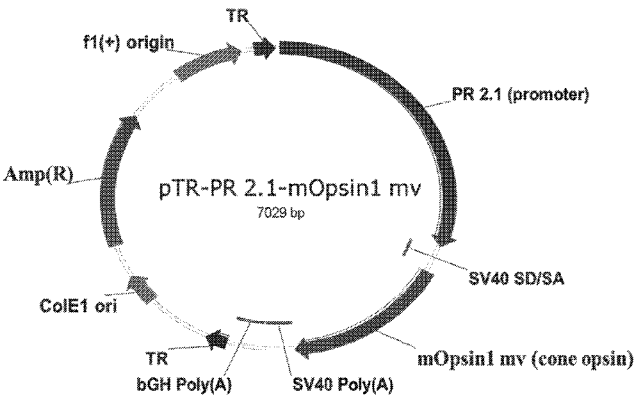


FIG. 1A

(57) Abstract: Provided are nucleic acid sequences, recombinant adeno-associated viral particles, compositions, and methods related to treating cone monochromacies, such as blue cone monochromacy (BCM). Specifically, the nucleic acid sequences, recombinant adeno-associated viral particles, compositions, and methods involve use of an M-opsin gene operably linked to a cone-specific promoter. Further disclosed is the sequence of a cone-specific PR2.1 promoter.

WO 2016/149664 A1

METHODS AND COMPOSITIONS FOR RESTORATION OF CONE FUNCTION IN BCM

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. provisional application number 62/134,822, filed March 18, 2015, the content of which is incorporated herein by reference in its entirety.

BACKGROUND

Human photoreceptors have three kinds of cones to distinguish among red, green and blue colors. Blue Cone Monochromacy (BCM) patients lack functional long wavelength-sensitive and medium wavelength-sensitive cones in the retina required for detection of red and green colors, which leaves the patients legally blind. There is currently no treatment for BCM.

SUMMARY

Aspects of the disclosure relate to nucleic acids, recombinant adeno-associated virus (rAAV) particles, compositions, and methods related to gene therapy for cone monochromacy and other eye disorders such as protanopia and deuteranopia.

As described herein, an M-opsin gene driven by the cone-specific promoter PR2.1 was delivered to the eye of a mouse model of BCM using rAAV particles. It was found that eyes treated with the rAAV particles were able to express the M-opsin gene in cone cells and that M-opsin function was restored in the treated eyes. As disclosed herein, such expression vectors and rAAV particles are useful for treatment of cone monochromacy and other eye disorders such as protanopia and deuteranopia, including in humans.

In some aspects, the disclosure provides a nucleic acid comprising an expression construct containing an M-opsin gene operably linked to a cone-specific promoter, wherein the expression construct is flanked by inverted terminal repeat (ITR) sequences (*e.g.*, an ITR sequence at each end of the expression construct). In some embodiments, the ITR sequence is an AAV2 ITR sequence. In some embodiments, the cone-specific promoter is a PR2.1

promoter having the sequence of SEQ ID NO: 1. In some embodiments, the expression construct further contains nucleic acid segments that encode an SV40 polyadenylation signal and a bovine growth hormone polyadenylation signal, wherein the nucleic acid segments are positioned 3' to the M-opsin gene. In some embodiments, the nucleic acid comprises or consists of the sequence of SEQ ID NO: 2. In some embodiments, the nucleic acid is a recombinant adeno-associated virus (rAAV) vector. In some embodiments, the nucleic acid is a plasmid, e.g., containing one or more of an origin of replication and a resistance marker.

Other aspects of the disclosure relate to a recombinant adeno-associated virus (rAAV) particle comprising the nucleic acid of any one of the above embodiments or as otherwise described herein. In some embodiments, the rAAV particle is an AAV5 particle (e.g., a particle that has AAV5 capsid proteins).

Yet other aspects of the disclosure relate to a composition comprising a plurality of the rAAV particle of any one of the above embodiments or as otherwise described herein. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

Other aspects of the disclosure relate to a method of treating a cone monochromacy, protanopia, or deuteranopia in a subject, the method comprising administering a therapeutically effective amount of the rAAV particle of any one of the above embodiments or as otherwise described herein or the composition of any one of the above embodiments or as otherwise described herein to a subject having a cone monochromacy, protanopia, or deuteranopia. In some embodiments, the rAAV particle or composition are administered via subretinal injection. In some embodiments, the subject has blue cone monochromacy. In some embodiments, the subject is a human subject.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A is a schematic drawing of the exemplary plasmid containing the exemplary

construct that was packaged into AAV5 in Example 1.

FIG. 1B shows the sequence of the exemplary plasmid. The sequence corresponds to SEQ ID NO: 2.

FIG. 2 is a series of traces showing scotopic ERG, photopic ERG, and M-cone ERG in *Opn1mw* knock-out (KO) mice two months after P14 treatment with AAV5-PR2.1-mouse M-opsin. Tx= treated eye. UnTx= untreated eye. The left-most traces are from wild-type mice and the middle and right-most traces are from *Opn1mw* KO mice.

FIG. 3 is a series of photographs showing M-opsin (left column), S-opsin (middle column), or an overlay of M-opsin, S-opsin and DAPI (right column) in the inferior retina of a treated eye (Tx) or untreated eye (UnTx) from *Opn1mw* knock-out (KO) mice.

FIG. 4 is a series of photographs showing M-opsin (left column), S-opsin (middle column), or an overlay of M-opsin, S-opsin and DAPI (right column) in the superior retina of a treated eye (Tx) or untreated eye (UnTx) from *Opn1mw* knock-out (KO) mice.

DETAILED DESCRIPTION

Aspects of the disclosure relate to nucleic acids, recombinant AAV (rAAV) particles and nucleic acids, compositions, and methods of treating an ocular disorder, such as blue cone monochromacy (BCM).

In some embodiments, a nucleic acid is provided, the nucleic acid comprising an expression construct containing an opsin gene (e.g., an M-opsin gene) operably linked to a promoter. In some embodiments, promoters suitable for use with the methods and compositions described herein include constitutive promoters, strong promoters (e.g., CMV promoters), inducible promoters, and tissue-specific or cell-specific promoters (e.g., promoters that preferentially facilitate expression in a limited number of tissues or cell types). In some embodiments, tissue-specific or cell-specific promoters include eye-specific promoters (*e.g.*, cone-specific promoters, rod-specific promoters, retina-specific promoters, retinal cell-specific promoters, photoreceptor cell-specific promoters, and the like). In some embodiments, the promoter is an mGluR6 promoter, a GNA01 promoter, a CBA/smCBA (fusion of the CMV immediate early enhancer and bovine beta actin promoter plus intro1-

exon1 junction) promoter, CBA promoter (chicken beta actin), CMV promoter, RSV promoter, SV40 promoter, MoMLV promoter, or derivatives, mutants and/or fragments thereof. Other promoters that may similarly be utilized within the context of the present invention include cell or tissue specific promoters (*e.g*, a rod, cone, or ganglia derived promoter), or inducible promoters. Representative examples of suitable inducible promoters include inducible promoters sensitive to an antibiotic, e.g., tetracycline-responsive promoters such as "tet-on" and/or "tet-off" promoters. Inducible promoters may also include promoters sensitive to chemicals other than antibiotics.

In some embodiments, a nucleic acid is provided, the nucleic acid comprising an expression construct containing an opsin gene (e.g., an M-opsin gene) operably linked to a cone-specific promoter. Exemplary, non-limiting cone-specific promoters include PR2.1, red/green opsin promoter, IRBPe/GNAT2 promoter, and synGNAT2/GNAT2 (see, e.g., Dyka et al. Cone specific promoter for use in gene therapy of retinal degenerative diseases. Adv Exp Med Biol. 2014;801:695-701 and U.S. Patent Application Publication No. US20140275231). In some embodiments, the expression construct is flanked on each side by an inverted terminal repeat sequence. In some embodiments, the cone-specific promoter is a PR2.1 promoter having the sequence of SEQ ID NO: 1.

PR2.1 promoter sequence:

CCCCGGGGGATCCTCTAGAGTCGACAGGCCTACAGCAGCCAGGGTGAGATTATGAGGCTGAGCTGAGA
ATATCAAGACTGTACCGAGTAGGGGGCCTTGGCAAGTGTGGAGAGCCCGGCAGCTGGGGCAGAGGGCG
GAGTACGGTGTGCGTTTACGGACCTCTTCAAACGAGGTAGGAAGGTCAGAAGTCAAAAAGGGAACAAA
TGATGTTTAACCACACAAAAATGAAAATCCAATGGTTGGATATCCATTCCAAATACACAAAGGCAACG
GATAAGTGATCCGGGCCAGGCACAGAAGGCCATGCACCCGTAGGATTGCACTCAGAGCTCCCAAATGC
ATAGGAATAGAAGGGTGGGTGCAGGAGGCTGAGGGGTGGGGAAAGGGCATGGGTGTTTCATGAGGACA
GAGCTTCCGTTTCATGCAATGAAAAGAGTTTGGAGACGGATGGTGGTGACTGGACTATACACTTACAC
ACGGTAGCGATGGTACACTTTGTATTATGTATATTTTACCACGATCTTTTTAAAGTGTCAAAGGCAAA
TGGCCAAATGGTTCCTTGTCCTATAGCTGTAGCAGCCATCGGCTGTTAGTGACAAAGCCCCTGAGTCA
AGATGACAGCAGCCCCCATAACTCCTAATCGGCTCTCCCGCGTGGAGTCATTTAGGAGTAGTCGCATT
AGAGACAAGTCCAACATCTAATCTTCCACCCTGCCAGGGCCCCAGCTGGCAGCGAGGGTGGGAGACTC

CGGGCAGAGCAGAGGGCGCTGACATTGGGGCCCGGCCTGGCTTGGGTCCCTCTGGCCTTTCCCCAGGG
GCCCTCTTTCCTTGGGGCTTTCTTGGGCCGCCACTGCTCCCGCTCCTCTCCCCCCATCCCACCCCCTC
ACCCCCTCGTTCTTCATATCCTTCTCTAGTGCTCCCTCCACTTTCATCCACCCTTCTGCAAGAGTGTG
GGACCACAAATGAGTTTTCACCTGGCCTGGGGACACACGTGCCCCCACAGGTGCTGAGTGACTTTCTA
GGACAGTAATCTGCTTTAGGCTAAAATGGGACTTGATCTTCTGTTAGCCCTAATCATCAATTAGCAGA
GCCGGTGAAGGTGCAGAACCTACCGCCTTTCCAGGCCTCCTCCCACCTCTGCCACCTCCACTCTCCTT
CCTGGGATGTGGGGGCTGGCACACGTGTGGCCCAGGGCATTGGTGGGATTGCACTGAGCTGGGTCATT
AGCGTAATCCTGGACAAGGGCAGACAGGGCGAGCGGAGGGCCAGCTCCGGGGCTCAGGCAAGGCTGGG
GGCTTCCCCCAGACACCCCACTCCTCCTCTGCTGGACCCCCACTTCATAGGGCACTTCGTGTTCTCAA
AGGGCTTCCAAATAGCATGGTGGCCTTGGATGCCCAGGGAAGCCTCAGAGTTGCTTATCTCCCTCTAG
ACAGAAGGGGAATCTCGGTCAAGAGGGAGAGGTCGCCCTGTTCAAGGCCACCCAGCCAGCTCATGGCG
GTAATGGGACAAGGCTGGCCAGCCATCCCACCCTCAGAAGGGACCCGGTGGGGCAGGTGATCTCAGAG
GAGGCTCACTTCTGGGTCTCACATTCTTGGATCCGGTTCCAGGCCTCGGCCCTAAATAGTCTCCCTGG
GCTTTCAAGAGAACCACATGAGAAAGGAGGATTCGGGCTCTGAGCAGTTTCACCACCCACCCCCCAGT
CTGCAAATCCTGACCCGAGGGTCCACCTGCCCCAAAGGCGGACGCAGGACAGTAGAAGGGAACAGAGA
ACACATAAACACAGAGAGGGCCACAGCGGCTCCCACAGTCACCGCCACCTTCCTGGCGGGGATGGGTG
GGGCGTCTGAGTTTGGTTCCCAGCAAATCCCTCTGAGCCGCCCTTGCGGGCTCGCCTCAGGAGCAGGG
GAGCAAGAGGTGGGAGGAGGAGGTCTAAGTCCCAGGCCCAATTAAGAGATCAGGTAGTGTAGGGTTTG
GGAGCTTTTAAGGTGAAGAGGCCCGGGCTGATCCCACAGGCCAGTATAAAGCGCCGTGACCCTCAGGT
GATGCGCCAGGGCCGGCTGCCTGCGGGGACAGGGCTTTCCATAGCCATGAGGATCCGAATTCGGATCC
C (SEQ ID NO: 1)

Exemplary M-opsin genes include mouse M-opsin and human M-opsin. Exemplary nucleotide sequences of M-opsin genes are provided below.

Exemplary mouse m-opsin nucleotide sequence:

CAGAGACAGT TTTCTACAGC CATGGCCCAA AGGCTTACAG GTGAACAGAC ACTGGACCAC
TATGAGGATA GCACCCATGC AAGCATCTTC ACCTATACCA ACAGCAACAG CACCAAAGGT
CCCTTTGAAG GCCCCAATTA TCACATTGCT CCCAGGTGGG TGTACCACCT CACCAGCACC
TGGATGATTC TTGTGGTCGT TGCATCTGTC TTCACTAATG GACTTGTGCT GGCAGCCACC
ATGAGATTCA AGAAGCTGCG CCATCCACTG AACTGGATTC TGGTGAACTT GGCAGTTGCT
GACCTAGCAG AGACCATTAT TGCCAGCACT ATCAGTGTTG TGAACCAAAT CTATGGCTAC
TTCGTTCTGG GACACCCTCT GTGTGTCATT GAAGGCTACA TTGTCTCATT GTGTGGAATC
ACAGGCCTCT GGTCCCTGGC CATCATTTCC TGGGAGAGAT GGCTGGTGGT CTGCAAGCCC
TTTGGCAATG TGAGATTTGA TGCTAAGCTG GCCACTGTGG GAATCGTCTT CTCCTGGGTC

TGGGCTGCTA TATGGACGGC CCCACCAATC TTTGGTTGGA GCAGGTACTG GCCTTATGGC
CTGAAGACAT CCTGTGGCCC AGACGTGTTC AGCGGTACCT CGTACCCCGG GGTTCAGTCT
TATATGATGG TCCTCATGGT CACGTGCTGC ATCTTCCCAC TCAGCATCAT CGTGCTCTGC
TACCTCCAAG TGTGGCTGGC CATCCGAGCA GTGGCAAAGC AACAGAAAGA ATCTGAGTCC
ACTCAGAAGG CCGAGAAGGA GGTGACACGC ATGGTGGTGG TGATGGTCTT CGCATACTGC
CTCTGCTGGG GACCCTATAC TTTCTTTGCA TGCTTTGCTA CTGCCCACCC TGGCTATGCC
TTCCACCCTC TTGTGGCCTC CCTACCATCC TACTTTGCCA AAAGTGCCAC TATCTACAAC
CCCATTATCT ATGTCTTTAT GAACCGGCAG TTTCGAAACT GCATCTTACA TCTCTTTGGA
AAGAAGGTTG ATGATAGCTC TGAACTTTCC AGCACCTCCA AGACAGAAGT CTCATCTGTC
TCTTCAGTGT CACCTGCATA A (SEQ ID NO: 3)

Exemplary human M-opsin nucleotide sequence:

ATGGCCCA GCAGTGGAGC CTCCAAAGGC TCGCAGGCCG CCATCCGCAG GACAGCTATG
AGGACAGCAC CCAGTCCAGC ATCTTCACCT ACACCAACAG CAACTCCACC AGAGGCCCCT
TCGAAGGCCC GAATTACCAC ATCGCTCCCA GATGGGTGTA CCACCTCACC AGTGTCTGGA
TGATCTTTGT GGTCATTGCA TCCGTCTTCA CAAATGGGCT TGTGCTGGCG GCCACCATGA
AGTTCAAGAA GCTGCGCCAC CCGCTGAACT GGATCCTGGT GAACCTGGCG GTCGCTGACC
TGGCAGAGAC CGTCATCGCC AGCACTATCA GCGTTGTGAA CCAGGTCTAT GGCTACTTCG
TGCTGGGCCA CCCTATGTGT GTCCTGGAGG GCTACACCGT CTCCCTGTGT GGGATCACAG
GTCTCTGGTC TCTGGCCATC ATTTCCTGGG AGAGATGGAT GGTGGTCTGC AAGCCCTTTG
GCAATGTGAG ATTTGATGCC AAGCTGGCCA TCGTGGGCAT TGTCTTCTCC TGGATCTGGT
CTGCTGTGTG GACAGCCCCG CCCATCTTTG GTTGGAGCAG GTACTGGCCC CACGGCCTGA
AGACTTCATG CGGCCCAGAC GTGTTCAGCG GCAGCTCGTA CCCCGGGGTG CAGTCTTACA
TGATTGTCCT CATGGTCACC TGCTGCATCA CCCCACTCAG CATCATCGTG CTCTGCTACC
TCCAAGTGTG GCTGGCCATC CGAGCGGTGG CAAAGCAGCA GAAAGAGTCT GAATCCACCC
AGAAGGCAGA GAAGGAAGTG ACGCGCATGG TGGTGGTGAT GGTCCTGGCA TTCTGCTTCT
GCTGGGGACC ATACGCCTTC TTCGCATGCT TTGCTGCTGC CAACCCTGGC TACCCCTTCC
ACCCTTTGAT GGCTGCCCTG CCGGCCTTCT TTGCCAAAAG TGCCACTATC TACAACCCCG
TTATCTATGT CTTTATGAAC CGGCAGTTTC GAAACTGCAT CTTGCAGCTT TTCGGGAAGA
AGGTTGACGA TGGCTCTGAA CTCTCCAGCG CCTCCAAAAC GGAGGTCTCA TCTGTGTCCT
CGGTATCGCC TGCATGA (SEQ ID NO: 4)

In some embodiments, the M-opsin gene comprises a nucleotide sequence that encodes an M-opsin protein, such as mouse M-opsin or human M-opsin protein. Exemplary M-opsin protein sequences are provided below.

Mouse m-opsin amino acid sequence:

MAQRLTGEQTLDHYEDSTHASIFTYTNSNSTKGPFEGPNYHIAPRWVYHLTSTWMILVVVASVFTN
GLVLAATMRFKKLRHPLNWILVNLAVADLAETIIASTISVVNQIYGYFVLGHPLCVIEGYIVSLCG
ITGLWSLAIISWERWLVVCKPFGNVRFDAKLATVGIVFSWVWAAIWTAPPIFGWSRYWPYGLKTSC
GPDVFSGTSYPGVQSYMMVLMVTCCIFPLSIIVLCYLQVWLAIRAVAKQQKESESTQKAEKEVTRM
VVVMVFAYCLCWGPYTFFACFATAHPGYAFHPLVASLPSYFAKSATIYNPIIYVFMNRQFRNCILH
LFGKKVDDSSELSSTSKTEVSSVSSVSPA (SEQ ID NO: 5)

Human m-opsin amino acid sequence:

MAQQWSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWVYHLTSVWMIFVVIASV
FTNGLVLAATMKFKKLRHPLNWILVNLAVADLAETVIASTISVVNQVYGYFVLGHPMCVLEGYTVSLC
GITGLWSLAIISWERWMVVCKPFGNVRFDAKLAIVGIVFSWIWSAVWTAPPIFGWSRYWPHGLKTSCG
PDVFSGSSYPGVQSYMIVLMVTCCITPLSIIVLCYLQVWLAIRAVAKQQKESESTQKAEKEVTRMVVV
MVLAFCFCWGPYAFFACFAAANPGYPFHPLMAALPAFFAKSATIYNPVIYVFMNRQFRNCILQLFGKK
VDDGSELSSASKTEVSSVSSVSPA (SEQ ID NO: 6)

In some embodiments, the expression construct further contains nucleic acid segments that encode one or more (e.g., one, two, or three) polyadenylation signals. In some embodiments, the expression construct further contains nucleic acid segments that encode an SV40 polyadenylation signal and/or a bovine growth hormone (BGH) polyadenylation signal. In some embodiments, the nucleic acid segments are positioned 3' to the opsin gene (e.g., the M-opsin gene) in the expression construct. Exemplary SV40 and BGH polyadenylation sequences are provided below.

Exemplary SV40 polyA sequence

CGGGGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAA
AATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAA
GTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTT (SEQ ID NO: 7)

Exemplary BGH polyA sequence

GCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCT
GAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACA
ATAGCAGGCATGCTGGGGA (SEQ ID NO: 8)

In some embodiments, the nucleic acid is a plasmid. In some embodiments, the nucleic acid comprises or consists of the sequence of SEQ ID NO: 2.

Exemplary plasmid sequence

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCC
GGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTA
GGGGTTCCTAGATCTGAATTCGGTACCCCCGGGGGATCCTCTAGAGTCGACAGGCCTACAGCAGCCAG
GGTGAGATTATGAGGCTGAGCTGAGAATATCAAGACTGTACCGAGTAGGGGGCCTTGGCAAGTGTGGA
GAGCCCGGCAGCTGGGGCAGAGGGCGGAGTACGGTGTGCGTTTACGGACCTCTTCAAACGAGGTAGGA
AGGTCAGAAGTCAAAAAGGGAACAAATGATGTTTAACCACACAAAAATGAAAATCCAATGGTTGGATA
TCCATTCCAAATACACAAAGGCAACGGATAAGTGATCCGGGCCAGGCACAGAAGGCCATGCACCCGTA
GGATTGCACTCAGAGCTCCCAAATGCATAGGAATAGAAGGGTGGGTGCAGGAGGCTGAGGGGTGGGGA
AAGGGCATGGGTGTTTCATGAGGACAGAGCTTCCGTTTCATGCAATGAAAAGAGTTTGGAGACGGATG
GTGGTGACTGGACTATACACTTACACACGGTAGCGATGGTACACTTTGTATTATGTATATTTTACCAC
GATCTTTTTAAAGTGTCAAAGGCAAATGGCCAAATGGTTCCTTGTCCTATAGCTGTAGCAGCCATCGG
CTGTTAGTGACAAAGCCCCTGAGTCAAGATGACAGCAGCCCCCATAACTCCTAATCGGCTCTCCCGCG
TGGAGTCATTTAGGAGTAGTCGCATTAGAGACAAGTCCAACATCTAATCTTCCACCCTGCCAGGGCCC
CAGCTGGCAGCGAGGGTGGGAGACTCCGGGCAGAGCAGAGGGCGCTGACATTGGGGCCCGGCCTGGCT
TGGGTCCCTCTGGCCTTTCCCCAGGGGCCCTCTTTCCTTGGGGCTTTCTTGGGCCGCCACTGCTCCCG
CTCCTCTCCCCCCATCCCACCCCCTCACCCCCTCGTTCTTCATATCCTTCTCTAGTGCTCCCTCCACT
TTCATCCACCCTTCTGCAAGAGTGTGGGACCACAAATGAGTTTTCACCTGGCCTGGGGACACACGTGC
CCCCACAGGTGCTGAGTGACTTTCTAGGACAGTAATCTGCTTTAGGCTAAAATGGGACTTGATCTTCT
GTTAGCCCTAATCATCAATTAGCAGAGCCGGTGAAGGTGCAGAACCTACCGCCTTTCCAGGCCTCCTC
CCACCTCTGCCACCTCCACTCTCCTTCCTGGGATGTGGGGGCTGGCACACGTGTGGCCCAGGGCATTG
GTGGGATTGCACTGAGCTGGGTCATTAGCGTAATCCTGGACAAGGGCAGACAGGGCGAGCGGAGGGCC
AGCTCCGGGGCTCAGGCAAGGCTGGGGGCTTCCCCCAGACACCCCACTCCTCCTCTGCTGGACCCCCA
CTTCATAGGGCACTTCGTGTTCTCAAAGGGCTTCCAAATAGCATGGTGGCCTTGGATGCCCAGGGAAG
CCTCAGAGTTGCTTATCTCCCTCTAGACAGAAGGGGAATCTCGGTCAAGAGGGAGAGGTCGCCCTGTT
CAAGGCCACCCAGCCAGCTCATGGCGGTAATGGGACAAGGCTGGCCAGCCATCCCACCCTCAGAAGGG
ACCCGGTGGGGCAGGTGATCTCAGAGGAGGCTCACTTCTGGGTCTCACATTCTTGGATCCGGTTCCAG
GCCTCGGCCCTAAATAGTCTCCCTGGGCTTTCAAGAGAACCACATGAGAAAGGAGGATTCGGGCTCTG
AGCAGTTTCACCACCCACCCCCCAGTCTGCAAATCCTGACCCGAGGGTCCACCTGCCCCAAAGGCGGA
CGCAGGACAGTAGAAGGGAACAGAGAACACATAAACACAGAGAGGGCCACAGCGGCTCCCACAGTCAC
CGCCACCTTCCTGGCGGGGATGGGTGGGGCGTCTGAGTTTGGTTCCCAGCAAATCCCTCTGAGCCGCC
CTTGCGGGCTCGCCTCAGGAGCAGGGGAGCAAGAGGTGGGAGGAGGAGGTCTAAGTCCCAGGCCCAAT
TAAGAGATCAGGTAGTGTAGGGTTTGGGAGCTTTTAAGGTGAAGAGGCCCGGGCTGATCCCACAGGCC

AGTATAAAGCGCCGTGACCCTCAGGTGATGCGCCAGGGCCGGCTGCCTGCGGGGACAGGGCTTTCCAT
AGCCATGAGGATCCGAATTCGGATCCCCATGTCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAG
AAAGTTAACTGGTAAGTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAAT
CAAAGAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAGGCCTGTACGGAAGTGTTACTTCTGCTCT
AAAAGCTGCGGAATTGTACCCGCGGCCGCCACCATGGCCCAAAGGCTTACAGGTGAACAGACACTGGA
CCACTATGAGGATAGCACCCATGCAAGCATCTTCACCTATACCAACAGCAACAGCACCAAAGGTCCCT
TTGAAGGCCCCAATTATCACATTGCTCCCAGGTGGGTGTACCACCTCACCAGCACCTGGATGATTCTT
GTGGTCGTTGCATCTGTCTTCACTAATGGACTTGTGCTGGCAGCCACCATGAGATTCAAGAAGCTGCG
CCATCCACTGAACTGGATTCTGGTGAACTTGGCAGTTGCTGACCTAGCAGAGACCATTATTGCCAGCA
CTATCAGTGTTGTGAACCAAATCTATGGCTACTTCGTTCTGGGACACCCTCTGTGTGTCATTGAAGGC
TACATTGTCTCATTGTGTGGAATCACAGGCCTCTGGTCCCTGGCCATCATTTCCTGGGAGAGATGGCT
GGTGGTCTGCAAGCCCTTTGGCAATGTGAGATTTGATGCTAAGCTGGCCACTGTGGGAATCGTCTTCT
CCTGGGTCTGGGCTGCTATATGGACGGCCCCACCAATCTTTGGTTGGAGCAGGTACTGGCCTTATGGC
CTGAAGACATCCTGTGGCCCAGACGTGTTCAGCGGTACCTCGTACCCCGGGGTTCAGTCTTATATGAT
GGTCCTCATGGTCACGTGCTGCATCTTCCCACTCAGCATCATCGTGCTCTGCTACCTCCAAGTGTGGC
TGGCCATCCGAGCAGTGGCAAAGCAACAGAAAGAATCTGAGTCCACTCAGAAGGCCGAGAAGGAGGTG
ACACGCATGGTGGTGGTGATGGTCTTCGCATACTGCCTCTGCTGGGGACCCTATACTTTCTTTGCATG
CTTTGCTACTGCCCACCCTGGCTATGCCTTCCACCCTCTTGTGGCCTCCCTACCATCCTACTTTGCCA
AAAGTGCCACTATCTACAACCCCATTATCTATGTCTTTATGAACCGGCAGTTTCGAAACTGCATCTTA
CATCTCTTTGGAAAGAAGGTTGATGATAGCTCTGAACTTTCCAGCACCTCCAAGACAGAAGTCTCATC
TGTCTCTTCAGTGTCACCTGCATAAGCGGCCGCGGGGATCCAGACATGATAAGATACATTGATGAGTT
TGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTT
TATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAG
GTTCAGGGGGAGGTGTGGGAGGTTTTTTAGTCGACTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTC
TAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCA
CTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGG
GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAGAGATC
TAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCG
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AAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTA
TTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGAT
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AAATACCGCATCAGGAAATTGTAAACGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATC

AGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGAT
AGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAG
GGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGG
TCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAA
GCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTG
TAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCGCGC
CATTCGCCATTCAGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCA
GGCTGCAGGGGGGGGGGGGGGGGG (SEQ ID NO: 2)

In some embodiments, the nucleic acid is a recombinant adeno-associated virus (rAAV) vector. Exemplary rAAV nucleic acid vectors useful according to the disclosure include single-stranded (ss) or self-complementary (sc) AAV nucleic acid vectors.

In some embodiments, a recombinant rAAV particle comprises a nucleic acid vector, such as a single-stranded (ss) or self-complementary (sc) AAV nucleic acid vector. In some embodiments, the nucleic acid vector contains an expression construct comprising an opsin gene (e.g., an M-opsin gene) operably linked to a cone-specific promoter and one or more regions comprising inverted terminal repeat (ITR) sequences (e.g., wild-type ITR sequences or engineered ITR sequences) flanking the expression construct. In some embodiments, the nucleic acid is encapsidated by a viral capsid. In some embodiments, a nucleic acid vector is a linear nucleic acid vector (*e.g.*, a single-stranded or double-stranded linear nucleic acid, for example containing an expression cassette flanked by ITRs).

Accordingly, in some embodiments, an rAAV particle comprises a viral capsid and a nucleic acid vector as described herein, which is encapsidated by the viral capsid. In some embodiments, the viral capsid comprises 60 capsid protein subunits comprising VP1, VP2 and VP3. In some embodiments, the VP1, VP2, and VP3 subunits are present in the capsid at a ratio of approximately 1:1:10, respectively.

The ITR sequences of a nucleic acid or nucleic acid vector described herein can be derived from any AAV serotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) or can be derived from more than one serotype. In some embodiments of the nucleic acid or nucleic acid vector provided herein, the ITR sequences are derived from AAV2. ITR sequences and plasmids

containing ITR sequences are known in the art and commercially available (see, e.g., products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; and Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):14082-7; and Curtis A. Machida. Methods in Molecular Medicine™. Viral Vectors for Gene Therapy Methods and Protocols. 10.1385/1-59259-304-6:201 © Humana Press Inc. 2003. Chapter 10. Targeted Integration by Adeno-Associated Virus. Matthew D. Weitzman, Samuel M. Young Jr., Toni Cathomen and Richard Jude Samulski; U.S. Pat. Nos. 5,139,941 and 5,962,313, all of which are incorporated herein by reference).

In some embodiments, the nucleic acid or nucleic acid vector comprises a pTR-UF-11 plasmid backbone, which is a plasmid that contains AAV2 ITRs, or a nucleic acid region of the pTR-UF-11 plasmid that comprises the ITRs. This plasmid is commercially available from the American Type Culture Collection (ATCC MBA-331).

In some embodiments, the expression construct is no more than 6 kilobases, no more than 5 kilobases, no more than 4 kilobases, or no more than 3 kilobases in size. In some embodiments, the expression construct is between 4 and 6 kilobases in size, e.g., 4-6 kilobases, 4-5 kilobases, or 4.2-4.7 kilobases in size.

In some embodiments, the expression construct comprises one or more regions comprising a sequence that facilitates expression of the opsin gene, e.g., expression control sequences operably linked to the opsin gene. Non-limiting examples of expression control sequences include promoters, insulators, silencers, response elements, introns, enhancers, initiation sites, termination signals, and poly(A) tails. Any combination of such control sequences is contemplated herein (e.g., a promoter and an enhancer). In some embodiments, the promoter is a cone-specific promoter, such as a PR2.1 promoter as described herein. In some embodiments, the expression construct contains a splice donor/acceptor site, such as between the promoter and opsin gene.

In some embodiments, a nucleic acid described herein, such as a plasmid, may also

contain marker or reporter genes, e.g., LacZ or a fluorescent protein, and an origin of replication (e.g. an f1(+) origin and/or a ColE1 origin).

The rAAV particle may be of any AAV serotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), including any derivative or pseudotype. Non-limiting examples of derivatives and pseudotypes include AAV2-AAV3 hybrid, AAVrh.10, AAVhu.14, AAV3a/3b, AAVrh32.33, AAV-HSC15, AAV-HSC17, AAVhu.37, AAVrh.8, CHt-P6, AAV2.5, AAV6.2, AAV2i8, AAV-HSC15/17, AAVM41, AAV9.45, AAV6(Y445F/Y731F), AAV2.5T, AAV-HAE1/2, AAV clone 32/83, AAVShH10, AAV2 (Y->F), AAV8 (Y733F), AAV2.15, AAV2.4, AAVM41, and AAVr3.45. Such AAV serotypes and derivatives/pseudotypes, and methods of producing such derivatives/pseudotypes are known in the art (see, e.g., Mol Ther. 2012 Apr;20(4):699-708. doi: 10.1038/mt.2011.287. Epub 2012 Jan 24. The AAV vector toolkit: poised at the clinical crossroads. Asokan A1, Schaffer DV, Samulski RJ.). In some embodiments, the rAAV particle is a pseudotyped rAAV particle, which comprises (a) a nucleic acid vector comprising ITRs from one serotype (e.g., AAV2) and (b) a capsid comprised of capsid proteins derived from another serotype (e.g., AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10). Methods for producing and using pseudotyped rAAV vectors are known in the art (see, e.g., Duan et al., J. Virol., 75:7662-7671, 2001; Halbert et al., J. Virol., 74:1524-1532, 2000; Zolotukhin et al., Methods, 28:158-167, 2002; and Auricchio et al., Hum. Molec. Genet., 10:3075-3081, 2001).

In some embodiments, the rAAV particle is AAV5. In some embodiments, the rAAV particle comprises AAV5 capsid proteins. In some embodiments, the rAAV particle comprises wild-type AAV5 capsid proteins. In some embodiments, the rAAV particle comprises variant AAV5 capsid proteins, *e.g.*, AAV5 capsid proteins that contain one or more modifications compared to the wild-type capsid protein sequence. Such one or more modifications can include but are not limited to, one or more amino acid modifications (*e.g.*, one or more amino acid substitutions, one or more amino acid insertions, one or more amino acid deletions, or some combination thereof) with respect to the wild-type AAV5 capsid protein sequence. In some embodiments, the AAV5 capsid protein comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more amino acid modifications (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or

more amino acid substitutions; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more amino acid insertions; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more amino acid deletions; or some combination thereof) with respect to the wild-type AAV5 capsid protein sequence. An exemplary, non-limiting wild-type AAV5 capsid protein sequence is provided below.

Exemplary AAV5 capsid protein

1 MSFVDHPPDW LEEVGEGLRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN
51 YLGPGNGLDR GEPVNRADEV AREHDISYNE QLEAGDNPYL KYNHADAEFQ
101 EKLADDTSFG GNLGKAVFQA KKRVLEPFGL VEEGAKTAPT GKRIDDHFPK
151 RKKARTEEDS KPSTSSDAEA GPSGSQQLQI PAQPASSLGA DTMSAGGGGP
201 LGDNNQGADG VGNASGDWHC DSTWMGDRVV TKSTRTWVLP SYNNHQYREI
251 KSGSVDGSNA NAYFGYSTPW GYFDFNRFHS HWSPRDWQRL INNYWGFRPR
301 SLRVKIFNIQ VKEVTVQDST TTIANNLTST VQVFTDDDYQ LPYVVGNGTE
351 GCLPAFPPQV FTLPQYGYAT LNRDNTENPT ERSSFFCLEY FPSKMLRTGN
401 NFEFTYNFEE VPFHSSFAPS QNLFKLANPL VDQYLYRFVS TNNTGGVQFN
451 KNLAGRYANT YKNWFPGPMG RTQGWNLGSG VNRASVSAFA TTNRMELEGA
501 SYQVPPQPNG MTNNLQGSNT YALENTMIFN SQPANPGTTA TYLEGNMLIT
551 SESETQPVNR VAYNVGGQMA TNNQSSTTAP ATGTYNLQEI VPGSVWMERD
601 VYLQGPIWAK IPETGAHFHP SPAMGGFGLK HPPPMMLIKN TPVPGNITSF
651 SDVPVSSFIT QYSTGQVTVE MEWELKKENS KRWNPEIQYT NNYNDPQFVD
701 FAPDSTGEYR TTRPIGTRYL TRPL (SEQ ID NO: 9)

Methods of producing rAAV particles and nucleic acid vectors are also known in the art and commercially available (see, e.g., Zolotukhin et al. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods 28 (2002) 158–167; and U.S. Patent Publication Numbers US20070015238 and US20120322861, which are incorporated herein by reference; and plasmids and kits available from ATCC and Cell Biolabs, Inc.). For example, the nucleic acid vector may be combined with one or more helper plasmids, e.g., that contain a *rep* gene (e.g., encoding Rep78, Rep68, Rep52 and Rep40) and a *cap* gene (encoding VP1, VP2, and VP3), and transfected into a producer cell

line such that the rAAV particle can be packaged and subsequently purified.

In some embodiments, the one or more helper plasmids includes a first helper plasmid comprising a *rep* gene and a *cap* gene and a second helper plasmid comprising other genes that assist in AAV production, such as a E1a gene, a E1b gene, a E4 gene, a E2a gene, and a VA gene. In some embodiments, the rep gene is a *rep* gene derived from AAV2 and the *cap* gene is derived from AAV5. Helper plasmids, and methods of making such plasmids, are known in the art and commercially available (see, e.g., pDM, pDG, pDP1rs, pDP2rs, pDP3rs, pDP4rs, pDP5rs, pDP6rs, pDG(R484E/R585E), and pDP8.ape plasmids from PlasmidFactory, Bielefeld, Germany; other products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; pxx6; Grimm et al. (1998), Novel Tools for Production and Purification of Recombinant Adenoassociated Virus Vectors, Human Gene Therapy, Vol. 9, 2745-2760; Kern, A. et al. (2003), Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids, Journal of Virology, Vol. 77, 11072-11081.; Grimm et al. (2003), Helper Virus-Free, Optically Controllable, and Two-Plasmid-Based Production of Adeno-associated Virus Vectors of Serotypes 1 to 6, Molecular Therapy,Vol. 7, 839-850; Kronenberg et al. (2005), A Conformational Change in the Adeno-Associated Virus Type 2 Capsid Leads to the Exposure of Hidden VP1 N Termini , Journal of Virology, Vol. 79, 5296-5303; and Moullier, P. and Snyder, R.O. (2008), International efforts for recombinant adenoassociated viral vector reference standards, Molecular Therapy, Vol. 16, 1185-1188).

An exemplary, non-limiting, rAAV particle production method is described next. One or more helper plasmids are produced or obtained, which comprise rep and cap ORFs for the desired AAV serotype and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. HEK293 cells (available from ATCC®) are transfected via $CaPO_4$-mediated transfection, lipids or polymeric molecules such as Polyethylenimine (PEI) with the helper plasmid(s) and a plasmid containing a nucleic acid vector described herein. Alternatively, in another example, Sf9-based producer stable cell lines are infected with a single recombinant baculovirus containing the nucleic acid vector. As a further alternative, in another example HEK293 or BHK cell lines are infected with a

HSV containing the nucleic acid vector and optionally one or more helper HSVs containing rep and cap ORFs as described herein and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The HEK293, BHK, or Sf9 cells are then incubated for at least 60 hours to allow for rAAV particle production. The rAAV particles can then be purified using any method known the art or described herein, e.g., by iodixanol step gradient, CsCl gradient, chromatography, or polyethylene glycol (PEG) precipitation.

The disclosure also contemplates host cells that comprise at least one of the disclosed rAAV particles or nucleic acid vectors. Such host cells include mammalian host cells, with human host cells being preferred, and may be either isolated, in cell or tissue culture. In the case of genetically modified animal models (e.g., a mouse), the transformed host cells may be comprised within the body of a non-human animal itself.

Compositions

Aspects of the disclosure relate to compositions comprising rAAV particles or nucleic acids described herein. In some embodiments, rAAV particles described herein are added to a composition, e.g., a pharmaceutical composition.

In some embodiments, the composition comprises a pharmaceutically acceptable carrier. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the rAAV particle is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers. Non-limiting examples of pharmaceutically acceptable carriers include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, saline, syrup, methylcellulose, ethylcellulose, hydroxypropylmethylcellulose, polyacrylic acids, lubricating agents (such as talc, magnesium stearate, and mineral oil), wetting agents, emulsifying agents, suspending agents, preserving agents (such as methyl-, ethyl-, and

propyl-hydroxy-benzoates), and pH adjusting agents (such as inorganic and organic acids and bases). Other exemplary carriers that might be used include saline (e.g., sterilized, pyrogen-free saline), saline buffers (e.g., citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. USP grade carriers and excipients are particularly useful for delivery of rAAV particles to human subjects. Such compositions may further optionally comprise a liposome, a lipid, a lipid complex, a microsphere, a microparticle, a nanosphere, or a nanoparticle, or may be otherwise formulated for administration to the cells, tissues, organs, or body of a subject in need thereof. Methods for making such compositions are well known and can be found in, for example, Remington: The Science and Practice of Pharmacy, 22nd edition, Pharmaceutical Press, 2012.

Typically, such compositions may contain at least about 0.1% of the therapeutic agent (e.g., rAAV particle) or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of therapeutic agent(s) (e.g., rAAV particle) in each therapeutically-useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In some embodiments, a composition described herein may be administered to a subject in need thereof, such as a subject having BCM. In some embodiments, a method described herein may comprise administering a composition comprising rAAV particles as described herein to a subject in need thereof. In some embodiments, the subject is a human subject. In some embodiments, the subject has or is suspected of having a disease that may be treated with gene therapy, such as BCM.

Methods

Aspects of the disclosure relate to treatment of ocular disorders such as cone monochromacies (e.g., blue cone monochromacy) and cone dichromacies (e.g., protanopia or deuteranopia). In some embodiments, the method comprises administering a therapeutically effective amount of an rAAV particle or a composition as described herein to a subject having a cone monochromacy or dichromacy.

To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject. The compositions described above are typically administered to a subject in an effective amount, that is, an amount capable of producing a desirable result. The desirable result will depend upon the active agent being administered. For example, an effective amount of rAAV particles may be an amount of the particles that are capable of transferring an expression construct to a host organ, tissue, or cell. A therapeutically acceptable amount may be an amount that is capable of treating a disease, e.g., BCM. As is well known in the medical and veterinary arts, dosage for any one subject depends on many factors, including the subject's size, body surface area, age, the particular composition to be administered, the active ingredient(s) in the composition, time and route of administration, general health, and other drugs being administered concurrently.

The rAAV particle or nucleic acid vector may be delivered in the form of a composition, such as a composition comprising the active ingredient, such as an rAAV particle described herein, and a pharmaceutically acceptable carrier as described herein. The rAAV particles or nucleic acid vectors may be prepared in a variety of compositions, and may also be formulated in appropriate pharmaceutical vehicles for administration to human or animal subjects.

In some embodiments, the number of rAAV particles administered to a subject may be on the order ranging from 10^6 to 10^{14} particles/mL or 10^3 to 10^{15} particles/mL, or any values therebetween for either range, such as for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} particles/mL. In one embodiment, rAAV particles of higher than 10^{13} particles/mL are administered. In some embodiments, the number of rAAV particles

administered to a subject may be on the order ranging from 10^6 to 10^{14} vector genomes(vgs)/mL or 10^3 to 10^{15} vgs/mL, or any values therebetween for either range, such as for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} vgs/mL. In one embodiment, rAAV particles of higher than 10^{13} vgs/mL are administered. The rAAV particles can be administered as a single dose, or divided into two or more administrations as may be required to achieve therapy of the particular disease or disorder being treated. In some embodiments, 1 μL to 100 μLs are delivered to a subject. In some embodiments, 10 μL to 1000 μLs are delivered to a subject. In some embodiments, 0.5 mL to 10 mLs are delivered to a subject.

If desired, rAAV particles may be administered in combination with other agents as well, such as, e.g., proteins or polypeptides or various pharmaceutically-active agents, including one or more systemic or topical administrations of therapeutic polypeptides, biologically active fragments, or variants thereof. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The rAAV particles may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized.

In certain circumstances it will be desirable to deliver the rAAV particles in suitably formulated pharmaceutical compositions disclosed herein either subcutaneously, intraocularly, intravitreally, subretinally, parenterally, intravenously, intracerebroventricularly, intramuscularly, intrathecally, orally, intraperitoneally, by oral or nasal inhalation, or by direct injection to one or more cells, tissues, or organs by direct injection. In some embodiments, the administration is a route suitable for the eye, such as intraocular, intravitreal, or subretinal administration. The pharmaceutical forms of the rAAV particle compositions suitable for injectable use include sterile aqueous solutions or dispersions. In some embodiments, the form is sterile and fluid to the extent that easy syringability exists. In some embodiments, the form is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms, such as bacteria and fungi.

The carrier can be a solvent or dispersion medium containing, for example, water, saline, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, intravitreal, subretinal, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by, e.g., FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the rAAV particles in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization or another sterilization technique. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The amount of rAAV particle or nucleic acid vector compositions and time of

administration of such compositions will be within the purview of the skilled artisan having benefit of the present teachings. It is likely, however, that the administration of therapeutically-effective amounts of the disclosed compositions may be achieved by a single administration, such as for example, a single injection of sufficient numbers of infectious particles to provide therapeutic benefit to the patient undergoing such treatment. Alternatively, in some circumstances, it may be desirable to provide multiple, or successive administrations of the rAAV particle compositions, either over a relatively short, or a relatively prolonged period of time, as may be determined by the medical practitioner overseeing the administration of such compositions.

The composition may include rAAV particles, either alone, or in combination with one or more additional active ingredients, which may be obtained from natural or recombinant sources or chemically synthesized.

Toxicity and efficacy of the compositions utilized in methods of the disclosure can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD50 (the dose lethal to 50% of the population). The dose ratio between toxicity and efficacy the therapeutic index and it can be expressed as the ratio LD50/ED50. Those compositions that exhibit large therapeutic indices are preferred. While those that exhibit toxic side effects may be used, care should be taken to design a delivery system that minimizes the potential damage of such side effects. The dosage of compositions as described herein lies generally within a range that includes an ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

Subjects

Aspects of the disclosure relate to methods for use with a subject, such as human or non-human primate subjects. Non-limiting examples of non-human primate subjects include macaques (e.g., cynomolgus or rhesus macaques), marmosets, tamarins, spider monkeys, owl monkeys, vervet monkeys, squirrel monkeys, baboons, gorillas, chimpanzees, and orangutans. In some embodiments, the subject is a human subject. Other exemplary subjects

include domesticated animals such as dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and other animals such as mice, rats, guinea pigs, and hamsters.

In some embodiments, the subject has or is suspected of having a disease that may be treated with gene therapy. In some embodiments, the subject has or is suspected of having an ocular disorders such as a cone monochromacy (e.g., blue cone monochromacy) or cone dichromacy (e.g., protanopia or deuteranopia). Blue cone monochromacy (BCM) is characterized by the absence of L- and M-cone function in the retina due to the loss of functional long wavelength-sensitive and medium wave-length sensitive cones in the retina. BCM may result from mutations in a single red or red–green hybrid opsin gene, mutations in both the red and the green opsin genes, rearrangements of the L and M opsin genes, or deletions within the adjacent LCR (locus control region) on the X chromosome. Subjects with BCM have severe vision impairment, poor visual acuity, nystagmus, and photophobia. Protanopia is caused by the absence of red retinal photoreceptors. Deuteranopia is caused by the absence of green retinal photoreceptors. Subjects with BCM or other ocular disorders can be identified by the skilled practitioner using methods known in art, e.g., by clinical ophthalmological examination, electroretinography (ERG), psychophysical testing (e.g., color vision, dark adaptometry), and/or genetic testing.

In some embodiments, the subject has or is suspected of having one or more of Retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), Age-related macular degeneration (AMD), recessive RP, Dominant retinitis pigmentosa, X-linked retinitis pigmentosa, Incomplete X-linked retinitis pigmentosa, dominant, Dominant Leber congenital amaurosis, Recessive ataxia, posterior column with retinitis pigmentosa, Recessive retinitis pigmentosa with para-arteriolar preservation of the RPE, Retinitis pigmentosa RP12, Usher syndrome, Dominant retinitis pigmentosa with sensorineural deafness, Recessive retinitis punctata albescens, Recessive Alström syndrome, Recessive Bardet-Biedl syndrome, Dominant spinocerebellar ataxia w/macular dystrophy or retinal degeneration, Recessive abetalipoproteinemia, Recessive retinitis pigmentosa with macular degeneration, Recessive Refsum disease (adult form), Recessive Refsum disease (infantile form), Recessive enhanced S-cone syndrome, Retinitis pigmentosa with mental retardation, Retinitis pigmentosa with

myopathy, Recessive Newfoundland rod-cone dystrophy, Retinitis pigmentosa sinpigmento, Sector retinitis pigmentosa, Regional retinitis pigmentosa, Senior-Loken syndrome, Joubert syndrome, Stargardt disease (juvenile), Stargardt disease (late onset), Dominant macular dystrophy Stargardt type, Dominant Stargardt-like macular dystrophy, Recessive macular dystrophy, Recessive fundus flavimaculatus, Recessive cone-rod dystrophy, X-linked progressive cone-rod dystrophy, Dominant cone-rod dystrophy, Cone-rod dystrophy, de Grouchy syndrome, Dominant cone dystrophy, X-linked cone dystrophy, Recessive cone dystrophy, Recessive cone dystrophy with supernormal rod electroretinogram, X-linked atrophic macular dystrophy, X-linked retinoschisis, Dominant macular dystrophy, Dominant radial, macular drusen, Dominant macular dystrophy (bull's-eye), Dominant macular dystrophy (butterfly-shaped), Dominant adult vitelliform macular dystrophy, Dominant macular dystrophy (North Carolina type), Dominant retinal-cone dystrophy 1, Dominant macular dystrophy (cystoid), Dominant macular dystrophy (atypical vitelliform), Foveomacular atrophy, Dominant macular dystrophy (Best type), Dominant macular dystrophy (North Carolina-like with progressive), Recessive macular dystrophy (juvenile with hypotrichosis), Recessive foveal hypoplasia and anterior segment dysgenesis, Recessive delayed cone adaptation, Macular dystrophy in blue cone monochromacy, Macular pattern dystrophy with type II diabetes and deafness, Flecked Retina of Kandori, Pattern Dystrophy, Dominant Stickler syndrome, Dominant Marshall syndrome, Dominant vitreoretinal degeneration, Dominant familial exudative vitreoretinopathy, Dominant vitreoretinochoroidopathy, Dominant neovascular inflammatory vitreoretinopathy, Goldmann-Favre syndrome, Recessive achromatopsia, Dominant tritanopia, Recessive rod monochromacy, Congenital red-green deficiency, Deuteranopia, Protanopia, Deuteranomaly, Protanomaly, Recessive Oguchi disease, Dominant macular dystrophy (late onset), Recessive gyrate atrophy, Dominant atrophia greata, Dominant central areolar choroidal dystrophy, X-linked choroideremia, Choroidal atrophy, Central areolar, Central, Peripapillary, Dominant progressive bifocal chorioretinal atrophy, Progresive bifocal Choroioretinal atrophy, Dominant Doyne honeycomb retinal degeneration (Malattia Leventinese), Amelogenesis imperfecta, Recessive Bietti crystalline corneoretinal dystrophy, Dominant hereditary

vascular retinopathy with Raynaud phenomenon and migraine, Dominant Wagner disease and erosive vitreoretinopathy, Recessive microphthalmos and retinal disease syndrome, Recessive nanophthalmos, Recessive retardation, spasticity and retinal degeneration, Recessive Bothnia dystrophy, Recessive pseudoxanthoma elasticum, Dominant pseudoxanthoma elasticum, Recessive Batten disease (ceroid-lipofuscinosis) (juvenile), Dominant Alagille syndrome, McKusick-Kaufman syndrome, hypoprebetalipoproteinemia, acanthocytosis, palladial degeneration, Recessive Hallervorden-Spatz syndrome, Dominant Sorsby's fundus dystrophy, Oregon eye disease, Kearns-Sayre syndrome, Retinitis pigmentosa with developmental and neurological abnormalities, Basseb Korenzweig Syndrome, Hurler disease, Sanfilippo disease, Scieie disease, Melanoma associated retinopathy, Sheen retinal dystrophy, Duchenne macular dystrophy, Becker macular dystrophy, Birdshot Retinochoroidopathy, Multiple Evanescent White-dot syndrome, Acute Zonal Occult Outer Retinopathy, Retinal vein occlusion, Retinal artery occlusion, Diabetic retinopathy, Retinal toxicity, Retinal injury, Retinal traumata, Retinal laser lesions, and Fundus Albipunctata.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

Example 1: AAV-mediated Gene Therapy Restores M-Cone Function in S-opsin only Opn1mw KO Mice

Color vision is facilitated by cones expressing different opsin photopigments. Cones containing M- or S-opsin are sensitive to middle or short wavelength visible light, respectively. *Opn1mw* knock-out (M-opsin KO) mice lack of M-opsin expression while

maintaining a normal number of cones and S-opsin expression. Similarly, Blue Cone Monochromacy (BCM) patients lack functioning middle and long wavelength opsins and only express S-opsin. Therefore, the *Opn1mw* KO mouse is a good model to establish pre-clinical efficacy and safety data in future BCM gene therapy clinical trial. It was therefore tested whether AAV-mediated M-opsin expression in cones can restore M-cone function/structure in this model.

Opn1mw KO mice were generated by inserting a gene trap into intron 2 of the mouse Opn1m*w* gene. A plasmid containing a PR2.1-M-opsinconstruct was generated (FIGs. 1A and 1B). The construct contained the mouse cone opsin middle wavelength cDNA driven by the cone-specific PR2.1 promoter. The cDNA was followed by a SV40 polyadenylation signal and a bovine growth hormone polyadenylation signal. The expression cassette was flanked by inverted terminal repeats (ITRs). The expression construct was packaged into AAV5 using standard techniques. At postnatal day 14 (P14), one µl of AAV5-PR2.1-mouse-M-opsin vector (10^{13} vector genome particles/mL) was injected subretinally into one eye of each Opn1m*w* KO mouse. The other eye was uninjected and served as a control. M-cone mediated ERGs were recorded at two months after injection. Treated and untreated eyes were harvested immediately after ERG recording for immunohistochemical studies.

Two months after treatment, nearly normal dark-adapted rod ERG waveforms were recorded in either treated or untreated *Opn1mw* KO eyes with a mild reduction of ERG amplitudes in treated eyes, likely due to slight subretinal injection-related damage (FIG. 2). Cone mediated light-adapted ERGs or S-cone mediated ERGs were also recorded from treated and untreated eyes; M-cone mediated ERGs were restored only in treated but not in untreated *Opn1mw* KO eyes. Cone-opsin staining showed that S-opsin was present in both treated and untreated eyes, mainly in central and inferior retina (FIG. 3, middle column). No M-opsin was evident in untreated eyes (FIG. 3, left column, bottom row). In contrast, abundant M-opsin positive cones were observed in treated eyes throughout the retina (FIG. 3, left column, top row). AAV-mediated M-opsin expression not only co-localized with S-opsin expression in central and inferior cone outer segments, but was also found in the superior retina which has little or no S-opsin (FIG. 4, top row).

Since *Opn1mw* KO mice express only S-opsin, they serve as a model for human BCM. AAV5-PR2.1-mouse-M-opsin mediated gene therapy restored M-opsin expression and M-opsin function in *Opn1mw* KO mice. These results serve as a baseline for studying long-term M-cone rescue in *Opn1mw* KO mice and for developing a BCM gene therapy.

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be

understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in

a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as

"comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03. It should be appreciated that embodiments described in this document using an open-ended transitional phrase (e.g., "comprising") are also contemplated, in alternative embodiments, as "consisting of" and "consisting essentially of" the feature described by the open-ended transitional phrase. For example, if the disclosure describes "a composition comprising A and B", the disclosure also contemplates the alternative embodiments "a composition consisting of A and B" and "a composition consisting essentially of A and B".

What is claimed is:

CLAIMS

1. A nucleic acid comprising an expression construct containing an M-opsin gene operably linked to a cone-specific promoter, wherein the expression construct is flanked by inverted terminal repeat sequences.

2. The nucleic acid of claim 1, wherein the cone-specific promoter is a PR2.1 promoter having the sequence of SEQ ID NO: 1.

3. The nucleic acid of claim 1 or claim 2, wherein the expression construct further contains nucleic acid segments that encode an SV40 polyadenylation signal and a bovine growth hormone polyadenylation signal, wherein the nucleic acid segments are positioned 3' to the M-opsin gene.

4. The nucleic acid of any one of claims 1 to 3, wherein the nucleic acid comprises the sequence of SEQ ID NO: 2.

5. The nucleic acid of any one of claims 1 to 4, wherein the nucleic acid is a recombinant adeno-associated virus (rAAV) vector.

6. A recombinant adeno-associated virus (rAAV) particle comprising the nucleic acid of claim 5.

7. The rAAV particle of claim 6, wherein the rAAV particle is an AAV5 particle.

8. A composition comprising a plurality of the rAAV particle of claim 6 or claim 7.

9. The composition of claim 8, further comprising a pharmaceutically acceptable carrier.

10. A method of treating a cone monochromacy in a subject, the method comprising:

administering a therapeutically effective amount of the rAAV particle of claim 6 or claim 7 or the composition of claim 8 or claim 9 to a subject having a cone monochromacy.

11. The method of claim 10, wherein the rAAV particle or composition are administered via subretinal injection.

12. The method of claim 10 or claim 11, wherein the subject has blue cone monochromacy.

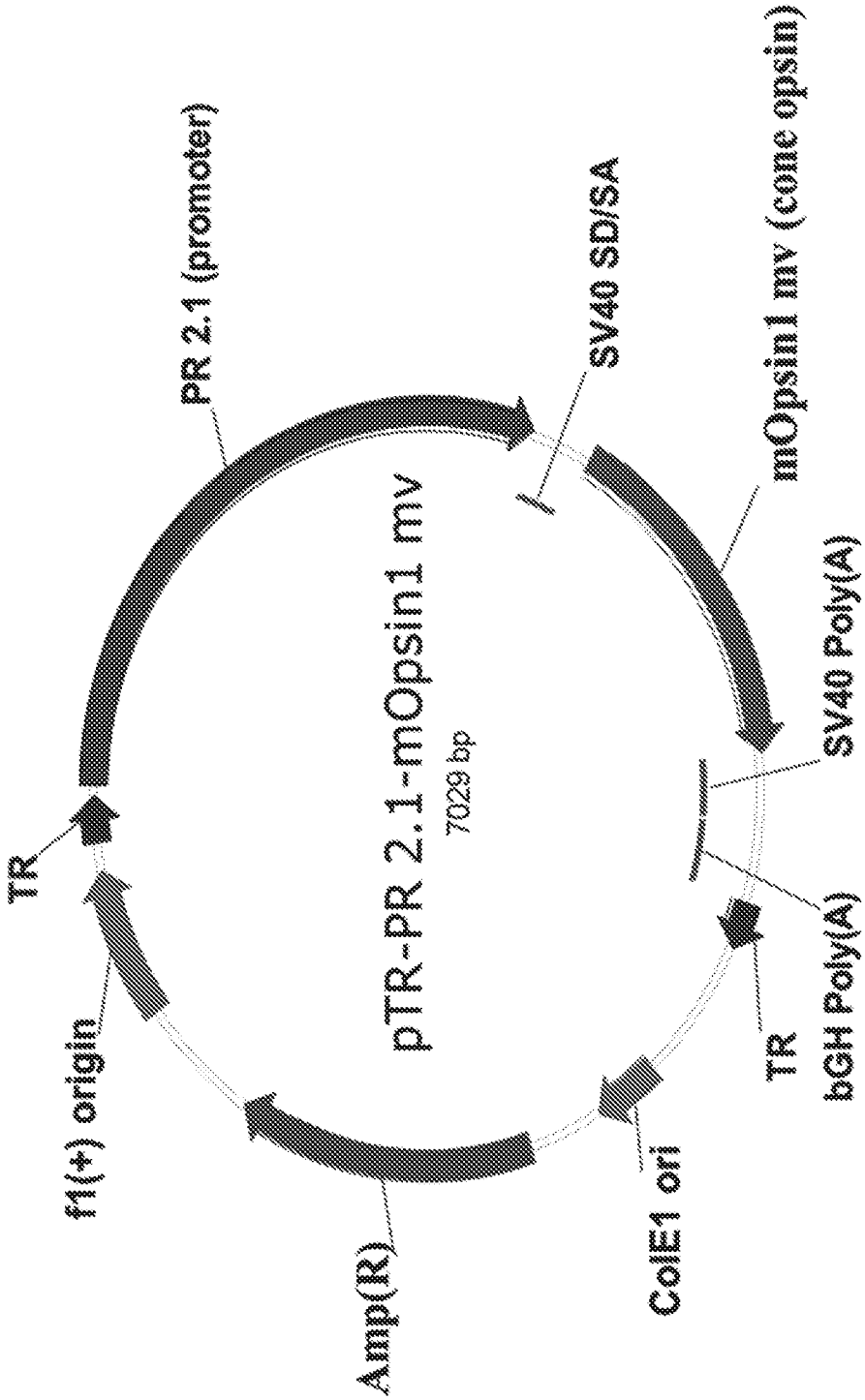

pTR-PR 2.1-mOpsin1 mv
7029 bp
PR 2.1 (promoter)
SV40 SD/SA
mOpsin1 mv (cone opsin)
SV40 Poly(A)
TR
bGH Poly(A)
ColE1 ori
Amp(R)
f1(+) origin
TR

FIG. 1A

Feature Map:

ITR 5'	Start: 1	End: 143
PR 2.1 (promoter)	Start: 163	End: 2271
mOpsin1mv(cone ospin)	Start: 2478	End: 3561
SV40 Poly(A)	Start: 3569	End: 3767
bGH Poly(A)	Start: 3784	End: 4006
ITR 3'	Start: 4019	End: 4161
ColE1 ori	Start: 4607	End: 4832
AMP(R)	Start: 5082	End: 6082
f1(+) origin	Start: 6486	End: 6945

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCC
GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTAGATCTGAATTCG
GTACCCCCGGGGGATCCTCTAGAGTCGACAGGCCTACAGCAGCCAGGGTGAGATTATGAGGCTGAGCTGAGAATATCAA
GACTGTACCGAGTAGGGGGCCTTGGCAAGTGTGGAGAGCCCGGCAGCTGGGGCAGAGGGCGGAGTACGGTGTGCGTTTA
CGGACCTCTTCAAACGAGGTAGGAAGGTCAGAAGTCAAAAAGGGAACAAATGATGTTTAACCACACAAAAATGAAAATC
CAATGGTTGGATATCCATTCCAAATACACAAAGGCAACGGATAAGTGATCCGGGCCAGGCACAGAAGGCCATGCACCCG
TAGGATTGCACTCAGAGCTCCCAAATGCATAGGAATAGAAGGGTGGGTGCAGGAGGCTGAGGGGTGGGGAAAGGGCATG
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AAATGGTTCCTTGTCCTATAGCTGTAGCAGCCATCGGCTGTTAGTGACAAAGCCCCTGAGTCAAGATGACAGCAGCCCC
CATAACTCCTAATCGGCTCTCCCGCGTGGAGTCATTTAGGAGTAGTCGCATTAGAGACAAGTCCAACATCTAATCTTCC
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CAGTAATCTGCTTTAGGCTAAAATGGGACTTGATCTTCTGTTAGCCCTAATCATCAATTAGCAGAGCCGGTGAAGGTGC
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CTCCCTCTAGACAGAAGGGGAATCTCGGTCAAGAGGGAGAGGTCGCCCTGTTCAAGGCCACCCAGCCAGCTCATGGCGG
TAATGGGACAAGGCTGGCCAGCCATCCCACCCTCAGAAGGGACCCGGTGGGGCAGGTGATCTCAGAGGAGGCTCACTTC
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TCAGGAGCAGGGGAGCAAGAGGTGGGAGGAGGAGGTCTAAGTCCCAGGCCCAATTAAGAGATCAGGTAGTGTAGGGTTT
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FIG. 1B

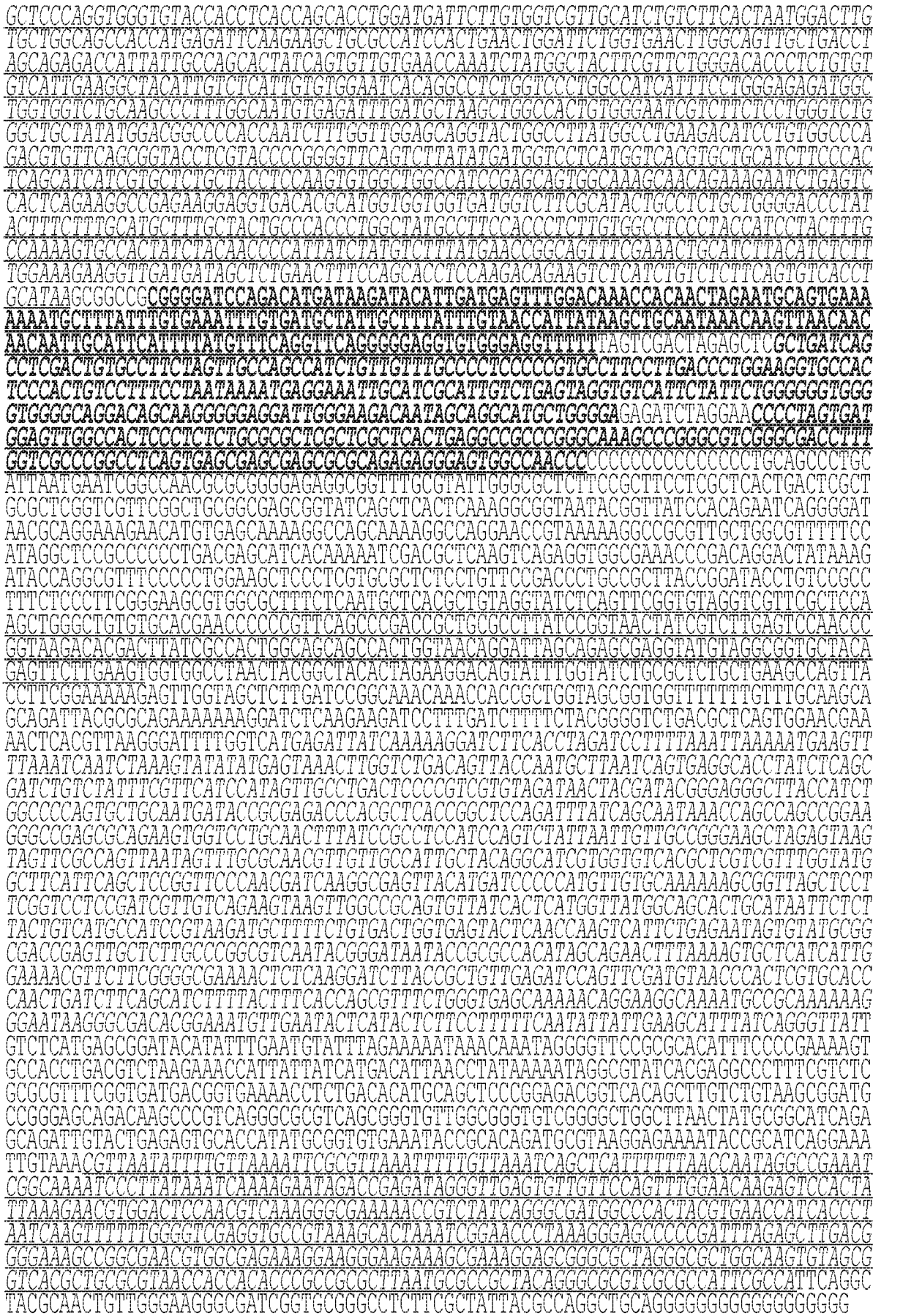


FIG. 1B (CONTINUED)

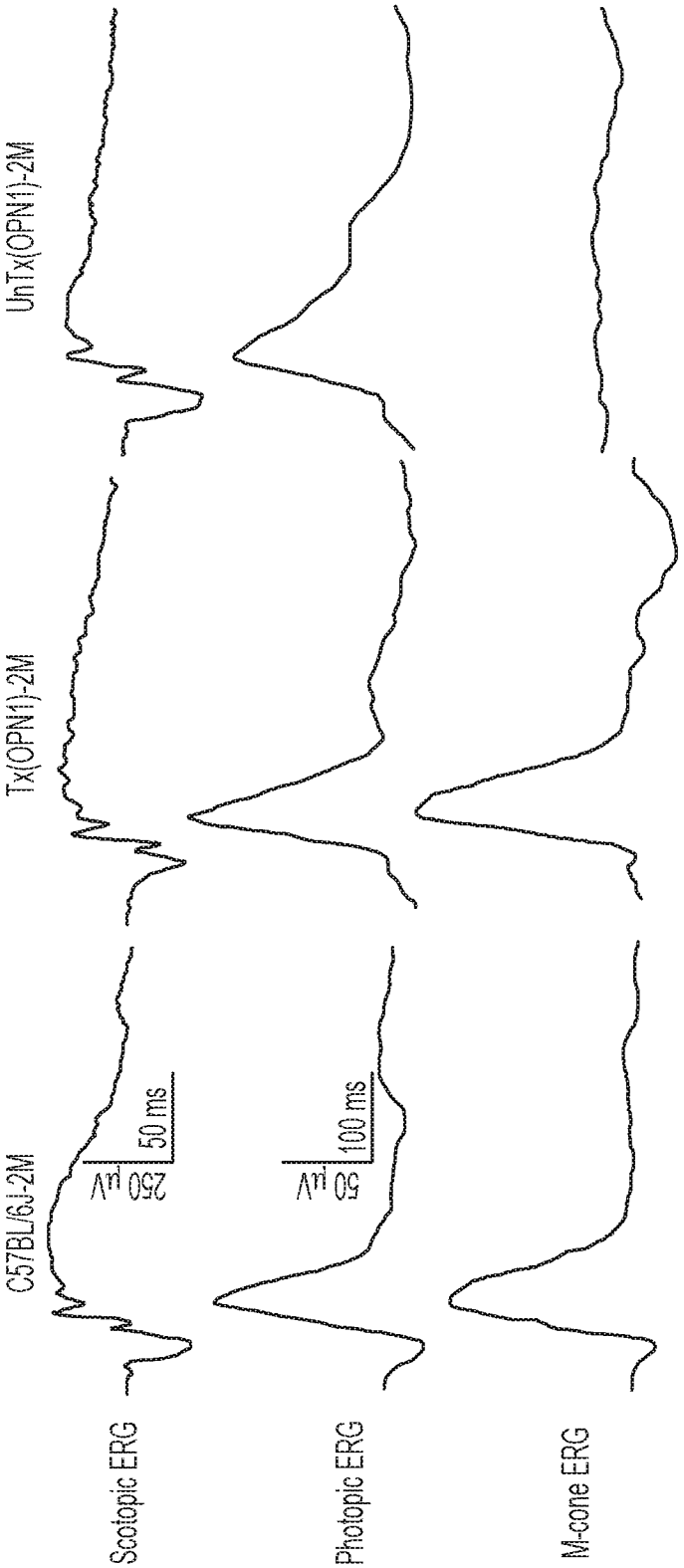

C57BL/6J-2M
Tx(OPN1)-2M
UnTx(OPN1)-2M
250 µV
50 ms
50 µV
100 ms
Scotopic ERG
Photopic ERG
M-cone ERG

FIG. 2

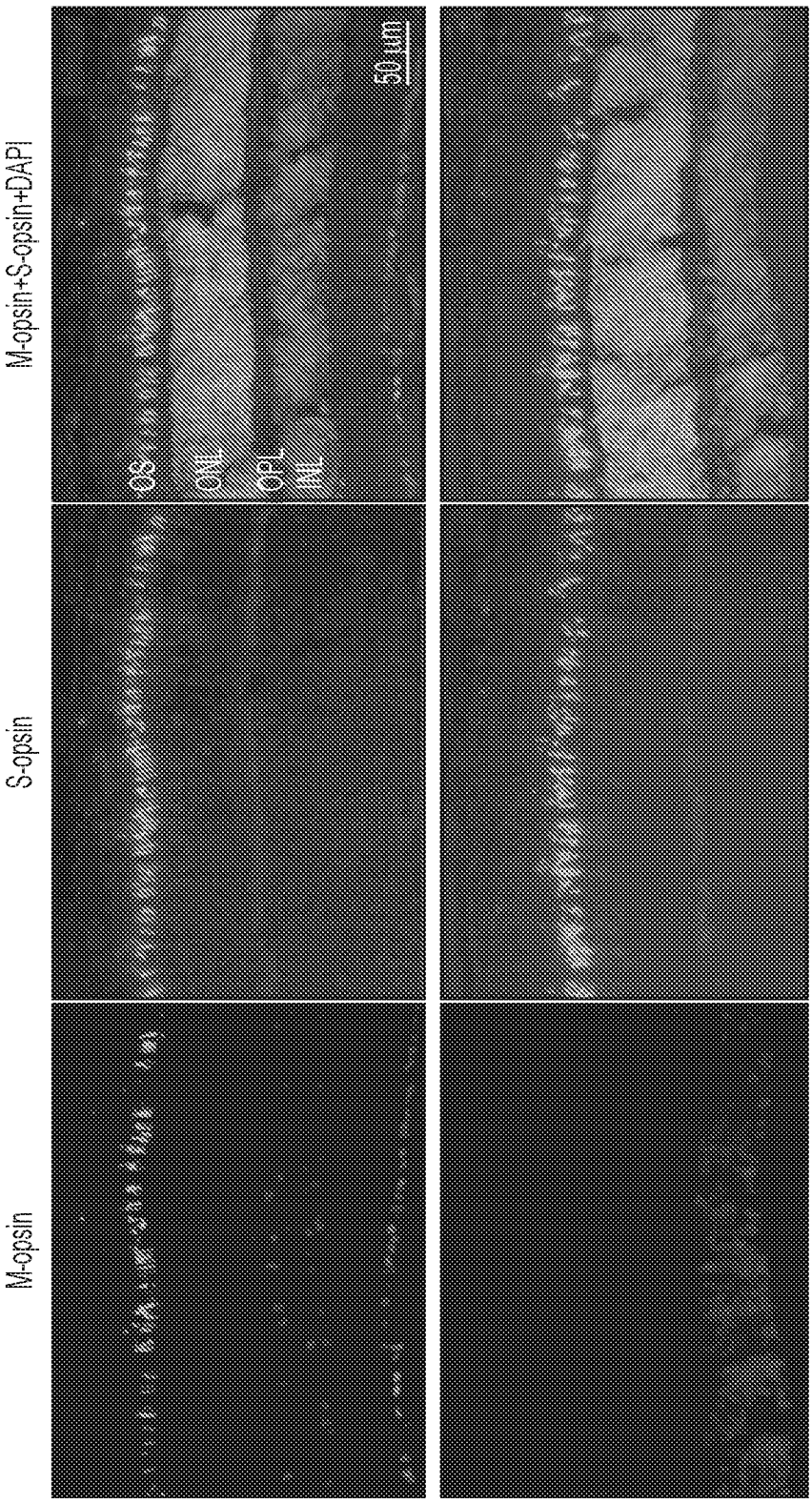

M-opsin
S-opsin
M-opsin+S-opsin+DAPI
OS
ONL
OPL
INL
50 um
Tx (OPN1)
2M (Inferior)
UnTx (OPN1)
2M (Inferior)

FIG. 3

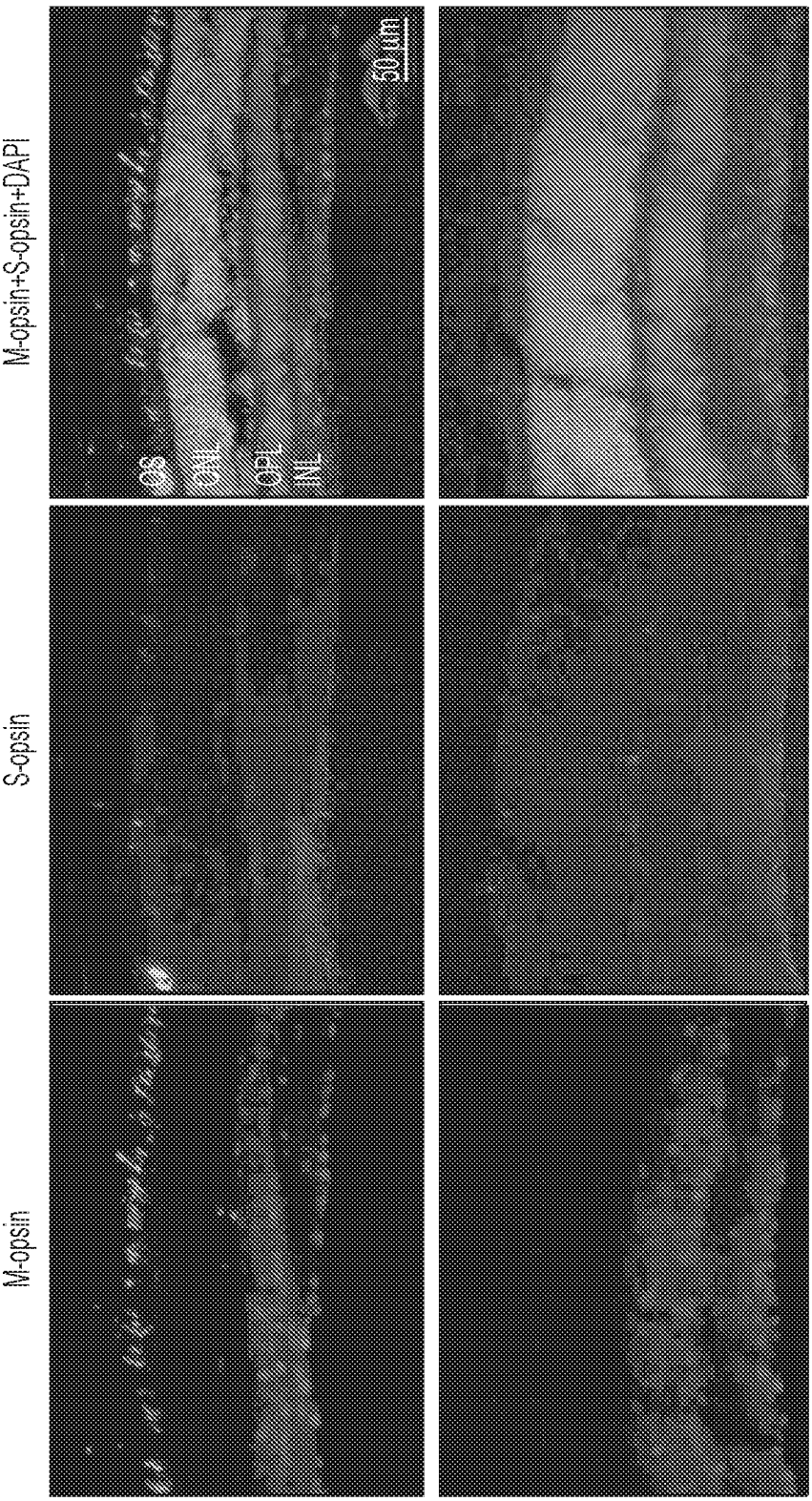

M-opsin
S-opsin
M-opsin+S-opsin+DAPI
50 um
OS
ONL
OPL
INL
Tx (OPN1)
2M (Superior)
UnTx (OPN1)
2M (Superior)

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/23252

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/12; A61K 48/00; C12N 15/00; C07H 21/04 (2016.01)
CPC - C07K 14/005; A61K 2039/5256; A61K 2039/53; C12N 15/85
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/12; A61K 48/00; C12N 15/00; C07H 21/04 (2016.01)
CPC - C07K 14/005; A61K 2039/5256; A61K 2039/53; C12N 15/85; USPC - 424/199.1; 514/44R; 435/320.1; 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 39/12; A61K 48/00; C12N 15/00; C07H 21/04 (2016.01) - see keyword below
CPC - C07K 14/005; A61K 2039/5256; A61K 2039/53; C12N 15/85; USPC - 424/199.1; 514/44R; 435/320.1; 536/24.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: M-opsin, Opn1mw, green opsin, cone, promoter, PR2.1 promoter, gene, vector, construct, terminal repeat sequence, LTR, flank, SV40, bovine growth hormone, BGH, polyA, polyadenylation signal, poly(A), deliver, retina, eye, nucleic acid, nucleotide, DNA, polynucleotide, operably, Middle-wavelengt

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2014/0080900 A1 (NEITZ et al.) 20 March 2014 (20.03.2014), para [0005], [0009], [0011], [0019], [0034], [0042], [0071], [0072], [0076], [0104], and [0138]	1 --- 3/(1) --- 2, 3/(2)
Y --- A	US 2013/0259833 A1 (Pan) 03 October 2013 (03.10.2013), Abstract, para [0007], [0018]-[0022], [0028], [0107], and claim 2	3/(1) --- 2, 3/(2)
A	US 2014/0364486 A1 (Ye) 11 December 2014 (11.12.2014), para [0004], [0007], [0008], and SEQ ID NO: 4	2, 3/(2)
A	DANIELE et al., A mouse M-opsin monochromat: Retinal cone photoreceptors have increased M-opsin expression when S-opsin is knocked out. Vision Res. 2011, Vol. 51(4), p. 447-58. Abstract	1-3
A	AZZONI et al., The impact of polyadenylation signals on plasmid nuclease-resistance and transgene expression. J Gene Med. 2007, Vol. 9(5), p. 392-402. Abstract	1-3
A	MANCUSO et al., Gene therapy for red-green colour blindness in adult primates. Nature. 2009, Vol. 461(7265), p. 784-787. PDF File: pg 1-11. Entire documentation, especially Abstract	1-3

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 May 2016 (10.05.2016)	13 JUL 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/23252

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-12
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.