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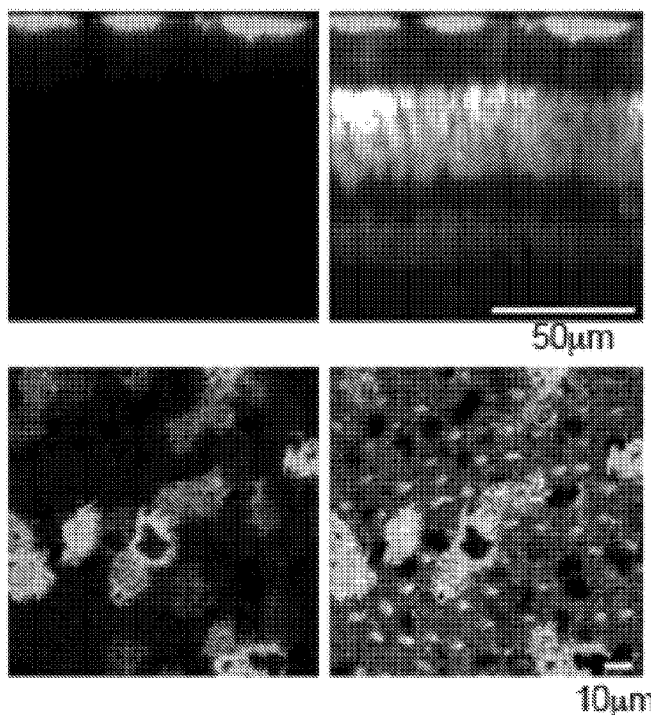


Figure 1

(57) Abstract: The present invention to a method for expressing an exogenous gene specifically in cells of the retinal pigment epithelium of a primate, this method comprising the step of delivering an isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid sequence of at least 400 bp having at least 80% overall identity to the sequence of SEQ ID NO:1, to cells of the retinal pigment epithelium of the primate, wherein this isolated nucleic acid molecule specifically leads to the expression of an exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid sequence coding for the exogenous gene is operatively linked to this isolated nucleic acid molecule.



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## 5 **Primate retinal pigment epithelium cell-specific promoter**

### FIELD OF THE INVENTION

10 The present invention relates to a nucleic acid sequence leading to the expression of genes specifically in cells of the retinal pigment epithelium (RPE).

### BACKGROUND OF THE INVENTION

15 For expression purposes recombinant genes are usually transfected into the target cells, cell populations or tissues, as cDNA constructs in the context of an active expression cassette to allow transcription of the heterologous gene. The DNA construct is recognized by the cellular transcription machinery in a process that involves the activity of many trans-acting transcription factors (TF) at cis-regulatory elements, including enhancers, silencers, insulators and promoters (herein globally referred to as "promoters").

20 Gene promoter are involved in all of these levels of regulation, serving as the determinant in gene transcription by integrating the influences of the DNA sequence, transcription factor binding and epigenetic features. They determines the strength of e.g. transgene expression which is encoded by a plasmid vector as well as in which cell type or types said transgene will be expressed.

25 The most common promoters used for driving heterologous gene expression in mammalian cells are the human and mouse cytomegalovirus (CMV) major immediate early promoter. They confer a strong expression and have proved robust in several cell types. Other viral promoters such as the SV40 immediate early promoter and the Rous Sarcoma Virus (RSV) long-terminal-repeat (LTR) promoter are also used frequently in expression cassettes.

30 Instead of viral promoters, cellular promoters can also be used. Among known promoters are those from house-keeping genes that encode abundantly transcribed cellular transcripts, such as beta-actin, elongation factor 1-alpha (EF-1alpha), or ubiquitin. Compared to viral promoters, eukaryotic gene expression is more complex and requires a precise coordination of many

35 different factors.

One of the aspects concerning the use of endogenous regulatory elements for transgene expression is the generation of stable mRNA and that expression can take place in the native environment of the host cell where trans-acting transcription factors are provided accordingly. Since expression of eukaryotic genes is controlled by a complex machinery of cis- and trans-acting regulatory elements, most cellular promoters suffer from a lack of extensive functional

40 characterization. Parts of the eukaryotic promoter are usually located immediately upstream of its transcribed sequence and serves as the point of transcriptional initiation. The core promoter immediately surrounds the transcription start site (TSS) which is sufficient to be recognized by the transcription machinery. The proximal promoter comprises the region

- 5 upstream of the core promoter and contains the TSS and other sequence features required for transcriptional regulation. Transcription factors act sequence-specific by binding to regulatory motifs in the promoter and enhancer sequence thereby activating chromatin and histone modifying enzymes that alter nucleosome structure and its position which finally allows initiation of transcription. The identification of a functional promoter is mainly
- 10 dependent on the presence of associated upstream or downstream enhancer elements. Another crucial aspect concerning the use of endogenous regulatory elements for transgene expression is that some promoters can act in a cell specific manner and will lead to the expression of the transgene on in cells of a specific type or, depending on the promoter, in cells of a particular subset.
- 15 Therefore, one goal of the present invention is to obtain new sequences suitable for expressing recombinant genes in mammal cells of the retina with high expression levels and in a cell type specific manner.
- Such sequence address a need in the art for retinal cells specific promoter to develop
- 20 systems for the study of neurodegenerative disorders, vision restoration, drug discovery, tumor therapies and diagnosis of disorders.

#### SUMMARY OF THE INVENTION

- 25 The present inventors have now serendipitously found that an artificial promoter which was known to drive gene expression specifically in retinal amacrine cells in a murine model as described in WO 2015/118507, has unexpectedly and atypically a totally different specificity in primates. In primates, this promoter specifically drives gene expression in cells of the retinal pigment epithelium (RPE). This specificity is extremely useful to address and target gene
- 30 expression in retinal pigment epithelium cells in diseased retina of e.g. age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy or retinal pigment epithelium hypertrophy patients.

The nucleic acid sequence of this promoter is:

- 35 TCAAGCCTCCTACCGCCTTTGTTATGCAAACATATCAAACGCCCTCCTTTGTTATGCAAAA  
GGGCTGGAACGGGGCCTTTGTTATGCAAATCGCCCTCCCGATCCCTTTGTTATGCAAA  
TTTGACGAATCCACCTTTGTTATGCAAACAAATCTCCTACCCTCCTTTGTTATGCAAAG  
TGAGAGGGGCTGCACCTTTGTTATGCAAATGCGGCCCTGAGACCTTTGTTATGCAAA  
AACCATGTACGCTTGCTTTGTTATGCAAACCGCCTGTTGCTTGGCCTTTGTTATGCAAA  
40 GCCACGCGATTGGCGCCTTTGTTATGCAAAGCTCGGTTATGTACACCTTTGTTATGCAAA  
GCTACTTTAACTTGCTTTGTTATGCAAATCACGACCTGACCGTCCTTTGTTATGCAAAA  
ACGGTTGAAATAGTCCTTTGTTATGCAAATGATATTGAATAGTCCTTTGTTATGCAAAA  
TTTAGATGCCGACCCTTTGTTATGCAAAGGAATGGGCGTGCTGCCTTTGTTATGCAAATT  
TTCGCTGCGACAGCCTTTGTTATGCAAACATGCTCGCCACTCACCTTTGTTATGCAAAGG

5 TCTAACAATGACCCCTTTGTTATGCAAACCTACGTGGAATAGATCCTTTGTTATGCAAACCC  
 CGAGTTTTTTGAACCTTTGTTATGCAAAATCAGTAACTTCATTCCTTTGTTATGCAAAATGT  
 GACTTAACCTCCCTTTGTTATGCAAA (SEQ ID NO:1).

10 The present invention hence provides method for expressing an exogenous gene specifically  
 in cells of the retinal pigment epithelium of a primate, said method comprising the step of  
 delivering an isolated nucleic acid molecule comprising, or consisting of, the nucleic acid  
 sequence of SEQ ID NO:1, or consisting of a nucleic acid sequence of at least 400 bp having  
 at least 80% overall identity to said sequence of SEQ ID NO:1, to cells of the retinal pigment  
 epithelium of said primate, wherein said isolated nucleic acid molecule specifically leads to  
 15 the expression of an exogenous gene in cells of the retinal pigment epithelium of primates  
 when a nucleic acid sequence coding for said exogenous gene is operatively linked to said  
 isolated nucleic acid molecule. The isolated nucleic acid molecule can further comprise,  
 operatively linked to said isolated nucleic acid molecule, a minimal promoter, e.g. the minimal  
 promoter of SEQ ID NO:2. The isolated nucleic acid molecule can be part of an expression  
 20 cassette, which in turn can be part of a vector, for instance a viral vector.

The present invention also provides the use of an isolated nucleic acid molecule comprising,  
 or consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid  
 sequence of at least 400 bp having at least 80% overall identity to said sequence of SEQ ID  
 25 NO:1, wherein said isolated nucleic acid molecule specifically leads to the expression of an  
 exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid  
 sequence coding for said exogenous gene is operatively linked to said isolated nucleic acid  
 molecule, to express an exogenous gene specifically in cells of the retinal pigment epithelium  
 of a primate. In this use, the isolated nucleic acid molecule can further comprise a minimal  
 30 promoter, e.g. the minimal promoter of SEQ ID NO:2. The isolated nucleic acid molecule can  
 be part of an expression cassette, which can be part of a vector, e.g. a viral vector.

The present invention further provides an isolated nucleic acid molecule comprising, or  
 consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid  
 35 sequence of at least 400 bp having at least 80% overall identity to said sequence of SEQ ID  
 NO:1, wherein said isolated nucleic acid molecule specifically leads to the expression of an  
 exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid  
 sequence coding for said exogenous gene is operatively linked to said isolated nucleic acid  
 molecule, for use in treating a disease associated with retinal pigment epithelium. The  
 40 disease associated with retinal pigment epithelium can be selected from the group consisting  
 of age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy and retinal  
 pigment epithelium hypertrophy.

5 The isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of  
SEQ ID NO:1 or a nucleic acid sequence of at least 400 bp having at least 80% overall  
identity to said nucleic acid sequence of SEQ ID NO:1, wherein said isolated nucleic acid  
molecule specifically leads to the expression in cells of the retinal pigment epithelium of  
primates of a gene operatively linked to said nucleic acid sequence coding for said gene can  
10 be at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 750 bp, at least  
800 bp at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp,  
at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at  
least 1900 bp, or at least 2000 bp, and has at least 80 % identity to said nucleic acid  
sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400  
15 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least  
1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500  
bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp,  
and has at least 85 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some  
embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at  
20 least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200  
bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at  
least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 90 % overall identity to  
said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid  
sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp,  
25 at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least  
1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900  
bp, or at least 2000 bp, and has at least 95 % overall identity to said nucleic acid sequence of  
SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least  
500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at  
30 least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least  
1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at  
least 96 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some  
embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at  
least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200  
35 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at  
least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 97 % overall identity to  
said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid  
sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp,  
at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least  
40 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900  
bp, or at least 2000 bp, and has at least 98 % overall identity to said nucleic acid sequence of  
SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least  
500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at  
least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least

- 5 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 99 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has 100 % overall identity to said nucleic acid sequence of SEQ ID NO:1.

The isolated nucleic acid molecule of the invention can comprise a minimal promoter, for instance a SV40 minimal promoter, e.g. the SV40 minimal promoter

- 15 GCTCGAGATCTGCGATCTGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTC  
CGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATCGCTGACTA  
ATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAG  
TGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAA (SEQ ID NO:2), preferably  
operatively linked to said isolated nucleic acid molecule.

- A typical gene which can be operatively linked to the promoter of the invention is a gene  
20 encoding for a photoreceptor. For instance it can be a photosensitive molecule such as a channelrhodopsin or a halorhodopsin.

#### BRIEF DESCRIPTION OF THE DRAWINGS

- 25 **Figure 1: Targeted expression to retinal pigmented epithelium cells in primate retina from a promoter of the comprising SEQ ID NO:1.** Laser-scanning confocal microscope images of EGFP expression from the promoter comprising SEQ ID NO:1 3 months after subretinal injection of AAV-synP12-ChR2-EGFP in adult macaque (*Macaca mulatta*) eyes. Induced expression in retinal pigmented epithelium cells can be observed. Green = EGFP driven by the SEQ ID NO:3.  
30 White = Hoechst.

#### DETAILED DESCRIPTION OF THE INVENTION

- 35 The present inventors have now serendipitously found that an artificial promoter, which was known to drive gene expression specifically in retinal amacrine cells in a murine model as described in WO 2015/118507, has unexpectedly and atypically a totally different specificity in primates. In primates, this promoter specifically drives gene expression in cells of the retinal pigment epithelium. This specificity is extremely useful to address and target gene expression  
40 in retinal pigment epithelium cells in diseased retina of e.g. age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy or retinal pigment epithelium hypertrophy patients.

The nucleic acid sequence of this promoter is:

TCAAGCCTCCTACCGCCTTTGTTATGCAAACATATCAAACGCCCTCCTTTGTTATGCAAAA

5 GGGCTGGAACGGGGCCTTTGTTATGCAAATCGCCCTCCCGATCCCTTTGTTATGCAAA  
 TTTGACGAATTCCCACCTTTGTTATGCAAACAAATCTCCTACCCTCCTTTGTTATGCAAAG  
 TGAGAGGGGCTGCACCTTTGTTATGCAAATGCGGGCCCTGAGACCTTTGTTATGCAAA  
 AACCATGTACGCTTGCCTTTGTTATGCAAACCGCCTGTTGCTTGGCCTTTGTTATGCAAA  
 GCCACGCGATTGGCGCCTTTGTTATGCAAAGCTCGGTTATGTACACCTTTGTTATGCAAA  
 10 GCTACTTTAACTTGCCCTTTGTTATGCAAATCACGACCTGACCGTCCTTTGTTATGCAAAA  
 ACGGTTGAAATAGTCCTTTGTTATGCAAATGATATTGAATAGTCCTTTGTTATGCAAAA  
 TTTAGATGCCGACCCTTTGTTATGCAAAGGAATGGGCGTGCTGCCTTTGTTATGCAAAAT  
 TTCGCTGCGACAGCCTTTGTTATGCAAACATGCTCGCCACTCACCTTTGTTATGCAAAGG  
 TCTAACAATGACCCCTTTGTTATGCAAACACTACGTGGAATAGATCCTTTGTTATGCAAACCC  
 15 CGAGTTTTTTGAACCTTTGTTATGCAAAAATCAGTAACTTCATTCTTTGTTATGCAAAAATGT  
 GACTTAACCTCCCTTTGTTATGCAAA (SEQ ID NO:1).

The present invention hence provides method for expressing an exogenous gene specifically  
 in cells of the retinal pigment epithelium of a primate, said method comprising the step of  
 20 delivering an isolated nucleic acid molecule comprising, or consisting of, the nucleic acid  
 sequence of SEQ ID NO:1, or consisting of a nucleic acid sequence of at least 400 bp having  
 at least 80% overall identity to said sequence of SEQ ID NO:1, to cells of the retinal pigment  
 epithelium of said primate, wherein said isolated nucleic acid molecule specifically leads to  
 the expression of an exogenous gene in cells of the retinal pigment epithelium of primates  
 25 when a nucleic acid sequence coding for said exogenous gene is operatively linked to said  
 isolated nucleic acid molecule. The isolated nucleic acid molecule can further comprise a  
 minimal promoter, e.g. the minimal promoter of SEQ ID NO:2. The isolated nucleic acid  
 molecule can be part of an expression cassette, which in turn can be part of a vector, for  
 instance a viral vector.

30 The present invention also provides the use of an isolated nucleic acid molecule comprising,  
 or consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid  
 sequence of at least 400 bp having at least 80% overall identity to said sequence of SEQ ID  
 NO:1, wherein said isolated nucleic acid molecule specifically leads to the expression of an  
 35 exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid  
 sequence coding for said exogenous gene is operatively linked to said isolated nucleic acid  
 molecule, to express an exogenous gene specifically in cells of the retinal pigment epithelium  
 of a primate. In this use, the isolated nucleic acid molecule can further comprise a minimal  
 promoter, e.g. the minimal promoter of SEQ ID NO:2. The isolated nucleic acid molecule can  
 40 be part of an expression cassette, which can be part of a vector, e.g. a viral vector.

The present invention further provides an isolated nucleic acid molecule comprising, or  
 consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid  
 sequence of at least 400 bp having at least 80% overall identity to said sequence of SEQ ID



- 5 NO:1, wherein said isolated nucleic acid molecule specifically leads to the expression of an exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid sequence coding for said exogenous gene is operatively linked to said isolated nucleic acid molecule, for use in treating a disease associated with retinal pigment epithelium. The disease associated with retinal pigment epithelium can be selected from the group consisting of age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy and retinal pigment epithelium hypertrophy.

The isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of SEQ ID NO:1 or a nucleic acid sequence of at least 400 bp having at least 80% overall identity to said nucleic acid sequence of SEQ ID NO:1, wherein said isolated nucleic acid molecule specifically leads to the expression in cells of the retinal pigment epithelium of primates of a gene operatively linked to said nucleic acid sequence coding for said gene can be at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 750 bp, at least 800 bp at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 80 % identity to said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 85 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 90 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 95 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 96 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 97 % overall identity to

- 5 said nucleic acid sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 98 % overall identity to said nucleic acid sequence of
- 10 SEQ ID NO:1. In some embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has at least 99 % overall identity to said nucleic acid sequence of SEQ ID NO:1. In some
- 15 embodiments, the nucleic acid sequence is at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, or at least 2000 bp, and has 100 % overall identity to said nucleic acid sequence of SEQ ID NO:1.
- 20 The isolated nucleic acid molecule of the invention can comprise a minimal promoter, for instance a SV40 minimal promoter, e.g. the SV40 minimal promoter
- GCTCGAGATCTGCGATCTGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTC  
CGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCATCGCTGACTA  
ATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAG
- 25 TGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAA (SEQ ID NO:2).

A typical gene which can be operatively linked to the promoter of the invention is a gene encoding for a photoreceptor. For instance it can be a photosensitive molecule such as a channelrhodopsin or a halorhodopsin.

- 30 Expression of exogenous genes using the promoter of the invention can be done *in vitro*, *in vivo* or *ex vivo*.

- As used herein, the term “promoter” refers to any cis-regulatory elements, including enhancers, silencers, insulators and promoters. A promoter is a region of DNA that is
- 35 generally located upstream (towards the 5' region) of the gene that is needed to be transcribed. The promoter permits the proper activation or repression of the gene which it controls. In the context of the present invention, the promoters lead to the specific expression of genes operably linked to them in the interneurons. “Specific expression”, also referred to as “expression only in a certain type of cell” means that at least more than 75% of the cells
- 40 expressing the gene of interest are of the type specified, i.e. cells of the retinal pigment epithelium (RPE) in the present case.

The pigmented layer of retina or retinal pigment epithelium (RPE), also known as the pigmentum nigrum, is the pigmented cell layer just outside the neurosensory retina that

5     nourishes retinal visual cells, and is firmly attached to the underlying choroid and overlying  
retinal visual cells. The RPE is composed of a single layer of hexagonal cells that are densely  
packed with pigment granules. At the ora serrata, the RPE continues as a membrane passing  
over the ciliary body and continuing as the back surface of the iris. This generates the fibers  
of the dilator. Directly beneath this epithelium is the neuroepithelium (i.e., rods and cones)  
10    passes jointly with the RPE. Both, combined, are understood to be the ciliary epithelium of the  
embryo. The front end continuation of the retina is the posterior iris epithelium, which takes on  
pigment when it enters the iris. When viewed from the outer surface, these cells are smooth  
and hexagonal in shape. When seen in section, each cell consists of an outer non-pigmented  
part containing a large oval nucleus and an inner pigmented portion which extends as a  
15    series of straight thread-like processes between the rods, this being especially the case when  
the eye is exposed to light. The RPE has several functions, namely, light absorption, epithelial  
transport, spatial ion buffering, visual cycle, phagocytosis, secretion and immune modulation.

Expression cassettes are typically introduced into a vector that facilitates entry of the  
20    expression cassette into a host cell and maintenance of the expression cassette in the host  
cell. Such vectors are commonly used and are well known to those of skill in the art.  
Numerous such vectors are commercially available, e. g., from Invitrogen, Stratagene,  
Clontech, etc., and are described in numerous guides, such as Ausubel, Guthrie, Strathern, or  
Berger, all supra. Such vectors typically include promoters, polyadenylation signals, etc. in  
25    conjunction with multiple cloning sites, as well as additional elements such as origins of  
replication, selectable marker genes (e. g., LEU2, URA3, TRP 1, HIS3, GFP), centromeric  
sequences, etc.

Viral vectors, for instance an AAV, a PRV or a lentivirus, are suitable to target and deliver  
30    genes to cells of the retinal pigment epithelium using a promoter of the invention.

The output of retinal cells can be measured using an electrical method, such as a multi-  
electrode array or a patch-clamp, or using a visual method, such as the detection of  
fluorescence.

35    The methods using nucleic acid sequence of the invention can be used for identifying  
therapeutic agents for the treatment of a neurological disorder or of a disorder of the retina  
involving cells of the RPE, said method comprising the steps of contacting a test compound  
with retinal pigment epithelium cells expressing one or more transgene under a promoter of  
40    the invention, and comparing at least one output of retinal pigment epithelium cells obtained  
in the presence of said test compound with the same output obtained in the absence of said  
test compound.

Moreover, the methods using promoters of the invention can also be used for *in vitro* testing of vision restoration, said method comprising the steps of contacting retinal pigment epithelium cells expressing one or more transgene under the control of a promoter of the invention with an agent, and comparing at least one output obtained after the contact with said agent with the same output obtained before said contact with said agent.

Channelrhodopsins are a subfamily of opsin proteins that function as light-gated ion channels. They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis, i.e. movement in response to light. Expressed in cells of other organisms, they enable the use of light to control intracellular acidity, calcium influx, electrical excitability, and other cellular processes. At least three “natural” channelrhodopsins are currently known:

Channelrhodopsin-1 (ChR1), Channelrhodopsin-2 (ChR2), and Volvox Channelrhodopsin (VChR1). Moreover, some modified/improved versions of these proteins also exist. All known Channelrhodopsins are unspecific cation channels, conducting H<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> ions. Halorhodopsin is a light-driven ion pump, specific for chloride ions, and found in phylogenetically ancient “bacteria” (archaea), known as halobacteria. It is a seven-transmembrane protein of the retinylidene protein family, homologous to the light-driven proton pump bacteriorhodopsin, and similar in tertiary structure (but not primary sequence structure) to vertebrate rhodopsins, the pigments that sense light in the retina. Halorhodopsin also shares sequence similarity to channelrhodopsin, a light-driven ion channel.

Halorhodopsin contains the essential light-isomerizable vitamin A derivative all-trans-retinal. Halorhodopsin is one of the few membrane proteins whose crystal structure is known. Halorhodopsin isoforms can be found in multiple species of halobacteria, including *H. salinarum*, and *N. pharaonis*. Much ongoing research is exploring these differences, and using them to parse apart the photocycle and pump properties. After bacteriorhodopsin, halorhodopsin may be the best type I (microbial) opsin studied. Peak absorbance of the halorhodopsin retinal complex is about 570 nm. Recently, halorhodopsin has become a tool in optogenetics. Just as the blue-light activated ion channel channelrhodopsin-2 opens up the ability to activate excitable cells (such as neurons, muscle cells, pancreatic cells, and immune cells) with brief pulses of blue light, halorhodopsin opens up the ability to silence excitable cells with brief pulses of yellow light. Thus halorhodopsin and channelrhodopsin together enable multiple-color optical activation, silencing, and desynchronization of neural activity, creating a powerful neuroengineering toolbox.

In some embodiments, the promoter is part of a vector targeted a retina, said vector expressing at least one reporter gene which is detectable in living retinal pigment epithelium cells. Such reporter genes can be indicative of a functioning neural circuit. Examples of such vectors are activity sensors or rainbow viruses (*Nature Methods* **6**, 127 - 130 (2009)). Examples of such viruses are retrograde, transsynaptic pseudorabies viruses (PRVs) with genetically encoded activity sensors that optically report the activity of connected neurons

5 among spatially intermingled neurons in the brain. Such activity sensor can be an isolated transsynaptic virus expressing an exogenous fluorescent activity sensor. The transsynaptic virus can be a rhabdovirus, e.g. rabies virus, or a herpesvirus, for instance an alphaherpesvirus, e.g. pseudorabies virus.

The fluorescent exogenous activity sensor can be a fluorescent protein  $\text{Ca}^{2+}$  sensor, e.g.

10 yellow cameleon, camgaroo, G-CaMP/Pericam, or TN-L15, or a fluorescent protein voltage sensor, e.g. FlaSh, SPARC, or a VSP, preferably VSP1.

Suitable viral vectors for the invention are well-known in the art. For instance an AAV, a PRV or a lentivirus, are suitable to target and deliver genes to retinal pigment epithelium cells.

15

When working with isolated retina, optimal viral delivery for photoreceptors can be achieved by mounting the ganglion cell side downwards, so that the photoreceptor side of the retina is exposed and can thus be better transfected. Another technique is slicing, e.g. with a razor

blade, the inner limiting membrane of the retina, such that the delivering viruses can

20 penetrate the inner membranes. A further way is to embed the retina in agar, slicing said retina and applying the delivery viruses from the side of the slice.

The output of transfected cells can be measured using well-known methods, for instance

25 using an electrical method, such as a multi-electrode array or a patch-clamp, or using a visual method, such as the detection of fluorescence. In some cases, the inner limiting membrane is removed by micro-surgery the inner limiting membrane. In other cases, recording is achieved through slices performed to the inner limiting membrane.

The source of retinal cells to be used for the present invention is a primate. In some

30 embodiments of the invention, the retinal cells come from, or are in, a human retina. In other embodiments, the retina is from another primate from any of the two distinct lineages, strepsirrhines and haplorhines. Human retina can be easily obtained from cornea banks

where said retinas are normally discarded after the dissection of the cornea. Adult human retina has a large surface (about 1100 mm<sup>2</sup>) and can therefore be easily separated to a

35 number of experimentally subregions. Moreover, retinas can also be used as an exquisite model for synaptic communication since the retina has synapses that are identical to the rest of the brain.

As used herein, the term "animal" is used herein to include all animals. In some

40 embodiments, the non-human animal is a vertebrate. Examples of animals are human, mice, rats, cows, pigs, horses, chickens, ducks, geese, cats, dogs, etc. The term "animal" also includes an individual animal in all stages of development, including embryonic and fetal stages. A "genetically-modified animal" is any animal containing one or more cells bearing genetic information altered or received, directly or indirectly, by deliberate genetic

5 manipulation at a sub-cellular level, such as by targeted recombination, microinjection or  
infection with recombinant virus. The term "genetically-modified animal" is not intended to  
encompass classical crossbreeding or *in vitro* fertilization, but rather is meant to encompass  
animals in which one or more cells are altered by, or receive, a recombinant DNA molecule.  
This recombinant DNA molecule may be specifically targeted to a defined genetic locus, may  
10 be randomly integrated within a chromosome, or it may be extrachromosomally replicating  
DNA. The term "germ-line genetically-modified animal" refers to a genetically-modified animal  
in which the genetic alteration or genetic information was introduced into germline cells,  
thereby conferring the ability to transfer the genetic information to its offspring. If such  
offspring in fact possess some or all of that alteration or genetic information, they are  
15 genetically-modified animals as well.

The alteration or genetic information may be foreign to the species of animal to which the  
recipient belongs, or foreign only to the particular individual recipient, or may be genetic  
information already possessed by the recipient. In the last case, the altered or introduced  
gene may be expressed differently than the native gene, or not expressed at all.

20 The genes used for altering a target gene may be obtained by a wide variety of techniques  
that include, but are not limited to, isolation from genomic sources, preparation of cDNAs from  
isolated mRNA templates, direct synthesis, or a combination thereof.

A type of target cells for transgene introduction is the ES cells. ES cells may be obtained from  
pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al. (1981),  
25 Nature 292:154-156; Bradley et al. (1984), Nature 309:255-258; Gossler et al. (1986), Proc.  
Natl. Acad. Sci. USA 83:9065-9069; Robertson et al. (1986), Nature 322:445-448; Wood et al.  
(1993), Proc. Natl. Acad. Sci. USA 90:4582- 4584). Transgenes can be efficiently introduced  
into the ES cells by standard techniques such as DNA transfection using electroporation or by  
retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be  
30 combined with morulas by aggregation or injected into blastocysts from a non-human animal.  
The introduced ES cells thereafter colonize the embryo and contribute to the germline of the  
resulting chimeric animal (Jaenisch (1988), Science 240:1468-1474). The use of gene-  
targeted ES cells in the generation of gene-targeted genetically-modified mice was described  
1987 (Thomas et al. (1987), Cell 51:503-512) and is reviewed elsewhere (Frohman et al.  
35 (1989), Cell 56:145-147; Capecchi (1989), Trends in Genet. 5:70-76; Baribault et al. (1989),  
Mol. Biol. Med. 6:481-492; Wagner (1990), EMBO J. 9:3025-3032; Bradley et al. (1992),  
Bio/Technology 10:534-539).

Techniques are available to inactivate or alter any genetic region to any mutation desired by  
using targeted homologous recombination to insert specific changes into chromosomal  
40 alleles.

As used herein, a "targeted gene" is a DNA sequence introduced into the germline of a non-  
human animal by way of human intervention, including but not limited to, the methods  
described herein. The targeted genes of the invention include DNA sequences which are  
designed to specifically alter cognate endogenous alleles.

5

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogeneous solution containing the clone and other members of the library) or a chromosome removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), or a preparation of randomly sheared genomic DNA or a preparation of genomic DNA cut with one or more restriction enzymes is not "isolated" for the purposes of this invention. As discussed further herein, isolated nucleic acid molecules according to the present invention may be produced naturally, recombinantly, or synthetically.

"Polynucleotides" can be composed of single-and double-stranded DNA, DNA that is a mixture of single-and double-stranded regions, single-and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single-and double-stranded regions. In addition, polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. Polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The expression "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

"Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml

5 denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 50 degree C. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37 degree C in a  
10 solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or  
15 substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

20 The terms "fragment," "derivative" and "analog" when referring to polypeptides means polypeptides which either retain substantially the same biological function or activity as such polypeptides. An analog includes a pro-protein which can be activated by cleavage of the pro-protein portion to produce an active mature polypeptide.

25 The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region "leader and trailer" as well as intervening sequences (introns) between individual coding segments (exons).

30 Polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in  
35 more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides  
40 may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include, but are not limited to, acetylation, acylation, biotinylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent



- 5 attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, derivatization by known protecting/blocking groups, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, linkage to an antibody molecule or other cellular ligand, methylation, myristoylation, oxidation, pegylation, proteolytic processing (e.g., cleavage), phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

20 A polypeptide fragment "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of the original polypeptide, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the original polypeptide (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, in some embodiments, not more than about tenfold less activity, or not more than about three-fold less activity relative to the original polypeptide.)

30 Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

"Variant" refers to a polynucleotide or polypeptide differing from the original polynucleotide or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the original polynucleotide or polypeptide.

35 As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in

- 5 percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=-1, Joining Penalty=-30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=-5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 impaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. Nevertheless, in the particular case, overall identity refers only to the portions of a sequence which have at least some degrees of similarities with the sequence of e.g. SEQ ID NO:1. In other words, sequence identity of a 2000bp query sequence would only be calculated for the part of the sequence comprising SEQ ID NO:1 or a very similar part thereof.
- 40 By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino

5 acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one  
10 or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for instance, the amino acid sequences shown in a sequence or to the amino acid sequence encoded by deposited DNA clone can be  
15 determined conventionally using known computer programs. A preferred method for determining, the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject  
20 sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=-1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=-5, Gap Size Penalty=-0.05, Window Size=500 or the length of the subject amino  
25 acid sequence, whichever is shorter. If the subject sequence is shorter than the query sequence due to N-or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N-and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N-and C-termini, relative to the query  
30 sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N-and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the  
35 above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N-and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest  
40 N-and C-terminal residues of the subject sequence. Only residue positions outside the N-and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

5 Naturally occurring protein variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes 11, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

10

"Label" refers to agents that are capable of providing a detectable signal, either directly or through interaction with one or more additional members of a signal producing system. Labels that are directly detectable and may find use in the invention include fluorescent labels. Specific fluorophores include fluorescein, rhodamine, BODIPY, cyanine dyes and the like.

15

A "fluorescent label" refers to any label with the ability to emit light of a certain wavelength when activated by light of another wavelength.

20

"Fluorescence" refers to any detectable characteristic of a fluorescent signal, including intensity, spectrum, wavelength, intracellular distribution, etc.

25

"Detecting" fluorescence refers to assessing the fluorescence of a cell using qualitative or quantitative methods. In some of the embodiments of the present invention, fluorescence will be detected in a qualitative manner. In other words, either the fluorescent marker is present, indicating that the recombinant fusion protein is expressed, or not. For other instances, the fluorescence can be determined using quantitative means, e. g., measuring the fluorescence intensity, spectrum, or intracellular distribution, allowing the statistical comparison of values obtained under different conditions. The level can also be determined using qualitative methods, such as the visual analysis and comparison by a human of multiple samples, e. g., samples detected using a fluorescent microscope or other optical detector (e. g., image analysis system, etc.). An "alteration" or "modulation" in fluorescence refers to any detectable difference in the intensity, intracellular distribution, spectrum, wavelength, or other aspect of fluorescence under a particular condition as compared to another condition. For example, an "alteration" or "modulation" is detected quantitatively, and the difference is a statistically significant difference. Any "alterations" or "modulations" in fluorescence can be detected using standard instrumentation, such as a fluorescent microscope, CCD, or any other fluorescent detector, and can be detected using an automated system, such as the integrated systems, or can reflect a subjective detection of an alteration by a human observer.

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The "green fluorescent protein" (GFP) is a protein, composed of 238 amino acids (26.9 kDa), originally isolated from the jellyfish *Aequorea victoria*/*Aequorea aequorea*/*Aequorea forskalea* that fluoresces green when exposed to blue light. The GFP from *A. victoria* has a major excitation peak at a wavelength of 395 nm and a minor one at 475 nm. Its emission peak is at 509 nm which is in the lower green portion of the visible spectrum. The GFP from the sea

5 pansy (*Renilla reniformis*) has a single major excitation peak at 498 nm. Due to the potential for widespread usage and the evolving needs of researchers, many different mutants of GFP have been engineered. The first major improvement was a single point mutation (S65T) reported in 1995 in Nature by Roger Tsien. This mutation dramatically improved the spectral characteristics of GFP, resulting in increased fluorescence, photostability and a shift of the major excitation peak to 488nm with the peak emission kept at 509 nm. The addition of the 10 37°C folding efficiency (F64L) point mutant to this scaffold yielded enhanced GFP (EGFP). EGFP has an extinction coefficient (denoted  $\epsilon$ ), also known as its optical cross section of  $9.13 \times 10^{-21}$  m<sup>2</sup>/molecule, also quoted as 55,000 L/(mol•cm). Superfolder GFP, a series of mutations that allow GFP to rapidly fold and mature even when fused to poorly folding 15 peptides, was reported in 2006.

The “yellow fluorescent protein” (YFP) is a genetic mutant of green fluorescent protein, derived from *Aequorea victoria*. Its excitation peak is 514nm and its emission peak is 527nm.

20 As used herein, the singular forms “a”, “an,” and “the” include plural reference unless the context clearly dictates otherwise.

A “virus” is a sub-microscopic infectious agent that is unable to grow or reproduce outside a host cell. Each viral particle, or virion, consists of genetic material, DNA or RNA, within a 25 protective protein coat called a capsid. The capsid shape varies from simple helical and icosahedral (polyhedral or near-spherical) forms, to more complex structures with tails or an envelope. Viruses infect cellular life forms and are grouped into animal, plant and bacterial types, according to the type of host infected.

30 The term “transsynaptic virus” as used herein refers to viruses able to migrate from one neurone to another connecting neurone through a synapse. Examples of such transsynaptic virus are rhabdoviruses, e.g. rabies virus, and alphaherpesviruses, e.g. pseudorabies or herpes simplex virus. The term “transsynaptic virus” as used herein also encompasses viral sub-units having by themselves the capacity to migrate from one neurone to another 35 connecting neurone through a synapse and biological vectors, such as modified viruses, incorporating such a sub-unit and demonstrating a capability of migrating from one neurone to another connecting neurone through a synapse.

40 Transsynaptic migration can be either anterograde or retrograde. During a retrograde migration, a virus will travel from a postsynaptic neuron to a presynaptic one. Accordingly, during anterograde migration, a virus will travel from a presynaptic neuron to a postsynaptic one.

5 Homologs refer to proteins that share a common ancestor. Analogs do not share a common ancestor, but have some functional (rather than structural) similarity that causes them to be included in a class (e.g. trypsin like serine proteinases and subtilisin's are clearly not related - their structures outside the active site are completely different, but they have virtually geometrically identical active sites and thus are considered an example of convergent evolution to analogs).

There are two subclasses of homologs - orthologs and paralogs. Orthologs are the same gene (e.g. cytochrome 'c'), in different species. Two genes in the same organism cannot be orthologs. Paralogs are the results of gene duplication (e.g. hemoglobin beta and delta). If two genes/proteins are homologous and in the same organism, they are paralogs.

As used herein, the term "disorder" refers to an ailment, disease, illness, clinical condition, or pathological condition.

As used herein, the term "pharmaceutically acceptable carrier" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredient, is chemically inert, and is not toxic to the patient to whom it is administered.

As used herein, the term "pharmaceutically acceptable derivative" refers to any homolog, analog, or fragment of an agent, e.g. identified using a method of screening of the invention, that is relatively non-toxic to the subject.

The term "therapeutic agent" refers to any molecule, compound, or treatment, that assists in the prevention or treatment of disorders, or complications of disorders.

Compositions comprising such an agent formulated in a compatible pharmaceutical carrier may be prepared, packaged, and labeled for treatment.

If the complex is water-soluble, then it may be formulated in an appropriate buffer, for example, phosphate buffered saline or other physiologically compatible solutions.

Alternatively, if the resulting complex has poor solubility in aqueous solvents, then it may be formulated with a non-ionic surfactant such as Tween, or polyethylene glycol. Thus, the compounds and their physiologically acceptable solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, rectal administration or, in the case of tumors, directly injected into a solid tumor.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution

5 with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e. g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e. g., lecithin or acacia); non-aqueous vehicles (e. g., almond oil, oily esters, or fractionated vegetable oils); and preservatives (e. g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e. g., pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (e. g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e. g., magnesium stearate, talc or silica); disintegrants (e. g., potato starch or sodium starch glycolate); or wetting agents (e. g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

20 For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e. g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e. g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

30 The compounds may be formulated for parenteral administration by injection, e. g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e. g., in ampoules or in multi-dose containers, with an added preservative.

35 The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e. g., sterile pyrogen-free water, before use.

The compounds may also be formulated as a topical application, such as a cream or lotion.

40 In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, intraocular, subcutaneous or intramuscular) or by intraocular injection.

5 Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophilic drugs.

10 The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

15 The invention also provides kits for carrying out the therapeutic regimens of the invention. Such kits comprise in one or more containers therapeutically or prophylactically effective amounts of the compositions in pharmaceutically acceptable form.

20 The composition in a vial of a kit may be in the form of a pharmaceutically acceptable solution, e. g., in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, the complex may be lyophilized or desiccated; in this instance, the kit optionally further comprises in a container a pharmaceutically acceptable solution (e. g., saline, dextrose solution, etc.), preferably sterile, to reconstitute the complex to form a solution for injection purposes.

25 In another embodiment, a kit further comprises a needle or syringe, preferably packaged in sterile form, for injecting the complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of compositions by a clinician or by the patient.

30 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will  
35 control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

## EXAMPLES

### *Gene construct*

40 The promoter used in this example (964 bp; SEQ ID NO:3) consists of the one of SEQ ID NO:1 coupled to the minimal promoter SV40 promoter of SEQ ID NO:2. ChR2-eGFP coding sequence was inserted immediately after this promoters and the optimized Kozak sequence (GCCACC), and followed by a woodchuck hepatitis virus posttranscriptional regulatory



- 5 element (*WPRE*) and *SV40* polyadenylation site. Retinal neurons were targeted using AAVs serotype 2/8 with a titer of  $2.9 \times 10^{10}$  GC/mL.

*Viral transfection and tissue preparation*

- For AAV administration, the eyes of anesthetized animals were punctured in the sclera close to the lens by a sharp 30 gauge needle. 2 microL of AAV particle suspension were injected subretinally by a Hamilton syringe. After 3 weeks, the isolated retinas were fixed for 30 min in 4% PFA in PBS, followed by a washing step in PBS at 4 degree C. Whole retinas were treated with 10% normal donkey serum (NDS), 1% BSA, 0.5% Triton X-100 in PBS for 1h at room temperature. Treatment with monoclonal rat anti-GFP Ab (Molecular Probes Inc.; 1:500) and polyclonal goat anti-ChAT (Millipore: 1:200) in 3% NDS, 1% BSA, 0.5% Triton X-100 in PBS was carried out for 5 days at room temperature. Treatment with secondary donkey anti-rat Alexa Fluor-488 Ab (Molecular Probes Inc.; 1:200), anti-goat Alexa Fluor-633 and Hoechst, was done for 2 hr. Sections were washed, mounted with ProLong Gold antifade reagent (Molecular Probes Inc.) on glass slides, and photographed using a Zeiss LSM 700 Axio Imager Z2 laser scanning confocal microscope (Carl Zeiss Inc.).

5

CLAIMS

1. A method for expressing an exogenous gene specifically in cells of the retinal pigment epithelium of a primate, said method comprising the step of delivering an isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid sequence of at least 400 bp having at least 80% overall identity to said sequence of SEQ ID NO:1, to cells of the retinal pigment epithelium of said primate, wherein said isolated nucleic acid molecule specifically leads to the expression of an exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid sequence coding for said exogenous gene is operatively linked to said isolated nucleic acid molecule.
2. The method of claim 1, wherein said isolated nucleic acid molecule further comprises a minimal promoter, e.g. the minimal promoter of SEQ ID NO:2.
3. The method of claim 1 or of claim 2, wherein said isolated nucleic acid molecule is part of an expression cassette.
4. The method of claim 3, wherein said expression cassette is part of a vector.
5. The method of claim 4, wherein said vector is a viral vector.
6. Use of an isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of SEQ ID NO:1, or consisting of a nucleic acid sequence of at least 400 bp having at least 80% overall identity to said sequence of SEQ ID NO:1, wherein said isolated nucleic acid molecule specifically leads to the expression of an exogenous gene in cells of the retinal pigment epithelium of primates when a nucleic acid sequence coding for said exogenous gene is operatively linked to said isolated nucleic acid molecule, to express an exogenous gene specifically in cells of the retinal pigment epithelium of a primate.
7. The use of claim 6, wherein said isolated nucleic acid molecule further comprises a minimal promoter, e.g. the minimal promoter of SEQ ID NO:2.
8. The use of claim 6 or of claim 7, wherein said isolated nucleic acid molecule is part of an expression cassette.
9. The use of claim 8, wherein said expression cassette is part of a vector.

- 5      10.      The use of claim 9, wherein said vector is a viral vector.
11.      An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid  
sequence of SEQ ID NO:1, or consisting of a nucleic acid sequence of at least 400 bp  
having at least 80% overall identity to said sequence of SEQ ID NO:1, wherein said  
10      isolated nucleic acid molecule specifically leads to the expression of an exogenous  
gene in cells of the retinal pigment epithelium of primates when a nucleic acid  
sequence coding for said exogenous gene is operatively linked to said isolated  
nucleic acid molecule, for use in treating a disease associated with retinal pigment  
epithelium.
- 15      12.      The isolated nucleic acid molecule for use of claim 11, wherein said disease  
associated with retinal pigment epithelium is selected from the group consisting of  
age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy and  
retinal pigment epithelium hypertrophy.
- 20

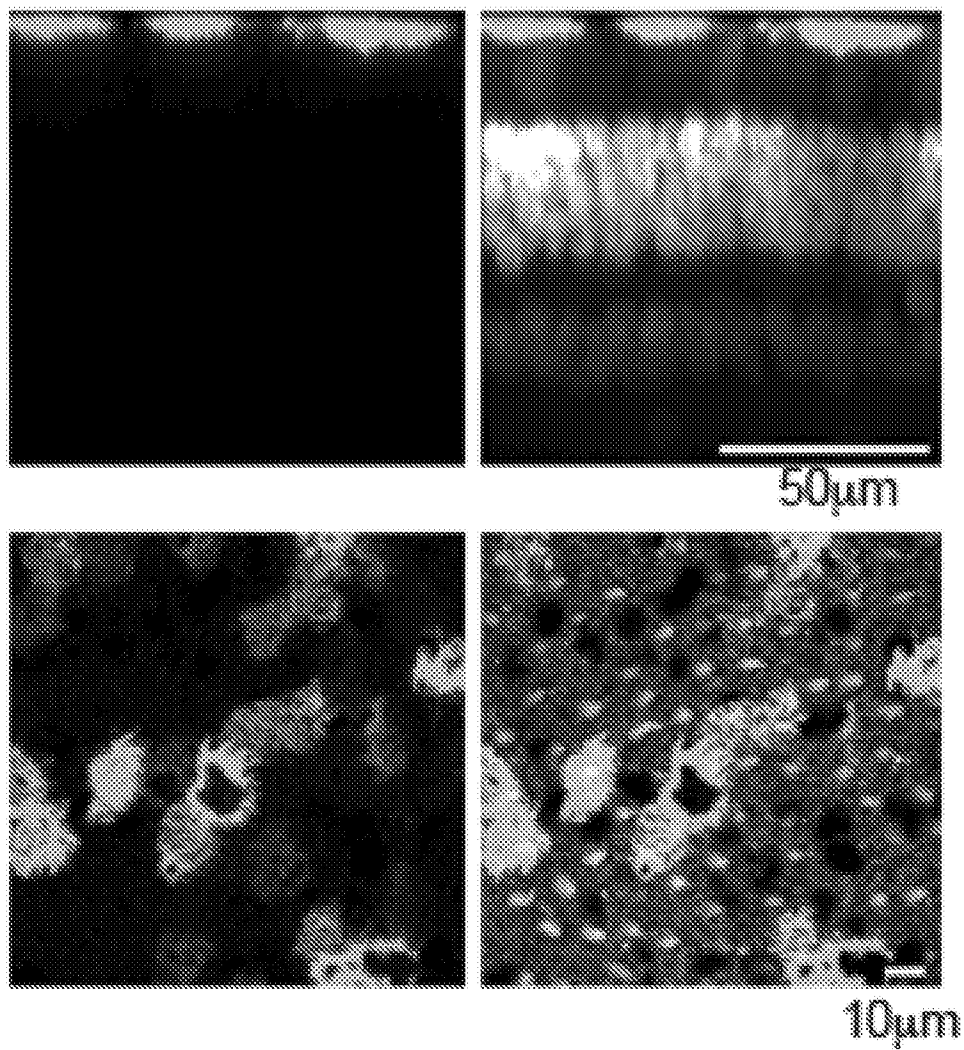


Figure 1

# INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2018/059015

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A01K67/027 A61K48/00 C12N15/85  
ADD.

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)

A01K A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/118507 A1 (FRIEDRICH MIESCHER INST FOR BIOMEDICAL RES [CH]; ROSKA BOTOND [CH]; JU) 13 August 2015 (2015-08-13) cited in the application claims 1-11	11,12
Y	WO 2012/051599 A2 (EOS NEUROSCIENCE INC [US]; UNIV SOUTHERN CALIFORNINA [US]; HORSAGER AL) 19 April 2012 (2012-04-19) paragraphs [0043], [0048], [0066], [0243]; claims 1-59 ----- -/-	11,12

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

☒ See patent family annex.

\* 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

8 January 2019

Date of mailing of the international search report

15/01/2019

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# INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2018/059015

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CARVALHO LIVIA S ET AL: "Promising and delivering gene therapies for vision loss", VISION RESEARCH, vol. 111, 2 August 2014 (2014-08-02), pages 124-133, XP029176591, ISSN: 0042-6989, DOI: 10.1016/J.VISRES.2014.07.013 the whole document -----	11,12
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Information on patent family members

International application No

PCT/IB2018/059015

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