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The invention relates to gene suppression and replacement. In particular, the invention relates to enhanced expression of suppression agents for suppressing gene expression in a cell and in vivo and replacement nucleic acids that are not inhibited by the suppression agent. Regulatory elements are included in expression vectors to optimize expression of the suppression agent and/or replacement nucleic acid.

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(57) Abstract: The invention relates to gene suppression and replacement. In particular, the invention relates to enhanced expression of suppression agents for suppressing gene expression in a cell and in vivo and replacement nucleic acids that are not inhibited by the suppression agent. Regulatory elements are included in expression vectors to optimize expression of the suppression agent and/or replacement nucleic acid.

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Genetic Suppression and Replacement

Field of the Invention

The invention relates to mutation independent suppression and replacement of disease-causing mutant genes.

Background of the Invention

Many mutation-based diseases are more genetically diverse than can be predicted from clinical presentation. Some mutation-based diseases are Mendelian and involve the inheritance of a single mutant gene, others are polygenic or multifactorial and involve multiple genetic insults. In the case of some Mendelian disorders, many different mutations within the same gene can give rise to, or can predispose an individual to, a disease. Similarly, for some multifactorial disorders, many different mutations within one or more genes can predispose an individual to a disease or can act in an additive manner with other genetic and environmental influences to give rise to a disease. This mutational heterogeneity underlying the molecular etiologies of many diseases represents a significant barrier to the development of therapies for such diseases. Moreover, genetic strategies for suppressing and replacing a mutant protein face many challenges with regard to the effectiveness of the machinery used to deliver and regulate the expression of the suppressor and replacement nucleic acids *in vivo*. Therefore, a need exists for effective mutation-independent therapeutics that achieve effective suppression and replacement.

Summary of the Invention

The invention relates to gene suppression and replacement. In particular, the invention relates to enhanced expression of suppression agents for suppressing gene expression in a cell and *in vivo* and of replacement nucleic acids that are not inhibited and/or are partially inhibited by the suppression agent. Expression vectors used to express the suppression agent(s) and replacement nucleic acids comprise regulatory elements to optimize expression of the suppression agent(s) and or replacement nucleic acids.

The invention embodies use of replacement genes using sequences to enhance expression of replacement genes from viral and or non-viral vectors. In a further aspect the invention relates to enhanced expression of suppression agent(s) and or replacement genes from viral or and non-viral vectors. In a further embodiment the invention relates to enhanced expression of suppression agent(s) and or replacement genes and or genes encoding neurotrophic factors from viral and or non-viral vectors.

In one aspect the invention relates to use of conserved sequences from retinal genes to enhance expression of suppression agent(s) and or replacement genes and or genes encoding neurotrophic factors. The use of such conserved sequences has been found to result in surprisingly efficient expression. In a particular aspect the invention relates to use of conserved sequences from retinal genes to enhance expression of suppression agent(s) and or replacement genes and or genes encoding neurotrophic factors from adeno associated virus (AAV) vectors. In another aspect the invention provides vectors for expression of suppression agent(s) and or replacement gene(s) and or genes encoding neurotrophic factors using regulatory sequences from retinal gene(s) and or non-retinal gene(s) and or ubiquitously expressing genes to enhance expression from vectors.

In one aspect, the invention provides vectors for expressing a suppression agent for a disease causing gene and/or a replacement nucleic acid that is not recognized or is partially recognized by the suppression agent.

In an embodiment, the vector comprises an enhancer sequence, such as, for example, a sequence of SEQ ID NOs: 402-413 or functional variants or equivalents thereof. In another embodiment, the vector comprises at least one regulatory element selected from the group consisting of a promoter, a stuffer, an insulator, a silencer, an intron sequence, a post translational regulatory element, a polyadenylation site, and a transcription factor binding site.

In another embodiment, the vector comprises at least one of conserved regions A through I from the rhodopsin gene, as represented by SEQ ID NOs: 92-99, or functional variant or equivalent thereof. In another embodiment, the vector comprises at least one transcription factor binding site sequence selected from the group consisting of SEQ ID NOs: 100-401, or functional variant or equivalent thereof.

The suppression agent may be a nucleic acid, protein, amino acid(s), antibody, aptamer, or any such agent that can bind to and inhibit a DNA, RNA, or protein. In an embodiment, the suppression agent is a siRNA selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35-67, 75, 77, 79, 81, 83, 85, and 414 – 421 or functional variant or equivalent thereof.

The replacement nucleic acid is not recognized or is recognized partially by the suppression effector, because its sequence has been altered such that it cannot bind or binds less efficiently to the suppression agent but still encodes a normal or enhanced gene product. In an embodiment, the replacement nucleic acid is a siRNA selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 68, 76, 78, 80, 82, 84, and 86, or functional variant or equivalent thereof.

In an embodiment, the invention provides vectors, such as viral vectors, that comprise a suppression agent and/or a replacement nucleic acid. For example, the vector comprises at least one suppression agent nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35-67, 75, 77, 79, 81, 83, and 85, or functional variant or equivalent thereof, and at least one replacement nucleic acid nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 68, 76, 78, 80, 82, 84, and 86, or functional variant or equivalent thereof.

In another aspect, the invention provides therapeutic compositions comprising at least one vector comprising at least one suppression agent nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35-67, 75, 77, 79, 81, 83, 85 and 414-421 or functional variant or equivalent thereof, and at least one replacement nucleic acid nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 68, 76, 78, 80, 82, 84, and 86, or functional variant or equivalent thereof. In an embodiment, the vector of the therapeutic composition further comprises a regulatory element selected from the group consisting of an enhancer, a promoter, a stuffer, an insulator, a silencer, an antirepressor, an intron sequence, a post translational regulatory element, a polyadenylation signal (e.g. minimal poly A), a conserved region A through I, and a transcription factor binding site.

In another aspect, the invention provides an enhanced viral expression vector, wherein said vector is a vector for expression of at least one of a suppression agent for a disease-causing gene and a replacement nucleic acid that is not recognized by the suppression agent, wherein said vector is an adeno associated virus (AAV) vector, wherein the vector comprises: conserved region B from the rhodopsin gene, wherein said conserved region B has the sequence set forth by SEQ ID NO: 93 or SEQ ID NO: 408; or a sequence which provides enhanced expression when operatively linked to said suppression agent and / or replacement nucleic acid, wherein the sequence shares at least 90% identity over the entire length of a sequence set forth by SEQ ID NO: 93 or SEQ ID NO: 408; or a sequence which provides enhanced expression when operatively linked to said suppression agent and / or replacement nucleic acid, wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence set forth by SEQ ID NO: 93 or SEQ ID NO:408.

In another aspect, the invention provides suppression and replacement in conjunction with provision of a gene encoding a neurotrophic / neuroprotective factor(s).

In another aspect, the invention provides a therapeutic composition comprising at least one enhanced viral expression vector of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention provides a cell comprising the enhanced viral expression vector of the invention.

In another aspect, the invention provides a transgenic animal comprising the nucleic acids and vectors of the invention.

In yet another aspect, the invention provides methods of suppressing the expression of a mutant gene and replacing expression of the mutant gene with a replacement nucleic acid, the method comprising administering to a mammal a therapeutic composition of the invention.

In yet another aspect, the invention provides methods of suppressing the expression of a mutant gene and replacing expression of the mutant gene with a replacement nucleic acid in conjunction with a gene encoding a neurotrophic / neuroprotective factor(s), the method comprising administering to a mammal a therapeutic composition of the invention.

In another aspect, the invention provides the enhanced viral expression vector of the invention for use in the treatment of ocular disease.

In another aspect, the invention provides use of the enhanced viral expression vector of the invention for the treatment of ocular disease.

In another aspect, the invention provides use of the enhanced viral expression vector of the invention for the manufacture of a therapeutic composition for the treatment of ocular disease.

Brief Description of the Drawings

The foregoing and other objects, features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments when read together with the accompanying drawings, in which:

Figure 1 illustrates *RHO* suppression and replacement constructs. Figure 1A is a diagrammatic representation of a *RHO* suppressor-EGFP construct shBB-EGFP (shQ1-EGFP and shNT-EGFP have the same format). shRNAs were expressed from the H1 promoter and EGFP from the CMV immediate early promoter. The SV40 polyadenylation signal was located at the 3' end of the EGFP gene. Figure 1B illustrates a two component suppression and replacement construct shBB-rBB (shQ1-rQ1 and shNT-rBB have the same format). Suppressors were expressed from the H1 promoter and replacement *RHO* cDNAs from a 1.7 kb mouse rhodopsin promoter

(rhoP). Polyadenylation signals of the *RHO* gene were included in the 1829 bp fragment. HGH int: human growth hormone intron. For tissue culture and retinal explant experiments these constructs were maintained in pEGFP-1 (A) or a CMV-promoterless derivative of pcDNA-3.1- (B) and for *in vivo* experiments in the AAV vector. Restriction enzyme sites used for cloning are indicated. Promoters were separated by spacer DNA fragments. Numbers indicate molecular sizes (bp) and arrows indicate direction of transcription.

Figure 2 illustrates *RHO* suppression in HeLa cells. HeLa cells were transiently co-transfected three times in triplicate with wild type *RHO* and *RHO*-targeting siRNAs (siB, siBB, siC, siCC, siQ1 or siQ2) or control siRNAs (siEGFP or siNT). Following transfection, *RHO* mRNA and protein levels were evaluated by real time RT-PCR (A), ELISA (A) and Alexa Fluor 568-labeled immunocytochemistry (B). Cell nuclei were counterstained with DAPI. Error bars represent SD values.

Figure 3 illustrates replacement of *RHO* expression in conjunction with suppression in HeLa cells. Replacement *RHO* sequences were generated with altered degenerate nucleotides at siRNA target sites. HeLa cells were transiently co-transfected three times in triplicate with a replacement *RHO* expression vector (rBB, rCC or rQ1) and a *RHO*-targeting siRNA (siBB, siCC or siQ1) or a non-targeting siRNA (siNT). Replacement *RHO* mRNA levels were evaluated by real time RT-PCR. Error bars represent SD values.

Figure 4 illustrates *RHO* suppression in retinal explants. Mouse retinas (n=6), dissected from newborn NHR+/- rho-/- pups (transgenic mice expressing a human rhodopsin transgene NHR on a mouse rhodopsin knockout background rho-/-), were electroporated with a construct co-expressing a shRNA targeting *RHO* or a non-targeting shRNA and EGFP (shBB-EGFP, shQ1-EGFP or shNT-EGFP). Negative control explants were not electroporated. Two week organotypic cultures were dissociated with trypsin and FACS analysed. Red and blue dots (right and left populations respectively in each of A1 and A2) represent gated and ungated populations of dissociated explants. Scatterplots of forward- (FSC) versus side-scatter (SSC) and histograms of EGFP fluorescence of the gated population of non-electroporated (A1 and A2, EGFP-negative) and electroporated (A3 and A4, EGFP-positive) retinas are given. The bar chart indicates *RHO* mRNA levels in retinal

explant cells expressing sNT-EGFP, sBB-EGFP and sQ1-EGFP, quantified by real time RT-PCR. Error bars represent SD values.

Figure 5 illustrates *RHO* suppression in photoreceptor cells *in vivo*. Adult transgenic NHR+/- rho-/- mice were subretinally injected with 3 μ l 2×10^{12} vp/ml AAV co-expressing a *RHO*-targeting or non-targeting shRNA and EGFP (AAV-shBB-EGFP or AAV-shNT-EGFP). Retinas were analysed two weeks post-injection. Expression of the 21 nucleotide (nt) shRNA BB, detected by RNase protection in two transduced retinas, is depicted in lanes L1 and L2 (A). *RHO* RNA probes were labelled with P³²- γ ATP and protected RNA separated on 15% denaturing acrylamide gels (A). M: size marker indicates 10, 20 and 30 nt. Bars represent *RHO* mRNA levels in FACS sorted cells from dissociated retinas (n=6) transduced with either AAV-shBB-EGFP or AAV-shNT-EGFP (B). Suppression levels were determined by real time RT-PCR. Error bars represent SD values. Rhodopsin immunocytochemistry (Cy3-labeled) and EGFP protein expression in cells from dissociated retinas, transduced with either AAV-shBB-EGFP (arrows) or AAV-shNT-EGFP (arrow heads), are depicted (C). Cell nuclei were counterstained with DAPI.

Figure 6A-D illustrates retinal histology and ERG analysis of *RHO*-M mouse. Two month old rho+/- (wild type), rho+/-, NHR+/- rho-/- and *RHO*-M+/- rho-/- mice were analysed by retinal histology and ERG (n=8). A, B and C: rhodopsin immunocytochemistry (Cy3) showing similar rod outer segment (ROS) labelling in rho+/-, NHR+/- rho-/- and *RHO*-M+/- rho-/- retinas respectively. Nuclear layers were stained with DAPI. D: representative rod-isolated ERG responses. ONL: outer nuclear layer. INL: inner nuclear layer. GCL: ganglion cell layer.

Figure 6E illustrates RNAi-mediated suppression of human rhodopsin in *RHO*-M mice. *RHO*-M mice were subretinally injected with AAV2/5 vectors carrying an shRNA-based suppressor and an EGFP reporter gene. Mice were sacrificed 14 days post-injection, retinas taken and retinal cells dissociated as in Palfi et al. 2006. RNAi-mediated suppression was evaluated using real-time RT-PCR assays. Retinal cells transduced with AAV-shBB-EGFP, AAV-shCC-EGFP and AAV-shQ1-EGFP vs AAV-shNT-EGFP were FACS sorted from adult *Rho*M mouse retinas, 14 days post subretinal injection. Of note is that AAV-shCC-EGFP suppresses *RHO* less in *RHO*-M mice due to the presence of a 2bp mismatch in the human rhodopsin transgenic in *RHO*-M animals. Levels of rhodopsin expression were shCC: 59.73%;

shBB: 8.77%; shQ1: 20.6% when compared to the non-targeting control shNT which was set at 100% expression.

Figure 6F illustrates depression of the ERG response in RHO-M eyes that have received AAV-shBB-EGFP or AAV-shQ1-EGFP when compared to eyes subretinally injected with AAV-shNT-EGFP. The top tracing in each panel represents the right eye which received the targeting AAV-shRNA vector and the bottom tracing in each panel represents the left eye which received the control non-targeting AAV-shNT vector. In contrast no reduction / depression of the ERG was observed in RHO-M mice subretinally injected with AAV-shCC-EGFP vector.

Figure 7 illustrates the expression of replacement *RHO* *in vivo*. Ten day old rho-/- mice were subretinally injected with a 1:1 mixture of 2 μ l 2×10^{12} vp/ml of two AAV vectors, AAV-EGFP (also termed AAV-CMV-EGFP) and AAV-shBB-rBB (also termed AAV-BB8). Rhodopsin, EGFP protein and nuclei were detected by Cy3-labeled immunocytochemistry, native fluorescence and nuclear DAPI staining respectively. Low magnification images show a cross section of a whole injected eye with arrowheads indicating the transduced area (A and B). High magnification laser scanning micrographs show transduced (C and D) and non-transduced (E and F) areas. INL: inner nuclear layer. GCL: ganglion cell layer. ROS: rod outer segments. ONL: outer nuclear layer. Figure 7 provides evidence of rhodopsin protein expression from replacement genes in retinal sections obtained from rho-/- mice subretinally injected with AAV2/5 suppression and replacement vectors.

Figure 8 illustrates the histology of AAV-transduced Pro23His retinas. Newborn Pro23His+/- rho+/- mice were subretinally injected with 1 μ l 2×10^{12} vp/ml AAV-shBB-rBB or AAV-EGFP (n=6). Ten days post-transduction eyes were processed for semi-thin sectioning and stained with toluidine blue. Approximately 40 measurements in three layers per eye of outer nuclear layer (ONL) thickness (μ m) were taken. A: bars represent ONL thickness, of the central meridian of the eye, of the lowest and highest 15% values (p<0.01). B and C: representative images of AAV-shBB-rBB- and AAV-EGFP- (control) injected sections corresponding to highest ONL thickness values. Yellow arrows indicate ONL thickness. INL: inner nuclear layer. GCL: ganglion cell layer. Error bars represent SD values.

Figure 9 illustrates suppression and/or replacement constructs used to generate recombinant AAV2/5 viruses using the procedures provided in Example 1. *RHO* suppression and or replacement constructs, pAAV-BB8, pAAV-BB9, pAAV-BB10, pAAV-BB11, pAAV-BB12, pAAV-BB13, pAAV-BB18, pAAV-BB26/Q26, pAAV-BB16, pAAV-BB24 and pAAV-BB27. Illustrations of some control constructs are also provided (pAAV-*rho*-EGFP and pAAV-CMV-EGFP). Suppression constructs with EGFP reporter genes are also provided (pAAV-shBB-EGFP, pAAV-shQ1-EGFP, pAAV-shCC-EGFP). Suppressors were expressed from the H1 promoter and replacement *RHO* cDNAs from differently sized mouse rhodopsin promoter sequences. HGH int: human growth hormone intron. CRX-NRL indicates enhancer element SEQ ID NO: 94. Restriction enzyme sites used for cloning are indicated. Promoters were separated by spacer DNA fragments. Numbers indicate molecular sizes (bp) and arrows indicate direction of transcription. Notably, any combination of the elements and conserved regions outlined and indeed other elements that can modulate gene expression could be used in the invention to exert control over expression of suppression and or replacement components.

Figure 10 illustrates a comparison of levels of expression from the Rho-M transgene versus that obtained from the suppression and replacement constructs in AAV2/5 and represented in Figure 9, using RNase protection. Figure 10 illustrates that the suppression and replacement constructs (see Figure 9) engineered into AAV2/5, AAV-BB8, AAV-BB10, AAV-BB11, AAV-BB12, AAV-BB13 and AAV-BB16 express the human rhodopsin replacement gene in RNA extracted from 129 wild type mice subretinally injected with suppression and or replacement constructs. (Lanes with material from mouse eyes injected with AAV-BB8 are indicated by BB8, AAV-BB10 by BB10, AAV-BB11 by BB11 etc. The plasmid constructs used to generate AAV vectors are written in the format pAAV as presented in Figure 9). BB8, BB10 and BB11 express rhodopsin at lower levels than BB12, BB13 and BB16.

Figure 11 provides a comparative analysis of rhodopsin expression from rAAV2/5 suppression and replacement vectors using real time RT-PCR. Figure 11 illustrates replacement rhodopsin expression levels in RNA extracted from 129 wild type mice subretinally injected with suppression and/or replacement constructs. Expression levels were also determined in Rho-M transgenic mice which express a rhodopsin replacement construct rCC and display normal retinal function.

Suppression and replacement constructs BB12, BB13, BB16 and BB18 express approximately in the same order of magnitude as levels of replacement rhodopsin transcript in Rho-M mice, indicating that enhanced replacement constructs with enhancer elements and conserved regions may express sufficient levels of rhodopsin to sustain a functional retina *in vivo*. (Lanes with material from mouse eyes injected with AAV-BB8 are indicated by BB8, AAV-BB10 by BB10, AAV-BB11 by BB11 etc.)

Figure 12 illustrates retinal histology of adult wild type retinas were subretinally injected with 2 μ l of 2×10^{12} particle/ml of different replacement-RHO AAV vectors (see Figure 9). Two weeks post-injection transduced eyes were removed, fixed in 4% paraformaldehyde and cryosectioned (12 μ m). Subsequently, sections were stained with human specific anti-RHO antibody to visualize expression of replacement-RHO using Cy3 label (red) on the secondary antibody; cell nuclei were counterstained with DAPI (blue). A: AAV-BB8, B: AAV-BB13, C: AAV-BB24, D: AAV-Q8, E: AAV-Q26, F: retina from uninjected RhoM transgenic mouse expressing RHO (positive control). Sections indicate different levels of RHO expression in the sections. OS: photoreceptor outer segments; IS: photoreceptor inner segments; ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer.

Figure 13 illustrates retinal histology of adult NHR transgenic mice on a rho-/- background, therefore expressing normal human RHO but not mouse rho. These mice were transduced by subretinal injection of 2 μ l of 2×10^{12} particle/ml of AAV-shQ1-EGFP (A) or AAV-shNT-EGFP (B). Two weeks after injection, eyes were removed, fixed in 4% paraformaldehyde and cryosectioned AAV-shQ1-EGFP expresses shRNA-Q1, which targets RHO, while AAV-shNT-EGFP expresses a non-targeting shRNA (Figure 9 illustrates exemplary constructs). Both constructs express EGFP allowing tracking the transduced cell populations (green). Sections were counterstained DAPI (blue) to label position of the nuclear layers. A significant reduction in the photoreceptor cell number in the transduced part of the outer nuclear layer is apparent in the AAV-shQ1-EGFP injected (A) retinas compared to those of injected with AAV-shNT-EGFP (B). IS: photoreceptor inner segments; ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer.

Figure 14A-C illustrates retinal histology of adult RHO-347 transgenic mice carrying a dominant RHO mutation on a mouse rho+/+ background causing retinal degeneration were subretinally injected with 2 ul of 2×10^{12} particle/ml of AAV-shNT-EGFP (A) or AAV-shQ1-EGFP (B) vectors. Two weeks post-injection transduced eyes were removed, fixed in 4% paraformaldehyde and cryosectioned (12 um). AAV-shQ1-EGFP expresses shRNA-Q1-EGFP, which targets RHO, while AAV-shNT-EGFP expresses a non-targeting shRNA. Both constructs express EGFP allowing tracking of the transduced part of the retina (green). Sections were counterstained with DAPI (blue) to indicate positions of the nuclear layers. A significant reduction of the photoreceptor cell numbers in the transduced part of the outer nuclear layer in the AAV-shNT-EGFP injected or the uninjected (C) retinas are apparent due to the degenerative effects of RHO-347 transgene. A significantly preserved outer nuclear layer is detected in the AAV-shQ1-EGFP transduced retinas, where shRNA-Q1-EGFP effectively suppresses the RHO-347 transcript therefore reducing retinal degeneration. Note, that mouse rho (expressed in these retinas) is refractory to suppression by shRNA-Q1-EGFP due to the presence of nucleotide changes at the target site for Q1 siRNA suppression. Suppression and replacement using the degeneracy of the genetic code provided therapeutic benefit at a histological level. Figure 14D provides evidence of an improvement in the electroretinogram (ERG) in RHO-347 eyes treated with AAV-shQ1-EGFP versus eyes treated with AAV-shNT-EGFP. Figure 14D provides a representative maximum ERG response of a RHO-347 mouse, containing a human rhodopsin transgene with a mutation at codon 347, subretinally injected with AAV2/5 constructs. This RHO-347 mouse normally displays a phenotype similar to autosomal dominant RP. The top panel in Figure 14D is the response of the right eye, which received an injection of AAV-shQ1, a AAV2/5 vector containing suppressor siRNA Q1 driven by an H1 promoter (shQ1) and a CMV-driven EGFP gene. The left eye received an AAV-shNT, a AAV2/5 containing a non-targeting (control) siRNA driven by an H1 promoter (shNT) and a CMV-driven EGFP gene. As can be seen in Figure 14D, the maximum response is significantly greater in the treated right eye than in the control left eye, indicating that suppression of the mutant rhodopsin transgene leads to some rescue at the ERG level.

Figure 15 illustrates exemplary constructs utilising chromatin opening elements to optimise expression are presented. Components utilised to enhance expression may be cloned into vectors such as AAV vectors. Elements to optimise

expression of a given gene may be combined with other promoter elements such as the rhodopsin promoter and / or enhancer sequences or alternatively sequences that modulate chromatin structures and drive gene expression may be utilised alone to facilitate optimisation of expression of a target gene.

Figure 16 shows sequences of exemplary elements that can facilitate modulation of chromatin structures.

Figure 17 shows nucleotide and amino acid sequences of a number of exemplary neurotrophic factors.

Figure 18 illustrates exemplary suppression and replacement constructs containing other genetic elements which are beneficial for photoreceptor cell survival. In the example pAAV-BB18 has been combined with neurotrophic factor GDNF, driven by a small UCOE (chromatin opening element. A Thrasher, Abstract 36, British Society for Gene Therapy 5th Annual Conference 2008) promoter). Other neurotrophic factors such as, for example, Neurturin may also be used in combination with any of the suppression and replacement constructs described. In addition, other beneficial genes, other than neurotrophic factors may also be combined with suppression and replacement constructs such as for example, a second suppression element, a second replacement element, VEGF and others. In example A, the additional element, in this case GDNF is co-located with the suppression and replacement construct within the two AAV inverted terminal repeat sequences, ITR1 and ITR2. In the second example, B, the GDNF gene and its promoter are not co-located with the suppression and replacement elements within ITR1 and ITR2, but are located within the backbone of the plasmid used to generate AAV. Since a small proportion of the backbone is packaged during AAV production, this would result in a mixed population of AAVs with the majority containing the suppression and replacement elements and a minority the GDNF elements. In this case, other beneficial genes, other than neurotrophic factors may also be combined with suppression and replacement constructs such as for example, a second suppression element, a second replacement element, VEGF and others.

Detailed Description of the Invention

The instant invention utilises efficient gene suppression in conjunction with gene replacement to overcome the challenge of mutational heterogeneity. The

suppression agent does not necessarily target a mutation (although it can encompass the site of a mutation), but is rather mutation independent. Suppression can involve one or both alleles of an endogenous gene. In conjunction with suppression, a replacement gene is provided that has been modified such that the replacement gene is refractory or partially refractory to suppression. The invention uses the degeneracy of the genetic code to modify the replacement gene. Alteration of “wobble” bases makes it possible for replacement nucleic acids to escape suppression at least in part, but does not change the protein product expressed from the replacement nucleic acids. Alternatively, replacement genes are modified in such a way that they encode altered amino acids but still encode a functional or partially functional protein that does not lead to pathology (e.g., because the amino acid changes are silent mutations or polymorphisms). Replacement has been demonstrated using rhodopsin nucleic acids, however, other genes or combinations of genes can be made and used in the practice of the invention. In particular, the invention relates to modulating and optimizing the expression levels of the suppression agents and/or replacement nucleic acids using one or more of the untranslated regions (UTRs) of a gene, intronic sequences, the degeneracy of the genetic code and/or polymorphisms to alter the sequence of replacement nucleic acids such that they are refractory or partially refractory to suppression.

In one aspect, the invention provides methods for preparing and using a suppression agent and replacement nucleic acid. The suppression agent binds to a coding region of a mature RNA or DNA encoding a mutant allele and inhibits expression of the mutant allele. The replacement nucleic acid encodes a wild-type or non-disease causing allele and comprises at least one degenerate / wobble nucleotide that is altered so that the replacement nucleic acid is not suppressed, or is only partially suppressed, by the suppression of one or both alleles of a gene.

The invention provides for replacement genes using sequences to enhance expression of replacement genes from viral and or non-viral vectors. In particular the invention relates to enhanced expression of suppression agent(s) and or replacement genes from viral or and non-viral vectors. The invention relates to use of conserved sequences from retinal genes to enhance expression of suppression agent(s) and or replacement genes. In a particular aspect the invention relates to use of conserved sequences from retinal genes to enhance expression of suppression agent(s) and or

replacement genes from adeno associated virus (AAV) vectors. In another aspect the invention provides vectors for expression of suppression agent(s) and or replacement gene(s) using regulatory sequences from retinal gene(s) and or non-retinal gene(s) and or ubiquitously expressing genes such as those provided in the Tables below to enhance expression from viral and non-viral vectors.

In another aspect, the invention provides a composition comprising a suppression agent that binds to the coding region of a mature and/or immature RNA or DNA encoding a mutant allele to inhibit expression of the mutant allele and a replacement nucleic acid that encodes a wild-type or non-disease causing allele and comprises at least one degenerate / wobble nucleotide that is altered so that the replacement nucleic acid is not suppressed, or is only partially suppressed, by the suppression agent.

In yet another aspect, the invention provides a kit comprising a suppression agent that suppresses the expression of a mature and or immature RNA or DNA encoding a mutant allele and a replacement nucleic acid that encodes a wild-type or non-disease causing allele that is not suppressed, or is only partially suppressed, by the suppression agent and differs from the mutant allele in at least one degenerate / wobble nucleotide.

Suppression is achieved using a wide variety of molecular tools, such as, for example, RNA interference (RNAi) including non-coding RNAs such as small interfering RNA (siRNA), short hairpin RNA (shRNA), microRNAs (miRNA), or other nucleotide-based molecules. In an embodiment, siRNAs in the order of 14-27 nucleotides in length are used for gene suppression. ShRNAs can be used to express functional siRNAs intracellularly and to achieve suppression *in vitro* and *in vivo*. Other suppression molecules include, for example, sense and antisense nucleic acids (single or double stranded), ribozymes, peptides, DNazymes, peptide nucleic acids (PNAs), triple helix forming oligonucleotides, antibodies, and aptamers and modified form(s) thereof directed to sequences in gene(s), RNA transcripts, or proteins.

In an embodiment, the invention relates to vector(s) for supplying an endogenously generated suppression agent, such as, for example, a dsRNA in the form of a short hairpin (shRNA) which can be processed intracellularly into siRNA. dsRNA may be locally or systemically delivered. Expression vectors are used to

generate functional siRNAs in cells and in animals typically using polymerase III promoters to drive expression, although polymerase II promoters are also used. For example, miRNA structures can be used to express double stranded RNAs from polymerase II promoters to enable tissue specific expression of double stranded RNA or polymerase II promoters can be juxtaposed to shRNA sequences to be expressed.

Suppression agents may be modified to alter the potency of the suppression agent, the target affinity of the suppression agent, the safety profile of the suppression agent and/or the stability of the suppression agent, for example, to render them resistant or partially resistant to intracellular degradation. Modifications, such as phosphorothioates, for example, can be made to oligonucleotides to increase resistance to nuclease degradation, binding affinity and/or uptake. In addition, hydrophobization and bioconjugation enhances siRNA delivery and targeting (De Paula et al., RNA. 13(4):431-56, 2007) and siRNAs with ribo-difluorotoluy nucleotides maintain gene silencing activity (Xia et al., ASC Chem. Biol. 1(3):176-83, (2006). siRNAs with amide-linked oligoribonucleosides have been generated which are more resistant to S1 nuclease degradation (Iwase R et al. 2006 Nucleic Acids Symp Ser 50: 175-176). In addition, modification of siRNA at the 2'-sugar position and phosphodiester linkage confers improved serum stability without loss of efficacy (Choung et al., Biochem. Biophys. Res. Commun. 342(3):919-26, 2006). In one study, 2'-deoxy-2'-fluoro-beta-D-arabinonucleic acid (FANA)- containing antisense oligonucleotides compared favourably to phosphorothioate oligonucleotides, 2'-O-methyl-RNA/DNA chimeric oligonucleotides and siRNAs in terms of suppression potency and resistance to degradation (Ferrari N et al. 2006 Ann N Y Acad Sci 1082: 91-102.)

Antisense and ribozyme suppression strategies have led to the reversal of a tumor phenotype by reducing expression of a gene product or by cleaving a mutant transcript at the site of the mutation (Carter and Lemoine Br. J. Cancer. 67(5):869-76, 1993; Lange et al., Leukemia. 6(11):1786-94, 1993; Valera et al., J. Biol. Chem. 269(46):28543-6, 1994; Dosaka-Akita et al., Am. J. Clin. Pathol. 102(5):660-4, 1994; Feng et al., Cancer Res. 55(10):2024-8, 1995; Quattrone et al., Cancer Res. 55(1):90-5, 1995; Lewin et al., Nat Med. 4(8):967-71, 1998). For example, neoplastic reversion was obtained using a ribozyme targeted to an H-ras mutation in bladder carcinoma cells (Feng et al., Cancer Res. 55(10):2024-8, 1995). Ribozymes have also

been proposed as a means of both inhibiting gene expression of a mutant gene and of correcting the mutant by targeted trans-splicing (Sullenger and Cech *Nature* 371(6498):619-22, 1994; Jones et al., *Nat. Med.* 2(6):643-8, 1996). Ribozyme activity may be augmented by the use of, for example, non-specific nucleic acid binding proteins or facilitator oligonucleotides (Herschlag et al., *Embo J.* 13(12):2913-24, 1994; Jankowsky and Schwenzer *Nucleic Acids Res.* 24(3):423-9, 1996). Multitarget ribozymes (connected or shotgun) have been suggested as a means of improving efficiency of ribozymes for gene suppression (Ohkawa et al., *Nucleic Acids Symp Ser.* (29):121-2, 1993).

Triple helix approaches have also been investigated for sequence-specific gene suppression. Triplex forming oligonucleotides have been found in some cases to bind in a sequence-specific manner (Postel et al., *Proc. Natl. Acad. Sci. U.S.A.* 88(18):8227-31, 1991; Duval-Valentin et al., *Proc. Natl. Acad. Sci. U.S.A.* 89(2):504-8, 1992; Hardenbol and Van Dyke *Proc. Natl. Acad. Sci. U.S.A.* 93(7):2811-6, 1996; Porumb et al., *Cancer Res.* 56(3):515-22, 1996). Similarly, peptide nucleic acids have been shown to inhibit gene expression (Hanvey et al., *Antisense Res. Dev.* 1(4):307-17, 1991; Knudsen and Nielson *Nucleic Acids Res.* 24(3):494-500, 1996; Taylor et al., *Arch. Surg.* 132(11):1177-83, 1997). Minor groove binding polyamides can bind in a sequence-specific manner to DNA targets and hence may represent useful small molecules for future suppression at the DNA level (Trauger et al., *Chem. Biol.* 3(5):369-77, 1996). In addition, suppression has been obtained by interference at the protein level using dominant negative mutant peptides and antibodies (Herskowitz *Nature* 329(6136):219-22, 1987; Rimsky et al., *Nature* 341(6241):453-6, 1989; Wright et al., *Proc. Natl. Acad. Sci. U.S.A.* 86(9):3199-203, 1989). In some cases suppression strategies have lead to a reduction in RNA levels without a concomitant reduction in proteins, whereas in others, reductions in RNA have been mirrored by reductions in protein.

The diverse array of suppression strategies that can be employed includes the use of DNA and/or RNA aptamers that can be selected to target, for example, a protein of interest such as rhodopsin. In the case of age related macular degeneration (AMD), anti-VEGF aptamers have been generated and have been shown to provide clinical benefit in some AMD patients (Ulrich H, et al. *Comb. Chem. High Throughput Screen* 9: 619-632, 2006). Suppression and replacement using aptamers

for suppression in conjunction with a modified replacement gene and encoded protein that is refractory or partially refractory to aptamer-based suppression could be used in the invention.

Recent evidence suggests that control of gene expression occurs endogenously in part by the activity of small non-coding RNAs, one broad category of which is termed microRNAs (miRNAs). miRNAs are expressed from polymerase II promoters, but can also be expressed from polymerase III promoters. miRNAs are processed intracellularly from larger transcripts to form small molecules approximately 20 nucleotides in length. miRNA structures can be used to express small double stranded RNAs and thus can be used to express the double stranded RNAs of the current invention.

Suppression targeted to coding sequence holds the advantage that such sequences are present in both precursor and mature RNAs, thereby enabling suppressor effectors to target all forms of RNA. A combined approach using a number of suppression effectors directed to multiple targets on an RNA or to multiple RNAs may also be used in the invention. As with suppression, multiple replacement nucleic acids can be used in the invention. For some disorders, it may be necessary to block expression of a disease allele completely to prevent disease symptoms whereas for others low levels of mutant protein may be tolerated. The invention can thus provide partial or complete suppression.

In one embodiment of the invention, suppressors are targeted to genes that are involved in the regulation of other genes. Suppression of these genes therefore may lead to up- or down-regulation of other genes.

In another embodiment, the invention relates to suppression of the expression of mutated genes that give rise to a dominant or deleterious effect or disease. A suppression effector may target either the disease allele or the normal allele. In another embodiment, the suppression effector targets both the disease allele and the normal allele.

In an embodiment of the invention, a replacement nucleic acid is provided that is altered at one or more degenerate or wobble bases from the endogenous wild type gene but that encodes the identical amino acids as the wild type or a non-disease causing gene. In another embodiment, the replacement nucleic acid encodes a

beneficial replacement nucleic acid (e.g., a more active or stable product than that encoded by the wild-type gene). The replacement nucleic acid provides expression of a normal or non-disease causing protein product when required to ameliorate pathology associated with reduced levels of wild type protein. The same replacement nucleic acid can be used in conjunction with the suppression of many different disease mutations within a given gene. In addition, multiple replacement nucleic acids can be used in the invention.

Although the instant application provides numerous exemplary suppression agents and replacement nucleic acid sequences, these are only examples and other such sequences can be determined as described herein for the same targets or for any desired target. "Functional variant" includes any variant nucleic acid or other suppression agent that may have one or more nucleic acid substitutions but that does not have a materially different function than, or that can still hybridize under stringent hybridization conditions (0.2X SCC, .1% SDS) to, or that shares at least 70% identity, for example 80%, such as at least 90% or at least 95% sequence identity with the nucleic acid indicated.

In another embodiment of the invention, suppression effectors are targeted to the untranslated regions (either 5'UTR or 3'UTR) of at least one allele of a gene. In another embodiment of the invention replacement nucleic acids are provided that have been altered at the suppression site, such that replacement nucleic acids provide functional or partially functional protein and escape or partially escape from suppression by suppressors.

In another embodiment of the invention, suppression effectors are targeted to intronic sequences. In another embodiment, replacement nucleic acids are provided which have been altered at one or more nucleotides of the targeted site of the intron so that transcripts from the replacement nucleic acids escape or partially escape suppression by suppressors. In another embodiment the whole targeted intron may not be present in replacement nucleic acids.

In another embodiment of the invention, suppression effectors are targeted to polymorphic sites and at least one allele of the gene is suppressed or partially suppressed. In another embodiment, replacement nucleic acids are provided for the alternative polymorphic variant such that replacement nucleic acids encode functional

or partially functional protein and escape or partially escape from suppression by suppressors.

In another embodiment of the invention the suppression agent and/or replacement nucleic acid is expressed from one or more promoter sequences. The invention provides promoter sequences that have been demonstrated to promote ubiquitous expression of nucleotides and/or promoters that have been demonstrated to exert tissue specific, temporal, inducible, and/or quantitative control of gene expression. The invention also provides enhancer sequences (Table 1) and/or post-translational regulatory elements and/or other regulatory elements and/or epigenetic elements that provide optimized expression of suppression agents and/or replacement nucleic acids.

Table 1: Exemplary Enhancer Elements

Enhancer Element	Reference
Chicken ovalbumin upstream promoter transcription factor II	Eguchi et al., Biochimie 89(3):278-88, 2007
Mouse dystrophin muscle promoter/enhancer	Anderson et al., Mol. Ther. 14(5):724-34, 2006
Tobacco eIF4A-10 promoter elements	Tian et al., J. Plant Physiol. 162(12):1355-66, 2005
Immunoglobulin (Ig) enhancer element HS1,2A	Frezza et al., Ann. Rheum. Dis. March 28, 2007
Col9a1 enhancer element	Genzer and Bridgewater Nucleic Acids Res. 35(4):1178-86, 2007
Gata2 intronic enhancer	Khandekar et al., Development March 29, 2007
TH promoter enhancer	Gao et al., Brain Res. 1130(1):1-16, 2007
CMV enhancer	InvivoGen cat# pdrive-cag 05A13-SV
Woodchuck hepatitis virus posttranscriptional regulatory element	Donello et al., J. Virol. 72(6):5085-92, 1998

Enhancer Element	Reference
Woodchuck hepatitis virus posttranscriptional regulatory element	Schambach et al., Gene Ther. 13(7):641-5, 2006
IRBP	Ying et al., Curr. Eye Res. 17(8):777-82, 1998
CMV enhancer and chicken β -actin promoter	InvivoGen cat# pdrive-cag 05A13-SV
CMV enhancer and chicken β -actin promoter and 5'UTR	InvivoGen cat# pdrive-cag 05A13-SV
CpG-island	Antoniou et al., Genomics 82:269-279, 2003

In a particular embodiment, sequences that influence chromatin structure, such as but not exclusive to insulator, antirepressor, cis-acting modulators of nucleosome positioning and/or silencer elements, sometimes termed epigenetic elements, are used to modulate expression of suppression agents and/or replacement nucleic acids. Exemplary epigenetic elements such as insulator and antirepressor sequences are provided in Table 2. It is clear that chromatin structures influence gene expression, for example, chromatin structures influence the ability of the transcriptional machinery to access promoter and/or enhancer elements amongst other sequence motifs. The inclusion of sequences which influence chromatin structures in viral and/or non-viral vectors and/or administered in conjunction with suppression and/or replacement nucleic acids can be used to optimize expression of either or both suppressors and replacement nucleic acids. In addition, chemical entities which influence chromatin structures can be used to optimize expression such as histone deacetylase (HDAC) inhibitors and/or DNA methyl transferase inhibitors and/or histone methyl transferase inhibitors. Such entities can be supplied in the form of DNA and/or RNA and/or protein amongst other forms. Similarly attracting enzymes and/or supplying enzymes (in the form of DNA and/or RNA and or protein) involved in chromatin remodelling such as but not exclusive to histone acetyl transferases to nucleic acids to be expressed and their associated regulatory regions can be used to optimize expression of suppression and/or replacement nucleic acids.

Table 2: Exemplary Epigenetic Elements

Epigenetic elements	Reference
Mcp Insulators	Kyrchanova et al., Mol. Cell Biol. 27(8):3035-43, 2007
CpG-island region of the HNRPA2B1 locus	Williams et al., BMC Biotechnol. 5:17, 2005
Chicken b-globin 5'hypersensitive site 4 (cHS4)	Kwaks and Otte 2006 Trends in Biotechnology 24: 137-142
Ubiquitous chromatin opening elements (UCOE)	Kwaks and Otte 2006 Trends in Biotechnology 24: 137-142
Matrix associated regions (MARs)	Kwaks and Otte 2006 Trends in Biotechnology 24: 137-142
Stabilising and antirepressor elements (STAR)	Kwaks and Otte 2006 Trends in Biotechnology 24: 137-142
Human growth hormone gene silencer	Trujillo MA et al. 2006 Mol Endocrinol 20: 2559
S/MAR	Liebich et al., Nucleic Acids Res. 30: 3433-42, 2002

In another embodiment, expression of a suppression agent and/or replacement nucleic acid is optimized to enable efficient suppression in conjunction with sufficient replacement. In an additional embodiment, suppression and/or replacement nucleic acids are provided with agents that aid vector transfection, transduction, and/or expression of suppression and replacement nucleic acids.

The invention circumvents the need for a specific therapy for every disease-causing mutation within a given gene. Notably, the invention has the advantage that the same suppression agents can be used to suppress many mutations in a gene. This is particularly relevant when any one of a large number of mutations within a single gene can cause disease pathology. The compositions and methods of the invention allow greater flexibility in choice of target sequence for suppression of expression of a disease allele. Furthermore, the compositions and methods of the invention allow greater flexibility in terms of controlling expression of the suppression and/or replacement of a given gene and or allele of a gene.

Suppression and replacement can be undertaken in conjunction with each other or separately. Suppression and replacement utilizing the degeneracy of the genetic code may be undertaken in test tubes, in cells, in animals, or in plants and may be used for experimental research (e.g., for the study of development or gene expression) or for therapeutic purposes. Suppression and replacement may be used in conjunction with agents to promote cell transfection or cell transduction such as, for example, lipids and polymers. Suppression and replacement may be provided to consumers in a kit.

The suppression and replacement agents of the invention can be delivered to a target cell and or tissue and or animal and or plant using 'naked' reagents such as DNA, RNA, peptides or other reagents. Alternatively viral and or non-viral vectors can be used with or without 'naked' reagents.

In an embodiment, suppression and/or replacement construct(s) can be delivered to a cell using an AAV2/5 recombinant virus, however, other viral and non-viral vectors, such as other AAV serotypes, adenovirus, herpes virus, SV40, HIV, SIV and other lentiviral vectors, RSV and non-viral vectors including naked DNA, plasmid vectors, peptide-guided gene delivery, terplex gene delivery systems, calcium phosphate nanoparticles, magnetic nanoparticles, colloidal microgels and/or the integrase system from bacteriophage phiC31 may be utilised in the invention, for example. Suppression and replacement components may be found on separate vectors or may be incorporated into the same vector. Viral vectors useful in the invention include, but are not limited to, those listed in Table 3. Non-viral vectors useful in the invention include, but are not limited to, those listed in Table 4. Cationic lipid-based non-viral vectors can include glycerol-based (e.g. DOTMA, DOTAP, DMRIE, DOSPA), non-glycerol-based (e.g. DOGS, DOTIM) and/or cholesterol-based cationic lipids (e.g. BGTC, CTAP; Karmali PP and Chaudhuri A 2006 Med Res Rev). Viral and non-viral vector delivery may be accompanied by other molecules such as cationic lipids and/or polymers and/or detergents and/or agents to alter pH, such as, for example, polyethelene glycol (PEG), to enhance cellular uptake of vectors and/or to enhance expression from vectors and/or to evade the immune system. For example, polycationic molecules have been generated to facilitate gene delivery including but not exclusive to cationic lipids, poly-amino acids, cationic block co-polymers, cyclodextrins amongst others. Pegylation of vectors with polyethelene

glycol (PEG) can shield vectors from, for example, the extracellular environment. Vectors may be used in conjunction with agents to avoid or minimise cellular immune responses such as PEG or as a Polyplex with Poly(L-Lysine). Vector delivery may be undertaken using physical methodologies such as electroporation, nucleofection and/or iontophoresis, either alone or in combination with molecules to enhance delivery. Vectors may be used in conjunction with agents to promote expression of suppression and/or replacement components incorporated into vectors, for example, using histone deacetylase inhibitors (HDAC) and/or DNA methyl transferase inhibitors and/or histone methyl transferase inhibitors to modulate chromatin structures thereby aiding expression. HDAC inhibitors include but are not exclusive to short chain fatty acids such as valproic acid and sodium butyrate, ketones, benzamides, cyclic and non-cyclic hydroxamates such as suberoyl anilide hydroxamic acids (SAHA), trichostatin A (TSA), cyclic peptides or tetrapeptides amongst others (Liu T et al. 2006 Cancer Treatment Reviews 32: 157-165). DNA methyl transferase inhibitors including, for example, 5-AC, decitabine and zebularine can be used to modulate chromatin structures. In addition, histone methyl transferase inhibitors can influence chromatin states, for example, BIX-01294 (diazepin-quinazolin-amine derivative). In addition, to the chemical entities referred to above, nucleic acids-based inhibitors can be used to suppress expression of proteins and/or non-coding RNAs involved in chromatin remodelling. In one embodiment of the invention vectors are optimized to specifically transduce target cell type(s) or target tissue type(s). Viral and/or non-viral vectors may be modified to target specific cell types and/or to prevent targeting of some cell types. For example, the inclusion of the capsid from AAV serotype 5 in an AAV2/5 hybrid virus facilitates transduction of photoreceptor cells. Similarly, for example, peptides may be included in viral vectors to facilitate targeting. Synthetic non-viral vectors can be modified to include ligands to facilitate targeting of vectors to specific cell and/or tissue types, for example, folate can be conjugated to liposomes to target tumour cells which over express the folate receptor (Hattori Y et al. 2005; Curr Drug Deliv 3: 243-52). In another embodiment of the invention, suppression and replacement vectors are designed to optimize the generation and/or production of vector, for example, to optimize viral titre and/or to optimize the number or type of nucleotides incorporated into vector(s). For example, vector genomes may be modified such that large transgenes may be incorporated into vectors, for example, 'gutless' adenovirus vectors have an increased capacity in terms

of size than previous generations of adenovirus vectors. Components of vectors can be modified to optimize generation and production of vectors, for example, genes involved in replication of AAV can be modified to optimize replication and/or self complementary AAV vectors can be used to optimize rates of transgene expression. In an additional embodiment, vectors are designed to optimize suppression in conjunction with replacement, to enable optimal expression of all components of a therapeutic. For example, to optimize expression of both elements of suppression and replacement from a given vector, additional sequences can be included in the vector. For example, inclusion of nucleotides to separate the ITRs of AAV and the shRNA sequences of an RNAi-based suppression agent can result in optimisation of expression of the suppression component. Nucleotides encoding suppressors and/or replacement nucleic acids can be juxtaposed or separated from each other and/or can be in the same orientation or opposing orientations. In addition, the suppressor(s) can be 5' and/or 3' to the replacement nucleic acids. Nucleotides encoding suppressors and/or replacement nucleic acids can be juxtaposed to nucleotides comprising vector(s) or can be separated from nucleotides comprising vector(s). Nucleotides encoding suppressors and/or replacement nucleic acids may be cloned within the backbone of the plasmid used to generate AAV and or may be cloned between the AAV ITRs and not within the plasmid backbone of the plasmid, and/or may be cloned in a combination of these positions. Additional sequences, such as, for example, stuffer sequences can be included in vectors to optimize vector design. In addition, multiple suppressors and/or replacement nucleic acids may be used in one vector.

Table 3: Exemplary Viral Vectors

Delivery Method	Serotype	Reference
AAV	All serotypes, including but not limited to 1,2,3,4,5,6,7,8,9,10,11,12,	Lebkowski et al., Mol. Cell Biol. 8(10) :3988-96, 1988 Flannery et al., Proc. Natl. Acad. Sci. U.S.A. 94(13) :6916-21, 1997
Lentivirus (for example but not exclusively Feline – FIV, Equine – EIAV, Bovine – BIV and Simian – SIV).	VSV-G Rabies-G Further serotypes**	Pang et al., Mol. Vis. 12 :756-67, 2006 Takahashi Methods Mol. Biol. 246 :439-49, 2004 Balaggan et al., J. Gene

		Med. 8(3) :275-85, 2006
Adenovirus	Various	Bennett et al., Nat. Med. 2(6) :649-54, 1996
Simian papovirus SV40	Various	Kimchi-Sarfaty et al., Hum. Gene Ther. 13(2) :299-310, 2002
Semliki Forest Virus	Various	DiCiommo et al., Invest. Ophthalmol. Vis. Sco. 45(9) :3320-9, 2004
Sendai Virus	Various	Ikeda et al., Exp. Eye Res. 75(1) : 39-48, 2002

The list provided is not exhaustive; other viral vectors and derivatives, natural or synthesized could be used in the invention.

Table 4: Exemplary Non-Viral Vectors or Delivery Methods

Delivery Method	Reference
Cationic liposomes	Sakurai et al., Gene Ther. 8(9) :677-86, 2001
HVJ liposomes	Hangai et al., Arch. Ophthalmol. 116(3) :342-8, 1998
Polyethylenimine	Liao and Yau Biotechniques 42(3) :285-6, 2007
DNA nanoparticles	Farjo et al., PloS ONE 1 :e38, 2006
Dendrimers	Marano et al., Gene Ther. 12(21) :1544-50, 2005
Bacterial	Brown and Giaccia Cancer Res. 58(7) :1408-16, 1998
Macrophages	Griffiths et al., Gene Ther. 7(3) :255-62, 2000
Stem cells	Hall et al., Exp. Hematol. 34(4) :433-42, 2006
Retinal transplant	Ng et al., Chem. Immunol. Allergy 92 :300-16, 2007
Marrow/Mesenchymal stromal cells	Kicic et al., J. Neurosci. 23(21) :7742-9,

Delivery Method	Reference
	2003 Chng et al., J. Gene Med. 9(1) :22-32, 2007
Implant (e.g., Poly(imide)uncoated or coated)	Montezuma et al., Invest. Ophthalmol. Vis. Sci. 47(8) :3514-22, 2006
Electroporation	Featherstone A. Biotechnol. Lab. 11(8) :16, 1993
Targeting peptides (for example but not exclusively Tat)	Trompeter et al., J. Immunol Methods. 274(1-2) :245-56, 2003
Lipid mediated (e.g., DOPE, PEG)	Nagahara et al., Nat. Med. 4(12) :1449-52, 1998 Zeng et al., J. Virol. 81(5) :2401-17, 2007
	Caplen et al., Gene Ther. 2(9) :603-13, 1995 Manconi et al., Int. J. Pharm. 234(1-2) :237-48, 2006 Amrite et al., Invest. Ophthalmol. Vis. Sci. 47(3) :1149-60, 2006
	Chalberg et al., Invest. Ophthalmol. Vis. Sci. 46(6) :2140-6, 2005

The list provided is not exhaustive. Other non-viral vectors and derivatives, natural or synthesized and other delivery methods could be used with the invention.

In an embodiment, the replacement nucleic acid encodes mammalian rhodopsin, collagen 1A1, collagen 1A2, collagen 7A1, or peripherin. In another embodiment, the replacement nucleic acid encodes a protein that has been mutated to cause an autosomal or X-linked dominant retinitis pigmentosa, such as those listed in Table 5. Suppression agents and replacement nucleic acids may be generated for one or more of these genes, for example.

Table 5: Genes known to be involved in retinitis pigmentosa (Table adapted from RETNET) (<http://www.sph.uth.tmc.edu/Retnet/>)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
LCA9	1p36	recessive Leber congenital amaurosis	Keen et al., Hum. Mol. Genet. 3:367-368 (1994)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
NPHP4, SLSN4;	1p36.31	recessive Senior-Loken syndrome; recessive nephronophthisis, juvenile; protein: nephronophthisis 4 protein	Mollet et al., Nat. Genet. 32:300-305 (2002); Otto et al., Am. J. Hum. Genet. 71:1161-1167 (2002); Schuermann et al., Am. J. Hum. Genet. 70:1240-1246 (2002)
RP32;	1p34.3-p13.3	recessive RP, severe	Zhang et al., Hum. Genet. 118:356-365 (2005)
RPE65, LCA2, RP20;	1p31.2	recessive Leber congenital amaurosis; recessive RP; protein: retinal pigment epithelium-specific 65 kD protein	Acland Nat. Genet. 28:92-95 (2001)
ABCA4, ABCR, RP19, STGD1;	1p22.1	recessive Stargardt disease, juvenile and late onset; recessive MD; recessive RP; recessive fundus flavimaculatus; recessive cone-rod dystrophy; protein: ATP-binding cassette transporter - retinal	Lewis et al., Am. J. Hum. Genet. 64:422-434 (1999)
COL11A1, STL2;	1p21.1	dominant Stickler syndrome, type II; dominant Marshall syndrome; protein: collagen, type XI, alpha 1	Annunen et al., Am. J. Hum. Genet. 65:974-983 (1999)
GNAT2, ACHM4;	1p13.3	recessive achromatopsia; protein: guanine nucleotide binding protein (G protein) cone-specific transducin alpha subunit	Aligianis et al., J. Med. Genet. 39:656-660 (2002)
PRPF3, HPRP3, PRP3, RP18;	1q21.2	dominant RP; protein: human homolog of yeast pre-mRNA splicing factor 3	Chakarova et al., Hum. Mol. Genet. 11:87-92 (2002)
SEMA4A, SEMAB;	1q22	dominant RP; dominant cone-rod dystrophy; protein: semaphorin 4A	Abid et al., J. Med. Genet. 43:378-381 (2005)
CORD8;	1q23.1-q23.3	recessive cone-rod dystrophy	Ismail et al., J. Hum. Genet. 51:827-831 (2006)
AXPC1	1q31-q32	recessive ataxia, posterior column with RP	Higgins et al., Neurol. 52:146-150 (1999)
ARMD1, FIBL6, FBLN6;	1q31.1	dominant MD, age-related; protein: hemicentin 1 (fibulin 6)	Schultz et al., Hum. Mol. Genet. 12:3315-3123 (2003)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
CFH, HF1;	1q31.3	age-related macular degeneration, complex etiology; protein: complement factor H	Edwards et al., Science 308:421-424 (2005)
CRB1, RP12;	1q31.3	recessive RP with para-arteriolar preservation of the RPE (PPRPE); recessive RP; recessive Leber congenital amaurosis; dominant pigmented paravenous chorioretinal atrophy; protein: crumbs homolog 1	Jacobson et al., Hum. Mol. Genet. 9:1073-1078 (2003)
RD3, C1ORF36;	1q32.3	recessive Leber congenital amaurosis; protein: RD3 protein	Friedman et al., Am. J. Hum. Genet. 79:1059-1070 (2006)
USH2A;	1q41	recessive Usher syndrome, type 2a; recessive RP; protein: usherin	Seyedahmadi et al., Exp. Eye. Res. 79:167-173 (2004)
RP28;	2p16-p11	recessive RP	Kumar et al., Mol. Vis. 10:399-402 (2004)
EFEMP1, DHRD, MTLV, FBLN3;	2p16.1	dominant radial, macular drusen; dominant Doyme honeycomb retinal degeneration (Malattia Leventinese); protein: EGF-containing fibrillin-like extracellular matrix protein 1 (fibulin 3)	Kermani et al., Hum. Genet. 104:77-82 (1999)
ALMS1, ALSS	2p13.1	recessive Alström syndrome; protein: ALMS1 protein	Hearn et al., Nat. Genet. 31:79-83 (2002)
RP33	2cen-q12.1	dominant RP	Zhao et al., Hum. Genet. 119:617-623 (2006)
LOC619531	2q11	recessive cone-rod dystrophy and amelogenesis imperfecta	Michaelides et al., J. Med. Genet. 41:468-473 (2004)
CNGA3, ACHM2, CNCG3, RMCH2	2q11.2	recessive achromatopsia; protein: cone photoreceptor cGMP-gated cation channel alpha subunit	Nishiguchi et al., Hum. Mutat. 25:248-258 (2005)
MERTK	2q13	recessive RP; protein: c-mer protooncogene receptor tyrosine kinase	Vollrath et al., Proc. Natl. Acad. Sci. USA 98:12584-12589 (2001)
NPHP1,	2q13	recessive Senior-Loken	Hildebrandt et al., Nat.

Symbols; OMIM Numbers	Location	Diseases; Protein	References
JBTS4, SLSN1		syndrome; recessive nephronophthisis, juvenile; recessive Joubert syndrome; protein: nephronophthisis 3 protein	Genet. 17:149-153 (1997)
BBS5	2q31.1	recessive Bardet-Biedl syndrome; protein: flagellar apparatus-basal body protein DKFZp7621194	Li et al., Cell. 117:541- 552 (2004)
CERKL, RP26	2q31.3	recessive RP; protein: ceramide kinase-like protein	Tuson et al., Am. J. Hum. Genet. 74:128- 138 (2004)
SVD	2q36	dominant vitreoretinal degeneration, snowflake	Jiao et al., Invest. Ophthalmol. Vis. Sci. 45:4498-503 (2004)
SAG	2q37.1	recessive Oguchi disease; recessive RP; protein: arrestin (s- antigen)	Nakazawa et al., Arch. Ophthalmol. 116:498- 501 (1998)
USH2B, DFNB6	3p24.2-p23	recessive Usher syndrome, type 2; recessive sensorineural deafness without RP	Hmani et al., Eur. J. Hum. Genet. 7:363-367 (1999)
CRV, HERNS, HVR	3p21.3-p21.1	dominant hereditary vascular retinopathy with Raynaud phenomenon and migraine	Ophoff et al., Am. J. Hum. Genet. 69:447- 453 (2001)
GNAT1	3p21.31	dominant CSNB, Nougaret type; protein: rod transducin alpha subunit	Dryja et al., Nat. Genet. 13:358-360 (1996)
ATXN7, ADCA2, OPCA3, SCA7	3p14.1	dominant spinocerebellar ataxia w/ MD or retinal degeneration; protein: ataxin 7	Aleman et al., Exp. Eye. Res. 74:737-745 (2002)
ARL6, BBS3	3q11.2	recessive Bardet-Biedl syndrome; protein: ADP-ribosylation factor- like 6	Fan et al., Nat. Genet. 36:989-993 (2004)
IQCB1, NPHP5, SLSN5	3q13.33	recessive Senior-Loken syndrome; protein: IQ motif containing B1 protein	Otto et al., Nat. Genet. 37:282-288 (2005)
NPHP3, SLSN3	3q22.1	recessive Senior-Loken syndrome; recessive nephronophthisis, adolescent; protein: nephronophthisis 3 protein	Olbrich et al., Nat. Genet. 34:455-459 (2003)
RHO, RP4	3q22.1	dominant RP; dominant CSNB; recessive RP; protein: rhodopsin	Dryja et al., Nat. Genet. 4:280-283 (1993)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
RP5	same as RHO	not distinct from RHO/RP4	Farrar et al., Hum. Mol. Genet. 1:769-771 (1992)
USH3A, USH3	3q25.1	recessive Usher syndrome, type 3; protein: clarin-1	Joensuu et al., Am. J. Hum. Genet. 69:673-684 (2001)
OPA1	3q29	dominant optic atrophy, Kjer type; dominant optic atrophy with sensorineural hearing loss; protein: OPA1 protein	Aung et al., Hum. Genet. 110:52-56 (2002)
STGD4	4p	dominant Stargardt-like macular dystrophy	Kniazeva et al., Am. J. Hum. Genet. 64:1394-1399 (1999)
MCDR2	4p16.3-p15.2	dominant MD, bull's-eye	Michaelides et al., Invest. Ophthalmol. Vis. Sci. 44:1657-1662 (2003)
PDE6B, CSNB3	4p16.3	recessive RP; dominant CSNB; protein: rod cGMP phosphodiesterase beta subunit	Pearce-Kelling et al., Mol. Vis. 7:42-47 (2001)
WFS1, DFNA38	4p16.1	recessive Wolfram syndrome; dominant low frequency sensorineural hearing loss; protein: wolframin	Hum. Mol. Genet. 10:2501-2508 (2001)
PROML1	4p15.32	recessive retinal degeneration; protein: prominin (mouse)-like 1	Maw et al., Hum. Mol. Genet. 9:27-34 (2000)
CNGA1, CNCG, CNCG1	4p12	recessive RP; protein: rod cGMP-gated channel alpha subunit	Dryja et al., Proc. Natl. Acad. Sci. USA 92:10177-10181 (1995)
WFS2	4q22-q24	recessive Wolfram syndrome; dominant	El-Shanti et al., Am. J. Hum. Genet. 66:1229-1236 (2000)
MTP, ABL	4q23	recessive abetalipoproteinemia; protein: microsomal triglyceride transfer protein	Narcisi et al., Am. J. Hum. Genet. 57: 1298-1310 (1995)
BBS7, BBS2L1	4q27	recessive Bardet Biedl syndrome; protein: BBS7 protein	Badano et al., Am. J. Hum. Genet. 72:650-658 (2003)
BBS12, FLJ35630	4q27	recessive Bardet-Biedl syndrome; protein: BBS12 protein	Stoetzel et al., Am. J. Hum. Genet. 80:1-11 (2007)
RP29	4q32-q34	recessive RP	Hameed et al., Invest. Ophthalmol. Vis. Sci.

Symbols; OMIM Numbers	Location	Diseases; Protein	References
			42:1436-1438 (2001)
LRAT	4q32.1	recessive RP, severe early-onset; recessive Leber congenital amaurosis; protein: lecithin retinol acyltransferase	Thompson et al., Nat. Genet. 128:123-124 (2001)
CYP4V2, BCD	4q35.2	recessive Bietti crystalline corneoretinal dystrophy; protein: cytochrome P450 4V2	Li et al., Am. J. Hum. Genet. 74:817-826 (2004)
MCDR3	5p15.33-p13.1	dominant MD	Michaelides et al., Invest. Ophthalmol. Vis. Sci. 44:2178-2183 (2003)
CSPG2, WGN1, ERVR	5q14.3	dominant Wagner disease and erosive vitreoretinopathy; protein: chondroitin sulfate proteoglycan 2 (versican)	Kloeckener-Gruissem et al., Mol. Vis. 12:350- 355 (2006)
MASS1, USH2C, VLGR1	5q14.3	recessive Usher syndrome, type 2; dominant/recessive febrile convulsions; protein: monogenic audiogenic seizure susceptibility 1 homolog	Weston et al., Am. J. Hum. Genet. 74:357- 366 (2004)
BSMD	5q21.2-q33.2	dominant MD, butterfly-shaped	den Hollander et al., J. Med. Genet. 41:699- 702 (2004)
PDE6A	5q33.1	recessive RP; protein: cGMP phosphodiesterase alpha subunit	Dryja et al., Invest. Ophthalmol. Vis. Sci. 40:1859-1865 (1999).
GRM6	5q35.3	recessive CSNB; protein: metabotropic glutamate receptor 6	Dryja et al., Proc. Natl. Acad. Sci. USA 102:4884-4889 (2005)
C2	6p21.32	age-related macular degeneration, complex etiology; protein: complement component 2	Gold et al., Nat. Genet. 38:458-462 (2006)
CFB, BF, BFD	6p21.32	age-related macular degeneration, complex etiology; protein: complement factor B, properdin	Gold et al., Nat. Genet. 38:458-462 (2006)
TULP1, RP14	6p21.31	recessive RP; recessive Leber congenital amaurosis; protein: tubby-like protein 1	Banerjee et al., Nat. Genet. 18:177-179 (1998)
RDS, RP7	6p21.2	dominant RP; dominant MD; digenic RP with ROM1; dominant adult vitelliform MD; protein: peripherin 2	Hum. Mutat. 10:301- 309 (1997)

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GUCA1A, COD3, GCAP1	6p21.1	dominant cone dystrophy; dominant cone-rod dystrophy; protein: guanylate cyclase activating protein 1A	Payne et al., Am. J. Hum. Genet. 61:A290 (1997)
GUCA1B, GCAP2	6p21.1	dominant RP; dominant MD; protein: guanylate cyclase activating protein 1B	Sato et al., Graefes Arch. Clin. Exp. Ophthalmol. 243:235- 242 (2004)
BCMAD	6p12.3-q16	dominant MD, benign concentric annular	van Lith-Verhoeven et al., Invest. Ophthalmol. Vis. Sci. 45: 30-35 (2004)
RP25	6cen-q15	recessive RP	Abd El-Aziz et al., Ann. Hum. Genet. (2006)
LCA5	6q11-q16	recessive Leber congenital amaurosis	Dharmaraj et al., Am. J. Hum. Genet. 66:319- 326 (2000)
COL9A1	6q13	recessive Stickler syndrome; dominant multiple epiphyseal dysplasia (MED); protein: collagen, type IX, alpha-1	Van Camp et al., Am. J. Hum. Genet. 79:449- 457 (2006)
RIMS1, CORD7, RIM1	6q13	dominant cone-rod dystrophy; protein: regulating synaptic membrane exocytosis protein 1 or rab3A-interacting molecule	Kelsell et al., Am. J. Hum. Genet. 63:274- 279 (1998)
MCDR1, PBCRA	6q14-q16.2	dominant MD, North Carolina type; dominant progressive bifocal chorioretinal atrophy	Small et al., Mol. Vis. 5:38 (1999)
ELOVL4, STGD3	6q14.1	dominant MD, Stargardt-like; protein: elongation of very long fatty acids protein	Edwards et al., Invest. Ophthalmol. Vis. Sci. 42:2652-2663 (2001)
AHI1, JBTS3	6q23.3	recessive Joubert syndrome; protein: Abelson helper integration site 1	Parisi et al., J. Med. Genet. 43:334-339 (2006)
PEX7, PTS2R, RCDP1,	6q23.3	recessive Refsum disease, adult form; protein: peroxisome biogenesis factor 7	van den Brink Oet al., Am. J. Hum. Genet. 72:471-477 (2003)
RCD1	6q25-q26	dominant retinal-cone dystrophy 1	OMIM 07
MDDC, CYMD	7p21-p15	dominant MD, cystoid	Inglehearn et al., Am. J. Hum. Genet. 55:581- 582 (1994)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
PTHB1, BBS9, PHTB1	7p14.3	recessive Bardet Biedl syndrome; protein: parathyroid hormone- responsive B1 protein	Nishimura et al., Am. J. Hum. Genet. 77:1021- 1033 (2005)
RP9, PAPI, PIM1K	7p14.3	dominant RP; protein: RP9 protein or PIM1-kinase associated protein 1	Sullivan et al., Invest. Ophthalmol. Vis. Sci. 47:3052-3064 (2006)
PEX1, IRD	7q21.2	recessive Refsum disease, infantile form; protein: peroxisome biogenesis factor 1	Portsteffen et al., Nat. Genet. 17:449-452 (1997)
IMPDH1, RP10	7q32.1	dominant RP; dominant Leber congenital amaurosis; protein: inosine monophosphate dehydrogenase 1	Mortimer et al., Biochem. J. 390:41-47 (2005)
OPN1SW, BCP, CBT	7q32.1	dominant tritanopia; protein: blue cone opsin	Fitzgibbon et al., Hum. Genet. 93:79-80 (1994)
CORD9	8p11	recessive cone-rod dystrophy	Danciger et al., Invest. Ophthalmol. Vis. Sci. 42:2458-2465 (2001)
RP1	8q12.1	dominant RP; recessive RP; protein: RP1 protein	Bowne et al., Hum. Mol. Genet. 11:2121- 2128 (1999)
TTPA	8q12.3	recessive RP and/or recessive or dominant ataxia; protein: alpha- tocopherol-transfer protein	Yokota et al., New Eng. J. Med. 335:1770- 1771 (1996)
ROA1	8q21-q22	recessive optic atrophy	Barbet et al., Eur. J. Hum. Genet. 11: 966- 971 (2003)
PXMP3, PAF1, PEX2, PMP35	8q21.13	recessive Refsum disease, infantile form; protein: peroxisomal membrane protein 3	Gartner et al., Nat. Genet. 1:16-23 (1992)
CNGB3, ACHM3	8q21.3	recessive achromatopsia Pingelapese; recessive, progressive cone dystrophy; protein: cone cyclic nucleotide- gated cation channel beta 3 subunit	Kohl et al., Eur. J. Hum. Genet. 13:302- 308 (2005)
VMD1	not 8q24	dominant MD, atypical vitelliform	Sohocki et al., Am. J. Hum. Genet. 61:239- 241 (1997)
RP31	9p22-p13	dominant RP	Papaioannou et al., Hum. Mut. 118:501- 503 (2005)
KCNV2	9q24.2	recessive cone dystrophy with	Wu et al., Am. J. Hum.

Symbols; OMIM Numbers	Location	Diseases; Protein	References
		supernormal rod electroretinogram; protein: potassium channel subfamily V member 2	Genet. 79:574-579 (2006)
INVS, NPHP2	9q31.1	recessive Senior-Loken syndrome; recessive nephronophthisis; protein: inversion	O'Toole et al., Nephrol. Dial. Transplant. 21:1989-1991 (2006)
DFNB31	9q32	recessive Usher syndrome, type 2; recessive deafness without RP; protein: whirlin	Ebermann et al., Hum. Genet. (2006)
TLR4	9q33.1	age-related macular degeneration, complex etiology; protein: toll- like receptor 4	Zarepari et al., Hum. Mol. Genet. 14:1449- 1455 (2005)
TRIM32, BBS11, HT2A	9q33.1	recessive Bardet-Biedl syndrome; recessive limb-girdle muscular dystrophy; protein: tripartite motif-containing protein 32	Chiang et al., Proc. Natl. Acad. Sci. USA 103:6287-6292 (2006)
RP21, RP8	not 9q34-qter	dominant RP with sensorineural deafness	Mansergh et al., Am. J. Hum. Genet. 64:971- 985 (1999)
JBTS1, CORS1	9q34	recessive Joubert syndrome	Saar et al., Am. J. Hum. Genet. 65:1666-1671 (1999)
PHYH, PAHX, RDPA	10p13	recessive Refsum disease, adult form; protein: phytanoyl-CoA hydroxylase	Jansen et al., Nat. Genet. 17:190-193 (1997)
RNANC	10q21	recessive nonsyndromal congenital retinal nonattachmen	Ghahsvand et al., Am. J. Med. Genet. 90:165- 168 (2000)
PCDH15, DFNB23, USH1F	10q21.1	recessive Usher syndrome, type 1f; recessive deafness without RP; protein: protocadherin 15	Ahmed et al., Hum. Mol. Genet. 12:3215- 3223 (2003)
CDH23, DFNB12, USH1D	10q22.1	recessive Usher syndrome, type 1d; recessive deafness without RP; protein: cadherin-like gene 23	Astuto et al., Am. J. Hum. Genet. 71:262- 275 (2002)
RGR	10q23.1	recessive RP; dominant choroidal sclerosis; protein: RPE-retinal G protein-coupled receptor	Morimura et al., Nat. Genet. 23:393-394 (1999)
RBP4	10q23.33	recessive RPE degeneration; protein: retinol-binding protein 4	Seeliger et al., Invest. Ophthalmol. Vis. Sci. 40:3-11 (1999)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
PAX2, ONCR	10q24.31	dominant renal-coloboma syndrome; protein: paired homeotic gene 2 protein	Favor et al., Proc. Natl. Acad. Sci. USA 93:13870-13875 (1996)
HTRA1, PRSS11	10q26.13	age-related macular degeneration, complex etiology; protein: HtrA serine peptidase 1	DeWan et al., Science 314:989-992 (2006)
LOC387715	10q26.13	age-related macular degeneration, complex etiology; protein: hypothetical protein with Entrez ID 387715	Jakobsdottir et al., Am. J. Hum. Genet. 77:389-407 (2005)
OAT	10q26.13	recessive gyrate atrophy; protein: ornithine aminotransferase	D Valle, O Simell. In 'The Metabolic and Molecular Bases of Inherited Disease', 8th Ed. CR Scriver, <i>et al.</i> eds., McGraw-Hill. (2000)
TEAD1, AA, TCF13, TEF1	11p15.3	dominant atrophica areata; protein: TEA domain family member 1 [Entrez]	Fossdal et al., Hum. Mol. Genet. 13:975-981 (2004)
USH1C, DFNB18	11p15.1	recessive Usher syndrome, Acadian; recessive deafness without RP; protein: harmonin	Ahmed et al., Hum. Genet. 110:527-531 (2002)
EVR3	11p13-p12	dominant familial exudative vitreoretinopathy	Downey et al., Am. J. Hum. Genet. 68:778-781 (2001)
CORS2, JBTS2	11p12-q13.3	recessive Joubert syndrome	Valente et al., Ann. Neurol. 57:513-519 (2005)
ROM1	11q12.3	dominant RP; digenic RP with RDS; protein: retinal outer segment membrane protein 1	Dryja et al., Invest. Ophthalmol. Vis. Sci. 18:1972-1982 (1997)
VMD2	11q12.3	dominant MD, Best type; dominant vitreoretinopathies; protein: bestrophin	Weber et al., Am. J. Hum. Genet. 55:1182-1187 (1994a)
BBS1	11q13	recessive Bardet-Biedl syndrome; protein: BBS1 protein	Myktyyn et al., Nat. Genet. 31:435-438 (2002)
VRNI	11q13	dominant neovascular inflammatory vitreoretinopathy	Stone et al., Hum. Mol. Genet. 1:685-689 (1992)
CABP4	11q13.1	recessive CSNB; protein: calcium	Zeit et al., Am. J.

Symbols; OMIM Numbers	Location	Diseases; Protein	References
		binding protein 4	Hum. Genet. 79:657-667 (2006)
LRP5, EVR4, HBM, OPPG	11q13.2	dominant familial exudative vitreoretinopathy; dominant high bone mass trait; recessive osteoporosis-pseudoglioma syndrome; recessive FEVR; protein: low density lipoprotein receptor-related protein 5	Jiao et al., Am. J. Hum. Genet. 75:878-884 (2004)
MYO7A, DFNB2, USH1B	11q13.5	recessive Usher syndrome, type 1; recessive congenital deafness without RP; recessive atypical Usher syndrome (USH3-like); protein: myosin VIIA	Gibbs et al., Natl. Acad. Sci. USA 100:6481-6486 (2003)
FZD4, EVR1, FEVR	11q14.2	dominant familial exudative vitreoretinopathy; protein: frizzled-4 Wnt receptor homolog	Müller et al., Genomics 20:317-319 (1994)
C1QTNF5, CTRP5	11q23.3	dominant MD, late onset; dominant MD with lens zonules; protein: C1q and tumor necrosis-related protein 5 collagen	Ayyagari et al., Invest. Ophthalmol. Vis. Sci. 46:3363-3371 (2005)
COL2A1, AOM, STL1	12q13.11	dominant Stickler syndrome, type I; dominant Wagner syndrome; dominant epiphyseal dysplasia; protein: collagen, type II, alpha 1	Snead et al., J. Med. Genet. 36:353-659 (1999)
RDH5, RDH1	12q13.2	recessive fundus albipunctatus; recessive cone dystrophy, late onset; protein: 11- <i>cis</i> retinol dehydrogenase 5	Cideciyan et al., Vis. Neurosci. 17:667-678 (2000)
BBS10, FLJ23560	12q21.2	recessive Bardet-Biedl syndrome; protein: BBS10 (C12orf58) chaperonin	Stoetzel et al., Nat. Genet. 38:521-524 (2006)
CEP290, JBTS5, NPHP6, SLSN6	12q21.32	recessive Senior-Loken syndrome; recessive Joubert syndrome; recessive Leber congenital amaurosis; protein: centrosomal protein 290 kDa	Chang et al., Hum. Mol. Genet. 15:1847-1857 (2006)
RB1	13q14.2	dominant germline or somatic retinoblastoma; benign retinoma; pinealoma; osteogenic sarcoma; protein: retinoblastoma protein 1	Lohmann et al., Am. J. Hum. Genet. 58:940-949 (1996)
GRK1, RHOK, RK	13q34	recessive CSNB, Oguchi type; protein: rhodopsin kinase	Cideciyan et al., Proc. Natl. Acad. Sci. USA 95:328-333 (1998)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
STGD2	not 13q34	dominant MD, Stargardt type	Zhang et al., Nat. Genet. 27:89-93 (2001)
ACHM1, RMCH	14	recessive rod monochromacy or achromatopsia	Pentao et al., Am. J. Hum. Genet. 50:690-699 (1992)
RP16	not 14	recessive RP	Bruford et al., Am. J. Hum. Genet. 55:A181 (1994)
MCDR4	14q	dominant MD, North Carolina-like with progressive sensorineural hearing loss	Francis et al., Br. J. Ophthalmol. 87:893-898 (2003)
NRL, RP27	14q11.2	dominant RP; recessive RP; protein: neural retina lucine zipper	Mears et al., Nat. Genet. 29:447-452 (2001)
RPGRIP1, LCA6	14q11.2	recessive Leber congenital amaurosis; protein: RPGR-interacting protein 1	Mellersh et al., Genomics 88:293-301 (2006)
LCA3	14q24	recessive Leber congenital amaurosis	Stockton et al., Hum. Genet. 103:328-333 (1998)
RDH12	14q24.1	recessive Leber congenital amaurosis with severe childhood retinal dystrophy; protein: retinol dehydrogenase 12	Janecke et al., Nat. Genet. 36:850-854 (2004)
USH1A, USH1	not 14q32	recessive Usher syndrome, French	Gerber et al., Am. J. Hum. Genet. 78:357-359 (2006)
TTC8, BBS8	14q32.11	recessive Bardet-Biedl syndrome; protein: tetratricopeptide repeat domain 8	Ansley et al., Nat. 425:628-633 (2003)
FBLN5	14q32.12	familial MD, age-related; protein: fibulin 5	Arch. Ophthalmol. 112:765-772 (1994)
NR2E3, ESCS, PNR	15q23	recessive enhanced S-cone syndrome; recessive RP in Portuguese Crypto Jews; Goldmann-Favre syndrome; protein: nuclear receptor subfamily 2 group E3	Sharon et al., Arch. Ophthalmol. 121:1316-1323 (2003)
MRST	15q24	recessive retardation, spasticity and retinal degeneration	Mitchell et al., Am. J. Hum. Genet. 62:1070-1076 (1998)
BBS4	15q24.1	recessive Bardet-Biedl syndrome; protein: BBS4 protein	Katsanis et al., Nat. Genet. 26:67-70 (2000)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
RLBP1, CRALBP	15q26.1	recessive RP; recessive Bothnia dystrophy; recessive retinitis punctata albescens; recessive Newfoundland rod-cone dystrophy; protein: retinaldehyde-binding protein 1	Burstedt et al., Invest. Ophthalmol. Vis. Sci. 40:995-1000 (1999)
ABCC6, ARA, MRP6, PXE	16p13.11	recessive pseudoxanthoma elasticum; dominant pseudoxanthoma elasticum; protein: ATP-binding cassette, subfamily C, member 6	Bergen et al., Nat. Genet. 25:228-231 (2000)
RP22	16p12.3-p12.1	recessive RP	Finckh et al., Genomics 48:341-345 (1998)
CLN3, JNCL	16p11.2	recessive Batten disease (ceroid-lipofuscinosis, neuronal 3), juvenile; protein: Batten disease protein	Kremmidiotis et al., Hum. Mol. Genet. 8:523-531 (1999)
BBS2	16q12.2	recessive Bardet-Biedl syndrome; protein: BBS2 protein	Beales et al., Am. J. Hum. Genet. 68:606-616 (2001)
CNGB1, CNCG2, CNCG3L, GAR1, GARP	16q13	recessive RP; protein: rod cGMP-gated channel beta subunit	Bareil et al., Hum. Genet. 108:328-334 (2001)
CDH3, CDHP, PCAD	16q22.1	recessive MD, juvenile with hypotrichosis; protein: cadherin 3, type 1, placental	Indelman et al., J. Invest. Dermatol. 119:1210-1213 (2002)
FHSD	16q23.2-q24.2	recessive foveal hypoplasia and anterior segment dysgenesis	Pal et al., J. Med. Genet. 41:772-777 (2004)
CACD	17p13	dominant central areolar choroidal dystrophy	Hughes et al., J. Med. Genet. 35:770-772 (1998)
PRPF8, PRPC8, RP13	17p13.3	dominant RP; protein: human homolog of yeast pre-mRNA splicing factor C8	Kojis et al., Am. J. Hum. Genet. 58:347-355 (1996)
AIPL1, LCA4	17p13.2	recessive Leber congenital amaurosis; dominant cone-rod dystrophy; protein: arylhydrocarbon-interacting receptor protein-like 1	Hanein et al., Hum. Mutat. 23:306-317 (2004)
GUCY2D, CORD6, LCA1,	17p13.1	recessive Leber congenital amaurosis; dominant cone-rod dystrophy; protein: retinal-	Hanein et al., Hum. Mutat. 23:306-317 (2004)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
RETGC, RETGC1		specific guanylate cyclase	
CORD5, RCD2	same as GUCY2D	dominant cone-rod dystrophy, progressive; recessive cone-rod dystrophy	Udar et al., Hum. Mut. 21:170-171 (2003)
CORD4	17q	cone-rod dystrophy	Klystra et al.,
UNC119, HRG4	17q11.2	dominant cone-rod dystrophy; protein: human homolog of <i>C. elegans</i> unc119 protein	Kobayashi et al., Invest. Ophthalmol. Vis. Sci. 41:3268-3277 (2000)
CA4, RP17	17q23.2	dominant RP; protein: carbonic anhydrase IV	Rebello et al., Proc. Natl. Acad. Sci. USA 101:6617-6622 (2004)
USH1G, SANS	17q24-q25	recessive Usher syndrome; protein: human homolog of mouse scaffold protein containing ankyrin repeats and SAM domain	Kikkawa et al., Hum. Mol. Genet. 12:453- 461 (2003)
RGS9	17q24.1	recessive delayed cone adaptation; protein: regulator of G-protein signalling 9	Nishiguchi et al., Nature 427:75-78 (2004)
PRCD	17q25.1	recessive RP; protein: progressive rod-cone degeneration protein	Zangerl et al., Genomics (2006)
FSCN2, RP30	17q25.3	dominant RP; dominant MD; protein: retinal fascin homolog 2, actin bundling protein	Wada et al., Arch. Ophthalmol. 121:1613- 1620 (2003)
OPA4	18q12.2-q12.3	dominant optic atrophy, Kjer type	Kerrison et al., Arch. Ophthalmol. 117:805- 810 (1999)
CORD1	18q21.1-q21.3	cone-rod dystrophy; de Grouchy syndrome	Manhant et al., Am. J. Hum. Genet. 57:A96 (1995)
R9AP	19q13.12	recessive delayed cone adaptation; protein: regulator of G-protein signalling 9-binding protein	Nishiguchi et al., Nature 427:75-78 (2004)
MCDR5	19q13.31- q13.32	dominant macular dystrophy	Yang et al., Science 314:992-993 (2006)
CRX, CORD2	19q13.32	dominant cone-rod dystrophy; recessive, dominant and <i>de novo</i> Leber congenital amaurosis; dominant RP; protein: cone-rod otx-like photoreceptor homeobox transcription factor	Hanein et al., Hum. Mutat. 23:306-317 (2004)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
OPA3, MGA3	19q13.32	recessive optic atrophy with ataxia and 3-methylglutaconic aciduria; protein: OPA3 protein	Anikster et al., Am. J. Hum. Genet. 69:1218-1224 (2001)
PRPF31, PRP31, RP11	19q13.42	dominant RP; protein: human homolog of yeast pre-mRNA splicing factor 31	Sullivan et al., Invest. Ophthalmol. Vis. Sci. 47:4579-4588 (2006)
JAG1, AGS	20p12.2	dominant Alagille syndrome; protein: Jagged protein 1	Li et al., Nat. Genet. 16:243-251 (1997)
MKKS, BBS6	20p12.2	recessive Bardet-Biedl syndrome; protein: McKusick-Kaufman syndrome protein	Beales et al., Am. J. Hum. Genet. 68:606-616 (2001)
PANK2, HARP, PKAN	20p13	recessive HARP (hypoprebetalipoproteinemia, acanthocytosis, RP, and pallidial degeneration); recessive Hallervorden-Spatz syndrome; protein: pantothenate kinase 2	Hartig et al., Ann. Neurol. 59:248-256 (2006)
USH1E	21q21	recessive Usher syndrome, type 1	Chäib et al., Hum. Mol. Genet. 6:27-31 (1997)
OPA5	22q12.1-q12.3	dominant optic atrophy	Rozet et al., Invest. Ophthalmol. Vis. Sci. 46:E-Abstract 2292 (2005)
TIMP3, SFD	22q12.3	dominant Sorsby's fundus dystrophy; protein: tissue inhibitor of metalloproteinases-3	Felbor et al., Am. J. Hum. Genet. 60:57-62 (1997)
RP23	Xp22	X-linked RP	Hardcastle et al., Invest. Ophthalmol. Vis. Sci. 41:2080-2086 (2000)
RS1, XLRS1	Xp22.13	retinoschisis; protein: retinoschisin	Grayson et al., Hum. Mol. Genet. 9:1873-1879 (2000)
(- - -)	Xp21-q21	RP with mental retardation	Aldred et al., Am. J. Hum. Genet. 55:916-922 (1994)
RP6	Xp21.3-p21.2	X-linked RP	Breuer et al., Invest. Ophthalmol. Vis. Sci. 41:S191 (2000)
DMD	Xp21.2-p21.1	Oregon eye disease (probably); protein: dystrophin	D'Souza et al., Hum. Mol. Genet. 5:837-842 (1995)
AIED, OA2	Xp11.4-q21	Åland island eye disease	Wutz et al., Eur. J.

Symbols; OMIM Numbers	Location	Diseases; Protein	References
			Hum. Genet. 10:449-456 (2002)
COD4	Xp11.4-q13.1	X-linked progressive cone-rod dystrophy	Jalkanen et al., J. Med. Genet. 40:418-423 (2003)
OPA2	Xp11.4-p11.2	X-linked optic atrophy	Assink et al., Am. J. Hum. Genet. 61:934-939 (1997)
NYX, CSNB1	Xp11.4	X-linked CSNB; protein: nyctalopin	Bech-Hansen et al., Nat. Genet. 26:319-323 (2000)
CSNB4	same as NYX	X-linked CSNB	Pusch et al., Nat. Genet. 26:324-327 (2000)
RPGR, RP3	Xp11.4	X-linked RP, recessive; X-linked RP, dominant; X-linked CSNB; X-linked cone dystrophy 1; X-linked atrophic MD, recessive; protein: retinitis pigmentosa GTPase regulator	Bader et al., Invest. Ophthalmol. Vis. Sci. 44:1458-1463 (2003)
COD1	same as RPGR	X-linked cone dystrophy 1	Demirci et al., Am. J. Hum. Genet. 70:1049-1053 (2002)
RP15	same as RPGR	X-linked RP, dominant	Mears et al., Am. J. Hum. Genet. 67:1000-1003 (2000)
PRD	Xp11.3-p11.23	retinal dysplasia, primary	Ravia et al., Hum. Mol. Genet. 8:1295-1297 (1993)
NDP, EVR2	Xp11.3	Norrie disease; familial exudative vitreoretinopathy; Coats disease; protein: Norrie disease protein	Black et al., Hum. Mol. Genet. 11:2021-2035 (1999)
CACNA1F, CSNB2, CSNBX2	Xp11.23	X-linked CSNB, incomplete; ÅIED-like disease; severe CSNB; protein: L-type voltage-gated calcium channel alpha-1 subunit	Nakamura et al., Arch. Ophthalmol. 121:1028-1033 (2003)
RP2	Xp11.23	X-linked RP; protein: novel XRP2 protein similar to human cofactor C	Hardcastle et al., Am. J. Hum. Genet. 64:1210-1215 (1999)
PGK1	Xq21.1	RP with myopathy; protein: phosphoglycerate kinase	Tonin et al., Neurol. 43:387-391 (1993)
CHM	Xq21.2	choroideremia; protein: geranylgeranyl transferase Rab	van den Hurk et al., Hum. Mutat. 9:110-117

Symbols; OMIM Numbers	Location	Diseases; Protein	References
		escort protein 1	(1997)
TIMM8A, DDP, DDP2, DFN1	Xq22.1	optic atrophy with deafness- dystonia syndrome; protein: inner mitochondrial membrane translocase 8 homolog A	Koehler et al., Proc. Natl. Acad. Sci USA 96:2141-1246 (1999)
RP24	Xq26-q27	X-linked RP	Gieser et al., Am. J. Hum. Genet. 63:1439- 1447 (1998)
COD2, XLPCD	Xq27	X-linked progressive cone dystrophy, 2	Bergen et al.,
RP34	Xq28-qter	X-linked RP	Melamud et al., J. Med. Genet. 43:e27 (2006)
OPN1LW, GCP, CBD	Xq28	deuteranopia and rare macular dystrophy in blue cone monochromacy with loss of locus control element; protein: green cone opsin	Ayyagari et al., Mol. Vis. 58:98-101 (1999)
OPN1MW, RCP, CBP	Xq28	protanopia and rare macular dystrophy in blue cone monochromacy with loss of locus control element; protein: red cone opsin	Ayyagari et al., Mol. Vis. 58:98-101 (1999)
KSS	mitochondrion	Kearns-Sayre syndrome including retinal pigmentary degeneration; protein: several mitochondrial proteins	al., Science 283:1482- 1488 (1999)
LHON, MTND1, MTND4, MTND6	mitochondrion	Leber hereditary optic neuropathy; protein: complex I, III or IV proteins	Brown et al., Am. J. Hum. Genet. 60:381- 387 (1997)
MTTL1, DMDF, TRNL1	mitochondrion	macular pattern dystrophy with type II diabetes and deafness; protein: leucine tRNA 1 (UUA/G), nt 3230-3304	Bonte et al., Retina 17:216-221 (1997)
MTATP6, ATP6, NARP	mitochondrion	RP with developmental and neurological abnormalities; Leigh syndrome; LHON; protein: complex V ATPase 6 subunit, nt 8527-9207	White et al., J. Inherit. Metab. Dis. 22:899-914 (1999)
MTTH, TRNH	mitochondrion	pigmentary retinopathy and sensorineural hearing loss; protein: histidine tRNA, nt 12138-12206	Crimi et al., Neurology 60:1200-1203 (2003)

Symbols; OMIM Numbers	Location	Diseases; Protein	References
MTTS2, TRNS2	mitochondrion	RP with progressive sensorineural hearing loss; protein: serine tRNA 2 (AGU/C), nt 12207-12265	Mansergh et al., Am. J. Hum. Genet. 64:971-985 (1999)

In an embodiment of the invention, suppression agents are siRNAs or shRNAs targeting human rhodopsin. Exemplary siRNAs and replacement rhodopsin sequences are provided in Table 6A.

Table 6A: Exemplary siRNA Sequences Targeting Human Rhodopsin and Replacement Rhodopsin Sequences

siRNA Target Site	SEQ ID NO	Replacement Site	SEQ ID NO
1. <u>TACGTCACCGTCCAGCACAAG</u>	1	TATGTGACGGTGCAACATAA	2
2. <u>CTCAACTACATCCTGCTCAAC</u>	3	CTGAATTATATTTTATTGAAT	4
3. <u>CAGCTCGTCTTCACCGTCAAG</u>	5	CAATTGGTGTTTACGGTGAAA	6
4. <u>ATCTATATCATGATGAACAAG</u>	7	ATTTACATTATGATGAATAAA	8
5. <u>GCCTACATGTTTCTGCTGATC</u>	9	GCTTATATGTTCTTATTAATT	10
6. <u>TACATGTTTCTGCTGATCGTG</u>	11	TATATGTTCTTATTAATTGTC	12
7. <u>CTGCGCACGCCTCTCAACTAC</u>	13	TTACGGACCCCCTTGAATTAT	14
8. <u>CGCACGCCTCTCAACTACATC</u>	15	CGGACCCCCTTGAATTATATT	16
9. <u>CTCAAGCCGGAGGTCAACAAC</u>	17	TTGAAACCCGAAGTGAATAAT	18
10. <u>CAGCTCGTCTTCACCGTCA</u>	19	CAATTGGTGTTTACGGTGA	20
11. <u>TACGCCAGCGTGGCATTCTAC</u>	21	TATGCTTCTGTCGCCTTTTAC	22
12. <u>CCAGCGTTCTTTGCCAAGA</u>	23	CCCGCCTTTTTCGCTAAAA	24
13. <u>GTCATCTATATCATGATGAAC</u>	25	GTGATTTACATTATGATGAAT	26

14. <u>A</u> ACTGCATGCTCACCACCATC	27	AATTGTATGTTGACGACGATT	28
15. <u>ACC</u> ATCTGCTGCGGCAAGA	29	ACGATTGTGTGTGGGAAAA	30
16. <u>GAC</u> GATGAGGCCTCTGCTA	31	GAGGACGAAGCTAGCGCCA	32
17. <u>CAC</u> CTCTCTGCATGGATACT	33	CACGAGCTTACACGGGTATT	34
siRNAs Targeting 5' UTR			
18. AGCTCAGGCCTTCGCAGCA	35		
19. CAGGCCTTCGCAGCATTCT	36		
siRNAs Targeting 3' UTR			
20. TCACTTTCTTCTCCTATAA	37		
21. TAGTTAATGTTGTGAATAA	38		
22. GCTCCTATGTTGGTATTAA	39		
23. AGTCACATAGGCTCCTTAA	40		
24. GATTCTTGCTTTCTGGAAA	41		
25. ACAGTAGGTGCTTAATAAA	42		
26. GAACATATCTATCCTCTCA	43		
27. CTGTACAGATTCTAGTTAA	44		
28. TGTGAATAACATCAATTAA	45		
29. CAATTAATGTAACTAGTTA	46		
30. TGATTATCACCTCCTGATA	47		
31. GCAGTCATCAGACCTGAAA	48		
32. TGTCATCCTTACTCGAAGA	49		

33. GAATTAAGCTGCCTCAGTA	50		
34. GCCAGAAGCTCTAGCTTTA	51		
35. AGCTCTGCCTGGAGACTAA	52		
siRNAs Targeting an Intron			
36. GATCTTATTTGGAGCAATA	53		
37. TGGCTGTGATCCAGGAATA	54		
38. GATGCATTCTTCTGCTAAA	55		
39. GCAATATGCGCTTGTCTAA	56		
40. TTGTCTAATTTACAGCAA	57		
41. TGTTTGTTCATTCAATAA	58		
42. CCAGAGCGCTAAGCAAATA	59		
43. GTCTTGCATTTAACAGGAA	60		
44. GGCTGTGATCCAGGAATAT	61		
45. TGCAGGAGGAGACGCTAGA	62		
46. CTITCACTGTTAGGAATGT	63		
47. TTTGGTTGATTAACTATAT	64		
48. TTA ACTATATGGCCACTCT	65		
49. AGATGTTCTGAATTCCATCA	66		
siRNAs Targeting a Polymorphism			
50. TCTTCACCGTCAAGGAGGTAT	67	TGTTTACGGTGAAAGAAGTAC	68

siRNA sequences 1-17 target the human rhodopsin coding sequence. siRNA sequences 18 and 19 target the human rhodopsin 5'UTR. siRNA sequences 20-35

target the human rhodopsin 3'UTR. siRNA sequences 36-49 target human rhodopsin intronic sequence. The sequence of the sense strand of the siRNA is given. Notably, siRNAs may also target a combination of these. For example, an siRNA target site may be in the 5'UTR and exon 1. Or an siRNA target site may be in the coding region and an intron. Or an siRNA target site may be in an exon and the 3'UTR. siRNA sequence 50 is an example of an siRNA that has a target site that spans Exon 3/intron 3 of the human rhodopsin gene. The site contains a known polymorphism in intron 3. If this site was used as an siRNA target, the replacement gene would have the wildtype base at the polymorphic site but degeneracy of the genetic code could be used to change other bases at the replacement site. The siRNA(s) may comprise all or part of the sequence provided. The sequences of replacement human rhodopsin nucleic acids over the target for siRNA-mediated suppression are provided for siRNA sequences 1-17. Replacement nucleic acids include at least one altered nucleotide(s) at degenerate position(s) over the siRNA target site (highlighted in bold print). Thus, replacement sequences here provide one of multiple replacement options. Some replacement constructs contain nucleotide changes in the coding sequence. These replacement constructs while altered in nucleotide sequence encode the same amino acids as the wild type rhodopsin protein. Other replacement constructs are altered at either silent or non-silent polymorphic sites. These replacement constructs encode wild type protein, with wild type function. For siRNAs targeting the UTRs or intronic sequence, no replacement constructs have been suggested because the number of base changes within the site is not limited to degenerate positions (as is the case for sequence coding for amino acids).

It is notable that suppression of a given gene such as rhodopsin may be evaluated in a variety of animal species. The siRNA sequences provided in Table 6B represent examples of RNAi sequences that are homologous between porcine and human rhodopsin. In some transgenic animal models the presence of the human transgene enables direct evaluation of sequences that target the human gene in that animal model. In other instances suppressor sequences may be chosen to maximise the homology between the human gene (for example, rhodopsin) and the endogenous gene in the animal under evaluation.

Table 6B: Exemplary siRNA Sequences Targeting Homologous Sequences Between Human and Porcine Rhodopsin

siRNA	Sequence	SEQ ID NO:	Position in NM_000539.2	Suppression levels in HeLa Cells
P1	ACCTCTCTGCATGGATA GT-TT	414	384-403	69%
P2	CATGTTCGTGGTCCACTT C-TT	415	713-732	81%

siRNA can be expressed in miR vectors using polymerase II promoters. For this purpose pcDNA6.2-GW/EmGFP-miR from Invitrogen is used where the cloned miR-155 gene is recombined in order to express the choice of siRNA. The antisense strand of the siRNA is kept intact followed by a modified terminal loop and the sense strand, which is modified by introducing a deletion of 2 central nucleotides in order to form an internal loop. See Catalogue no K4936-00, Block-IT, POLII, miR RNAi expression vector kits catalogue, Invitrogen, page 7 for figure showing the native miR-155 sequence and the converted sequence of siRNA-lacZ in the form of miR-lacZ.

Exemplary miRNA sequences targeting human rhodopsin:

CC miRNA oligos:

Top strand: 5' -

TGCTGCTTCTTGCTGGACGGTGACGTTTTGGCCACTGACTGACGTCACC
GTAGCACAAGAAG - 3' (SEQ ID NO: 416)

Bottom strand: 5' -

CCTGCTTCTTGCTACGGTGACGTCAGTCAGTGGCCAAAACGTCACCGTC
CAGCACAAGAAGC - 3' (SEQ ID NO: 417)

Q1 miRNA oligos:

Top strand: 5' -

TGCTGGTAGTAGTCGATTCCACACGAGTTTTGGCCACTGACTGACTCGTGT
GGTCGACTACTAC - 3' (SEQ ID NO: 418)

Bottom strand: 5' -

CCTGGTAGTAGTCGACCACACGAGTCAGTCAGTGGCCAAAACGTCGTGTGG
AATCGACTACTACC - 3' (SEQ ID NO: 419)

BB miRNA oligos:

Top strand: 5' –

TGCTGGTAGAGCGTGAGGAAGTTGATGTTTTGGCCACTGACTGACATCAA
CTTTCACGCTCTAC – 3' (SEQ ID NO: 420)

Bottom strand: 5' –

CCTGGTAGAGCGTGAAAGTTGATGTCAGTCAGTGGCCAAAACATCAACTT
CCTCACGCTCTACC – 3' (SEQ ID NO: 421)

In an embodiment of the invention, suppression agents and replacement genes are expressed in photoreceptor cells to alleviate disease pathology. In a further embodiment, replacement nucleic acids encode a gene which when mutated may cause retinal degeneration other than retinitis pigmentosa, for example, Stargarts Syndrome, glaucoma, cod-rod dystrophy, corneal dystrophy or Age-related Macular Degeneration (AMD) (Table 5).

In another aspect, the invention provides cells expressing a suppression effector such as a dsRNA, either transiently or stably, for experimental or therapeutic use. In an embodiment, the cells express an siRNA that targets rhodopsin. In another embodiment, the cells express a replacement nucleic acid expressing rhodopsin that is not targeted by the siRNA. In another embodiment, the cells comprise a vector encoding at least one or more siRNAs. In another embodiment, the cells comprise a vector encoding a replacement nucleic acid. In an additional embodiment, the cells comprise one or more vectors encoding siRNA(s) and replacement nucleic acid(s).

In another aspect, the invention provides transgenic animals and their experimental or therapeutic use. In an embodiment, the transgenic animal is a model for Retinitis Pigmentosa, for example, an animal with a mutation observed in humans such as the Pro23His and or Pro347ser mutations. In another embodiment, the transgenic animal expresses a dsRNA that targets human rhodopsin. In another embodiment, the transgenic animal expresses a replacement nucleic acid transgene that has been altered at one or more wobble position(s) such that it escapes suppression.

Suppression agents and replacement nucleic acids of the invention can be administered to cells, tissues, plants and/or animals, either separately or together. In yet another aspect administration of suppression agent and/or replacement nucleic

acid may be systemic or local. In yet another aspect, administration of suppression agent and replacement nucleic acid may be used in conjunction with chemical and/or physical agents to aid administration. In another aspect, the invention provides methods for suppressing rhodopsin expression in an animal by intraocular (e.g., subretinal or intravitreal) injection of a suppression agent into the animal. In another aspect intraocular administration (e.g., subretinal injection, intravitreal) is used to administer a suppression agent and/or replacement nucleic acid to an animal. In another embodiment, iontophoresis or electroporation is used to administer suppression agents and/or replacement nucleic acids. In another embodiment, suppression agents and/or replacement nucleic acids are administered using nanotechnology (Kawasaki and Player *Nanomedicine* 1(2):101-9, 2005; Silva *Surg. Neurol.* 67(2):113-6, 2007; Andrieu-Solar et al., *Mol. Vis.* 12:1334-47, 2006) or bacteria (Daudel et al., *Expert Rev. Vaccines* 6(1):97-110, 2007).

Suppression agents and replacement nucleic acids may be optimally combined with conserved regions A-I and/or transcription factor binding sites identified within conserved regions A-I and/or with enhancer elements and/or other regulatory elements (see Tables 1 and 2 above and Tables 9-12 below).

In one aspect of the invention, there is provided a vector for expression of a suppression agent for a disease causing gene and/or a replacement nucleic acid that is not recognized by the suppression agent, wherein the vector comprises at least one of the conserved regions selected from: conserved region B from the rhodopsin gene represented by SEQ ID NO: 93, or a variant or equivalent thereof; conserved region C from the rhodopsin gene represented by SEQ ID NO: 94, or a variant or equivalent thereof; conserved region F and G from the rhodopsin gene represented by SEQ ID NO: 97 or a variant or equivalent thereof; and conserved region A from the rhodopsin gene represented by SEQ ID NO: 92, or a variant or equivalent thereof. In a particular embodiment, the vector comprises at least one of the conserved regions selected from: conserved region B from the rhodopsin gene represented by SEQ ID NO: 93; conserved region C from the rhodopsin gene represented by SEQ ID NO: 94; conserved region F and G from the rhodopsin gene represented by SEQ ID NO: 97; and conserved region A from the rhodopsin gene represented by SEQ ID NO: 92.

In one embodiment of the invention the use of suppression and replacement constructs in combination with one or more factors to facilitate cell survival, cell

viability and / or cell functioning is contemplated. In relation to neurons, a range of neurotrophic and / or neuroprotective factors may be used *inter alia* brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurturin, ciliary derived neurotrophic factor (CNTF), nerve growth factor (NGF), fibroblast growth factors (FGF), insulin-like growth factors (IGF), pigment epithelium-derived factor (PEDG), hepatocyte growth factor (HGF), thyrotrophin releasing hormone (TRH) and rod derived cone viability factor (RDCVF) amongst others. There is substantial evidence in the literature that such factors may increase cell viability and / or cell survival for a range of cell types. For example, these factors have been shown to provide beneficial effects to a wide range of neuronal cell types including, for example, photoreceptors, when delivered either in protein or DNA forms (Buch et al., Mol. Ther., 2006; 14(5):700-709). The use of GDNF to augment gene-based therapies for recessive disease has been demonstrated in mice (Buch et al., Mol. Ther., 2006; 14(5):700-709). Genes encoding neurotrophic / neuroprotective factors may be expressed from general promoters such as the CBA promoter (Buch et al., Mol. Ther., 2006; 14(5):700-709) or from tissue specific promoters. Sequences to optimise expression of neurotrophic / neuroprotective factors such as those sequences identified in Tables 1, 2, 9-13 may be included in constructs.

Sequences of a number of exemplary neurotrophic factors are provided in Figure 17. DNA encoding one or more neurotrophic and / or neuroprotective factors may be utilised in conjunction with suppression and replacement. Figure 18 provides examples of constructs incorporating suppression and replacement sequences together with sequence encoding a factor promoting cell viability and / or cell functioning such as GDNF, neurturin, CNTF and / or RDCVF amongst others as described above. Well established art known methods involving DNA restriction digestion, DNA ligation into plasmids, bacterial transformation, characterization of transfected bacterial colonies, plasmid purification and DNA sequencing may be used to clone suppression and replacement and neuroprotection / neurotrophic sequences into DNA-based vectors. Examples of the design of such constructs are provided in Figure 18

Constructs incorporating suppression and replacement and neurotrophic / neuroprotective factor(s) may be delivered using viral and / or non-viral vectors using art known methods (Andrieu-Soler et al., Mil. Vis., 2006; 12:1334-47). Naked DNA, lipids, polymers, nanoparticles, electrotransfer amongst other methods have been used

to achieve gene / nucleotide delivery in cells and animals. For example, lentiviral vectors and / or adenoassociated viral (AAV) vectors may be used to deliver constructs incorporating the 3 components defined above (suppression, replacement and neurotrophism / neuroprotection). 3-component constructs in some instances may require vectors that have significant capacity in terms of size of DNA inserts. Many viral and non-viral vectors have been characterised that can facilitate large DNA fragments including *inter alia* lentiviral vectors and some of adenoassociated viral serotypes. For example, AAV serotype 2 capsid 5 vectors (AAV2/5) have been shown to accommodate 8-9 kilobases of DNA (Alberto Aurichio; British Society of Gene Therapy, 2008). One or more components (suppression, replacement, neurotrophism/neuroprotection) may, for example, in the case of AAV be cloned between the AAV ITRS and or one or more components may be cloned into the backbone of the plasmid used to generate AAV. Figure 18 provides key elements of the construct design (B). Utilisation of backbone plasmid sequences to carry components of a multi-component construct can be used to optimise the population of AAV vectors generated using that plasmid. Moreover, in relation to eye disease, it is notable that there is significant evidence that AAV2/5 transduces photoreceptor efficiently. Generation of AAV vectors carrying suppression and replacement sequences in conjunction with sequences encoding neurotrophic and / or neuroprotective agents is contemplated. While AAV may be of value as a vector to deliver 3-component constructs for some target tissues, a range of additional viral and non-viral vectors are available for this purpose, such as those described above, and vectors that are well known in the art.

While utilisation of a single vector to deliver 3-component constructs involving suppression and replacement and a neurotrophic / neuroprotective sequence to a cell, a tissue and or an animal is contemplated, the use of multiple vectors in combination to deliver all 3 components is also contemplated. The multivalent approach involving suppression, replacement and neuroprotection may involve the use of 1 or more vectors for delivery. In addition, the 3 components may be delivered using a combination of a vector or vectors incorporating DNA sequences together with RNA and or dsRNA and or protein. In the current invention, delivery of protein, of RNA encoding protein and / or of DNA encoding protein or a combination thereof

to achieve delivery of all 3 components, suppression, replacement and neuroprotection, is contemplated.

In another embodiment of the invention the size of the backbone of the AAV plasmid vector is either increased or decreased so as to increase expression from the virus. For example, it has been described in the art that increasing the AAV virus backbone in size such that it is larger than the insert cloned within ITR1 and ITR2 favours AAV packaging of the insert over packaging of the backbone, thereby increasing expression of DNA cloned within the ITR regions (Bennet et al., Reversal of visual defects in animal models of LCA within weeks of treatment with an optimized AAV. *Molecular Therapy* Vol. 15, supplement 1, s286).

In a further embodiment of the invention the size of the backbone is increased with a gene which is therapeutically beneficial driven by a promoter. In this embodiment a portion of packaged AAV consists of the backbone and hence a portion of AAV particles will express the gene encoded within the backbone. In one embodiment the therapeutically beneficial gene cloned in the backbone is a neurotrophic factor such as GDNF, Neurturin or others.

While the invention can be used for dominant and or polygenic disorders, it may also be practised for recessive disorders. For example, the art describes that when treating the recessive disorder phenylketonuria (PKU) with replacement genes, endogenous protein expressed from mutant genes interfered with protein from replacement genes (Described in a thesis submitted to the University of Florida in partial fulfilment of the requirements for the degree of Doctor of Philosophy, by Catherine Elisabeth Charron, August 2005 and entitled "Gene therapy for phenylketonuria: dominant-negative interference in a recessive disease"). Thus, suppression and replacement constructs may be targeted to recessive disorders which like PKU require suppression and replacement.

Suppression and replacement technology provides a strategy that may be applicable to a wide range of genetic disorders including disorders characterized by either a recessive, dominant, polygenic, multifactorial or a dominant negative pathology. In a further embodiment of the invention conserved regions identified in the promoter region of mammalian rhodopsin genes and/or enhancer elements and/or other regulatory elements and/or epigenetic elements such as listed in Table 5 may be combined with suppressors targeting genes with mutations other than rhodopsin and

providing replacement genes other than rhodopsin. Osteogenesis imperfecta, epidermolysis bullosa, autosomal dominant early onset Alzheimer's disease, autosomal dominant polycystic kidney disease, Rett syndrome, familial platelet disorder, dominant negative diabetes insipidus, autosomal dominant Stargardt like macular dystrophy, nemaline myopathy, familial pulmonary arterial hypertension, APC and p53 related cancers and several other disorders (OMIM) may potentially benefit from a suppression and replacement therapeutic approach. Triplet repeat disorders, 14 of which have been characterised to date, including Huntington's disease, spinocerebellar ataxia and myotonic dystrophy may benefit from a suppression and replacement approach. For each disorder, promoters of the endogenous gene or constitutive promoters or promotes from other genes, or inducible promoters may be used to express the suppression agent or replacement nucleic acid.

In another embodiment of the invention, promoter and/or enhancer elements and/or other regulatory elements and/or epigenetic elements may be combined with other promoters than rhodopsin in combination with suppression and/or replacement elements. For example, but not exclusively, promoter and enhancer elements can be combined with the COL1A1 and or COL1A2 and or COL7A1 and or Keratin 5 and or Keratin 14 and or peripherin and/or IMPDH1 promoters and/or genes. Depending upon the tissue in which the suppression agent and/or replacement nucleic acid is administered or active *in vivo*, tissue specific regulatory elements are used to enhance expression of the suppression agent and/or replacement nucleic acid.

The suppressors and/or replacement nucleic acids of the invention can be targeted to suppress and replace a gene where mutations in the gene can give rise, predispose or work in combination with other genetic factors and/or environmental factors to cause disease pathology. For example, in the case of dominant retinopathies the rhodopsin gene may be suppressed and replaced. For example, siRNAs targeting RHO- (NM_000539.2) can be designed and provided commercially. Likewise control siRNAs, for example, targeting EGFP (U57608) and or other reporter genes and or other non-targeting siRNAs can be designed and synthesised. siRNAs are chosen to target sequences which differed by at least one and preferable many more nucleotides from any known gene in mouse and human databases (<http://www.ncbi.nlm.nih.gov/blast>, BLASTN2.2.6, Altschul et al., Nuc Acids Res. 25: (17:3389-402, 1997). siRNAs can be cloned downstream of, for example,

polymerase III promoters such as the H1 or U6 promoters to generate short hairpin RNAs (shRNAs; Brummelkamp et al., Science 296: (5567:505-3, 2001). Alternatively, polymerase II promoters which drive expression in many or all cell or tissue types including the CMV promoter, ubiquitin promoter and or the β -actin promoter, for example, may be used to express shRNAs. Likewise tissue specific promoters such as the rhodopsin promoter, peripherin promoter and or enolase promoter amongst others may be used to express shRNAs. shRNA sequences can be cloned into vectors with a reporter gene to facilitate monitoring expression from vectors, for example, shRNAs can be cloned in pEGFP-1 amongst other plasmids (BD Biosciences, Clontech, Palo Alto, CA). Suppressors can be delivered to cells, tissues and or animals with or without replacement nucleic acids.

Replacement nucleic acids with nucleotide sequence changes over the target site for siRNA-mediated suppression, for example, at degenerative nucleotides can be generated by primer directed mutagenesis and cloned into vectors such as pcDNA3.1- (Invitrogen). Replacement nucleic acids may also be modified at the UTRs and or at polymorphic sites within the target gene. Ubiquitous promoters such as the CMV promoter and or the ubiquitin promoter and or the β -actin promoter amongst others can be used to drive expression of replacement nucleic acids. Alternatively, tissue specific promoters such as the rhodopsin promoter, peripherin promoter, Col1A1 promoter, Col1A2 promoter, Col1A7 promoter, Keratin promoters and/or the enolase promoter amongst others and/or inducible promoters such as a tetracycline responsive promoter can be used to drive expression of replacement nucleic acids. Replacement human rhodopsin nucleic acids which have been altered in nucleotide sequence at degenerate positions over siRNA target sites for example, replacement nucleic acids for siRNA sequences 1-17 are provided in Table 5. Replacement nucleic acids can be delivered to cells, tissues and or animals with or without suppressor agents.

Suppression and replacement in cells and tissues

Promoter driven replacement nucleic acids such as rhodopsin nucleic acids and siRNAs and/or shRNAs targeting rhodopsin can be co-transfected into cells, for example, HeLa and or Cos-7 cells amongst other cell types using art known methods. For example, 24 hours post-transfection of suppressor agents and/ or replacement nucleic acids, RNA and cytoplasmic protein can be isolated from cells using well established methodologies. Additionally, suppression and replacement can be

evaluated in tissues. In the case of retinal genes, for example, organotypic retinal explant cultures from mouse or rat, for example, can be prepared and maintained using art known methods and suppressor agents and or replacement nucleic acids can be delivered to organotypic cultures. For example, electroporation can be used to deliver siRNA and/ or shRNA constructs and/ or shRNA constructs and replacement nucleic acids to retinal explants as described in Palfi et al., Hum. Mutat. 27(3):260-8, 2006. Subsequent to electroporation of retinal explants, retinas can be treated with trypsin to expedite dissociation of cells. Retinal cell sub-populations within the dissociated cell population which have a particular feature, for example, that express a reporter gene such as EGFP can be identified. One method of identification that can be invoked is FACS (Palfi et al., Hum. Mutat. 27(3):260-8, 2006). Levels of suppression and replacement of a target gene can be evaluated in FACS isolated cell populations. For example, suppression and/or suppression and replacement can be evaluated in electroporated EGFP positive cells from retinal explants.

Evaluation of suppression and replacement using RNA assays

Suppression and replacement can be evaluated in cells, tissues and/or animals using RNA assays including real time RT-PCR, northern blotting, RNA *in situ* hybridisation and or RNase protection assays. RNA expression levels of suppressors and/or of endogenous genes and or replacement nucleic acids can be assessed by real time RT-PCR using, for example, a 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) and using, for example, a QuantiTect SYBR Green RT-PCR kit (Qiagen Ltd). RT-PCR assays are undertaken using levels of expression of housekeeping controls such as β -actin or GAPDH, for example, for comparative purposes. Levels of RNA expression can be evaluated using sets of primers targeting the nucleic acids of interest including suppressors, target genes and/or replacements, for example, the following primers can be used for the evaluation of levels of expression of human rhodopsin, β -actin and GAPDH.

Table 7: PCR Primers for measuring rhodopsin, β -actin, and GAPDH

Primer	Sequence	SEQ ID NO
RHO forward primer	5' CTTTCCTGATCTGCTGGGTG 3'	69
RHO reverse primer	5' GGCAAAGAACGCTGGGATG 3'	70

β -actin forward primer	5' TCACCCACACTGTGCCCATCTACGA 3'	71
β -actin reverse primer	5' CAGCGGAACCGCTCATTGCCAATGG 3'	72
GAPDH forward primer:	5'-CAGCCTCAAGATCATCAGCA-3'	73
GAPDH reverse primer:	5'-CATGAGTCCTTCCACGATAC-3'	74

Expression of replacement constructs and/or shRNAs may be confirmed, for example, by Northern blotting. RNA may also be detected by in situ hybridisations using single stranded RNA probes that have been labelled with, for example, DIG. To evaluate levels of expression of suppression agents and/or replacement nucleic acids and/or endogenous target genes, RNase protection assays can be performed using art known methods, such as that described in the Ambion mirVanaTM Probe and Marker kit manual (catalogue number 1554) and the Ambion RPAIIITM Ribonuclease protection assay kit manual (catalogue number 1414). For example, RNA probes approximately 15-25 nucleotides in length specific for transcripts from, for example, an endogenous target gene and/or a suppressor and/or a replacement nucleic acid can be synthesized. For example, RNA probes targeting mouse rhodopsin and/or human rhodopsin and/or suppression agents targeting rhodopsin and/or rhodopsin replacement nucleic acids can be synthesized using companies such as Sigma-Prologo or Ambion. RNA probes and size standards can be labelled to aid visualization after separation of samples on denaturing polyacrylamide gels. For example, RNA probes and DecadeTM size marker (Ambion Inc) can be 5' end-labelled with P³²- γ ATP (GE Healthcare) using the mirVanaTM probe and marker kit according to the manufacturer's protocol (Ambion Inc.). RNase protection assays can be performed using art known methods, for example, using the RPA IIITM Ribonuclease Protection Assay Kit and the manufacturer's protocol (Ambion Inc.).

Expression of suppressors and/or replacement nucleic acids and/or endogenous genes can be undertaken and determined in cells, in tissues and or in animals using, for example, the assays and associated methodologies provided above.

Evaluation of suppression and replacement using protein assays

Suppression and replacement can be evaluated in cells, tissues and/ or animals using protein assays including ELISA, western blotting and immunocytochemistry assays. ELISAs can be undertaken to evaluate levels of suppression by assessing levels of expression of a target endogenous gene and/or can be used to evaluate levels expression of replacement nucleic acids – such proteins assays are well know in the art and methods are provided in, for example, Palfi et al., Hum. Mutat. 27(3):260-8, 2006. For example, in the case of retinal genes such as the rhodopsin gene, ELISA is undertaken using a rhodopsin primary antibody which is typically used in a diluted form, for example, using a 1/10-1/10000 dilution (but possibly outside of this range) of an antibody for the target protein. In addition, Western Blotting may be undertaken to determine relative quantities of a specific protein, for example rhodopsin. Briefly, protein samples are separated using SDS-PAGE and transferred to a membrane. The membrane is incubated with generic protein (for example milk proteins) to bind to “sticky” places on the membrane. A primary antibody is added to a solution which is able to bind to its specific protein and a secondary antibody-enzyme conjugate, which recognizes the primary antibody is added to find locations where the primary antibody bound.

In addition to the protein assays referred to above, assays using antibodies in conjunction with microscopy can be used to evaluate protein levels. For example, in the case of rhodopsin immunocytochemistry (for example, using a 1/10-1:1000 dilution of a primary rhodopsin antibody) and fluorescent microscopy can be carried out as has been documented in Kiang et al., 2005 Mol. Ther. 12(3):555-61, 2005. Immunocytochemistry can be undertaken on cells and/or tissues. In the case of the retina, various modes of sectioning can be implemented to evaluate retinal sections. For example, frozen sections, agar embedded sections and/or resin embedded sections can be used. To obtain thin sections, for example of the retina, epon embedding and semi-thin sectioning can be performed using art known methods such as those provided in McNally et al., Hum. Mol. Genet. 11(9):1005-16, 2002. Immunocytochemistry may be used to evaluate suppression of a target gene and or expression of replacement nucleic acids. Additionally, histological analyses can be used to evaluate the histological effect(s) associated with the administration of suppressors and or replacement nucelic acids. In animal models of retinal

degenerations such as the rho-/-, rds, rhodopsin Pro23His, rhodopsin Pro2347Ser mice and others there is a degeneration of the photoreceptor cell layer over time. Histological analyses can be used to evaluate if this degeneration has been modulated subsequent to administration of suppression agents and/or replacement nucleic acids.

Delivery of suppression and replacement

Both non-viral and/or viral vectors can be used in the invention to deliver the suppression agents and/or replacement nucleic acids. For example, in the case of retina, recombinant adenoassociated virus (AAV) and more specifically AAV2/5 has previously been found to elicit efficient transduction of photoreceptor cells. Other AAV serotypes may also be used to deliver to retina, for example, AAV2/2 elicits efficient delivery to the retinal pigment epithelium (RPE) as does AAV4. AAV vectors can be generated using protocols with and without helper virus. For example, a helper virus free protocol using a triple transfection approach is well documented (Xiao et al., J. Virol. 72(3):2224-32, 1998). Expression cassettes carrying suppression and/or replacement elements can be cloned into plasmids such as pAAV-MCS provided by Stratagene Inc. Suppressors and/or replacement nucleic acids are cloned between the inverted terminal repeats of AAV2 and transfected into 293 cells (Stratagene; ATACC cat no CRL-1573) with two other plasmids, hence the term triple transfection. For example, the pRep2/Cap5 plasmid (Hildinger et al., J. Virol. 75(13):6199-203, 2001) together with the pHelper plasmid (Stratagene), at, for example, a ratio of 1:1:2, can be used to generate AAV2/5 vectors. Virus can be generated using a variety of art known procedures including the method outlined below. For example, to generate virus fifty 150mm plates of confluent HEK293 cells were transfected (50 µg DNA/plate) with polyethylenimine (Reed et al., J. Virol. Methods 138(1-2):85-98, 2006). 48hrs post-transfection crude viral lysates were cleared (Auricchio et al., 2001) and purified by CsCl₂ gradient centrifugation (Zolotukhin et al., Gene Ther. 6(6):973-85, 1999). The AAV containing fraction was dialyzed against PBS. Genomic titres, viral particles (vp/ml), were determined by quantitative real-time PCR using art known methods (Rohr et al., J. Virol. Methods 106(1):81-8, 2002). AAVs can be generated that contain, for example, either targeting shRNAs or control shRNAs and/or replacement nucleic acids such as rhodopsin and/or reporter nucleic acids such as EGFP and/or stuffer sequences and/or sequences aiding expression of suppression agents and/or replacement nucleic acids

such as promoter and/or enhancer sequences and/or other regulatory sequences and/or epigenetic elements.

Administration of suppression and replacement vectors

Animal models can be used to mirror human disorders. For example, animal models of human retinopathies or that express a human retinal gene have been generated, for example, rho^{-/-} mice (Humphries et al., Nat. Genet. 15(2):216-9, 1997), NHR +/- mice (Olsson et al., Neuron 9(5):815-30, 1992), Pro23His mice (Olsson et al., Neuron 9(5):815-30, 1992), Pro347Ser mice (Li et al., Proc. Natl. Acad. Sci. U.S.A. 95(20):11933-8, 1998) and RHO-M mice (see below). Mice typically are maintained under specific pathogen free (SPF) housing conditions and in a controlled light environment. The suppression agents and/or replacement nucleic acids of the invention can be administered to animals either locally and/ or systemically. Local administration can include direct injection to the target tissues and/or in the proximity of the target tissue as has been described in detail in the art in, for example, Xia et al. (ACS Chem. Biol. 1(3):176-83, 2004) delivered AAV vectors with shRNAs to brain to treat spinocerebellar ataxia. In the case of the retina, subretinal injection can be used to administer suppression agents and/or replacement nucleic acids according to the following procedure. For example, mice can be anaesthetised by intraperitoneal injection of Domitor and Ketalar (10 and 50 µg/g of body weight respectively). The pupils are dilated with phenylephrine and under local analgesia (amethocaine) a small puncture is made in the sclera. A micro-needle attached to a 10 µl syringe (Hamilton Company Europe) is inserted through the puncture to the subretinal space and 1-3 µl of vector is administered. For example, in the case of AAV 1-3µl of a 10¹²⁻¹⁴ vp/ml AAV vector preparation in PBS is administered. A reverse anaesthetic (antisedan, 50 µg/g of body weight) can be applied by intraperitoneal injection post-delivery. Body temperature during the procedure is sustained using a homeothermic heating device. In addition newborn mice can be prepared for subretinal injection according to Matsuda and Cepko (Proc. Natl. Acad. Sci. U.S.A. 101(1):16-22, 2004).

Assay for Function

To evaluate if suppression and/or replacement modulates the function of a target tissue and/ or cell type, one or more assays may be employed that are well described in the prior art. In the case of the retina, functional assays include but are

not limited to electrophysiology, such as pattern electroretinogram (ERG), full field ERG, and visual evoked potentials. In addition, visual field assessments, color vision assessments, and pupillometry may be performed. For example, electroretinography can be used to evaluate the response of the retina to light. This can be performed using, for example, the following procedure or an adapted procedure. Animals can be dark-adapted overnight and prepared for ERG under dim red light. Pupils are dilated with 1% cyclopentolate and 2.5% phenylephrine. Animals are anesthetized with ketamine and xylazine (16 and 1.6 $\mu\text{g}/10\text{g}$ body weight respectively) injected intraperitoneally. Standardized flashes of light are presented to the animal, for example a mouse, in a Ganzfeld bowl. ERG responses are recorded simultaneously from both eyes by means of contact lens electrodes (Medical Workshop, Netherlands) using 1% amethocaine as topical anesthesia. Reference and ground electrodes are positioned subcutaneously, approximately one mm from the temporal canthus and anterior to the tail respectively. Responses are analysed using a RetiScan RetiPort electrophysiology unit (Roland Consulting GmbH). The protocol is based on that approved by the International Clinical Standards Committee for human electroretinography. Rod-isolated responses are recorded using a dim white flash (-25 dB maximal intensity where maximal flash intensity was $3 \text{ candelas}/\text{m}^2/\text{s}$) presented in the dark-adapted state. Maximal combined rod-cone responses to the maximal intensity flash are then recorded. Following a 10 minute light adaptation to a background illumination of $30 \text{ candelas}/\text{m}^2$, cone-isolated responses are recorded to the maximal intensity flash presented initially as a single flash and subsequently as 10 Hz flickers. A-waves are measured from the baseline to the trough and b-waves from the baseline (in the case of rod-isolated responses) or from the a-wave to the trough.

The agents of the invention are administered in effective amounts. An effective amount is a dosage of the agent sufficient to provide a medically desirable result. An effective amount means that amount necessary to delay the onset of, inhibit the progression of or halt altogether the onset or progression of the particular condition or disease being treated. An effective amount may be an amount that reduces one or more signs or symptoms of the disease. When administered to a subject, effective amounts will depend, of course, on the particular condition being treated; the severity of the condition; individual patient parameters including age, physical condition, size and weight, concurrent treatment, frequency of treatment, and

the mode of administration. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

Actual dosage levels of active ingredients in the pharmaceutical compositions of the invention can be varied to obtain an amount of the agent(s) that is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level depends upon the activity of the particular agent, the route of administration, the severity of the condition being treated, the condition, and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the agent(s) at levels lower than required to achieve the desired therapeutic effort and to gradually increase the dosage until the desired effect is achieved.

The agents and pharmaceutical compositions of the invention can be administered to a subject by any suitable route. For example, the compositions can be administered orally, including sublingually, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically and transdermally (as by powders, ointments, or drops), buccally, or nasally. The term "parenteral" administration as used herein refers to modes of administration other than through the gastrointestinal tract, which include intravenous, intramuscular, intraperitoneal, intrasternal, intramammary, intraocular, retrobulbar, intrapulmonary, intrathecal, subcutaneous and intraarticular injection and infusion. Surgical implantation also is contemplated, including, for example, embedding a composition of the invention in the body such as, for example, in the brain, in the abdominal cavity, under the splenic capsule, brain, or in the cornea.

Agents of the present invention also can be administered in the form of liposomes. As is known in the art, liposomes generally are derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any nontoxic, physiologically acceptable, and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33, et seq.

Dosage forms for topical administration of an agent of this invention include powders, sprays, ointments, and inhalants as described herein. The agent is mixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives, buffers, or propellants which may be required. Ophthalmic formulations, eye ointments, powders, and solutions also are contemplated as being within the scope of this invention.

Pharmaceutical compositions of the invention for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions, or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water ethanol, polyols (such as, glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils (such, as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions also can contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It also may be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the agent, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This result can be accomplished by the use of a liquid suspension of crystalline or amorphous materials with poor water solubility. The rate of absorption of the agent then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug from is accomplished by dissolving or suspending the agent in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the agent in biodegradable polymers such a polylactide-polyglycolide. Depending upon the ratio of agent to polymer and the nature of the particular polymer employed, the

rate of agent release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations also are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The injectable formulations can be sterilized, for example, by filtration through a bacterial- or viral-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

The invention provides methods for oral administration of a pharmaceutical composition of the invention. Oral solid dosage forms are described generally in Remington's Pharmaceutical Sciences, 18th Ed., 1990 (Mack Publishing Co. Easton Pa. 18042) at Chapter 89. Solid dosage forms for oral administration include capsules, tablets, pills, powders, troches or lozenges, cachets, pellets, and granules. Also, liposomal or proteinoid encapsulation can be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Pat. No. 4,925,673). Liposomal encapsulation may include liposomes that are derivatized with various polymers (e.g., U.S. Pat. No. 5,013,556). In general, the formulation includes an agent of the invention and inert ingredients which protect against degradation in the stomach and which permit release of the biologically active material in the intestine.

In such solid dosage forms, the agent is mixed with, or chemically modified to include, a least one inert, pharmaceutically acceptable excipient or carrier. The excipient or carrier preferably permits (a) inhibition of proteolysis, and (b) uptake into the blood stream from the stomach or intestine. In a most preferred embodiment, the excipient or carrier increases uptake of the agent, overall stability of the agent and/or circulation time of the agent in the body. Excipients and carriers include, for example, sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, cellulose, modified dextrans, mannitol, and silicic acid, as well as inorganic salts such as calcium triphosphate, magnesium carbonate and sodium chloride, and commercially available diluents such as FAST-FLO[®], EMDEX[®], STA-RX 1500[®], EMCOMPRESS[®] and AVICEL[®], (b) binders such as, for example, methylcellulose ethylcellulose, hydroxypropylmethyl cellulose, carboxymethylcellulose, gums (e.g., alginates, acacia), gelatin, polyvinylpyrrolidone, and sucrose, (c) humectants, such as glycerol, (d) disintegrating agents, such as agar-

agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium carbonate, starch including the commercial disintegrant based on starch, EXPLOTAB[®], sodium starch glycolate, AMBERLITE[®], sodium carboxymethylcellulose, ultramylopectin, gelatin, orange peel, carboxymethyl cellulose, natural sponge, bentonite, insoluble cationic exchange resins, and powdered gums such as agar, karaya or tragacanth; (e) solution retarding agents such as a paraffin, (f) absorption accelerators, such as quaternary ammonium compounds and fatty acids including oleic acid, linoleic acid, and linolenic acid (g) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate, anionic detergent surfactants including sodium lauryl sulfate, dioctyl sodium sulfosuccinate, and dioctyl sodium sulfonate, cationic detergents, such as benzalkonium chloride or benzethonium chloride, nonionic detergents including laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65, and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose; (h) absorbents, such as kaolin and bentonite clay, (i) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils, waxes, CARBOWAX[®] 4000, CARBOWAX[®] 6000, magnesium lauryl sulfate, and mixtures thereof; (j) glidants that improve the flow properties of the drug during formulation and aid rearrangement during compression that include starch, talc, pyrogenic silica, and hydrated silicoaluminate. In the case of capsules, tablets, and pills, the dosage form also can comprise buffering agents.

Solid compositions of a similar type also can be employed as fillers in soft and hard-filled gelatin capsules, using such excipients as lactose or milk sugar, as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They optionally can contain opacifying agents and also can be of a composition that they release the active ingredient(s) only, or preferentially, in a part of the intestinal tract, optionally, in a delayed manner. Exemplary materials include polymers having pH sensitive solubility, such as the materials available as EUDRAGIT[®]. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds also can be in micro-encapsulated form, if appropriate,

with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms can contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol ethyl carbonate ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions also can include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, coloring, flavoring, and perfuming agents. Oral compositions can be formulated and further contain an edible product, such as a beverage.

Suspensions, in addition to the agent(s), can contain suspending agents such as, for example ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, and mixtures thereof.

Also contemplated herein is pulmonary delivery of the agent(s) of the invention. The agent(s) is delivered to the lungs of a mammal while inhaling, thereby promoting the traversal of the lung epithelial lining to the blood stream. See, Adjei et al., *Pharmaceutical Research* 7:565-569 (1990); Adjei et al., *International Journal of Pharmaceutics* 63:135-144 (1990) (leuprolide acetate); Braquet et al., *Journal of Cardiovascular Pharmacology* 13 (suppl.5): s.143-146 (1989)(endothelin-1); Hubbard et al., *Annals of Internal Medicine* 3:206-212 (1989)(α 1-antitrypsin); Smith et al., *J. Clin. Invest.* 84:1145-1146 (1989) (α 1-proteinase); Oswein et al., "Aerosolization of Proteins," *Proceedings of Symposium on Respiratory Drug Delivery II*, Keystone, Colorado, March, 1990 (recombinant human growth hormone); Debs et al., *The Journal of Immunology* 140:3482-3488 (1988) (interferon- γ and tumor necrosis factor α) and Platz et al., U.S. Pat. No. 5,284,656 (granulocyte colony stimulating factor).

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products,

including, but not limited to, nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially available devices suitable for the practice of the invention are the ULTRAVENT[®] nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, MO; the ACORN II[®] nebulizer, manufactured by Marquest Medical Products, Englewood, CO.; the VENTOL[®] metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, N.C.; and the SPINHALER[®] powder inhaler, manufactured by Fisons Corp., Bedford, MA.

All such devices require the use of formulations suitable for the dispensing of a agent(s) of the invention. Typically, each formulation is specific to the type of device employed and can involve the use of an appropriate propellant material, in addition to diluents, adjuvants, and/or carriers useful in therapy.

The composition is prepared in particulate form, preferably with an average particle size of less than 10 μm , and most preferably 0.5 to 5 μm , for most effective delivery to the distal lung.

Carriers include carbohydrates such as trehalose, mannitol, xylitol, sucrose, lactose, and sorbitol. Other ingredients for use in formulations may include lipids, such as DPPC, DOPE, DSPC and DOPC, natural or synthetic surfactants, polyethylene glycol (even apart from its use in derivatizing the inhibitor itself), dextrans, such as cyclodextran, bile salts, and other related enhancers, cellulose and cellulose derivatives, and amino acids.

Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, typically comprise a compound of the invention dissolved in water at a concentration of about 0.1 to 25 mg of biologically active protein per mL of solution. The formulation also can include a buffer and a simple sugar (e.g., for protein stabilization and regulation of osmotic pressure). The nebulizer formulation also can contain a surfactant to reduce or prevent surface-induced aggregation of the inhibitor composition caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered-dose inhaler device generally comprise a finely divided powder containing the agent suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this

purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid also can be useful as a surfactant.

Formulations for dispensing from a powder inhaler device comprise a finely divided dry powder containing the agent and also can include a bulking agent, such as lactose, sorbitol, sucrose, mannitol, trehalose, or xylitol, in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation.

Nasal delivery of the agent(s) and composition of the invention also is contemplated. Nasal delivery allows the passage of the agent or composition to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran. Delivery via transport across other mucous membranes also is contemplated.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the agent(s) of the invention with suitable nonirritating excipients or carriers, such as cocoa butter, polyethylene glycol, or suppository wax, which are solid at room temperature, but liquid at body temperature, and therefore melt in the rectum or vaginal cavity and release the active compound.

In order to facilitate delivery of the agent(s) across cell and/or nuclear membranes, compositions of relatively high hydrophobicity are preferred. The agent(s) can be modified in a manner which increases hydrophobicity, or the agent(s) can be encapsulated in hydrophobic carriers or solutions which result in increased hydrophobicity.

Practice of the invention will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

EXEMPLIFICATION

Example 1: siRNAs Targeting Human Rhodopsin and Rhodopsin Replacement Nucleic Acids

siRNAs targeting human rhodopsin were synthesized and evaluated for RNAi-mediated suppression (listed in Table 8). Suppression and replacement constructs

with suppressors targeting the human rhodopsin mRNA sequence and replacement rhodopsin genes that escape suppression by the suppressor due to subtle changes in the sequence were subsequently designed. These changes, while enabling replacement nucleic acids to escape suppression at least in part, did not change the protein product expressed from the replacement genes. Short hairpin RNAs (shRNAs) were used to demonstrate suppression *in vivo* (Figure 1). The sequence of the sense and antisense strands of the shRNAs is the same as the sequence used for the siRNAs. An intervening loop is included between the sense and antisense strands in the same manner as Brummelkamp et al., Science 296(5567):550-3, 2001. Notably, the number of nucleotides and the make up of the nucleotides in the intervening loop can vary. The construct(s) were delivered using an AAV2/5 recombinant virus. Non-targeting siRNA can be used as controls, for example, a non-targeting siRNA directed towards an EGFP reporter gene can be used – for example.

siRNAs were designed according to the method of Elbashir et al., Nature 411(6836):494-8, 2001, or by using the HiPerformance siRNA design algorithm (Qiagen Ltd. Crawley, UK). siRNA target sequences differed by at least 4 nucleotides from any non-rhodopsin sequences in mouse and human databases (<http://www.ncbi.nlm.nih.gov/blast>, BLAST2.2.6 (Altschul et al., Nucleic Acids Res. 25(17):3389-402, 1997). siBB, siQ1 and a non-targeting siRNA siNT (5' UUCUCCGAACGUGUCACGU 3'; SEQ ID NO:75) or EGFP (U57608), siEGFP (nt 256-277) were initially cloned downstream of the H1 promoter using BglII/BamHI and Hind III restriction sites to generate shRNAs and subsequently in pEGFP-1 (BD Biosciences, Clontech, Palo Alto, CA) using EcoRI and Hind III sites generating shBB-EGFP, shQ1-EGFP and shNT-EGFP (Figure 1A). The EGFP gene enabled viral transduction to be monitored. Six siRNAs sequences targeted the coding region of human rhodopsin. Replacement nucleic acids were cloned into pCDNA3.1-plasmid (Invitrogen, Karlsruhe, Germany). The CMV promoter was replaced with either the human ubiquitin C promoter (pUB6/V5-His, Invitrogen) or a 1.7 kb fragment of the mouse rho promoter (rhoP). Sequence alterations were introduced into replacement nucleic acids using primer directed PCR-based mutagenesis using art known methods. Replacement nucleic acids with sequence alterations over the target sites for siB, siBB, siC, siCC, siQ1 and siQ2 were termed rB, rBB, rC, rCC, rQ1, and rQ2. Altered nucleotides in the replacement rhodopsin sequences are at wobble

positions (highlighted in bold print). These replacement genes were designed to avoid suppression by the siRNAs yet encode wild type protein. Table 8 provides one replacement example for each siRNA target site; however, in each case there are several alternative possible replacement sequences because some amino acids have as many as six codons and others have four or three codons.

Table 8: siRNA Sequence and Replacement Rhodopsin Sequence

siRNA	Sequence	SEQ ID NO	Replacement rhodopsin sequence	SEQ ID NO	Position in NM_000539.2
siB	TCAACTTCCTCACGCTCTA	75	ATAAATTTTTTGACCCTGTA T	76	256-277
siBB	TCACCGTCCAGCACAAAGAA	77	CTGTATGTGACGGTGCAGCA C	78	254-274
siC	CGTGTGGAATCGACTACTA	79	AGCTGCGGTATAGATTATTA	80	270-292
siCC	CGCTCAAGCCGGAGGTCAA	81	ACCTTGAAACCCGAAGTGAA	82	274-294
siQ1	TCAACTTCCTCACGCTCTAC GT	83	CTGTATGTGACGGTGCAGCA C	84	650-670
siQ2	CTCTACGTCACCGTCCAGC ACAA	85	CTGTATGTGACGGTGCAGCA C	86	671-694

Suppression of RHO in HeLa Cells

RNAi-mediated suppression of *RHO* was initially evaluated in HeLa cells. siRNAs targeting *RHO* were co-transfected with a CMV promoter-driven wild type *RHO*. Transfections were carried out three times in quadruplicate using lipofectamine 2000 to aid transfections (Gibco-BRL). Real time RT-PCRs, performed on RNAs extracted from transfected cells 24 hours post-transfection, demonstrated up to 87% suppression ($p < 0.01$, Figure 2A) (see Table 7 for primer sequences). siRNAs siBB, siCC and siQ1 were selected for further analysis. Similar levels of rhodopsin protein suppression were quantified by ELISA (up to 88%, $p < 0.01$, Figure 2A) and demonstrated by immunocytochemistry 24 hours post-transfection (Figure 2B). Subsequently, replacement *RHO* constructs, rBB, rCC and rQ1, were generated incorporating nucleotide changes at degenerate positions over the target sites for siRNAs, siBB, siQ1 and siCC as described above and shown in Table 8. Transfections were performed three times in quadruplicate in HeLa cells according to

art known methods as described above. Results indicated that replacement *RHO* constructs were not suppressed by corresponding siRNAs, for example, rBB by siBB (Figure 3). However, significant levels of suppression were obtained with other non-corresponding siRNAs, for example siQ1 suppressed rBB and rCC (Figure 3).

Long Term Suppression of RHO in Retinal Explants

To provide long term *RHO* suppression, siBB and siQ1 were cloned as shRNAs into an EGFP expressing vector (shBB-EGFP and shQ1-EGFP, Figure 1A). Plasmids were electroporated into retinal explants from newborn NHR+/- rho-/- mice using the methods described in Matsuda and Cepko (2004) Proc. Natl. Acad. Sci. USA 101: 16-22. NHR+/- mice express a wild type human *RHO* gene and display a wild type phenotype. Cells from retinal explants (n=6) were dissociated two weeks post-electroporation and EGFP-positive cells isolated by FACS (Figure 4A). Real time RT-PCR was performed on RNA extracted from EGFP-positive FACS-isolated cells using the primers described in Table 8 and results obtained in explants mirrored those found in HeLa cells. Results indicated that *RHO* suppression of greater than 85% was achieved ($p < 0.001$, Figure 4B).

Long Term Suppression Using AAV Vectors

Long-term expression of therapies will be required for a progressive retinopathy such as adRP. To achieve long-term suppression *in vivo*, shBB-EGFP and the non-targeting shNT-EGFP were engineered into AAV vectors (AAV-shBB-EGFP and AAV-shNT-EGFP) (Figure 1A). Recombinant AAV2/5 viruses were generated using a helper virus free system. Expression cassettes were cloned into pAAV-MCS (Stratagene, La Jolla, CA, USA), between the inverted terminal repeats of AAV2, and transfected into HEK-293 cells (ATCC no. CRL-1573) with pRep2/Cap5 and pHelper (Stratagene), at a ratio of 1:1:2. Fifty 150 mm plates of confluent cells were transfected (50 μ g DNA/plate) with polyethylenimine. Forty eight hours post-transfection crude viral lysates were cleared and purified by CsCl₂ gradient centrifugation. AAV-containing fractions were dialysed against PBS. Genomic titres, i.e., viral particles (vp/ml), were determined by quantitative real time PCR. AAVs generated contained the shBB-EGFP and shNT-EGFP constructs (AAV-shBB-EGFP and AAV-shNT-EGFP, Figure 1A).

The EGFP gene enabled viral transduction to be monitored. Three μ l of AAV-shBB-EGFP (2×10^{12} vp/ml) or AAV-shNT-EGFP (3×10^{12} vp/ml) were subretinally injected into adult NHR+/- rho-/- mice. Two weeks post-injection two animals were sacrificed and expression of the 21 nucleotide shBB shown in two retinas using RNase protection (Figure 5A). Retinas were dissociated and EGFP-positive cells collected by FACS. RNAi-mediated suppression of *RHO*, as evaluated by real time RT-PCR (see Table 8 for primer sequences) two weeks post-injection (n=6), was approximately 90% ($p < 0.001$) in AAV-shBB-EGFP-transduced photoreceptor cells (Figure 5B). Four retinas were dissociated and significant suppression of rhodopsin protein expression was demonstrated *in vivo* in EGFP-positive transduced cells by immunocytochemistry (Figure 5C).

Suppression in Transgenic Animals

A transgenic mouse expressing a sequence-modified *RHO* gene was generated (*RHO-M*). *RHO-M*+/- rho-/- were evaluated at two months of age for rescue of the retinal pathology present in rho-/- mice by histology (Figure 6A-C) and ERG (Figure 6D). Rhodopsin immunolabeling in rod outer segments and the thickness of outer nuclear layers were similar in wild type rho+/+ (Figure 6A), NHR+/- rho-/- (Figure 6B) and *RHO-M*+/- rho-/- (Figure 6C) mice. Additionally, ERG responses were similar in wild type rho+/+, rho+/-, NHR+/- rho-/- and *RHO-M*+/- rho-/- mice. ERG b-waves of rod-isolated responses of 500-700 μ V were observed in mice of all genotypes (Figure 6D). The amplitudes and timings of the combined rod and cone responses to the maximal intensity flash presented in the dark-adapted state, as well as the light adapted cone-isolated responses both to single flash and 10Hz flickers, were equivalent in all the genotypes examined (data not shown). These results validate the use of the degeneracy of the genetic code to engineer codon-modified human *RHO* genes which can provide functional human rhodopsin protein.

AAV-delivered suppression and replacement of human RHO in vivo

Having established shBB and shQ1 as potent suppressors and rBB and rQ1 as being refractory to their corresponding suppressors, shBB-rBB and shQ1-rQ1 were cloned into AAV vectors using the triple plasmid system detailed above and viruses containing both elements of the therapeutics were generated (AAV-shBB-rBB (also

termed AAV-BB8) and AAV-shQ1-rQ1 (also termed AAV-Q1)) using the method detailed above. Three μ l of AAV-shBB-rBB was subretinally injected into adult wild type rho+/+ mice (n=12) and replacement *RHO* mRNA expression confirmed by RT-PCR and RNase protection using RNA extracted 10 days post-injection (data not shown). To demonstrate that AAV-delivered rBB is translated into protein, 2 μ l of a 1:1 mix of AAV-shBB-rBB and AAV-CMV-EGFP was subretinally injected into 10 day old rho-/- mice (n=6). Two weeks post-injection rhodopsin and EGFP protein expression were determined using fluorescent microscopy. Marked rhodopsin expression, overlapping with EGFP, was observed in transduced areas (Figure 7).

Subsequently, 1 μ l of AAV-shBB-rBB or AAV-shQ1-rQ1 was subretinally injected into newborn Pro23His+/- rho+/- mice (n=10) that present with a retinal degeneration resulting in complete loss of photoreceptors by two weeks of age. In all animals one eye was injected with therapeutic virus (either AAV-shBB-rBB or AAV-shQ1-rQ1) and the other with a control virus (AAV-EGFP). The early onset and rapid nature of the retinopathy in young Pro23His pups precluded use of ERG as a readout for benefit. However, at ten days of age retinal histology was evaluated in semi-thin resin embedded sections cut at approximately 50 μ m intervals throughout the central meridian of the eye (n=10). From each section approximately 40 measurements of ONL thickness were taken. Since only a part of the retina is transduced by a single subretinal injection of AAV (particularly in newborn pups), to identify the transduced area ONL measurements were ordered by thickness and the 15% highest and lowest values grouped for analysis. Lowest values represent thinnest ONL readings, most likely corresponding to peripheral areas of the retina and thus not in close proximity to injection sites. Highest values represent thickest ONL readings, most likely corresponding to central areas of the retina and thus in closer proximity to injection sites. Significant differences in ONL thickness between AAV-shBB-rBB- and AAV-EGFP-treated eyes were observed. The ONL of treated eyes was found to be approximately 33% ($p<0.001$) thicker than control injected counterparts for the highest value groupings (Figure 8A-C). In the lowest value groupings a difference of approximately 10% was observed (Figure 8A). These data provide evidence at the histological level that AAV2/5-delivered RNAi in conjunction with provision of a codon-modified replacement gene can beneficially modulate the retinopathy in Pro23His+/- rho-/- mice.

RNAi-mediated suppression was evaluated in retinal tissue after sub-retinal injection of AAV vectors expressing either a suppressor targeting rhodopsin (AAV-shBB-EGFP, AAV-shCC-EGFP and AAV-shQ1-EGFP) or a non-targeting control (AAV-shNT-EGFP). Mice expressing a human rhodopsin replacement gene (referred to as RHO-M mice and detailed in the section on suppression in transgenic animals) were subretinally injected with AAV vectors (AAV2/5), containing shRNA sequences for BB, CC and Q1 and an EGFP reporter gene (AAV-shBB-EGFP, AAV-shCC-EGFP and AAV-shQ1-EGFP). The presence of the EGFP reporter gene enabled isolation of the population of retinal cells that are EGFP positive and therefore have received AAV using FACS to isolate these cell populations. AAV-delivered RNAi-mediated suppression with each suppressor (BB, CC and Q1) was evaluated using real-time RT-PCR in cell populations characterised by FACS and was compared to suppression obtained using AAV with non-targeting control shRNA sequences (AAV-shNT-EGFP). Significant rhodopsin suppression was obtained with BB and Q1 suppressors, however, significantly lower levels of suppression were obtained with the CC suppressor (Figure 6E). The replacement gene in RHO-M mice was partially protected from suppression due to the presence of two nucleotide mismatches between the CC suppressor sequence and the target site for suppression in the human rhodopsin replacement gene. The replacement gene is partially protected from siRNA CC-based suppression by the introduction of two nucleotide changes at degenerate sites in the replacement gene. Figure 6F illustrates depression of the ERG response in RHO-M eyes that have received AAV-shBB-EGFP (panel 1) or AAV-shQ1-EGFP (panel 2) when compared to eyes subretinally injected with AAV-shNT-EGFP. The top tracing in each panel represents the right eye which received the targeting AAV-shRNA vector and the bottom tracing in each panel represents the left eye which received the control non-targeting AAV-shNT vector. In contrast no reduction / depression of the ERG was observed in RHO-M mice subretinally injected with AAV-shCC-EGFP (panel 3) vector; this is likely due to the reduced levels of rhodopsin suppression observed with AAV-shCC-EGFP (see Figure 6E above).

Example 2: Optimization of Expression of Suppression Agents and Replacement Nucleic Acids

Expression of suppression and/or replacement vectors was optimized by including in the vectors sequences that enhanced and/or modulate expression levels at the RNA and/or protein level. A list of exemplary sequence elements is provided in Table 1, however, the enhancing and/or modulating elements of the invention are not exclusive to this list. For example, one or more of a promoter, a stuffer, an insulator, a silencer, a chromatin remodelling sequence, an intron sequence, a poly adenylation signal, a post translational regulatory element, and a transcription factor binding site can be included in suppression and/or replacement constructs to modulate expression of suppression and/or replacement components relating to the invention. Such elements and derivatives thereof can be used to modulate levels of expression, tissue specificity, timing of expression, and/or induction of expression. Table 9 provides some exemplary sequences that can be used to modulate expression of suppression and/or replacement constructs relating to the invention. The sequences provided are within conserved regions as evaluated by comparison of sequences from multiple species. At any one position a nucleotide may not be conserved between all species – the sequences represent regions where overall there is a high degree of conservation. Such conserved sequences from any species such as human, mouse, rat, bacteria, virus and/or indeed a hybrid sequence from more than one species could be used in the invention.

Table 9: Exemplary Enhancer Sequences

CMV enhancer element amplified from pCDNA3.1 Invitrogen nt 308 – 734 http://www.invitrogen.com/ (SEQ ID NO:87)
CCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCG CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCA TTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGT GTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA TTAGTCATCGCTATTACCATGGTGATGCGTTTTTGGCAGTACATCAATGGGCGTGGA TAGCGGTTTGACTCACGGGGATTTC AAGTCTCCACCCCATTGACGTCAATGGGAGT TTG TTTTGGCACC AAAATCAACG GGAC
pAAV.BB11 The WPR element from pSin11 CMV GFPpre mut FL (Gene Therapy (7) : 641-5 (2006)) (SEQ ID NO:88)
GAGCAT CTTACCGCCATTTATTCCCA TATTTGTTCT GTTTTCTTG ATTTGGGTAT

ACATTTAAATGTTAATAAAA CAAAATGGTG GGGCAATCAT TTACATTTT
 AGGGATATGTAAATTACTAGT TCAGGTGTAT TGCCACAAGA CAAACATGTT
 AAGAAACTTTCCCGTTATTT ACGCTCTGTT CCTGTTAATC AACCTCTGGA
 TTACAAAATTTGTGAAAGAT TGA CTGATAT TCTTAACTAT GTTGCTCCTT
 TTACGCTGTGTGGATATGCT GCTTTATAGC CTCTGTATCT AGCTATTGCT
 TCCCGTACGGCTTTCGTTTT CTCCTCCTTG TATAAATCCT GGTTGCTGTC
 TCTTTTAGAGGAGTTGTGGC CCGTTGTCCG TCAACGTGGC GTGGTGTGCT
 CTGTGTTTGCTGACGCAACC CCCACTGGCT GGGGCATTGC CACCACCTGT
 CAACTCCTTTCTGGGACTTT CGCTTTCCCC CTCCCGATCG CCACGGCAGA
 ACTCATCGCCGCCTGCCTTG CCCGCTGCTG GACAGGGGCT AGGTTGCTGG
 GCACTGATAATCCGTGGTG TTGTC

pAAV.BB13 The WPR element from pBSK11 (Donello JE, et al. J. Virol. 1998 72(6):5085-92.)
 (SEQ ID NO:89)

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT
 GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTC
 CCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGG
 AGTTGTGGCCCCGTTGT CAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAA
 CCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTT
 CCCCCCTCCCTATTGCCACGGCGGAAC TCATCGCCGCCTGCCTTGCCCGCTGCTGGAC
 AGGGGCTCGGCTGTTGGGCACTGACAATCCGTGGTGTGTCGGGGAAGCTGACGTC
 CTTTCCATGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGC
 TACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTC
 TGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGC
 CGCCTCCCC

(Wild type woodchuck hepatitis B virus genome sequence ACCESSION J04514)

Example 3: Comparison of Rhodopsin Genes

In addition to adding enhancing and/or modulating elements to suppression and/or replacement vectors, the rhodopsin promoter was studied in detail. A comparison of rhodopsin genes present in different mammals resulted in identification of 9 highly conserved regions in the rhodopsin gene (conserved regions A through I, Sequence 1, Table 10). Regions A, B, C and D are in the rhodopsin promoter region, conserved region E is in intron 2 of the gene and conserved regions F, G, H and I are in the 3' region.

The following sequence (Sequence 1; Table 10) shows the conserved regions within the mouse promoter human intronic and exonic and 3' sequence. Notably, conserved sequences in the mouse promoter are nearly the same in the human rhodopsin promoter and it is contemplated that the human or other mammalian rhodopsin promoters and/or derivatives and/or hybrids thereof may be used in suppression and replacement constructs. Additionally, it is contemplated that other promoters could be combined with some or all of conserved regions A through I and used in suppression and/or replacement constructs, for example, other retinal promoter sequences may be used.

Table 10: Conserved Regions of Rhodopsin

Sequence 1: Mouse rhodopsin promoter sequence (upper case) ending at the Xho I site (highlighted in bold print), followed by the human rhodopsin 5'UTR, human rhodopsin exons and introns and human rhodopsin 3' region sequence (lower case). Conserved regions A - I are highlighted in bold print. (SEQ ID NO:90).

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GTTCCAGGGC CCAGGGGCTT CCAGCCATGA GGGCACCTAG ACTTGTAATC
CCTAGAGTCC TCCTGATGCC ACTGCCCAGG GACAGACAGC ACACAGCACC
CCTCCCCCAC TCTCTTAACA GGCAGAAGCA GGGAGATGGA GGCATGCTGA
AGATGTCCAT GTGAGGCTGG TGGTAGCATG CCCACTGCTG GGATGAAGAG
ATGGGGGCAA AGTGAGTGGC AGAGGCCAGG CCAGGTCCAG GCCCTTCCAG
GCTTCCTCTG CCACTGTGGA GATGAAAGAG GGAGCCAGGC AAGGTCCAGG
CCCTCCCCAC CCCCTCTGCC TCTATGGAGA TGAAGGGGGA ATGAAGAAGG
GAGCCAGACA GTTGTGCCAA CACAACCTCT CCGTCGAGTG TCTAATTGCT
TATGATCATG CATGCTCTCT CTCCCACTAA ACATTTATTA ATGTGTTAGG cons reg A
ATTTCCATTA GCGCGTGCCT TGAAGTGAAT TCATTTGCAT ATGGCTGGGA
AAAAGTGGGG TGAGGGAGGA AACAGTGCCA GCTCCCCAAC AGGCGTCAAT
CACAGTGACA GATCAGATGG TTTCTGGCTG GAGGCAGGGG GGCTGTCTGA
GATGGCGGCA TGCATCCTTT CAGTGCATAT CACAGAAATT CAGGTGACTC
CTGCTGGGAG CCAAGACCCT GAGGCTGAGC CTGGCCACAG CTCCAATAGC
TGCTGGATAT CATCATGTCT GGGCTGAGCA GCCTCTAGAG GTACCCTTTT

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ACAGATAGTA AAAGTGAGGC TCAGTGACTG CTGAGCCAAA GTTGGACCCA
 CCCACACTCA TTTGCAGACT GCCGTGGGCC ATGTTCTGAT CTCTCCCTA
 CCTGGACTCA GCCCAGCACA CTCGGCACAC AAGGCCCTTC TTCAGCTTGA
 ATACAGCGTC CTCAGCTATA GCCAGCATCT ATGAATGGAG CTCAGTGACC
 CTGACTGGAG GAAGTTAGGA CAGGGATTTT TTCTGGAGTT TTGGCAGGAA
 GAGGCCAGGG TCAGGTGACT GCTGGAGCAC ACAGCTTGGT AAGACTAGTC
 AGGACCTGCG TCCTGAGGCT ACATGTCATA TCCACAGTAA GGAAGTGAA
 GATGGGAGAT GACTGGCTGG GCCACAACCA GTGAGTGAA TGTCTTGTG
 CATCTTTGTT TCCTAACCTT CCCCTCTGTA GCTGCTGAAA CACACACACA
 CCCCATGCTC TGTTATGCCT CTTCCCTGGC CTGGGATTTC CATGGCTGAG
 GTGATGGGGC ACTGAGGCAC CGCCAGGAAA GGCTGTAACC CATCTGCTCC
 CCCATCCTTC ACCAGACTTC AAGCACCTAC CTAGAGCACA GGTGCAATTT
 TGTACCCTCC CTGTCTGGGA CCCACAGTGG TTCCTCAATG CCGGCCAACC
 AGACTCATAG GCCTGCCCAC AAGGCCCTTG GGGCTATCTG TCTGAGGCCT
 GCAGGTGCCC TCCTGGCCAC CTAGGCTCCT GTGAGACTTA GACTTCCATA
 GATTCTTCCT GAAAGACTAC TGAGGGCAGG AGCCCCAAG CCTCAGGGTT
 AGCTTTCCTC AGCCCTGCCT CTTTGCTAGC TCCGTTTCCA CATTGAAGGC
 AGGGCTGAGC AGGGCAGGCG CAGCGAGGAG CTAAGTGTG CTTCTCTCTC
 GTTCATTTGT CTGCTGCCCT GAGACGCCAC AGCACCTAAT AAGAGCATGT
 TATGTGTAGC AAACATTAGG CCTGTAAGGA AGGAAAGGAG TGACGTCCCT
 TGACGTCTC AGCTAGGCTG TGGTGACACA AGCAAGAGGA CTAAGCCACA
 GGTGAGGAGA AAGGGGGGGG GGGGTCTGCT GACCCAGCAA CACTCTTTCC cons reg B
 TTCTGAGGCT TAAGAGCTAT TAGCGTAGGT GACTCAGTCC CTAATCCTCC
 ATCAATGCC CTGTGACTGC CCCTGCTTCT GAAGGGCCAA CATGGCTACA
 GCTAGCTCCA GAGACAGCTT TTCAGGGCCC CAGCATCCAA GCATCTCACA
 GTTCTCCACT GACCACACTC CTGTGCAGCA CTGGGCTTTT CAATGCCCTT
 GACTTGAAGA GAACTCAAAC TGCAGGTCAA CTAGACTCTG CAAACTTCAC
 CTGTGCTGGG GGTTCTAGC CTGTGGGGAC AGTGTATCTT GAATACCTGC
 TGCTATGGAC CAAGAGCTGA ACACACAGAC AAACAGGCTC AGCTGGCCGG

CATTCTGGAA CCACAAATGA GTGTGGATGA GCAGGAGGGC AACAAAATGG
 TCTGGGTGTT GTCAACACAG TCAGTAAACA ATGCACGCAG TGGGGCTGGG
 CCCTGATGTG GAGCTAGGTG GGGTTGGCTC TCCTTGGA AAA CCTGAAGGGA
 GAAGGAGAGG GAGCGAGATG ATGAGGTTTA TCAGCCTGCA GAGGCAGGGG
 GTCAGGAAGG AGTGCCACTG TACTGACCCA GGACCTCTGT GGGACATCAA
 GCCATGCCAA GGAGCCATGG AGCCTCGATT GCACTGGCAG GGACAGGTTG
 TGATGCCCCA GAGTCCCCAG ACCCAGCAAA CAGAGGCCCA GAGTGGGAAG
 TGGAGCTTTC CAGGGTATCG GGGTGACTCA GAGACACAGG GTAGAATCTG
 CCTTGGGTGC TCACTGCCCT ATCTGAGTCC ACATGGCTCA GTCCCCAGGC
 CCTGTTCTCT AGTGACTGTT GCTTTGATGA GGTAGAGACA GGCAGCCCTC
 TTCTAAGAAC TATGTTTTGA TGGGGGACTC AGAGTTGGGG TGGGGTGGCA
 ATGAAATTCT GTAGACTGTG TGGTTATAAC CCTGGCTGTT ACTAGCTAGT
 TCTGTGACCT TGGTGACCCA CTTCAGACTC TAGGCCCTCAG CCTCTGTAAG
 TGCAGATACA CAGCGCCAAT CAGCCGATGA CTTCTAACAA TACTCTTAAC
 TCACACAGAG CTTGTCTCAC TGAGCCAACA CCCTGTACCC TCAGCTCAGT
 GACGGCTTTC AACCTGTGGG GCTGCCTCTG TTACCCAAGT GAGAGAGGGC
 CAGTGCTCCC AGAGGTGACC TTGTTTGCCC ATTCTCTCCC TGGGTCAGCC
 AGTGTATTATC TGTTGTATAC CCAGTCCACC CTGCAGGCTC ACATCAGAGC
 CTAGGAGATG GCTAGTGTCC CCGCGGAGAC CACGATGAAG CTTCCCAGCT HindIII
 GTCTCAAGCA CAAGCTGGCT GCAGAGGCTG CTGAGGCACT GCTAGCTGGG start 1.734kb
 GATGGGGGCA GGGTAGATCT GGGGCTGACC ACCAGGGTCA GAATCAGAAC
CTCCACCTTG ACCTCATTA CGCTGGTCTT AATCACCAAG CCAAGCTCCT cons reg C
TAAACTGCTA GTGGCCAACT CCCAGGCCCT GACACACATA CCTGCCCTGT
 GTTCCCAAAC AAGACACCTG CATGGAAGGA AGGGGGTTGC TTTCTAAGC
 AAACATCTAG GAATCCCGGG TGCAGTGTGA GGAGACTAGG CGAGGGAGTA
 CTTTAAGGGC CTCAAGGCTC AGAGAGGAAT ACTTCTTCCC TGGTTAGCCT
 CGTGCCTAGG CTCCAGGGTC TTTGTCTGCT CTGGATACCT ATGTGGCAAG
 GGGCATAGCA TTTCCCCAC CATCAGCTCT TAGCTCAACC TTATCTTCTC
 GGAAAGACTG CGCAGTGTA CAACACAGCA GAGACTTTTC TTTTGTCCCC

TGTCTACCCC TGTAAC TGCT ACTCAGAAGC ATCTTTCTCA CAGGGTACTG
 GCTTCTTGCA TCCAGAGTTT TTTGTCTCCC TCGGGCCCCC AGAATCAAAT
 TCTTCCTCTG GGACTCAGTG GATGTTTCAC ACACGTATCG GCCTGACAGT
 CATCCTGGAG CATCCTACAC AGGGGCCATC ACAGCTGCAT GTCAGAAATG
 CTGGCCTCAC ATCCTCAGAC ACCAGGCCTA GTGCTGGTCT TCCTCAGACT
 GGGCTCCCCA GCAGGCCAGT AGGATCATCT TTTAGCCTAC AGAGTTCTGA
 AGCCTCAGAG CCCCAGGTCC CTGGTCATCT TCTCTGCCCC TGAGATTTTT
 CCAAGTTGTA TGCCTTCTAG GTAAGGCAAA ACTTCTTACG CCCCTCCTCG
 TGGCCTCCAG GCCCCACATG CTCACCTGAA TAACCTGGCA GCCTGCTCCC
 TCATGCAGGG ACCACGTCCT GCTGCACCCA GCAGGCCATC CCGTCTCCAT
 AGCCCATGGT CATCCCTCCC TGGACAGGAA TGTGTCTCCT CCCCgggCTG
 AGTCTTGCTC AAGCTAGAAG CACTCCGAAC AGGGTTATGG GCGCCTCCTC
 CATCTCCCAA GTGGCTGGCT TATGAATGTT TAATGTACAT GTGAGTGAAC
 AAATTCCAAT TGAACGCAAC AAATAGTTAT CGAGCCGCTG AGCCGGGGGG
 CGGGGGGTGT GAGACTGGAG GCGATGGACG GAGCTGACGG CACACACAGC
 TCAGATCTGT CAAGTGAGCC ATTGTCAGGG CTTGGGGACT GGATAAGTCA
 GGGGGTCTCC TGGGAAGAGA TGGGATAGGT GAGTTCAGGA GGAGACATTG
 TCAACTGGAG CCATGTGGAG AAGTGAATTT AGGGCCCCAAA GGTTCAGTC
 GCAGCCTGAG GCCACCAGAC TGACATGGGG AGGAATTCCC AGAGGACTCT
 GGGGCAGACA AGATGAGACA CCCTTTCCTT TCTTTACCTA AGGGCCTCCA
 CCCGATGTCA CCTTGGCCCC TCTGCAAGCC AATTAGGCCC CGGTGGCAGC
 AGTGGGATTA GCGTTAGTAT GATATCTCGC GGATGCTGAA TCAGCCTCTG
 GCTTAGGGAG AGAAGGTCAC TTTATAAGGG TCTGGGGGGG GTCAGTGCCT
 GGAGTTGCGC TGTGGGAGCC GTCAGTGGCT GAGCTCGCCA AGCAGCCTTG
 GTCTCTGTCT ACGAAGAGCC CGTGGGGCAG **CCTCGAG**

XhoI

ggatcctgag tacctctcct cctgacctc aggtctctc **ctagtgtcac** **cttggccct** conserved region D

cttagaagcc **aattaggccc** **tcagttctg** **cagcggggat** **taatagtatt** **atgaacaccc**

ccaatctccc **agatgctgat** **tcagccagga** **gcttaggagg** **gggaggtcac** **tttataaggg**

tctggggggg **tcagaaccca** **gagtcaccca** **gctggagccc** **tgagtggctg** **agctcagggc**

ttcgcagcat tcttgggtgg gagcagccac gggtcagcca caagggccac agccatgaat
 ggacagaag gccctaactt ctacgtgcc ttctcaatg cgacgggtgt ggtacgcagc
 ccttcgagt acccacagta ctacctggct gagccatggc agttctccat gctggccgcc
 tacatgttc tctgatcgt gctgggcttc cccatcaact tctcacgct ctacgtacc
 gtccagcaca agaagctgcg cagcctctc aactacatcc tctcaacct agccgtggct
 gacctctca tggctcagg tggcttacc agcaccctct acacctctct gcatggatac
 ttctcttcg ggcccacagg atgcaattg gagggctct ttgccacct gggcggtatg
 agccgggtgt ggggtgggtg tgcaggagcc cgggagcatg gaggggtctg ggagagtccc
 gggcttggcg gtggtggctg agaggccttc tccctctcc tctctgtca atgttatcca
 aagccctcat atattcagtc aacaacacc attcatggtg atagccgggc tctgtttgt
 gcagggtgg cactgaacac tgccttgatc ttatttgag caatatgcgc ttgtctaatt
 tcacagcaag aaaactgagc tgaggctcaa aggccaagtc aagccctgc tggggcgta
 cacaggagc ggtgcagagt tgagtggaa gcccgcatct atctcgggcc atgtttgcag
 caccaagcct ctgtttccct tggagcagct gtgctgagtc agaccaggc tgggactga
 ggagagctg ggcaagccag acccctctc tctggggcc caagtcagg gtgggaagtg
 gattttccat tctcagta ttgggtctc cctgtgctgg gcaatgggt cggccccctc
 tggcatctc tgcctccct ctacgccct gtctcaggt gccctccag cctccctgcc
 gcttccaag tctctggtg ttgagaaccg caagcagccg ctctgaagca gtccctttt
 gcttagaat aatgtctgc attaacagg aaaacagatg ggggtctgca gggataacag
 atccactta acagagagga aaactgagc agggagaggg gaagagactc attagggat
 gtggccagc agcaacaaga gcctaggtct cctggctgtg atccaggaat atctctgtg
 agatgcagga ggagacgcta gaagcagcca ttgcaaagct gggtagcggg gagagcttac
 cgccagccac aagcgtctct ctgccagct tgcctgtct ccccatgtc caggctgtg
 cctcggtccc attctcagg aatctctggc cattgttggg tgtttgtgc attcaataat
 cacagatcac teagtctgg ccagaagggt ggtgtgccac ttacgggtgg ttgtctctg
 cagggtcagt cccagttac aaatattgtc ctttactg ttaggaatgt cccagtttg
 ttgattaact atatggccac tctccctatg aaactcatg ggggtgtgag caggacagat
 gtgcgaatc catcattcc ttctctcc tctgggcaaa acattgcaca ttgctcatg
 gctcctagga gagggccca catgtccggg ttatttcatt tccgagaag ggagagggag

gaaggactgc caattctggg ttccaccac ctctgcattc ctccaaca aggaactctg
 cccacatta gtagcattc ttctgctaaa cacacacaca cacacacaca cacacaacac
 acacacacac acacacacac acacacacac aaaactccct accgggttcc cagttaatc
 ctgacccctt gatctgattc gtgtccctta tgggcccaaga gcgctaagca aataacttcc
 cccattccct ggaatttctt tgcccagctc tctcagcgt gtgtccctc tgccccttcc
 cctctctccc agcaccaagc tctctcttc cccaaggcct cctcaaatcc ctctccact
 cctgggtgcc ttctagctca cctctccct gtctaggggg gtagtgaccc tcttaggca
 gtggggtctg tgctgaccgc ctgctgactg ccttcaggt gaaattgccc tgtgtctt
 ggtgtcctg gccatcgagc ggtacgtggt ggtgtgaag ccatgagca acttccgctt
 cggggagaac catgccatca tgggcgttc ctccactgg gtcattgggc tggcctgcgc
 cgcaccccca ctgcgggct ggtccagga atggcactga gcagaaggga agaagctccg
 ggggctctt gtagggtctt ccagtcagga ctcaaacca gtagtgtctg gttccaggca
 ctgacctgt atgtctctg gcccaatgc ccactcagg taggggtgta gggcagaaga
 agaaacagac tctaatttg ctacaaggc tggcccatc tctgagccc catgtcaaac conserved region E
agaatccaag acatccaac cttcacctt ggtgtgccc ctaatctca actaagtag
gcgcaattc caatctctt tggctagta ccccgggggc agccctctt aacctgggc
ctcagcaga ggggaggcca cacttcta gtgcaggtg ccatatttg gcccttga
 actgggtccc actcagctc taggcattg tctcctaag gggtgagat gagactcagt
 ggggacagt gtttgacaa taggactggt gactctggt cccagaggcc tcatgtcct
 ctgtctcag aaaattccca ctctacttc ctttctctc tcatgttgc taggttccat
 ttctaccct tctgaattt gagcccacc cctggactt tccccatct tctccaatct
 ggcctagttc tctctctgg aagcagagcc gctggacgt ctgggttcc tgaggccgt
 ccactgtac caatatcagg aaccattgcc acgtcctaag gacgtgcgt ggaagcctt
 agttccaga agctgcaca agatccctta gatactctgt gtgtccatct ttggcctgga
 aaatactct accctggggc taggaagacc tgggttgta caaacttct caaatgcaga
 gcctgagggc tctccccacc tctcaccaa cctctgcgt ggcattagccc tagcctcagc
 gggcagtga tcttggggt gggcatgcag ggagaggctg ggtggtgtca tctgtaacg
 cagccaccaa acaatgaag gacactgatt ccacaagggt catctgcat cccatctgat
 ccattccat ctgtaccca gccatgcaga cgttatgat ccccttcc agggaggga

tgtgaagccc cagaagggc cagcgtcgg cagccacctt ggctgtccc aagtcctca
 caggcaggggt ctcctacct gcctgtctc aggtacatcc ccgagggcct gcagtgtcgc
 tgtggaatcg actactacac gctcaagccg gaggtcaaca acgagtcttt tgtcatctac
 atgttcgtgg tccacttcac catcccatg attatcatct tttctgcta tgggcagctc
 gtcttcaccg tcaaggaggt acgggccggg ggggtggcgg cctcacggct ctgagggicc
 agccccagc atgcatctgc ggctcctgct ccctggagga gccatgtct ggacccgggt
 cccgtgtcct gcaggccgct gcccagcagc aggagtcagc caccacacag aaggcagaga
 aggaggtcac ccgcatggic atcatcatgg tcatcgcttt cctgatctgc tgggtgcct
 acgccagcgt ggcatctac atctcaccc accagggtc caactcggc cccatctca
 tgaccatccc agcgttctt gccaaagcgc ccgccatcta caaccctgc atctatatca
 tgatgaacaa gcaggtgcct actcggggtg ggaggggccc agtggcccag gccacaggcg
 ctgcctgccaggacaagct actccagggg caggggaggg gctccatcag ggttactggc
 agcagttctg ggtcagcagt ccaatgggg agtgtgtgag aaatgcagat tcctggcccc
 actcagaact gctgaatctc aggggtggcc caggaaactg cattccagc aagccctcca
 caggtggctc agatgtcac tcaggtggga gaagctccag tcagctagtt ctggaagccc
 aatgtcaaag tcagaaggac ccaagtcggg aatgggatgg gccagtcctc ataaagctga
 ataaggagct aaaaagtctt attctgaggg gtaaagggtt aaagggttcc tcggagaggt
 acctccgagg ggtaaacagt tgggtaaaca gtctctgaag tcagctctgc catttctag
 ctgtatggcc ctgggcaagt caatttctt ctctgtgctt tggtttctc atccatagaa
 aggtagaag ggcaaacac caactcttg gattacaaga gataattac agaaccct
 tggcacacag agggcaccat gaaatgtcac gggtgacaca gcccctgt gctcagtc
 tggcatctc aggggtgagg agcgtctgcc tagcaggttc ccaccaggaa gctggattg
 agtggatggg gcgctggaat cgtgaggggc agaagcaggc aaagggtcgg ggcgaacctc
 actaacgtgc cagttccaag cacactgtgg gcagccctgg cctgactca agcctctgc
 ctccagttc cggaactgca tgctcaccac catctgtgc ggcaagaacc cactgggtga
 cgatgaggcc tctgtaccg tgtccaagac ggagacgagc caggtggccc cggcctaaga
 cctgcctagg actctgtggc cgactatagg cgtctccat cccctacacc tccccagc
cacagccatc ccaccaggag cagcgcctgt gcagaatgaa cgaagtcaca taggtcctt conserved region F
aattttttt ttttttttaa gaaataatta atgaggtcctc tcaactacct gggacagcct

gagaagggac **atccaccaag** accfactgat **ctggagtecc** acgttccca aggccagcgg
 gatgtgtgcc cctcctcctc ccaactcacc **tttcaggaac** acgaggattc **ttgcittctg**
gaaaagtgc ccagcttagg gataagtgc tagcacagaa tggggcacac agtaggtgct conserved region G
taataaatgc tggatggatg caggaaggaa tggaggaatg aatgggaagg gagaacatat
 ctatectctc agaccctcgc agcagcagca actcactatt ggctaattgat atggagcagt
 tgttttccc tccctgggcc tcaatttctt ctctataaa atggaaatcc cagatccctg
 gtcctgccga cagcagcta ctgagaagac caaaagaggt gtgtgtgtgt ctatgtgtgt
 gtttcagcac ttgttaata gcaagaagct gtacagattc tagtaattgt tgtgaataac
 atcaattaat gtaactagti aattactatg attatcacct cctgatagtg aacatttga
 gattgggcat tcagatgatg gggtttcacc caaccttggg gcaggtttt aaaaattagc
 taggcatcaa gggcagacca gggctggggg ttgggctgta ggcagggaca gtcacaggaa
 tgcaggatgc agtcacaga cctgaaaaaa caacactggg ggaggggggac ggtgaaggcc
 aagttcccaa tgagggtgag attgggcctg gggctcacc cctagtgtgg gggcccaggt
 cccgtgcctc cccctcccaa tgtggcctat ggagagacag gcctttctct cagcctctgg
 aagccactg ctcttttct ctacacactg ggtcccagca tctagagcat **ggagcctcta**
gaagccatgc taccgcgcc acatttaatt aacagctgag tccctgatgt catccttact conserved region H
cgaagagctt agaacaaga agtgggaaat tccactgggc ctaccttct tggggatgtt
 catgggcccc agtttccagt ttcccttgc agacaagccc atcttcagca gttgctagtc
 cattctcat tctggagaat ctgctccaaa aagctggcca catctctgag gtgtcagaat
 taagctgcct cagtaactgc tccccctct ccatataagc aaagccagaa gctctagctt
 taccagctc tgcctggaga ctaaggcaaa ttgggccatt aaaagctcag ctctatgtt
ggtattaacg gtggtgggtt ttgttcttt cacactctat ccacaggata gattgaaact conserved region I
gccagcttcc acctgatccc tgacctggg atggctggat tgagcaatga gcagagccaa
gcagcacaga gtccttggg gctagagggtg gaggaggcag tcttgggaat gggaaaaacc
 ccaacttgg ggtcatagag gcacaggtaa ccataaaac tgcaacaag ctt

Conserved regions A through I and some sequence flanking the regions (5' and 3', were combined (Table 11, SEQ ID NO: 92 through SEQ ID NO: 99, Sequence 2). This sequence was analyzed using MatInspector Release Professional 7.4.1 to identify other regions that may be involved in transcriptional and/or translational

control of rhodopsin gene expression. (A small portion of the MatInspector results are presented in Table 12). This table illustrates some sequences within conserved regions A through I that are thought to be involved in the transcription and/or translation and/or stability of rhodopsin. Some of these sequences, such as the CRX binding element in conserved region D and the TATA box in region G are known in the art. Others, such as the CRX binding region in E, are not. The complete set of results from MatInspector are presented in Table 13. 302 putative transcription binding sites and/or regulatory sequences were identified and some are highlighted in bold. On the basis of the conserved nature of regions A through I and the important transcription factor binding sites thought to be located within these regions, the constructs in Figure 9 were generated. Construct BB16 contains conserved regions A, B, C, D, F and G. In addition an artificial CRX-NRL element (below) was inserted between conserved regions A and B. The components of the artificial CRX-NRL enhancer element include the CRX motif from conserved region D, the CRX motif from conserved region E and NRL binding sites are underlined.

TTTCTGCAGCGGGGATTAATATGATTATGAACACCCCAATCTCCCAGATG
CTGATTCAGCCAGGAGGTACC (SEQ ID NO:91)

All these constructs contain transcription binding sites identified within conserved regions A through I.

Sequence 2: Conserved regions A through I in the rhodopsin gene are highlighted in bold below. The nucleotides of these sequences and a small section of 5' and 3' sequence surrounding conserved regions have been numbered 1-1600. This sequence was analysed with MatInspector and the nucleotide numbering system of sequence 2 (1-1600) relates to the nucleotide numbering system in Table 13.

Table 11 (Conserved regions are in bold)

Conserved region A 1-210 (SEQ ID NO:92)
CACAACTCCT CCGTCGAGTG TCTAATTGCT TATGATCATG CATGCTCTCT CTCCCACTAA ACATTTATTA ATGTGTTAGG ATTTCCATTA GCGCGTGCCT TGAAGTGAAG TCATTTGCAT ATGGCTGGGA AAAAGTGGGG TGAGGGAGGA AACAGTGCCA

GCTCCCCAAC AGGCGTCAAT CACAGTGACA GATCAGATGG TTTCTGGCTG 210
Conserved region B 210-310 (SEQ ID NO:93)
AAGGGGGGGG GGGGTCTGCT GACCCAGCAA CACTCTTTCC TTCTGAGGCT TAAGAGCTAT TAGCGTAGGT GACTCAGTCC CTAATCCTCC ATTCAATGCC 310
Conserved region C 310-410 (SEQ ID NO:94)
GGGGCTGACC ACCAGGGTCA GAATCAGAAC CTCCACCTTG ACCTCATTA CGCTGGTCTT AATCACCAAG CCAAGCTCCT TAAACTGCTA GTGGCCAAC 410
Conserved region D 410-690 (SEQ ID NO:95)
aggttctc ctagtgteac cttggcccct cttagaagcc aattaggecc tcagtttctg cagcggggat taatatgatt atgaacaccc ccaatctccc agatgtgat tcagccagga gcttaggagg gggaggteac tttataaggg tctggggggg tcagaaccca gagtcacca getggagccc tgagtggctg agctcagccc ttcgagcat tcttggtgg gaggagccac gggtcagcca caagggccac agccatgaat ggcacagaag 690
Conserved region E 690-850 (SEQ ID NO:96)
tcctgagccc catgtcaaac agaatccaag acatccaac ccttcacctt ggetgtgecc ctaatectca actaagctag gcgcaaatc caatctctt tggctagta ccccgggggc agccccctct aaccttgggc ctcagcagca ggggaggcca 850

Conserved regions F and G 850-1220 (SEQ ID NO:97)
ccccacacc tccccccagc cacagccatc ccaccaggag cagcgccgtg gcagaatgaa cgaagtcaca taggctccft aattttttt ttttttttaa gaaataatta atgaggctcc tcactcacct gggacagcct gagaagggac atccaccaag acctactgat ctggagtccc acgttcccca aggccagcgg gatgtgtgcc cctcctctc ccaactcacc tttcaggaac acgaggattc ttgctttctg gaaaagtgc ccagcttagg gataagtgc tagcacagaa tggggcacac agtaggtgct taataaatgc tggatggatg caggaaggaa tggaggaatg aatgggaagg 1220
Conserved region H 1220 1230-1316 1330 (SEQ ID NO:98)
tctagagcat ggagcctcta gaagccatgc tcaccgccc acatttaatt aacagctgag tcctgatgt catccttact cgaagagctt agaaacaaag agtgggaaat 1330
Conserved region I 1330 1342-1425 1600 (SEQ ID NO:99)
gctctagctt taccagctc tgctggaga ctaaggcaaa ttgggccatt aaaagctcag ctcctatgtt ggtattaacg gtggtgggtt ttgttcttt cacactctat ccacaggata gattgaaact gccagcttec acctgatccc tgacctggg atggctggat tgagcaatga gcagagccaa gcagcacaga gtcacctggg gctagagggtg gaggaggcag tcttgggaat gggaaaaacc ccaactttgg ggtcatagag 1600

Table 12: Conserved sequence motifs in Rhodopsin

Conserved region	Position	Name
B	288-304	CRX
C	366-382	CRX
D	470-486	CRX

E	784-764	CRX
G	1172-1177	TATA box
D	500-520	Neuron-restrictive silencer factor
E	794-814	Neuron-restrictive silencer factor
E	831-851	Neuron-restrictive silencer factor

Table 13: Putative Rhodopsin Transcription Regulatory Factors

Family/matrix	Further Information	Opt.	Position	Str.	Core sim.	Matrix sim.	Sequence (red: ci-value > 60 capitals: core sequence)	SEQ ID NO
V\$PDX1/ISL1.01	Pancreatic and intestinal lim-homeodomain factor	0.82	14 - 34	(+)	1.000	0.860	tcgagtgtcTAA Ttgcttatg	100
V\$HOME/MSX.01	Homeodomain proteins MSX-1 and MSX-2	0.97	18 - 30	(+)	1.000	0.995	gtgtcTAATtgc t	101
V\$HOXF/GSH2.01	Homeodomain transcription factor Gsh-2	0.95	19 - 35	(+)	1.000	0.975	tgtcTAATtgctt atga	102
V\$GABF/GAGA.01	GAGA-Box	0.78	33 - 57	(-)	1.000	0.825	gtgggAGAGag agcatgcatgatca	103
V\$FKHD/FREAC2.01	Fork head related activator-2 (FOXF2)	0.84	52 - 68	(+)	1.000	0.884	icccacTAAAc atttat	104
V\$HOXF/HOXC13.01	Homeodomain transcription factor HOXC13	0.91	58 - 74	(-)	1.000	0.914	acattaaTAAAt gttta	105
V\$NKXH/HMX2.02	Hmx2/Nkx5-2 homeodomain transcription factor	0.82	58 - 72	(-)	0.750	0.835	attaatAAATggt ta	106
V\$SATB/SATB1.01	Special AT-rich sequence-binding protein 1, predominantly expressed in thymocytes, binds to matrix attachment regions (MARs)	0.94	58 - 72	(-)	1.000	0.956	attAATAaatggt ta	107
V\$BRNF/BRN3.02	Brn-3, POU-IV protein class	0.89	59 - 77	(-)	1.000	0.892	aacacatTAATa aatgttt	108
V\$PDX1/PDX1.01	Pdx1 (IDX1/IPF1) pancreatic and	0.74	59 - 79	(-)	1.000	0.744	ctaacacatTAA Taaatgttt	109

V\$PIT1/PIT1.01	intestinal homeodomain TF Pit1, GHF-1 pituitary specific pou domain transcription factor	0.84	61 - 73	(+)	1.000	0.857	acatTTATtaatg	110
V\$BRNF/BRN3.02	Brn-3, POU-IV protein class	0.89	62 - 80	(+)	1.000	0.893	catttatTAATgt	111
V\$LHXF/LMX1B.0 1	LIM- homeodomain transcription factor	0.91	62 - 76	(-)	1.000	0.946	gttagg acacatTAATAa atg	112
V\$HOXH/MEIS1B_ HOXA9.01	Meis1b and Hoxa9 form heterodimeric binding complexes on target DNA	0.78	64 - 78	(-)	0.750	0.823	TAACacattaat aaa	113
V\$HOXF/HOX1- 3.01	Hox-1.3, vertebrate homeobox protein	0.82	65 - 81	(+)	1.000	0.826	ttatTAATgtgtt agga	114
V\$OCT1/OCT1.04	Octamer-binding factor 1	0.80	77 - 91	(-)	0.846	0.866	ctAATGgaaatc cta	115
V\$HOXF/PHOX2.01	Phox2a (ARIX) and Phox2b	0.87	78 - 94	(-)	1.000	0.969	gcgcTAATgga aatcct	116
V\$AHRR/AHRARN T.01	Aryl hydrocarbon receptor / Arnt heterodimers	0.92	83 - 107	(+)	1.000	0.932	ttccattagcgCG TGccttgaactg	117
V\$MOKF/MOK2.02	Ribonucleoprotei n associated zinc finger protein MOK-2 (human)	0.98	85 - 105	(+)	1.000	0.988	ccattagcgcggtg CCTTgaac	118
V\$EBOX/MYCMA X.03	MYC-MAX binding sites	0.91	87 - 101	(-)	1.000	0.918	aaggcaCGCGc taat	119
V\$HESF/HELT.01	Hey-like bHLH- transcriptional repressor	0.91	87 - 101	(-)	1.000	0.947	aaggCACGcgc taat	120
V\$HOMF/EN1.01	Homeobox protein engrailed (en-1)	0.77	97 - 109	(+)	0.782	0.776	gccTTGAactg aa	121
V\$OCT1/OCT1.02	Octamer-binding factor 1	0.85	109 - 123	(-)	1.000	0.992	catATGCaaatg att	122
V\$OCTP/OCT1P.01	Octamer-binding factor 1, POU- specific domain	0.86	113 - 125	(-)	1.000	0.910	gccATATgcaa at	123
V\$AIRE/AIRE.01	Autoimmune regulator	0.86	119 - 145	(+)	0.916	0.862	atatggctgggaaa aagTGGGgtga gg	124
V\$RBPF/RBPJK.02	Mammalian transcriptional repressor RBP- Jkappa/CBF1	0.94	122 - 136	(+)	1.000	0.941	tggtGTTGGGaaa aagt	125
V\$RXRF/VDR_RX R.06	Bipartite binding site of VDR/RXR heterodimers: 4 spacer nucleotides	0.75	123 - 147	(+)	0.812	0.760	ggctgggaaaaag tgGGGTgaggg a	126

	between the two directly repeated motifs								
V\$NKXH/HMX3.01	H6 homeodomain HMX3/Nkx5.1 transcription factor	0.89	127 - 141	(+)	1.000	0.910	gggaaaAAGTg gggt	127	
V\$CIZF/NMP4.01	NMP4 (nuclear matrix protein 4) / CIZ (Cas- interacting zinc finger protein)	0.97	128 - 138	(+)	1.000	0.998	ggAAAAagtgg	128	
V\$EBOX/SREBP.01	Sterol regulatory element binding protein 1 and 2	0.90	132 - 146	(-)	1.000	0.960	cccTCACccca cttt	129	
V\$RXRF/VDR_RX R.02	VDR/RXR Vitamin D receptor RXR heterodimer site	0.86	134 - 158	(+)	1.000	0.878	agtggggtgagg GAGGaaacagt gc	130	
V\$ETSF/PU1.01	Pu.1 (Pu120) Ets- like transcription factor identified in lymphoid B- cells	0.89	141 - 157	(+)	1.000	0.895	tgagggaGGAA acagtg	131	
V\$NFAT/NFAT.01	Nuclear factor of activated T-cells	0.95	145 - 155	(+)	1.000	0.989	ggaGGAAaca g	132	
V\$AREB/AREB6.04	AREB6 (Atp1a1 regulatory element binding factor 6)	0.98	146 - 158	(-)	1.000	0.991	gcactGTTTcct c	133	
V\$COMP/COMP1.0 1	COMP1, cooperates with myogenic proteins in multicomponent complex	0.77	163 - 185	(-)	1.000	0.811	ctgtgATTGacg cctgtgggga	134	
V\$PAX6/PAX6.01	Pax-6 paired domain binding site	0.77	163 - 181	(-)	0.808	0.781	gaTTGAcgcct gttgggga	135	
V\$MYBL/CMYB.01	c-Myb, important in hematopoiesis, cellular equivalent to avian myoblastosis virus oncogene v-myb	0.90	165 - 177	(+)	1.000	0.945	ccCAACaggcg tc	136	
V\$CREB/CREB.02	cAMP- responsive element binding protein	0.89	167 - 187	(-)	1.000	0.902	cactgtgatTGA Cgcctgttg	137	
V\$WHZF/WHN.01	Winged helix protein, involved in hair keratinization and thymus epithelium differentiation	0.95	169 - 179	(-)	1.000	0.955	ttgACGCctgt	138	
V\$HOXC/PBX1.01	Homeo domain	0.78	170 - 186	(-)	1.000	0.840	actgtGATTgac	139	

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V\$PBXC/PBX1_MEIS1.02	factor Pbx-1 Binding site for a Pbx1/Meis1 heterodimer	0.77	170 - 186	(-)	1.000	0.875	gcctg actgTGATtgac gcctg	140
V\$AP1R/TCF11MAFG.01	TCF11/MafG heterodimers, binding to subclass of AP1 sites	0.81	177 - 201	(+)	1.000	0.838	caatcacagTGA Cagatcagatggt	141
V\$TALE/MEIS1.01	Binding site for monomeric Meis1 homeodomain protein	0.95	183 - 193	(-)	1.000	0.971	atcTGTCactg	142
V\$HOXH/MEIS1A_HOXA9.01	Meis1a and Hoxa9 form heterodimeric binding complexes on target DNA	0.77	186 - 200	(+)	1.000	0.770	TGACagatcag atgg	143
V\$GATA/GATA3.02	GATA-binding factor 3	0.91	187 - 199	(+)	1.000	0.950	gacAGATcaga tg	144
V\$AP4R/TAL1BETAE47.01	Tal-1beta/E47 heterodimer	0.87	189 - 205	(+)	1.000	0.955	cagatCAGAtg gtttct	145
V\$NEUR/NEUROG.01	Neurogenin 1 and 3 (ngn1/3) binding sites	0.92	191 - 203	(-)	1.000	0.925	aaaCCATctgat c	146
V\$ZBPF/ZBP89.01	Zinc finger transcription factor ZBP-89	0.93	205 - 227	(-)	1.000	0.966	agacccccccCC CCcttcagcca	147
V\$ZBPF/ZNF219.01	Kruppel-like zinc finger protein 219	0.91	207 - 229	(-)	1.000	0.997	gcagaccCCCC cccccttcagc	148
V\$INSM/INSM1.01	Zinc finger protein insulinoma-associated 1 (IA-1) functions as a transcriptional repressor	0.90	209 - 221nc	(+)	1.000	0.914	tgaagGGGGgg gg	149
V\$EKLF/KKLF.01	Kidney-enriched kruppel-like factor, KLF15	0.91	210 - 226nc	(+)	1.000	0.934	gaagggGGGG gggggtc	150
V\$EGRF/WT1.01	Wilms Tumor Suppressor	0.92	211 - 227nc	(+)	0.837	0.945	aagggGGGGg ggggtct	151
V\$SP1F/GC.01	GC box elements	0.88	211 - 225nc	(+)	0.819	0.897	aagggGGGGg ggggt	152
V\$EKLF/KKLF.01	Kidney-enriched kruppel-like factor, KLF15	0.91	212 - 228nc	(+)	1.000	0.949	agggggGGGG gggtctg	153
V\$SP1F/GC.01	GC box elements	0.88	213 - 227nc	(+)	0.819	0.908	gggggGGGGg ggtct	154
V\$EGRF/WT1.01	Wilms Tumor Suppressor	0.92	214 - 230nc	(+)	0.837	0.932	gggggGGGGg gtctgtct	155
V\$GLIF/ZIC2.01	Zinc finger transcription factor, Zic family member 2 (odd-paired homolog, Drosophila)	0.89	214 - 228	(-)	1.000	0.967	cagacccCCCC cccc	156

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V\$MAZF/MAZR.01	MYC-associated zinc finger protein related transcription factor	0.88	215 – 227nc	(+)	1.000	0.972	ggggggGGGG tct	157
V\$AP1R/BACH2.01	Bach2 bound TRE	0.89	221 - 245	(-)	0.813	0.897	gagtgttgcTGG Gtcagcagacccc	158
V\$AP1R/VMAF.01	v-Maf	0.82	221 - 245	(+)	1.000	0.957	gggggtctgcTGA Cccagcaacactc	159
V\$XBBF/MIF1.01	MIBP-1 / RFX1 complex	0.76	225 - 243	(-)	0.800	0.778	gtgttgctggGTC Agcaga	160
V\$XBBF/RFX1.01	X-box binding protein RFX1	0.89	227 - 245	(+)	1.000	0.907	tgctgacccaGC AAcactc	161
V\$NFAT/NFAT.01	Nuclear factor of activated T-cells	0.95	243 - 253	(-)	1.000	0.971	gaaGGAAaga g	162
V\$NKXH/HMX2.01	Hmx2/Nkx5-2 homeodomain transcription factor	0.83	253 - 267	(-)	1.000	0.911	gctCTTAagcct cag	163
V\$PAX8/PAX8.01	PAX 2/5/8 binding site	0.88	254 - 266	(-)	0.800	0.901	ctcTTAAgcctc a	164
V\$NKXH/HMX2.01	Hmx2/Nkx5-2 homeodomain transcription factor	0.83	256 - 270	(+)	1.000	0.931	aggCTTAagag ctat	165
V\$HOXF/PHOX2.01	Phox2a (ARIX) and Phox2b	0.87	260 - 276	(-)	1.000	0.898	acgcTAATagc tcttaa	166
V\$CLOX/CDPCR3.01	Cut-like homeodomain protein	0.73	266 - 284	(-)	0.880	0.770	agtcacctacgcta ATAGc	167
V\$EGRF/NGFIC.01	Nerve growth factor-induced protein C	0.80	269 - 285	(+)	1.000	0.855	attaGCGTaggt gactc	168
V\$AP1R/BACH2.01	Bach2 bound TRE	0.89	271 - 295	(-)	1.000	0.957	attagggacTGA Gtcacctacgcta	169
V\$CREB/TAXCRE B.02	Tax/CREB complex	0.71	274 - 294	(+)	1.000	0.744	cgtaggTGACtc agtcctaa	170
V\$AP1F/AP1.01	Activator protein 1	0.94	278 - 288	(+)	0.904	0.967	ggtgACTCagt	171
V\$AP1F/AP1.03	Activator protein 1	0.94	278 - 288	(-)	1.000	0.976	acTGAGtcacc	172
V\$HOXF/CRX.01	Cone-rod B homeobox-containing transcription factor / otx-like homeobox gene	0.94	288 - 304	(+)	1.000	0.972	tcccTAATcctc cattc	173
V\$SORY/HBP1.01	HMG box-containing protein 1	0.86	298 - 310	(-)	1.000	0.905	ggcattgAATG ga	174
V\$IRFF/IRF7.01	Interferon regulatory factor 7 (IRF-7)	0.86	329 - 347	(+)	0.936	0.865	caGAATcagaa cctccacc	175
V\$RORA/RORA1.01	RAR-related orphan receptor alpha1	0.93	342 - 360	(-)	1.000	0.953	ttaatgaGGTCa aggtgga	176
V\$CSEN/DREAM.01	Downstream regulatory element-antagonist	0.95	344 - 354	(-)	1.000	0.960	agGTCAaggtg	177

	modulator, Ca²⁺-binding protein of the neuronal calcium sensors family that binds DRE (downstream regulatory element) sites as a tetramer								
V\$E4FF/E4F.01	GLI-Krueppel- related transcription factor, regulator of adenovirus E4 promoter	0.82	345 - 357	(-)	0.789	0.824	atgAGGTcaag gt	178	
V\$HOXF/BARX2.0 1	Barx2, homeobox transcription factor that preferentially binds to paired TAAT motifs	0.95	347 - 363	(-)	1.000	0.980	gcgtTAATgag gtcaag	179	
V\$MYBL/VMYB.04	v-Myb, AMV v- myb	0.85	356 - 368	(+)	1.000	0.881	attAACGctggt c	180	
V\$HOXF/CRX.01	Cone-rod (C) homeobox- containing transcription factor / otx-like homeobox gene	0.94	366 - 382	(+)	1.000	0.962	gtctTAATcacc aagcc	181	
V\$RCAT/CLTR_CA AT.01	Mammalian C- type LTR CCAAT box	0.71	375 - 399	(+)	1.000	0.718	aCCAAgccaag ctccttaaactgct	182	
V\$ETSF/ETS1.01	c-Ets-1 binding site	0.92	409 - 425	(-)	1.000	0.921	actaggaGGAA gcctag	183	
V\$SF1F/FTF.01	Alpha (1)- fetoprotein transcription factor (FTF), liver receptor homologue-1 (LRH-1)	0.94	426 - 438	(-)	1.000	0.940	gggcCAAGgtg ac	184	
V\$BCL6/BCL6.02	POZ/zinc finger protein, transcriptional repressor, translocations observed in diffuse large cell lymphoma	0.77	436 - 452	(+)	1.000	0.785	ccccctctTAGAa gcca	185	
V\$HOXF/GSH2.01	Homeodomain transcription factor Gsh-2	0.95	443 - 459	(-)	1.000	1.000	ggccTAATtgg cttcta	186	
V\$CAAT/CAAT.01	Cellular and viral CCAAT box	0.90	445 - 459	(+)	1.000	0.949	gaagCCAAtta ggcc	187	
V\$NKXH/NKX25.0 2	Homeo domain factor Nkx- 2.5/Csx, tinman	0.88	446 - 460	(-)	1.000	0.938	gggccTAATtg gctt	188	

V\$HOMF/S8.01	homolog low affinity sites Binding site for S8 type	0.97	448 - 460	(-)	1.000	0.999	gggccTAATtg gc	189
V\$HOXF/CRX.01	homeodomains Cone-rod (D) homeobox- containing transcription factor / otx-like homeobox gene	0.94	470 - 486	(-)	1.000	0.985	atatTAATcccc gctgc	190
V\$MZF1/MZF1.01	Myeloid zinc finger protein MZF1	0.99	473 - 481	(+)	1.000	0.991	gcGGGGatt	191
V\$OCTB/TST1.01	POU-factor Tst- 1/Oct-6	0.90	475 - 487	(+)	1.000	0.947	ggggATTAAat at	192
V\$CART/CART1.01	Cart-1 (cartilage homeoprotein 1)	0.86	477 - 493	(-)	1.000	0.926	caTAATcatatt aatcc	193
V\$CART/CART1.01	Cart-1 (cartilage homeoprotein 1)	0.86	479 - 495	(+)	1.000	0.914	atTAATatgatta tgaa	194
V\$SATB/SATB1.01	Special AT-rich sequence-binding protein 1, predominantly expressed in thymocytes, binds to matrix attachment regions (MARs)	0.94	479 - 493	(+)	1.000	0.957	attAATAtgatta tg	195
V\$PDX1/PDX1.01	Pdx1 (IDX1/IPF1) pancreatic and intestinal homeodomain TF	0.74	480 - 500	(+)	0.826	0.775	ttaatatgaTTAT gaacaccc	196
V\$GLIF/ZIC2.01	Zinc finger transcription factor, Zic family member 2 (odd- paired homolog, Drosophila)	0.89	491 - 505	(+)	1.000	0.932	atgaacaCCCCc aat	197
V\$CAAT/ACAAT.0 1	Avian C-type LTR CCAAT box	0.83	497 - 511	(+)	1.000	0.905	acccCCAAtctc cca	198
V\$RREB/RREB1.01	Ras-responsive element binding protein 1	0.80	499 - 513	(+)	1.000	0.841	cCCCAatctccc aga	199
V\$NRSF/NRSF.01	Neuron- restrictive silencer factor	0.69	500 - 520	(-)	1.000	0.696	atcAGCAtctgg gagattggg	200
V\$IKRS/LYF1.01	LyF-1 (Ikaros 1), enriched in B and T lymphocytes	0.98	502 - 514	(-)	1.000	1.000	atcTGGGagatt g	201
V\$AP4R/TAL1ALP HAE47.01	Tal-1alpha/E47 heterodimer	0.87	505 - 521	(+)	1.000	0.905	tctccCAGAtgc tgatt	202
V\$RP58/RP58.01	Zinc finger protein RP58 (ZNF238), associated preferentially with	0.84	507 - 519	(-)	1.000	0.865	tcagCATCtggg a	203

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V\$AP1R/NFE2.01	heterochromatin NF-E2 p45	0.85	508 - 532	(+)	1.000	0.904	cccagatgCTG Attcagccaggag c	204
V\$AP1R/NFE2.01	NF-E2 p45	0.85	508 - 532	(-)	1.000	0.882	gctcctggCTGA atcagcatctggg	205
V\$BEL1/BEL1.01	Bel-1 similar region (defined in Lentivirus LTRs)	0.81	510 - 532	(-)	1.000	0.818	gctcctggctgaaT CAGcatctg	206
V\$NRLF/NRL.01	Neural retinal basic leucine zipper factor (bZIP)	0.85	511 - 529	(-)	1.000	0.991	cctggCTGAatc agcatct	207
V\$AP1F/AP1.03	Activator protein 1	0.94	515 - 525	(+)	0.885	0.970	gcTGATtcagc	208
V\$AP1F/AP1.03	Activator protein 1	0.94	515 - 525	(-)	0.857	0.963	gcTGAAtcagc	209
V\$HOXF/PTX1.01	Pituitary Homeobox 1 (Ptx1, Pitx-1)	0.94	523 - 539	(-)	1.000	0.944	ctcCTAAgctcc tggt	210
V\$ZBPF/ZNF219.01	Kruppel-like zinc finger protein 219	0.91	528 - 550	(-)	1.000	0.926	gtgacctCCCCc tcctaagctcc	211
V\$RXRF/VDR_RX R.01	VDR/RXR Vitamin D receptor RXR heterodimer site	0.85	531 - 555	(+)	1.000	0.889	gcttaggaggggG AGGtcactttat	212
V\$ZBPF/ZBP89.01	Zinc finger transcription factor ZBP-89	0.93	531 - 553	(-)	1.000	0.958	aaagtgacctCC CCctcctaagc	213
V\$EKLF/KKLF.01	Kidney-enriched kruppel-like factor, KLF15	0.91	534 - 550	(+)	1.000	0.913	taggagGGGGa ggtcac	214
V\$GLIF/ZIC2.01	Zinc finger transcription factor, Zic family member 2 (odd- paired homolog, Drosophila)	0.89	536 - 550	(-)	1.000	0.945	gtgacctCCCCc tcc	215
V\$RORA/TR2.01	Nuclear hormone receptor TR2, half site	0.92	538 - 556	(+)	1.000	0.950	agggggaGGTC actttata	216
V\$TBPF/TATA.01	Cellular and viral TATA box elements	0.90	543 - 559	(-)	1.000	0.915	ccttaTAAAgtg acctc	217
V\$SRFF/SRF.01	Serum response factor	0.66	545 - 563	(-)	1.000	0.722	agaccctTATAa agtgacc	218
V\$SRFF/SRF.01	Serum response factor	0.66	546 - 564	(+)	1.000	0.712	gtcactTATAa gggtctg	219
V\$TBPF/LTATA.01	Lentivirus LTR TATA box	0.82	550 - 566	(+)	1.000	0.829	cttTATAagggt ctggg	220
V\$MOKF/MOK2.01	Ribonucleoprotei n associated zinc finger protein MOK-2 (mouse)	0.74	552 - 572	(-)	1.000	0.772	gacccccccagac CCTTataa	221
V\$ZBPF/ZNF219.01	Kruppel-like zinc finger protein 219	0.91	553 - 575	(-)	1.000	0.948	tctgaccCCCCc agacccttata	222
V\$GLIF/ZIC2.01	Zinc finger	0.89	560 - 574	(-)	1.000	0.967	ctgacccCCCCa	223

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	transcription factor, Zic family member 2 (odd-paired homolog, Drosophila)						gac	
V\$MAZF/MAZR.01	MYC-associated zinc finger protein related transcription factor	0.88	561 - 573	(+)	1.000	0.919	tctgggGGGgta	224
V\$ZNFP/SZF1.01	SZF1, hematopoietic progenitor-restricted KRAB-zinc finger protein	0.82	579 - 603	(-)	0.801	0.829	tcaGGGctccagctggatgactctg	225
V\$AP4R/AP4.01	Activator protein 4	0.85	584 - 600UTR	(+)	1.000	0.916	tcataCAGctggagccc	226
V\$EBOX/ATF6.01	Member of b-zip family, induced by ER damage/stress, binds to the ERSE in association with NF-Y	0.93	596 - 610	(-)	1.000	0.970	cagCCACtcaggct	227
V\$CAAT/CAAT.01	Cellular and viral CCAAT box	0.90	597 - 611	(-)	0.826	0.937	tcagCCACtcaggc	228
V\$HEAT/HSF1.01	Heat shock factor 1	0.84	621 - 645	(-)	1.000	0.857	tgctcccaccaAGAAtgctgcgaacaGCATtcttgggtggg	229
V\$OAZF/ROAZ.01	Rat C2H2 Zn finger protein involved in olfactory neuronal differentiation	0.73	625 - 641	(+)	0.750	0.779		230
V\$OAZF/ROAZ.01	Rat C2H2 Zn finger protein involved in olfactory neuronal differentiation	0.73	626 - 642nc	(-)	0.750	0.744	tcCCACccaagaatgct	231
V\$EGRF/EGR2.01	Egr-2/Krox-20 early growth response gene product	0.79	631 - 647UTR	(+)	0.766	0.828	tcttGGGTgggagcagc	232
V\$EGRF/WT1.01	Wilms Tumor Suppressor	0.92	633 - 649	(+)	1.000	0.930	ttgggTGGGagcagcca	233
V\$RBPF/RBPJK.01	Mammalian transcriptional repressor RBP-Jkappa/CBF1	0.84	634 - 648UTR	(+)	1.000	0.847	tgggTGGGagcagcc	234
V\$OAZF/ROAZ.01	Rat C2H2 Zn finger protein involved in olfactory neuronal differentiation	0.73	641 - 657	(+)	0.750	0.818	gaGCAGccacgggtcag	235
V\$EBOX/USF.03	Upstream stimulating factor	0.89	643 - 657	(-)	1.000	0.904	ctgaccCGTGgctgc	236

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V\$EBOX/MYCMA X.03	MYC-MAX binding sites	0.91	644 - 658	(+)	0.842	0.919	cagccaCGGGt cagc	237
V\$PAX5/PAX5.03	PAX5 paired domain protein	0.80	659 – 687gen	(+)	0.894	0.833	cacaagggCCA Cagccatgaatgg cacag	238
V\$CLOX/CDPCR3. 01	Cut-like homeodomain protein	0.73	665 – 683gen	(+)	1.000	0.735	ggccacagccatg aATGGc	239
V\$PAX5/PAX5.03	PAX5 paired domain protein	0.80	674 – 702gen	(+)	1.000	0.800	catgaatgGCAC agaagtcctgagcc cca	240
V\$ZNFP/ZBRK1.01	Transcription factor with 8 central zinc fingers and an N- terminal KRAB domain	0.77	680 - 704	(-)	0.813	0.847	catggggcTCA Ggacttctgtgcc	241
V\$PBXC/PBX1_ME IS1.02	Binding site for a Pbx1/Meis1 heterodimer	0.77	699 - 715	(-)	0.750	0.860	attcTGTTtgaca tggg	242
V\$TALE/TGIF.01	TG-interacting factor belonging to TALE class of homeodomain factors	1.00	700 – 710nc	(+)	1.000	1.000	ccatGTCAaac	243
V\$SNAP/PSE.02	Proximal sequence element (PSE) of RNA polymerase III- transcribed genes	0.73	745 - 763	(+)	1.000	0.734	gtgccCCTAatc ctcaact	244
V\$HOXF/CRX.01	Cone-rod (E) homeobox- containing transcription factor / otx-like homeobox gene	0.94	748 - 764	(+)	1.000	0.965	ccccTAATcctc aacta	245
V\$FAST/FAST1.01	FAST-1 SMAD interacting protein	0.81	749 - 763	(-)	0.983	0.829	agttgagGATTa ggg	246
V\$NR2F/HPF1.01	HepG2-specific P450 2C factor-1	0.78	767 - 787	(+)	0.750	0.801	ctaggcgcAAA Ttccaatcct	247
V\$SORY/HMGIY.0 1	HMGI(Y) high- mobility-group protein I (Y), architectural transcription factor organizing the framework of a nuclear protein- DNA transcriptional complex	0.92	770 - 782	(-)	1.000	0.938	tggAATTtgccg c	248
V\$HMTB/MTBF.01	Muscle-specific Mt binding site	0.90	774 - 782	(-)	1.000	0.953	tggaATTTg	249
V\$LEFT/LEF1.01	TCF/LEF-1, involved in the Wnt signal transduction pathway	0.86	783 - 799	(-)	1.000	0.889	actagacCAAA gaggat	250
V\$NRSF/NRSE.01	Neural-	0.67	794 –	(+)	0.782	0.762	tctagtacccCGG	251

	restrictive-silencer-element		814nc				Gggcagcc	
V\$ZBPF/ZF9.01	Core promoter-binding protein (CPBP) with 3 Krueppel-type zinc fingers	0.87	803 – 825nc	(+)	0.769	0.878	ccgggggCAG Cccccctaacct	252
V\$HICF/HIC1.01	Hypermethylated in cancer 1, transcriptional repressor containing five Krüppel-like C2H2 zinc fingers, for optimal binding multiple binding sites are required.	0.93	804 - 816	(-)	1.000	0.970	ggggcTGCCcc cg	253
V\$NFKB/NFKAPP AB50.01	NF-kappaB (p50)	0.83	806 - 818	(-)	0.750	0.865	aggGGGctgcc cc	254
V\$STAF/ZNF76_14 3.01	ZNF143 is the human ortholog of Xenopus Staf, ZNF76 is a DNA binding protein related to ZNF143 and Staf	0.76	810 - 832	(+)	0.809	0.761	cagcCCCCtcta acctgggcct	255
V\$SF1F/SF1.01	SF1 steroidogenic factor 1	0.95	819 - 831	(-)	1.000	0.966	ggccCAAGgtt ag	256
V\$RXRF/VDR_RX R.06	Bipartite binding site of VDR/RXR heterodimers; 4 spacer nucleotides between the two directly repeated motifs	0.75	825 – 849nc	(+)	0.812	0.787	ttgggcctcagcag cAGGGgaggc c	257
V\$MYOD/MYF5.01	Myf5 myogenic bHLH protein	0.90	831 – 847nc	(+)	1.000	0.903	ctcagCAGCag gggagg	258
V\$NRSF/NRSF.01	Neuron-restrictive silencer factor	0.69	831 – 851nc?	(+)	1.000	0.705	ctcAGCAGcag gggaggccac	259
V\$ZBPF/ZF9.01	Core promoter-binding protein (CPBP) with 3 Krueppel-type zinc fingers	0.87	832 - 854	(-)	0.820	0.890	gggggtggCCTC ccctgctgctga	260
V\$ZBPF/ZF9.01	Core promoter-binding protein (CPBP) with 3 Krueppel-type zinc fingers	0.87	841 – 863nc	(+)	0.923	0.937	ggggaggCCA Cccctacacctc	261
V\$PLAG/PLAG1.01	Pleomorphic adenoma gene (PLAG) 1, a developmentally regulated C2H2 zinc finger	0.88	847 - 867	(-)	0.958	0.929	GGGGgaaggtg taggggtggc	262

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V\$ZBPF/ZNF202.01	protein Transcriptional repressor, binds to elements found predominantly in genes that participate in lipid metabolism	0.73	859 - 881	(+)	1.000	0.776	ccttccCCCAgc cacagccatcc	263
V\$INSM/INSM1.01	Zinc finger protein insulinoma-associated 1 (IA-1) functions as a transcriptional repressor	0.90	860 - 872	(-)	1.000	0.965	tggctGGGGga ag	264
V\$MZF1/MZF1.02	Myeloid zinc finger protein MZF1	0.99	860 - 868	(-)	1.000	0.994	tgGGGgaag	265
V\$HAML/AML3.01	Runt-related transcription factor 2 / CBFA1 (core-binding factor, runt domain, alpha subunit 1)	0.84	863 - 877	(-)	1.000	0.845	ggctGTGGctg gggg	266
V\$NRF1/NRF1.01	Nuclear respiratory factor 1 (NRF1), bZIP transcription factor that acts on nuclear genes encoding mitochondrial proteins	0.78	889 - 905nc	(-)	0.750	0.828	tctGCACaggc gctgct	267
V\$NRF1/NRF1.01	Nuclear respiratory factor 1 (NRF1), bZIP transcription factor that acts on nuclear genes encoding mitochondrial proteins	0.78	890 - 906nc	(+)	1.000	0.801	gcaGCGCctgt gcagaa	268
V\$SORY/HBP1.01	HMG box-containing protein 1	0.86	898 - 910nc	(+)	1.000	0.862	tgtgcagAATG aa	269
V\$BRNF/BRN2.03	Brn-2, POU-III protein class	0.92	923 - 941	(+)	1.000	0.932	ggctccttaATT Tttttt	270
V\$NKXH/NKX25.02	Homeo domain factor Nkx-2.5/Csx, tinman homolog low affinity sites	0.88	925 - 939	(+)	1.000	0.956	ctcctTAATtttt t	271
V\$CDXF/CDX1.01	Intestine specific homeodomain factor CDX-1	0.94	939 - 957	(+)	1.000	0.948	ttttttTTTAaga aataa	272
V\$HOXF/HOXB9.01	Abd-B-like homeodomain protein Hoxb-9	0.88	940 - 956	(-)	1.000	0.888	tatttctTAAaaa aaaa	273

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V\$CEBP/CEBPB.01	CCAAT/enhancer binding protein beta	0.94	943 - 957nc	(+)	1.000	0.942	tttttaaGAAAtaa	274
V\$HNF1/HNF1.01	Hepatic nuclear factor 1	0.80	947 - 963	(-)	0.790	0.824	cATTAAattattcttaa	275
V\$HOXF/BARX2.01	Barx2, homeobox transcription factor that preferentially binds to paired TAAT motifs	0.95	948 - 964	(-)	1.000	0.967	tcaTAAATtatttc tta	276
V\$BRNF/BRN3.01	Brn-3, POU-IV protein class	0.78	949 - 967	(-)	1.000	0.990	gcctcattaATTAttctt	277
V\$BRNF/BRN4.01	POU domain transcription factor brain 4	0.89	949 - 967nc	(+)	1.000	0.894	aagaaataatTAATgaggc	278
V\$LHXF/LMX1B.01	LIM-homeodomain transcription factor	0.91	949 - 963nc	(+)	1.000	0.962	aagaaaTAATta atg	279
V\$HOMF/S8.01	Binding site for S8 type homeodomains	0.97	950 - 962nc	(+)	1.000	0.997	agaaaTAATtaaa t	280
V\$HOXF/GSH1.01	Homeobox transcription factor Gsh-1	0.85	952 - 968nc	(+)	1.000	0.863	aaataatTAATgaggct	281
V\$LHXF/LMX1B.01	LIM-homeodomain transcription factor	0.91	952 - 966	(-)	1.000	0.946	cctcatTAATtat tt	282
V\$RBIT/BRIGHT.01	Bright, B cell regulator of IgH transcription	0.92	952 - 964nc	(+)	1.000	0.961	aaataATTAatga	283
V\$HOMF/S8.01	Binding site for S8 type homeodomains	0.97	953 - 965	(-)	1.000	0.992	ctcatTAATtatt	284
V\$LHXF/LHX3.01	Homeodomain binding site in LIM/Homeodomain factor LHX3	0.81	953 - 967nc	(+)	1.000	0.851	aataaTTAAtgaggc	285
V\$SORY/HBP1.01	HMG box-containing protein 1	0.86	953 - 965nc	(+)	1.000	0.876	aataattAATGagg	286
V\$HOXF/BARX2.01	Barx2, homeobox transcription factor that preferentially binds to paired TAAT motifs	0.95	955 - 971nc	(+)	1.000	0.987	taafTAATgaggctcct	287
V\$RXRF/VDR_RXR.02	VDR/RXR Vitamin D receptor RXR heterodimer site	0.86	960 - 984	(-)	1.000	0.871	tcccagggtgagtGAGGagcctcatt	288
V\$APIF/API.03	Activator protein 1	0.94	969 - 979	(-)	1.000	0.940	ggTGAGtgagg	289
V\$AREB/AREB6.01	AREB6 (Atp1a1 regulatory element binding	0.93	972 - 984nc	(+)	1.000	0.933	cactcACCTggga	290

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V\$PAX6/PAX6.02	factor 6) PAX6 paired domain and homeodomain are required for binding to this site	0.89	973 - 991	(-)	1.000	0.893	caggctgtcCCA Ggtgagt	291
V\$AP2F/AP2.01	Activator protein 2	0.90	1033 - 1047	(-)	1.000	0.911	ctgGCCTtggg gaac	292
V\$EREF/ERR.01	Estrogen related receptor	0.87	1033 - 1051nc	(+)	1.000	0.897	gttccccAAGG ccagcggg	293
V\$MZF1/MZF1.02	Myeloid zinc finger protein MZF1	0.99	1033 - 1041	(-)	1.000	0.994	ttGGGGaac	294
V\$SF1F/SF1.01	SF1 steroidogenic factor 1	0.95	1035 - 1047nc	(+)	1.000	0.992	tcccCAAGgcc ag	295
V\$TEAF/TEF.01	Thyrotrophic embryonic factor	0.88	1044 - 1060	(-)	0.968	0.894	ggcacaCATCc cgctgg	296
V\$SP1F/TIEG.01	TGFbeta- inducible early gene (TIEG) / Early growth response gene alpha (EGRalpha)	0.83	1046 - 1060nc	(+)	0.750	0.878	agcGGGAtgtgt gcc	297
V\$MAZF/MAZ.01	Myc associated zinc finger protein (MAZ)	0.90	1056 - 1068	(-)	1.000	0.909	ggagGAGGgg cac	298
V\$RXRF/VDR_RX R.03	Bipartite binding site of VDR/RXR heterodimers without a spacer between directly repeated motifs	0.74	1056 - 1080	(-)	0.823	0.750	gatgAGTTggg aggaggaggggc ac	299
V\$EVII/MEL1.02	MEL1 (MDS1/EVII- like gene 1) DNA-binding domain 2	0.99	1071 - 1087	(-)	1.000	0.997	cctgaaaGATG agttgg	300
V\$HEAT/HSF1.01	Heat shock factor 1	0.84	1073 - 1097	(-)	0.857	0.849	tcctcgtgtccTG AAagatgagtt	301
V\$MYT1/MYT1L.0 1	Myelin transcription factor 1-like, neuronal C2HC zinc finger factor 1	0.92	1073 - 1085	(-)	0.818	0.927	tgaaAGATgag tt	302
V\$STAT/STAT1.01	Signal transducer and activator of transcription 1	0.77	1075 - 1093	(-)	0.767	0.774	cgtgttcctGAA Agatgag	303
V\$STAT/STAT.01	Signal transducers and activators of transcription	0.87	1077 - 1095	(+)	1.000	0.911	catctttcaGGA Acacgag	304
V\$EBOX/NMYC.01	N-Myc	0.92	1085 - 1099	(-)	1.000	0.923	aatcctCGTGttc ct	305
V\$HEAT/HSF2.02	Heat shock factor 2	0.95	1089 - 1113	(-)	1.000	0.967	ttccagaaagcaA GAAtcctcgtgt	306

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V\$HEAT/HSF1.01	Heat shock factor 1	0.84	1097 - 1121	(-)	1.000	0.874	ggacacttttccA	307
V\$STAT/STAT1.01	Signal transducer and activator of transcription 1	0.77	1099 - 1117	(-)	0.767	0.798	GAAagcaagaat acttttccaGAA	308
V\$STAT/STAT.01	Signal transducers and activators of transcription	0.87	1101 - 1119	(+)	1.000	0.895	ttgctttctGGAA aagtgt	309
V\$BCL6/BCL6.02	POZ/zinc finger protein, transcriptional repressor, translocations observed in diffuse large cell lymphoma	0.77	1102 - 1118	(+)	0.800	0.808	tgctttcTGGAA aagtgt	310
V\$BNCF/BNC.01	Basonuclin, cooperates with USF1 in rDNA PolII transcription)	0.85	1107 - 1125	(+)	1.000	0.852	tctggaaaagTG TCccagc	311
V\$GATA/GATA2.01	GATA-binding factor 2	0.92	1127 - 1139	(+)	1.000	0.938	taggGATAagtg	312
V\$NKXH/NKX32.01	Homeodomain protein NKX3.2 (BAPX1, NKX3B, Bagpipe homolog)	0.96	1128 - 1142	(+)	1.000	0.962	agggataAGTG tcta	313
V\$PAX1/PAX1.01	Pax1 paired domain protein, expressed in the developing vertebral column of mouse embryos	0.62	1135 - 1153	(-)	0.750	0.696	cCATTctgtgtct agacact	314
V\$SORY/HBP1.01	HMG box-containing protein 1	0.86	1142 - 1154nc	(+)	1.000	0.860	agcacagAATG gg	315
V\$NKXH/NKX25.02	Homeo domain factor Nkx-2.5/Csx, tinman homolog low affinity sites	0.88	1166 - 1180	(+)	1.000	0.898	gtgctTAATAaa tgc	316
V\$HOXF/HOXC13.01	Homeodomain transcription factor HOXC13	0.91	1167 - 1183	(+)	1.000	0.944	tgcttaaTAAAt gctgg	317
V\$HOXC/HOX_PB X.01	HOX/PBX binding sites	0.81	1178 - 1194	(+)	0.944	0.862	tgctGGATggat gcagg	318
V\$AIRE/AIRE.01	Autoimmune regulator	0.86	1184 - 1210	(+)	1.000	0.877	atggatgcaggaa ggaaTGGAgga atg	319
V\$ETSF/ELF2.01	Ets - family member ELF-2 (NERF1a)	0.90	1186 - 1202	(+)	1.000	0.933	ggatgcaGGAA ggaatg	320
V\$GKLF/GKLF.01	Gut-enriched Krueppel-like factor	0.86	1191 - 1203	(+)	0.779	0.864	caggaaggaAT GG	321
V\$SORY/HBP1.01	HMG box-	0.86	1192 -	(+)	1.000	0.904	aggaaggAATG	322

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	containing protein 1		1204				ga	
V\$TEAF/TEF1.01	TEF-1 related muscle factor	0.84	1192 - 1208	(-)	1.000	0.859	ttccctCATTcct	323
V\$ETSF/PU1.01	Pu.1 (Pu120) Ets-like transcription factor identified in lymphoid B-cells	0.89	1198 - 1214	(+)	1.000	0.899	tcct gaatggaGGAA tgaatg	324
V\$SORY/HBP1.01	HMG box-containing protein 1	0.86	1200 - 1212	(+)	1.000	0.916	atggaggAATG aa	325
V\$TEAF/TEF1.01	TEF-1 related muscle factor	0.84	1200 - 1216	(-)	1.000	0.884	cccattCATTcct	326
V\$SORY/HBP1.01	HMG box-containing protein 1	0.86	1204 - 1216	(+)	1.000	0.949	ccat aggaatgAATG gg	327
V\$IRFF/IRF7.01	Interferon regulatory factor 7 (IRF-7)	0.86	1208 - 1226nc	(+)	0.936	0.885	atGAATgggaa ggtctaga	328
V\$RBP/RBPJK.02	Mammalian transcriptional repressor RBP-Jkappa/CBF1	0.94	1209 - 1223nc	(+)	1.000	0.942	tgaaTGGGaag gtct	329
V\$IKRS/IK1.01	Ikaros 1, potential regulator of lymphocyte differentiation	0.92	1210 - 1222nc	(+)	1.000	0.925	gaatGGAaagg tc	330
V\$RORA/NBRE.01	Monomers of the nur subfamily of nuclear receptors (nur77, nurr1, nor-1)	0.89	1212 - 1230nc	(+)	1.000	0.947	atgggAAGGtct agagcat	331
V\$ZFIA/ZID.01	Zinc finger with interaction domain	0.85	1225 - 1237	(-)	1.000	0.916	agGCTCcatgct c	332
V\$AIRE/AIRE.01	Autoimmune regulator	0.86	1238 - 1264	(-)	0.916	0.863	atgtgggcgggtg agcaTGGCttct ag	333
V\$EGRF/WT1.01	Wilms Tumor Suppressor	0.92	1246 - 1262	(-)	0.953	0.930	gtgggCGGGtg agcatg	334
V\$SP1F/SP1.01	Stimulating protein 1, ubiquitous zinc finger transcription factor	0.88	1250 - 1264	(-)	1.000	0.907	atgtGGGCgggt gag	335
V\$NKXH/HMX3.02	Hmx3/Nkx5-1 homeodomain transcription factor	0.92	1258 - 1272	(-)	1.000	0.933	ttaaTTAAatgtg gg	336
V\$CREB/E4BP4.01	E4BP4, bZIP domain, transcriptional repressor	0.80	1259 - 1279	(+)	0.758	0.801	ccacatttaaTTA Acagctga	337
V\$BRNF/BRN3.02	Brn-3, POU-IV protein class	0.89	1260 - 1278	(-)	1.000	0.940	cagctgtTAATt aatgtg	338
V\$LHXF/LHX3.01	Homeodomain binding site in	0.81	1260 - 1274	(+)	1.000	0.944	cacatTTAAtta aca	339

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	LIM/Homeodom ain factor LHX3							
V\$OCT1/OCT1.05	Octamer-binding factor 1	0.89	1260 - 1274	(+)	0.900	0.942	caCATTtaattaa ca	340
V\$HOMF/S8.01	Binding site for S8 type homeodomains	0.97	1261 - 1273	(+)	1.000	0.997	acattTAATtaa c	341
V\$HOXF/PHOX2.01	Phox2a (ARIX) and Phox2b	0.87	1262 - 1278	(+)	1.000	0.877	cattTAATtaac agctg	342
V\$NKXH/NKX25.0 2	Homeo domain factor Nkx- 2.5/Csx, tinman homolog low affinity sites	0.88	1262 - 1276	(-)	1.000	0.898	gctgtTAATtaa atg	343
V\$PBXC/PBX1_ME IS1.02	Binding site for a Pbx1/Meis1 heterodimer	0.77	1262 - 1278	(+)	0.750	0.781	cattTAATtaac agctg	344
V\$RBIT/BRIGHT.0 1	Bright, B cell regulator of IgH transcription	0.92	1262 - 1274	(-)	1.000	0.967	tgtaATTAAatg	345
V\$FAST/FAST1.01	FAST-1 SMAD interacting protein	0.81	1263 - 1277	(-)	0.850	0.845	agctgttAATTa aat	346
V\$LHXF/LHX3.01	Homeodomain binding site in LIM/Homeodom ain factor LHX3	0.81	1263 - 1277	(-)	1.000	0.870	agctgTTAAtta aat	347
V\$RBIT/BRIGHT.0 1	Bright, B cell regulator of IgH transcription	0.92	1263 - 1275	(+)	1.000	0.941	atttaATTAAaca g	348
V\$ZNFP/SZF1.01	SZF1, hematopoietic progenitor- restricted KRAB- zinc finger protein	0.82	1263 - 1287	(-)	0.875	0.866	tcaGGGActca gctgtaataaat	349
V\$ATBF/ATBF1.01	AT-binding transcription factor 1	0.79	1264 - 1280	(-)	1.000	0.812	ctcagctgttAAT Taaa	350
V\$HOMF/S8.01	Binding site for S8 type homeodomains	0.97	1264 - 1276	(-)	1.000	0.997	gctgtTAATtaa a	351
V\$HEN1/HEN1.02	HEN1	0.81	1265 - 1285	(-)	1.000	0.845	agggactcaGCT Gttaattaa	352
V\$NKXH/HMX3.02	Hmx3/Nkx5-1 homeodomain transcription factor	0.92	1265 - 1279	(+)	1.000	0.927	ttaaTTAAcagc tga	353
V\$AP4R/AP4.02	Activator protein 4	0.92	1267 - 1283	(-)	1.000	0.950	ggactcAGCTgt taatt	354
V\$AP1R/NFE2.01	NF-E2 p45	0.85	1268 - 1292	(+)	1.000	0.865	attaacagCTGA gtccctgatgca	355
V\$BEL1/BEL1.01	Bel-1 similar region (defined in Lentivirus LTRs)	0.81	1270 - 1292	(-)	1.000	0.842	tgacatcaggac TCAGctgtta	356
V\$CREB/CREBP1.0 1	cAMP- responsive element binding protein 1	0.85	1278 - 1298	(-)	1.000	0.851	taaggaTGACat cagggactc	357

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V\$CREB/ATF2.01	Activating transcription factor 2	0.87	1279 - 1299	(+)	0.814	0.871	agtcccTGATgt catccttac	358
V\$E4FF/E4F.01	GLI-Krueppel-related transcription factor, regulator of adenovirus E4 promoter	0.82	1284 - 1296	(+)	0.842	0.824	ctgATGTcatcc t	359
V\$HOXF/PTX1.01	Pituitary Homeobox 1 (Ptx1, Pitx-1)	0.94	1299 - 1315	(-)	1.000	0.949	tttCTAAgctctt cgag	360
V\$TBPF/ATATA.01	Avian C-type LTR TATA box	0.78	1302 - 1318	(-)	1.000	0.781	ttgtttcTAAGct cttc	361
V\$XBBF/RFX1.01	X-box binding protein RFX1	0.89	1302 - 1320	(+)	0.881	0.890	gaagagcttaGA AAcaaag	362
V\$LEFF/LEF1.01	TCF/LEF-1, involved in the Wnt signal transduction pathway	0.86	1309 - 1325nc	(+)	1.000	0.884	ttagaaaCAAA gagtgg	363
V\$RBPF/RBPJK.02	Mammalian transcriptional repressor RBP-Jkappa/CBF1	0.94	1319 - 1333 nc	(+)	1.000	0.977	agagTGGGaaa tgct	364
V\$CP2F/CP2.01	CP2	0.90	1331 - 1349	(-)	1.000	0.932	agCTGGgtaaa gctagagc	365
V\$SRFF/SRF.02	Serum response factor	0.84	1362 - 1380	(+)	0.888	0.842	taaggCAAAttg ggccatt	366
V\$CART/XVENT2.01	Xenopus homeodomain factor Xvent-2; early BMP signaling response	0.82	1366 - 1382	(+)	0.750	0.882	gcAAATtgggc cattaa	367
V\$CART/XVENT2.01	Xenopus homeodomain factor Xvent-2; early BMP signaling response	0.82	1367 - 1383	(-)	1.000	0.835	ttTAATggccca atttg	368
V\$PDX1/ISL1.01	Pancreatic and intestinal lim-homeodomain factor	0.82	1370 - 1390	(-)	1.000	0.875	ctgagctttTAAT ggcccaat	369
V\$NKXH/HMX3.02	Hmx3/Nkx5-1 homeodomain transcription factor	0.92	1372 - 1386	(-)	1.000	0.946	gcttTTAAAtggc cca	370
V\$HOXF/HOXC13.01	Homeodomain transcription factor HOXC13	0.91	1373 - 1389	(+)	1.000	0.932	gggccatTAAA agctca	371
V\$NKXH/HMX3.02	Hmx3/Nkx5-1 homeodomain transcription factor	0.92	1375 - 1389	(+)	1.000	0.953	gcccaTTAAaag ctca	372
V\$MYBL/VMYB.05	v-Myb, variant of AMV v-myb	0.90	1404 - 1416	(+)	1.000	0.990	attAACGgtgtgt g	373
V\$AHRR/AHRARN T.02	Aryl hydrocarbon / Arnt	0.77	1423 - 1447	(-)	0.750	0.781	cctgtggataGA GTgtgaaagcaa	374

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	heterodimers, fixed core						c	
V\$EVI1/EVI1.06	Ecotropic viral integration site 1 encoded factor, amino-terminal zinc finger domain	0.83	1440 - 1456	(+)	0.750	0.835	tccacaGGATa gattga	375
V\$HOXC/HOX_PB X.01	HOX/PBX binding sites	0.81	1442 - 1458	(+)	0.944	0.814	cacaGGATaga ttgaaa	376
V\$HOXC/PBX1.01	Homeo domain factor Pbx-1	0.78	1446 - 1462	(+)	1.000	0.809	ggataGATTga aactgc	377
V\$IRFF/ISRE.01	Interferon- stimulated response element	0.81	1447 - 1465	(+)	1.000	0.829	gatagattGAAA ctgccag	378
V\$HOXH/MEIS1B_ HOXA9.01	Meis1b and Hoxa9 form heterodimeric binding complexes on target DNA	0.78	1450 - 1464	(-)	0.750	0.781	TGGCagtttcaat ct	379
V\$NR2F/ARP1.01	Apolipoprotein AI regulatory protein 1, NR2F2	0.82	1469 - 1489	(-)	0.857	0.897	ccagggtcaggG ATCaggtgg	380
V\$MEF3/MEF3.01	MEF3 binding site, present in skeletal muscle- specific transcriptional enhancers	0.89	1474 - 1486	(-)	1.000	0.943	gggTCAGggat ca	381
V\$RORA/TR4.01	Nuclear hormone receptor TR4 homodimer binding site	0.84	1474 - 1492	(-)	1.000	0.841	atcccagGGTC agggatca	382
V\$CSEN/DREAM.0 1	Downstream regulatory element- antagonist modulator, Ca2+-binding protein of the neuronal calcium sensors family that binds DRE (downstream regulatory element) sites as a tetramer	0.95	1476 - 1486	(-)	1.000	0.974	ggGTCAGggat	383
V\$CP2F/CP2.01	CP2	0.90	1493 - 1511	(+)	1.000	0.969	ggCTGGattga gcaatgag	384
V\$HOXC/PBX1.01	Homeo domain factor Pbx-1	0.78	1493 - 1509	(+)	1.000	0.811	ggctgGATTga gcaatg	385
V\$CEBP/CEBPB.01	CCAAT/enhance r binding protein beta	0.94	1496 - 1510	(+)	1.000	0.984	tggattgaGCAA tga	386
V\$CAAT/NFY.03	Nuclear factor Y (Y-box binding factor)	0.81	1513 - 1527	(+)	1.000	0.873	agagCCAAGca gcac	387
V\$STAF/ZNF76_14	ZNF143 is the	0.76	1522 -	(-)	1.000	0.765	tagcCCCAggg	388

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3.01	human ortholog of Xenopus Staf, ZNF76 is a DNA binding protein related to ZNF143 and Staf		1544					gactctgtgctg	
V\$NOLF/OLF1.01	Olfactory neuron-specific factor	0.82	1526 - 1548	(+)	1.000	0.879	acagagTCCCct ggggctagagg	389	
V\$AP2F/AP2.02	Activator protein 2 alpha	0.92	1531 - 1545	(-)	0.905	0.941	ctaGCCCCcagg ggac	390	
V\$ZBPF/ZNF202.01	Transcriptional repressor, binds to elements found predominantly in genes that participate in lipid metabolism	0.73	1536 - 1558	(-)	0.761	0.739	gcctccTCCAcc tctagccccag	391	
V\$IKRS/IK1.01	Ikaros 1, potential regulator of lymphocyte differentiation	0.92	1561 - 1573	(+)	1.000	0.933	tcctGGGAatgg g	392	
V\$TEAF/TEF1.01	TEF-1 related muscle factor	0.84	1561 - 1577	(-)	1.000	0.855	ttttccCATTCcc agga	393	
V\$IRFF/IRF7.01	Interferon regulatory factor 7 (IRF-7)	0.86	1565 - 1583nc	(+)	0.936	0.895	ggGAATggga aaaaccca	394	
V\$LTUP/TAACC.01	Lentiviral TATA upstream element	0.71	1565 - 1587nc	(+)	1.000	0.721	gggaatgggaaa AACCccaactt	395	
V\$RBP/ RBPJK.02	Mammalian transcriptional repressor RBP-Jkappa/CBF1	0.94	1566 - 1580nc	(+)	1.000	0.947	ggaaTGGGaaa aacc	396	
V\$IKRS/IK1.01	Ikaros 1, potential regulator of lymphocyte differentiation	0.92	1567 - 1579nc	(+)	1.000	0.927	gaatGGGAaaa ac	397	
V\$NFKB/CREL.01	c-Rel	0.91	1571 - 1583	(-)	1.000	0.971	tgggggtttTTCCc	398	
V\$CIZF/NMP4.01	NMP4 (nuclear matrix protein 4) / CIZ (Cas-interacting zinc finger protein)	0.97	1572 - 1582nc	(+)	1.000	0.986	ggAAAAaccccc	399	
V\$SRFF/SRF.02	Serum response factor	0.84	1576 - 1594	(-)	0.888	0.881	gacccCAAAGt tgggggttt	400	
V\$MYT1/MYT1.02	MyT1 zinc finger transcription factor involved in primary neurogenesis	0.88	1578 - 1590	(-)	1.000	0.882	ccaAAGTtggg gt	401	

Cartharius K, et al. (2005) Bioinformatics 21, 2933-42.

Example 4

Utilising the data from Examples 2 and 3, a suite of constructs are generated containing various shRNA suppressors and/ or replacement rhodopsin nucleic acids enhanced with additional promoter sequences, known to be conserved between vertebrate species and various sequences known to enhance expression at RNA and/or protein levels. Figures 9 and 16 represents diagrammatically sequences cloned in suppression and/or replacement constructs. Notably, any combination of the elements and conserved regions outlined and indeed other elements that can modulate gene expression could be used in the invention to control expression of suppression and/or replacement components.

Suppression and/or replacement constructs (Figure 9) were then used to generate recombinant AAV2/5 viruses using the procedures provided in Example 1. AAV2/5 suppression and/or replacement vectors were evaluated in 129 wild type (WT) mice for levels of expression of suppressors and/or replacement nucleic acids at the RNA and protein levels as detailed in Example 1. Figure 10A illustrates a comparison using an RNase protection assay of levels of human rhodopsin expression from the RHO-M transgene in RHO-M mice (lane M) versus the rhodopsin expression obtained from the suppression and replacement constructs in rAAV2/5 subretinally injected into wild type 129 mice (lanes B8, B9, B11, B12, B13, B16, B8). Figure 10A illustrates that AAV-BB8, AAV-BB10, AAV-BB11, AAV-BB12, AAV-BB13 and AAV-BB16 express the human rhodopsin replacement gene in RNA extracted from 129 wild type mice subretinally injected with these suppression and or replacement constructs. AAV-BB8, AAV-BB10 and AAV-BB11 express human rhodopsin at lower levels than AAV-BB12, AAV-BB13 and AAV-BB16.

Further evaluation of suppression and replacement vectors was undertaken. Figure 11 provides a comparative analysis of human rhodopsin expression from rAAV2/5 suppression and replacement vectors using real time RT-PCR. Figure 11 illustrates replacement rhodopsin expression levels in RNA extracted from 129 wild type mice subretinally injected with suppression and/or replacement constructs. Expression levels were also determined in Rho-M transgenic mice which express a rhodopsin replacement construct termed rCC and display normal retinal function. Suppression and replacement vectors AAV-BB12, AAV-BB13, AAV-BB16 and AAV-BB18 express approximately in the same order of magnitude as levels of

replacement rhodopsin transcript in Rho-M mice, indicating that enhanced replacement constructs with enhancer elements and conserved regions may express sufficient levels of rhodopsin to sustain a functional retina *in vivo*.

Figure 12 illustrates retinal histology of adult wild type mouse retinas subretinally injected with 2 μ l of 2×10^{12} particle/ml of different suppression and replacement rhodopsin AAV vectors (see Figure 9). Two weeks post-injection of AAV vectors transduced eyes were removed, fixed in 4% paraformaldehyde and cryosectioned (12 μ m). Subsequently, sections were stained with human specific anti-RHO antibody to visualise expression of replacement-RHO using Cy3 label (red) on the secondary antibody; cell nuclei were counterstained with DAPI (blue). A: AAV-BB8, B: AAV-BB13, C: AAV-BB24, D: AAV-Q8, E: AAV-Q26, F: retina from uninjected Rho-M transgenic mouse expressing RHO (positive control). Clear evidence of human rhodopsin expression from AAV suppression and replacement vectors was obtained. Sections indicate different levels of human RHO expression from the AAV suppression and replacement vectors under evaluation. OS: photoreceptor outer segments; IS: photoreceptor inner segments; ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer.

To explore efficacy of the suppression component of the suppression and replacement approach delivered using AAV, a variety of suppression only vectors were generated with an EGFP reporter gene (see Figure 9). Adult NHR transgenic mice on a rho^{-/-} background, therefore expressing normal human RHO but not mouse rho, were transduced by subretinal injection of 2 μ l of 2×10^{12} particle/ml of AAV-shQ1-EGFP (A) or AAV-shNT-EGFP (B). Two weeks after injection, eyes were removed, fixed in 4% paraformaldehyde and cryosectioned (Figure 13). AAV-shQ1-EGFP expresses shRNA-Q1, which targets RHO, while AAV-shNT-EGFP expresses a non-targeting shRNA (see Figure 9 for constructs). Both constructs express EGFP allowing tracking of the transduced cell populations (green). Sections were counterstained with DAPI (blue) to label the position of the nuclear layers. A significant reduction in the photoreceptor cell number in the transduced part of the outer nuclear layer was apparent in the AAV-shQ1-EGFP injected (A) retinas compared to those of injected with AAV-shNT-EGFP (B) (Figure 13). IS: photoreceptor inner segments; ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer.

Adult RHO-347 transgenic mice carrying a dominant RHO mutation causing retinal degeneration akin to human RP, were subretinally injected with 2 μ l of 2×10^{12} particle/ml of AAV-shNT (A) or AAV-shQ1 (B) vectors (Figure 14A). Two weeks post-injection transduced eyes were removed, fixed in 4% paraformaldehyde and cryosectioned (12 μ m). AAV-shQ1 expresses shRNA-Q1, which targets RHO, while AAV-shNT expresses a non-targeting shRNA. Both constructs express EGFP allowing tracking of the transduced part of the retina (green). Sections were counterstained with DAPI (blue) to indicate positions of the nuclear layers. A significant reduction of the photoreceptor cell numbers in the transduced part of the outer nuclear layer in the AAV-shNT injected or the uninjected (C) retinas was apparent due to the degenerative effects of RHO-347 transgene (Figure 14A). A significantly preserved outer nuclear layer is detected in the AAV-shQ1 transduced retinas, where shRNA-Q1 effectively suppresses the RHO-347 transcript therefore reducing retinal degeneration (Figure 14A). Note that the mouse rhodopsin gene (expressed in these retinas) was refractory to suppression by shRNA-Q1 due to the presence of nucleotide changes at the target site for Q1 siRNA-based suppression. Suppression of human rhodopsin and replacement using the degeneracy of the genetic code provided therapeutic benefit at a histological level in RHO-347 mice.

In addition, Figure 14D provides evidence of an improvement in the electroretinogram (ERG) in RHO-347 eyes treated with AAV-shQ1-EGFP versus eyes treated with AAV-shNT-EGFP. In Figure 14D a representative maximum ERG response of a RHO-347 mouse, containing a human rhodopsin transgene with a mutation at codon 347, subretinally injected with AAV2/5 constructs is presented. This RHO-347 mouse normally displays a phenotype similar to autosomal dominant RP. The top figure is the response of the right eye, which received an injection of AAV-shQ1-EGFP, a AAV2/5 vector containing suppressor siRNA Q1 driven by an H1 promoter (shQ1) and a CMV-driven EGFP gene. The left eye received an AAV-shNT-EGFP, a AAV2/5 containing a non-targeting (control) siRNA driven by an H1 promoter (shNT) and a CMV-driven EGFP gene. As can be seen above, the maximum response is significantly greater in the treated right eye than in the control left eye, indicating that suppression of the mutant rhodopsin transgene leads to some rescue at the ERG level.

Example 5: Sequences of various elements designed to enhance expression of replacement constructs.

As described, enhancer elements, conserved regions A through I and/or transcription factor binding sites and/or other regulatory elements and/or epigenetic elements may be combined to improve expression of replacement constructs (see Figures 9 and 15 and Tables 1, 2, 9-13). These elements can be used in many different combinations to achieve optimum expression, as demonstrated in the Examples provided above. Additional examples include inter alia a construct comprising a human rhodopsin gene expressed from a composite promoter element containing the 484 bp mouse rhodopsin promoter together with the CMV enhancer, the rhodopsin promoter enhancer element, the rhodopsin promoter conserved region B and flanked at the 3' end of the gene by a woodchuck posttranscriptional regulatory element and a minimal poly A sequence. Another example is similar to the one above but instead of the CMV enhancer, it contains multiples of the CRX and/or NRL binding sites.

Example 6: Utilisation of neuroprotective / neurotrophic factors in conjunction with suppression and replacement.

As described above, there is evidence from the prior art that neurotrophic / neuroprotective factors can improve cell viability and or cell functioning, the sequences encoding a number of these factors are provided in Figure 17. Figure 18 provides suppression and replacement constructs containing genetic elements that are beneficial for neuronal cell survival. In the example, the suppression and replacement construct pAAV-BB18 (Figure 9) has been combined with the gene encoding the neurotrophic factor GDNF, driven by a small UCOE (chromatin opening element. A Thrasher, Abstract 36, British Society for Gene Therapy 5th Annual Conference 2008) promoter. Notably other neurotrophic factors and or genes encoding neurotrophic factors such as, for example, Neurturin may also be used in combination with any of the suppression and replacement constructs described. In example A (Figure 18), the additional element, in this case sequence encoding GDNF is co-located with the suppression and replacement construct within the two AAV inverted terminal repeat sequences, ITS1 and ITS2. In the second example, B (Figure 18), the GDNF gene and its promoter are not co-located with the suppression and replacement elements within ITS1 and ITS2, but are located within the backbone of the plasmid used to generate

AAV. Since a small proportion of the backbone is packaged during AAV production, this results in a mixed population of AAVs with the majority containing the suppression and replacement elements and a minority the GDNF elements.

AAV vectors generated to contain suppression, replacement and neurotrophic / neuroprotection components can be subretinally injected into wild typ mice and or into mice with inherited retinal degenerations such as the RHO-347 and Pro23His mice described in the Examples above.

Table 14: Enhancers

CMV Enhancer (SEQ ID NO:402)
CCGCGTTAC'A TAACTTACGG TAAATGGCCC GCCTGGCTG A CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT GGATAGCGGT TTGACTCACG GGGATTTCCTA AGTCTCCACC CCATTGACGT CAATGGGAGT TTGTTTTGGC ACCAAAATCA ACGGGAC

Rhodopsin promoter conserved REGION A (SEQ ID NO:403)
GAGTGTCTAATTGCTTATGATCATGCATGCTCTCTCTCCCACTAAACATTT
ATTAATGTGTTAGGATTTCATTAGCGCGTGCCTTGAAGTAAATCATT
GCATATGGCTGGGAAAAAGTGGGGTGAGGGAGGAAACAGTGCCAGCTCCC
CAACAGGCGTCAATCACAGTGACAGATCAGATGG
Rhodopsin Promoter Enhancer Element (contains Crx D(-) & CrxE (+) & NRL binding sites) (SEQ ID NO:404)
TTTCTGCAGCGGGGATTAATATGATTATG
AACACCCCAATCTCCCAGATGCTGATTCAGCCAGGAGGTACC
Crx D(-) (SEQ ID NO:405)
GCGGGGATTAATAT
CrxE (+)(SEQ ID NO:406)
TGAACACCCCAATCTC
NRL (SEQ ID NO:407)
TGCTGATTCAGC
Rhodopsin promoter conserved region B (SEQ ID NO:408)
TCTGCTGACCCAGCAACACTCTTTCCTTCTGAGGCTTAAGAGCTATTAGCGTAGGTG
ACTCAGTCCCTAATCCTCC

Human rhodopsin polyA region F (SEQ ID NO:409)

GACCTGCCTAGGACTCTGTGGCCGACTATAGGCGTCTCCCATCCCCTACACCTTCCCC
 CAGCCACAGCCATCCCACCAGGAGCAGCGCCTGTGCAGAATGAACGAAGT
 CACATAGGCTCCTTAATTTTTTTTTTTTTTTTAAGAAATAATTAATGAGG
 CTCCTCACTC

Human rhodopsin polyA region G (SEQ ID NO:410)

ACCTGGGACAGCCTGAGAAGGGACATCCACCAAGACCTAC
 TGATCTGGAGTCCCACGTTCCCCAAGGCCAGCGGGATGTGTGCCCCCTCCT
 CCTCCCAACTCATCTTTCAGGAACACGAGGATTCTTGCTTTCTGGAAAAG
 TGTCCCAGCTTAGGGATAAGTGTCTAGCACAGAATGGGGCACACAGTAGG
 TGCTTAATAAATGCTGGATGGATGCAGGAAGGAATGGAGGAATGAATGGG
 AAGGGAGAACATAGGATCC

SV40 Minimal polyA (SEQ ID NO:411)

AATAAAGGAAATTTATTTTCATGCAATAGTGTGTTGGTTTTTTGTGTG

WPRE from pSK11 (SEQ ID NO:412)

GGATCC AATCAACCTC
 TGGATTACAA AATTTGTGAA AGATTGACTG GTATTCTTAA CTATGTTGCT
 CCTTTTACGC TATGTGGATA CGCTGCTTAA ATGCCTTTGT ATCATGCTAT
 TGCTTCCCGT ATGGCTTTCA TTTTCTCCTC CTTGTATAAA TCCTGGTTGC
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WPRE from pSin11 (SEQ ID NO:413)

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 GCCTGCCTTG CCCGCTGCTG GACAGGGGCT AGGTTGCTGG GCACTGATAA
 TTCCGTGGTG TTGTC

Incorporation by Reference

The contents of all cited references (including literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, which are well known in the art.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are

therefore to be considered in all respects illustrative rather than limiting of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

Sequence Listing in Electronic Form

In accordance with section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 94058-10 Seq 08-12-17 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

Sequence Table

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<150> US 60/923,067

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 <223> siRNA sequence targeting an intron
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 ttaactatat ggccactct 19
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 <223> siRNA sequence targeting an intron
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 <210> 67
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 <223> siRNA sequence targeting a polymorphism
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 <210> 69
 <211> 20
 <212> DNA

<213> Artificial
 <220>
 <223> Forward primer for rhodopsin
 <400> 69
 ctttcctgat ctgctgggtg 20

<210> 70
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 <223> Reverse primer for rhodopsin
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<210> 71
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 <223> Forward primer for GAPDH
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 cagcctcaag atcatcagca 20

<210> 74
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 <223> Reverse primer for GAPDH

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19

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<210> 77
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<223> siRNA sequence targeting rhodopsin (siC)

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<223> Replacement rhodopsin sequence (rC)

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<210> 81

<211> 19

<212> DNA

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<223> siRNA sequence targeting rhodopsin (siCC)

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cgctcaagcc ggaggtcaa 19

<210> 82

<211> 20

<212> DNA

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<223> Replacement rhodopsin sequence (rCC)

<400> 82

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<210> 83

<211> 22

<212> DNA

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<223> siRNA sequence targeting rhodopsin (siQ1)

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<210> 84
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<223> Replacement rhodopsin sequence (rQ1)

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<210> 85
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tcaatgggtg gagtatttac ggtaaaactgc ccacttgga gtacatcaag tgtatcatat 180

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acgggac 427

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<210> 88
<211> 581
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<213> Artificial

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<220>

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<223> pAAV.BB11 - the WPR element from pSin11 CMV GFPpre mut FL

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<210> 89
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<213> Artificial

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<220>

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<223> pAAV.BB13 - the WPR element from pBSK11

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<210> 90

<211> 11840

<212> DNA

<213> Artificial

<220>

<223> Conserved regions A-I showing mouse rhodopsin promoter sequence (conserved regions A-D) followed by human rhodopsin 5'UTR, human rhodopsin exons and introns (conserved region E) and human rhodopsin 3' region sequence (conserved region F-I)

<400> 90

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 gctggattga gcaatgagca gagccaagca gcacagagtc ccctggggct agaggtggag 11760
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<210> 91
 <211> 72
 <212> DNA
 <213> Artificial

<220>
 <223> CRX-NRL element including the CRX motif from conserved region D,
 the CRX motif from conserved region E and NRL binding sites

<400> 91
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 ccaggaggta cc 72

<210> 92
 <211> 210
 <212> DNA
 <213> Artificial

<220>
 <223> DNA sequence (1-210) of conserved region A of the rhodopsin gene

<400> 92
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 acatttatta atgtgttagg atttccatta gcgcgtgcct tgaactgaaa tcatttgcatt 120
 atggctggga aaaagtgggg tgagggagga aacagtgccca gctccccaac aggcgtcaat 180
 cacagtgaca gatcagatgg tttctggctg 210

<210> 93
 <211> 100
 <212> DNA
 <213> Artificial

<220>

<223> Conserved region B of the rhodopsin gene (210-310)

<400> 93
 aagggggggg ggggtctgct gaccagcaa cactctttcc ttctgaggct taagagctat 60
 tagcgtaggc gactcagtc ctaatcctcc attcaatgcc 100

<210> 94
 <211> 100
 <212> DNA
 <213> Artificial

<220>

<223> Conserved region C of the rhodopsin gene (310-410)

<400> 94
 ggggctgacc accaggggtca gaatcagaac ctccaccttg acctcattaa cgctgggtctt 60
 aatcaccaag ccaagctcct taaactgcta gtggccaact 100

<210> 95
 <211> 280
 <212> DNA
 <213> Artificial

<220>

<223> Conserved region D of the rhodopsin gene (410-690)

<400> 95
 aggcttcttc ctagtgtcac cttggcccct cttagaagcc aattaggccc tcagtttctg 60
 cagcggggat taatatgatt atgaacaccc ccaatctccc agatgctgat tcagccagga 120
 gcttaggagg gggagggtcac tttataaggg tctggggggg tcagaacca gagtcatcca 180
 gctggagccc tgagtggctg agctcaggcc ttgcagcat tcttgggtgg gagcagccac 240
 gggtcagcca caagggccac agccatgaat ggcacagaag 280

<210> 96
 <211> 160
 <212> DNA
 <213> Artificial

<220>

<223> Conserved region E of the rhodopsin gene (690-850)

<400> 96
 tcctgagccc catgtcaaac agaatccaag acatcccaac ccttcacctt ggctgtgccc 60
 ctaatcctca actaagctag gcgcaaattc caatcctctt tggcttagta ccccgggggc 120
 agccccctct aaccttgggc ctcagcagca ggggaggcca 160

<210> 97
 <211> 370
 <212> DNA
 <213> Artificial

<220>
 <223> Conserved region F & G of the rhodopsin gene (850-1220)

<400> 97
 cccctacacc ttccccagc cacagccatc ccaccaggag cagcgctgt gcagaatgaa 60
 cgaagtcaca taggtcctt aatttttttt ttttttttaa gaaataatta atgaggctcc 120
 tcactcacct gggacagcct gagaaggagc atccaccaag acctactgat ctggagtccc 180
 acgttcccca aggccagcgg gatgtgtgcc cctcctcctc ccaactcatc tttcaggaac 240
 acgaggattc ttgctttctg gaaaagtgtc ccagcttagg gataagtgtc tagcacagaa 300
 tggggcacac agtaggtgct taataaatgc tggatggatg caggaaggaa tggaggaatg 360
 aatgggaagg 370

<210> 98
 <211> 110
 <212> DNA
 <213> Artificial

<220>
 <223> Conserved region H of the rhodopsin gene (1220 1230-1316 1330)

<400> 98
 tctagagcat ggagcctcta gaagccatgc tcacccgccc acatttaatt aacagctgag 60
 tccctgatgt catccttact cgaagagctt agaaacaaag agtgggaaat 110

<210> 99
 <211> 270
 <212> DNA
 <213> Artificial

<220>
 <223> Conserved region I of the rhodopsin gene (1330 1342-1425 1600)

<400> 99
 gctctagctt taccagctc tgcttgaga ctaaggcaaa ttgggccatt aaaagctcag 60

ctcctatgtt ggtattaacg gtggtgggtt ttgttgcttt cacactctat ccacaggata 120
gattgaaact gccagcttcc acctgatccc tgaccctggg atggctggat tgagcaatga 180
gcagagccaa gcagcacaga gtcccctggg gctagagggtg gaggaggcag tcctgggaat 240
gggaaaaacc ccaactttgg ggtcatagag 270

<210> 100
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<220>
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<400> 100
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<210> 101
<211> 13
<212> DNA
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<220>
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<400> 101
gtgtctaatt gct 13

<210> 102
<211> 17
<212> DNA
<213> Unknown

<220>
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<400> 102
tgtctaattg cttatga 17

<210> 103
<211> 25
<212> DNA
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<220>
<223> Putative Rhodopsin transcription regulatory factors

<400> 103
gtgggagaga gagcatgcat gatca 25

<210> 104
 <211> 17
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 <220>
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 <400> 104
 tcccactaaa catttat 17

<210> 105
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 <220>
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 <400> 105
 acattaataa atgttta 17

<210> 106
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 <400> 106
 attaataaat gttta 15

<210> 107
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 <220>
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 <400> 107
 attaataaat gttta 15

<210> 108
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 <400> 108

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<210> 109

<211> 21

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<213> Unknown

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<223> Putative Rhodopsin transcription regulatory factors

<400> 109

ctaacacatt aataaatgtt t 21

<210> 110

<211> 13

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<220>

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<400> 110

acatttatta atg 13

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catttattaa tgtgttagg 19

<210> 112

<211> 15

<212> DNA

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acacattaat aaatg 15

<210> 113

<211> 15

<212> DNA

<213> Unknown

<220>

<223> Putative Rhodopsin transcription regulatory factors

<400> 113
taacacatta ataaa 15

<210> 114
<211> 17
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<220>
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<400> 114
ttattaatgt gttagga 17

<210> 115
<211> 15
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<400> 115
ctaattggaaa tccta 15

<210> 116
<211> 17
<212> DNA
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<220>
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<400> 116
gcgctaattgg aaatcct 17

<210> 117
<211> 25
<212> DNA
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<220>
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<400> 117
ttccattagc gcgtgccttg aactg 25

<210> 118
<211> 21
<212> DNA

<213> Homo sapiens
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<210> 119
 <211> 15
 <212> DNA
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<220>
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<210> 120
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<220>
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 gccatatgca aat 13

<210> 124
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<210> 125
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<210> 126
 <211> 25
 <212> DNA
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 <400> 126
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<210> 129
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 ccctcacccc acttt 15

<210> 130
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<210> 131
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<210> 132
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 ggaggaaaca g 11

<210> 133
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<210> 134
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<210> 136
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<210> 141
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<210> 142
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<210> 143
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<210> 144
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<210> 148
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<210> 149
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<210> 153
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<210> 155
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<400> 157
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<210> 158
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<210> 159
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<210> 161
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 ctcttaagcc tca 13

<210> 165
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<210> 167
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 attagcgtag gtgactc 17

<210> 169
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 attagggact gagtcaccta cgcta 25

<210> 170
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 <220>
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 <400> 171
 ggtgactcag t 11

<210> 172
 <211> 11
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 <400> 172
 actgagtcac c 11

<210> 173
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 <400> 173
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<210> 174
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 ggcattgaat gga 13

<210> 175
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 <400> 175

cagaatcaga acctccacc 19

<210> 176

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<211> 11

<212> DNA

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<220>

<223> Putative Rhodopsin transcription regulatory factors

<400> 177

aggtcaaggt g 11

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<400> 178

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<400> 339

cacattttaat taaca

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<220>

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<400> 340

cacattttaat taaca

15

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<223> Putative Rhodopsin transcription regulatory factors

<400> 341

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13

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<400> 342

cattttaatta acagctg

17

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 agctgttaat taaat 15

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 gctgttaatt aaa 13

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<220>
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<220>

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gaagagctta gaaacaaag 19

<210> 363
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<220>
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<400> 364
agagtgggaa atgct 15

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<400> 365
agctgggtaa agctagagc 19

<210> 366
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<210> 367
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<400> 367

gcaaattggg ccattaa 17

<210> 368
 <211> 17
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<220>
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<400> 368
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<210> 369
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 <212> DNA
 <213> Xenopus sp.

<400> 369
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<210> 370
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<220>
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<220>
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<400> 372

gccattaaaa gctca 15

<210> 373

<211> 13

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<220>

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<210> 375

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<220>

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<400> 375

tccacaggat agattga 17

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<211> 17

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<400> 376

cacaggatag attgaaa 17

<210> 377

<211> 17

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<210> 383
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 gggtcaggga t 11

<210> 384
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 ggctggattg agcaatg 17

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 tggattgagc aatga 15

<210> 387
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gcctcctcca cctctagccc cag 23

<210> 392
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<400> 392
 tcctgggaat ggg 13

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<220>
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<400> 393
 ttttcccatt cccagga 17

<210> 394
 <211> 19
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 <213> Unknown

<220>
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<400> 394
 gggaatggga aaaacccca 19

<210> 395
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<220>
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<400> 395
 gggaatggga aaaaccccaa ctt 23

<210> 396
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<400> 396

ggaatgggaa aaac 14

<210> 397
 <211> 13
 <212> DNA
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<220>
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<400> 397
 gaatgggaaa aac 13

<210> 398
 <211> 13
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<220>
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<400> 398
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<400> 399
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<210> 400
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<220>
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<400> 400
 gaccccaaag ttgggggttt 19

<210> 401
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<220>

<223> Putative Rhodopsin transcription regulatory factors

<400> 401

ccaaagttgg ggt

13

<210> 402

<211> 427

<212> DNA

<213> Artificial

<220>

<223> Artificial Sequence

<400> 402

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attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt tccattgacg 120

tcaatgggtg gagtatttac ggtaaaactgc ccacttggca gtacatcaag tgtatcatat 180

gccaagtacg cccctattg acgtcaatga cggtaaatgg cccgcctggc attatgccc 240

gtacatgacc ttatgggact ttctacttg gcagtacatc tacgtattag tcatcgctat 300

taccatgggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggg ttgactcacg 360

gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc accaaaatca 420

acgggac 427

<210> 403

<211> 185

<212> DNA

<213> Artificial

<220>

<223> Sequence for rhodopsin promoter conserved Region A

<400> 403

gagtgtctaa ttgcttatga tcatgcatgc tctctctccc actaaacatt tattaatgtg 60

ttaggatttc cattagcgcg tgccttgaac tgaaatcatt tgcatatggc tgggaaaaag 120

tgggggtgagg gaggaaacag tgccagctcc ccaacaggcg tcaatcacag tgacagatca 180

gatgg 185

<210> 404

<211> 72

<212> DNA

<213> Artificial

<220>

<223> Sequence for rhodopsin promoter enhancer element (contains Crx

D(-) & CrxE (+) & NRL binding sites)

<400> 404
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 ccaggaggta cc 72

<210> 405
 <211> 14
 <212> DNA
 <213> Artificial

<220>
 <223> Sequence for Crx D(-) enhancer

<400> 405
 gcggggatta atat 14

<210> 406
 <211> 17
 <212> DNA
 <213> Artificial

<220>
 <223> Sequence for CrxE (+) Enhancer

<400> 406
 tgaacacccc caatctc 17

<210> 407
 <211> 12
 <212> DNA
 <213> Artificial

<220>
 <223> Sequence for NRL enhancer

<400> 407
 tgctgattca gc 12

<210> 408
 <211> 76
 <212> DNA
 <213> Artificial

<220>
 <223> Sequence for rhodopsin promoter conserved region B

<400> 408
 tctgctgacc cagcaacact ctttccttct gaggcttaag agctattagc gtaggtgact 60
 cagtcacctaa tctctc 76

<210> 409
 <211> 168
 <212> DNA
 <213> Homo sapiens

<400> 409
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 gccacagcca tcccaccagg agcagcgcct gtgcagaatg aacgaagtca cataggctcc 120
 ttaattttttt tttttttttt aagaaataat taatgaggct cctcactc 168

<210> 410
 <211> 259
 <212> DNA
 <213> Homo sapiens

<400> 410
 acctgggaca gcctgagaag ggacatccac caagacctac tgatctggag tcccacgttc 60
 cccaaggcca gcgggatgtg tgccccctcct cctcccaact catctttcag gaacacgagg 120
 attcttgctt tctggaaaag tgtcccagct tagggataag tgtctagcac agaatggggc 180
 acacagtagg tgcttaataa atgctggatg gatgcaggaa ggaatggagg aatgaatggg 240
 aagggagaac ataggatcc 259

<210> 411
 <211> 48
 <212> DNA
 <213> Simian virus 40

<400> 411
 aataaaggaa atttattttc atgcaatagt gtgttggttt tttgtgtg 48

<210> 412
 <211> 593
 <212> DNA
 <213> Plasmid pSK11

<400> 412
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 gttgctcctt ttacgctatg tggatacgtt gctttaatgc ctttgatatca tgctattgct 120
 tcccgatatg ctttcatttt ctctctcttg tataaatcct gggttgctgtc tctttatgag 180
 gagttgtggc ccgttgtcag gcaacgtggc gtggtgtgca ctgtgtttgc tgacgcaacc 240
 cccactgggtt ggggcattgc caccacctgt cagctccttt ccgggacttt cgctttcccc 300
 ctccctattg ccacggcgga actcatcgcc gcctgccttg ccgctgctg gacaggggct 360

cggtgttgg gcactgacaa ttccgtggtg ttgtcgggga agctgacgtc ctttccatgg 420
 ctgctcgcc ctgttgccac ctggattctg cgcgggacgt ccttctgcta cgtcccttcg 480
 gccctcaatc cagcggacct tccttccgc gccctgctgc cggctctgcg gcctcttcg 540
 cgtcttcgcc ttcgccctca gacgagtcgg atctccctt ggccgcctc ccc 593

<210> 413
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 <212> DNA
 <213> Plasmid pSin11

<400> 413
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 ttaaattgta ataaaacaaa atggtggggc aatcatttac attttaggg atatgtaatt 120
 actagttcag gtgtattgcc acaagacaaa catgttaaga aactttcccg ttatttacgc 180
 tctgttcctg ttaatcaacc tctggattac aaaatttgtg aaagattgac tgatattctt 240
 aactatgttg ctctttttac gctgtgtgga tatgctgctt tatagcctct gtatctagct 300
 attgcttccc gtacggcttt cgttttctcc tccttgata aatcctgggt gctgtctctt 360
 ttagaggagt tgtggcccg tgcctgcaa cgtggcggtg tgtgctctgt gtttgcgtgac 420
 gcaaccccc ctggctggg cattgccacc acctgtcaac tcctttctgg gactttcgt 480
 tccccctcc cgatcgccac ggcagaactc atcgccgct gccttgccg ctgctggaca 540
 ggggctagg tgcggggcac tgataattcc gtggtgtgt c 581

<210> 414
 <211> 21
 <212> DNA
 <213> Artificial

<220>
 <223> siRNA sequence targeting homologous sequence between human and porcine rhodopsin

<400> 414
 acctctctgc atggatagtt t 21

<210> 415
 <211> 21
 <212> DNA
 <213> Artificial

<220>
 <223> siRNA sequence targeting homologous sequence between human and

porcine rhodopsin

<400> 415
catgttcgtg gtccacttct t 21

<210> 416
<211> 64
<212> DNA
<213> Artificial

<220>
<223> Top strand CC miRNA oligonucleotide targeting human rhodopsin

<400> 416
tgctgcttct tgtgctggac ggtgacgttt tggccactga ctgacgtcac cgtagcacia 60
gaag 64

<210> 417
<211> 64
<212> DNA
<213> Artificial

<220>
<223> Bottom strand CC miRNA oligonucleotide targeting human rhodopsin

<400> 417
cctgcttctt gtgctacggt gacgtcagtc agtggccaaa acgtcaccgt ccagcacaag 60
aagc 64

<210> 418
<211> 64
<212> DNA
<213> Artificial

<220>
<223> Top strand Q1 miRNA oligonucleotide targeting human rhodopsin

<400> 418
tgctggtagt agtcgattcc acacgagttt tggccactga ctgactcgtg tggtcgacta 60
ctac 64

<210> 419
<211> 64
<212> DNA
<213> Artificial

<220>
<223> Bottom strand Q1 miRNA oligonucleotide targeting human rhodopsin

<400> 419

cctggtagta gtcgaccaca cgagtcagtc agtggccaaa actcgtgtgg aatcgactac 60
tacc 64

<210> 420
<211> 64
<212> DNA
<213> Artificial

<220>
<223> Top strand BB miRNA oligonucleotide targeting human rhodopsin

<400> 420
tgctggtaga gcgtgaggaa gttgatgttt tggccactga ctgacatcaa ctttcacgct 60
ctac 64

<210> 421
<211> 64
<212> DNA
<213> Artificial

<220>
<223> Bottom strand BB miRNA oligonucleotide targeting human rhodopsin

<400> 421
cctggtagag cgtgaaagtt gatgtcagtc agtggccaaa acatcaactt cctcacgctc 60
tacc 64

<210> 422
<211> 1551
<212> DNA
<213> Artificial

<220>
<223> DNA sequence of UCOE 1.5

<400> 422
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<400> 423

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 35 40 45

Leu Lys Arg Ser Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu
 50 55 60

Arg Asn Arg Gln Ala Ala Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys
65 70 75 80

Gly Arg Arg Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala
85 90 95

Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu
100 105 110

Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr
115 120 125

Thr Tyr Asp Lys Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val
130 135 140

Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp
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His Ser Ala Lys Arg Cys Gly Cys Ile
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<400> 427

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35 40 45

Asn Leu Asp Ser Ala Asp Gly Met Pro Val Ala Ser Thr Asp Gln Trp
50 55 60

Ser Glu Leu Thr Glu Ala Glu Arg Leu Gln Glu Asn Leu Gln Ala Tyr
65 70 75 80

Arg Thr Phe His Val Leu Leu Ala Arg Leu Leu Glu Asp Gln Gln Val
85 90 95

His Phe Thr Pro Thr Glu Gly Asp Phe His Gln Ala Ile His Thr Leu
100 105 110

Leu Leu Gln Val Ala Ala Phe Ala Tyr Gln Ile Glu Glu Leu Met Ile
115 120 125

Leu Leu Glu Tyr Lys Ile Pro Arg Asn Glu Ala Asp Gly Met Pro Ile
130 135 140

Asn Val Gly Asp Gly Gly Leu Phe Glu Lys Lys Leu Trp Gly Leu Lys
145 150 155 160

Val Leu Gln Glu Leu Ser Gln Trp Thr Val Arg Ser Ile His Asp Leu
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Arg Phe Ile Ser Ser His Gln Thr Gly Ile Pro Ala Arg Gly Ser His

180

185

190

Tyr Ile Ala Asn Asn Lys Lys Met
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Ala Tyr Pro Gly Val Arg Thr His Gly Thr Leu Glu Ser Val Asn Gly
 35 40 45

Pro Lys Ala Gly Ser Arg Gly Leu Thr Ser Leu Ala Asp Thr Phe Glu
 50 55 60

His Val Ile Glu Glu Leu Leu Asp Glu Asp Gln Lys Val Arg Pro Asn
 65 70 75 80

Glu Glu Asn Asn Lys Asp Ala Asp Leu Tyr Thr Ser Arg Val Met Leu
 85 90 95

Ser Ser Gln Val Pro Leu Glu Pro Pro Leu Leu Phe Leu Leu Glu Glu
 100 105 110

Tyr Lys Asn Tyr Leu Asp Ala Ala Asn Met Ser Met Arg Val Arg Arg
 115 120 125

His Ser Asp Pro Ala Arg Arg Gly Glu Leu Ser Val Cys Asp Ser Ile
 130 135 140

Ser Glu Trp Val Thr Ala Ala Asp Lys Lys Thr Ala Val Asp Met Ser
 145 150 155 160

Gly Gly Thr Val Thr Val Leu Glu Lys Val Pro Val Ser Lys Gly Gln
 165 170 175

Leu Lys Gln Tyr Phe Tyr Glu Thr Lys Cys Asn Pro Met Gly Tyr Thr
 180 185 190

Lys Glu Gly Cys Arg Gly Ile Asp Lys Arg His Trp Asn Ser Gln Cys
 195 200 205

Arg Thr Thr Gln Ser Tyr Val Arg Ala Leu Thr Met Asp Ser Lys Lys
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Leu Gly Pro Ala Leu Val Pro Leu His Arg Leu Pro Arg Thr Leu Asp
35              40              45

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Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala
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Pro Asp Ala Met Glu Leu Arg Glu Leu Thr Pro Trp Ala Gly Arg Pro
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Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg Ala
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Arg Leu Gly Ala Arg Pro Cys Gly Leu Arg Glu Leu Glu Val Arg Val
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Ser Glu Leu Gly Leu Gly Tyr Ala Ser Asp Glu Thr Val Leu Phe Arg
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Tyr Cys Ala Gly Ala Cys Glu Ala Ala Ala Arg Val Tyr Asp Leu Gly
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Leu Arg Arg Leu Arg Gln Arg Arg Arg Leu Arg Arg Glu Arg Val Arg
 145 150 155 160

Ala Gln Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe
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Leu Asp Ala His Ser Arg Tyr His Thr Val His Glu Leu Ser Ala Arg
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Glu Cys Ala Cys Val
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<210> 432
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<220>
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19

CLAIMS:

1. An enhanced viral expression vector, wherein said vector is a vector for expression of at least one of a suppression agent for a disease-causing gene and a replacement nucleic acid that is not recognized by the suppression agent, wherein said vector is an adeno associated virus (AAV) vector, wherein the vector comprises:

conserved region B from the rhodopsin gene, wherein said conserved region B has the sequence set forth by SEQ ID NO: 93 or SEQ ID NO: 408; or

a sequence which provides enhanced expression when operatively linked to said suppression agent and / or replacement nucleic acid, wherein the sequence shares at least 90% identity over the entire length of a sequence set forth by SEQ ID NO: 93 or SEQ ID NO: 408; or

a sequence which provides enhanced expression when operatively linked to said suppression agent and / or replacement nucleic acid, wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence set forth by SEQ ID NO: 93 or SEQ ID NO: 408.

2. The enhanced viral expression vector according to claim 1, wherein said vector further comprises at least one of the conserved regions selected from

(a) conserved region C from the rhodopsin gene comprising a sequence as set forth by SEQ ID NO: 94, or a sequence having function of a sequence as set forth by SEQ ID NO: 94 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence set forth by SEQ ID NO: 94, or that shares at least 90% sequence identity over the entire length of a sequence set forth by SEQ ID NO: 94;

(b) conserved region F and G from the rhodopsin gene comprising a sequence as set forth by SEQ ID NO: 97, or a sequence having function of a sequence set forth by SEQ ID NO: 97 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 97, or that shares

at least 90% sequence identity over the entire length of a sequence set forth by SEQ ID NO: 97; and

(c) conserved region A from the rhodopsin gene comprising a sequence as set forth by SEQ ID NO: 92 or SEQ ID NO: 403, or a sequence having function of a sequence as set forth by SEQ ID NO: 92 or SEQ ID NO: 403 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 92 or SEQ ID NO: 403, or that shares at least 90% sequence identity over the entire length of a sequence set forth by SEQ ID NO: 92 or SEQ ID NO: 403, respectively.

3. The enhanced viral expression vector according to claim 1, wherein the vector additionally comprises conserved region D from the rhodopsin gene comprising a sequence as set forth by SEQ ID NO: 95, or a sequence having function of a sequence as set forth by SEQ ID NO: 95 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 95, or that shares at least 90% sequence identity over the entire length of a sequence set forth by, SEQ ID NO: 95.

4. The enhanced viral expression vector according to any one of claims 1 to 3, wherein the vector additionally comprises at least one of conserved regions H and I from the rhodopsin gene; wherein conserved region H comprises a sequence as set forth by SEQ ID NO: 98, or a sequence having function of a sequence as set forth by SEQ ID NO: 98 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 98, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by SEQ ID NO: 98; and wherein conserved region I comprises SEQ ID NO: 99, or a sequence having function of a sequence as set forth by SEQ ID NO: 99 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 99, or that shares at least 90% sequence identity over the entire length of a sequence set forth by, SEQ ID NO: 99.

5. The enhanced viral expression vector according to any one of claims 1 to 4, wherein the vector comprises one of each of conserved regions B, C, D, E, F and G, H, I and A from the rhodopsin gene comprising a sequence as set forth by SEQ ID NOs: 93, 94, 95, 96, 97, 98, 99 and 92, or a sequence having function of any of a sequence as set forth by SEQ ID NOs: 93, 94, 95, 96, 97, 98, 99 or 92 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 93, 94, 95, 96, 97, 98, 99 or 92, or that share at least 90% sequence identity with, over the entire length of a sequence as set forth by SEQ ID NOs: 93, 94, 95, 96, 97, 98, 99 or 92, respectively.

6. The enhanced viral expression vector according to any one of claims 1 to 4, wherein the vector comprises

(a) conserved region B, from the rhodopsin gene comprising a sequence as set forth by SEQ ID NO: 408, or a sequence having function of a sequence as set forth by SEQ ID NO: 408 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 408, or that share at least 90% sequence identity with, over the entire length of a sequence as set forth by SEQ ID NO: 408, and

(b) conserved region A from the rhodopsin gene comprising a sequence as set forth by SEQ ID NO:403, or a sequence having function of a sequence as set forth by SEQ ID NO: 403 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NO: 403, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by SEQ ID NO:403.

7. The enhanced viral expression vector according to any one of claims 1 to 6, wherein the vector comprises one or more sequences to optimize expression of the suppression agent, wherein said sequence to optimize expression is an enhancer sequence or a transcription factor binding site.

8. The enhanced viral expression vector according to any one of claims 1 to 7, wherein the vector comprises one or more sequences to optimize expression of the replacement nucleic acid, wherein said sequence to optimize expression is an enhancer sequence or a transcription factor binding site.
9. The enhanced viral expression vector of claim 8, wherein said sequence to optimize expression is an enhancer sequence.
10. The enhanced viral expression vector of claim 8 or 9, wherein the sequence(s) to optimize expression is selected from any of a sequence as set forth by SEQ ID NOs: 87-89, or a sequence having function of any of SEQ ID NOs: 87-89 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 87-89, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by SEQ ID NO: 87, 88, or 89, respectively.
11. The enhanced viral expression vector of claim 8 or 9, wherein the sequence(s) to optimize expression is selected from any of a sequence as set forth by SEQ ID NOs: 402-413, or a sequence having function of any of SEQ ID NOs: 402-413 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 404-413, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by any one of SEQ ID NOs: 402-413, respectively.
12. The enhanced viral expression vector of any one of claims 1 to 11, wherein the vector comprises at least one transcription factor binding site sequence selected from any of a sequence as set forth by SEQ ID NOs: 100-401, or a sequence having function of any of SEQ ID NOs: 100-401 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 100-401, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by any one of SEQ ID NOs: 100-401, respectively.

13. The enhanced viral expression vector according to any one of claims 1 to 12, wherein the vector comprises a chromatin opening element.

14. The enhanced viral expression vector according to any one of claims 1 to 13, wherein the vector comprises a sequence encoding a neurotrophic or neuroprotective factor.

15. The enhanced viral expression vector according to any one of claims 1 to 14, wherein the vector comprises at least one suppression agent.

16. The enhanced viral expression vector according to claim 15, wherein said suppression agent comprises a nucleotide sequence selected from the group consisting of a sequence as set forth by SEQ ID NOs: 75, 77, 79, 81, 83, 85, 414, 415, 416, 417, 418, 419, 420 and 421, or a sequence having function of any of a sequence as set forth by SEQ ID NOs: 75, 77, 79, 81, 83, 85, 414, 415, 416, 417, 418, 419, 420 and 421 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 75, 77, 79, 81, 83, 85, 414, 415, 416, 417, 418, 419, 420 and 421 or that shares at least 90% sequence identity over the entire length of a sequence as set forth by, any one of SEQ ID NOs: 75, 77, 79, 81, 83, 85, 414, 415, 416, 417, 418, 419, 420 and 421, respectively.

17. The enhanced viral expression vector according to claim 15, wherein said suppression agent comprises a nucleotide sequence selected from the group consisting of a sequence as set forth by SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33, or a sequence having function of any of a sequence as set forth by SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33, or that shares at least 90% sequence identity over the entire length of a sequence a sequence as set forth by, any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33, respectively.

18. The enhanced viral expression vector according to claim 15, wherein said suppression agent comprises a nucleotide sequence selected from the group consisting of a sequence as set forth by SEQ ID NOs: 35-67, or sequence having function of any of SEQ ID NOs: 35-67 wherein the complement of said sequence can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 35-67, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by any one of SEQ ID NOs: 35-67, respectively.

19. The enhanced viral expression vector according to any one of claims 1 to 18 wherein the vector comprises at least one replacement nucleic acid.

20. The enhanced viral expression vector according to claim 19, wherein said replacement nucleic acid comprises a nucleotide sequence selected from the group consisting of a sequence as set forth by SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 68, or a sequence having function of any of a sequence as set forth by SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 68 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 68, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by, any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 68, respectively.

21. The enhanced viral expression vector according to claim 20, wherein said replacement nucleic acid comprises a nucleotide sequence selected from the group consisting of a sequence as set forth by SEQ ID NOs: 76, 78, 80, 82, 84, and 86, or a sequence having function of any of SEQ ID NOs: 76, 78, 80, 82, 84, and 86 wherein the complement thereof can hybridize at stringent hybridization conditions (0.2SCC, 0.1% SDS) to a sequence as set forth by SEQ ID NOs: 76, 78, 80, 82, 84, and 86, or that shares at least 90% sequence identity over the entire length of a sequence as set forth by any one of SEQ ID NOs: 76, 78, 80, 82, 84, and 86, respectively.

22. The enhanced viral expression vector according to any one of claims 1 to 21, wherein said disease is a disease of the eye.
23. The enhanced viral expression vector according to any one of claims 1 to 22, wherein the replacement nucleic acid encodes a rhodopsin gene.
24. A therapeutic composition comprising at least one enhanced viral expression vector according to any one of claims 1 to 23 and a pharmaceutically acceptable carrier.
25. A cell comprising the enhanced viral expression vector of any one of claims 1 to 23.
26. The enhanced viral expression vector according to any one of claims 1 to 23 for use in the treatment of ocular disease.
27. Use of the enhanced viral expression vector according to any one of claims 1 to 23 for the treatment of ocular disease.
28. Use of the enhanced viral expression vector according to any one of claims 1 to 23 for the manufacture of a therapeutic composition for the treatment of ocular disease.

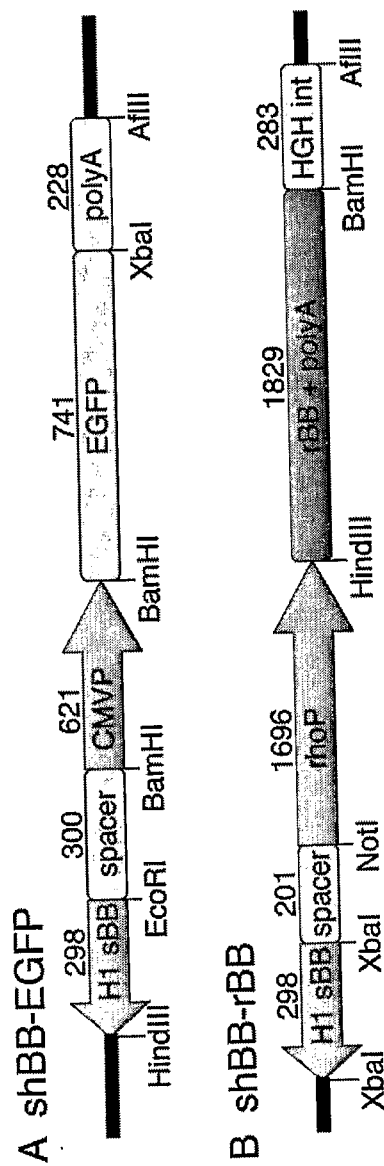


Figure 1

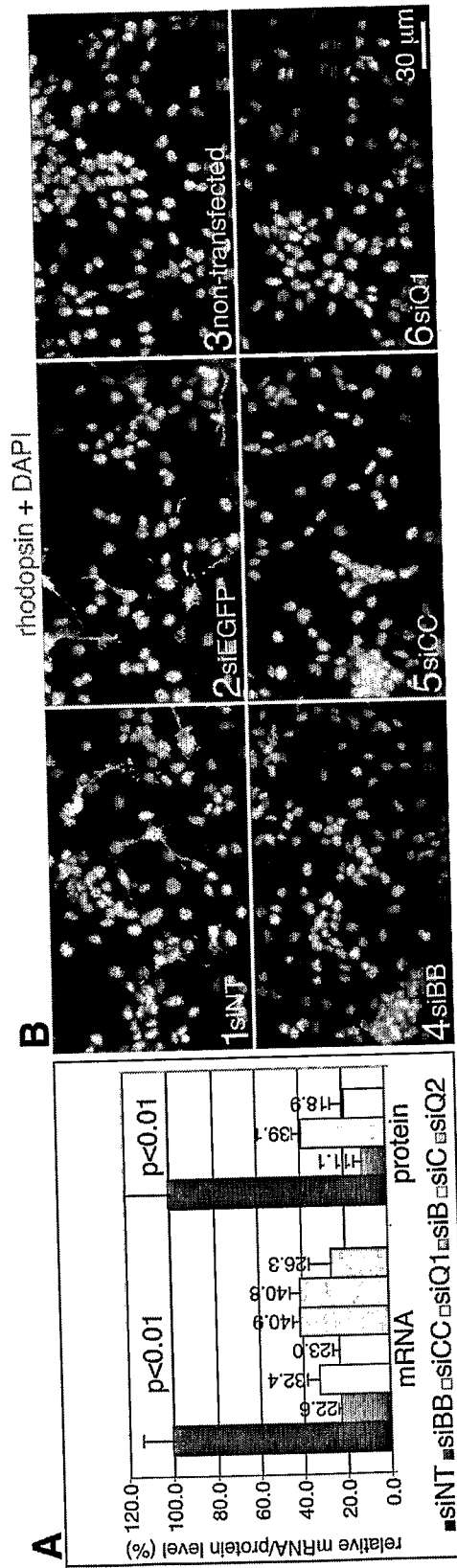


Figure 2

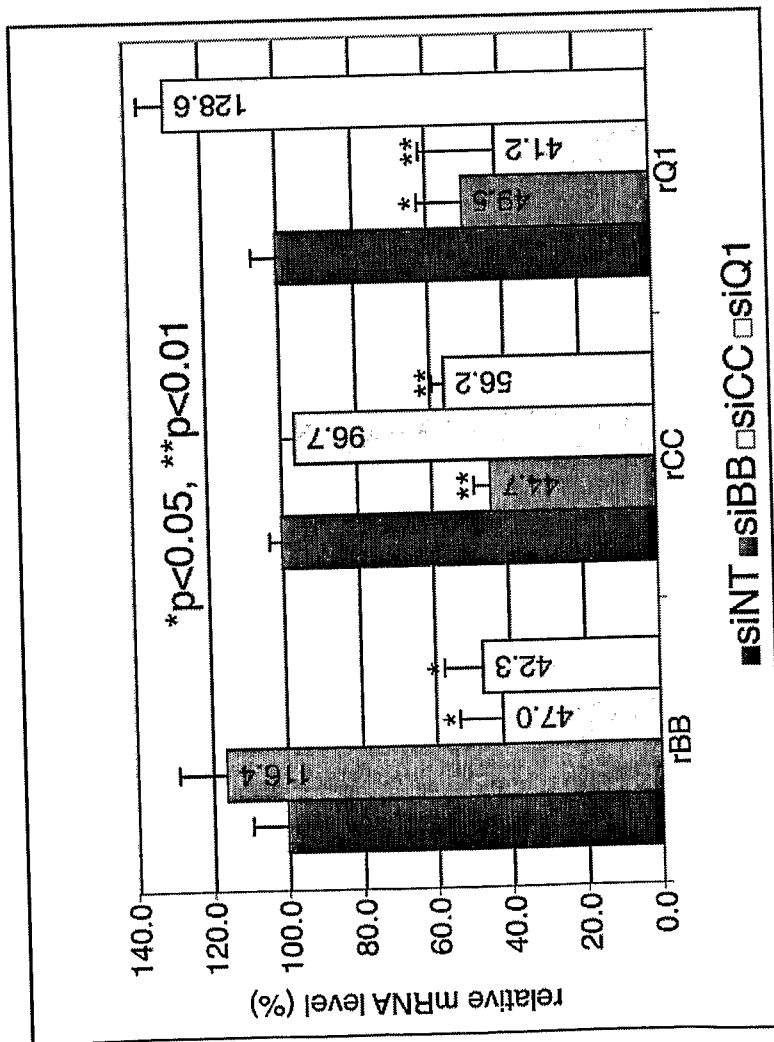


Figure 3

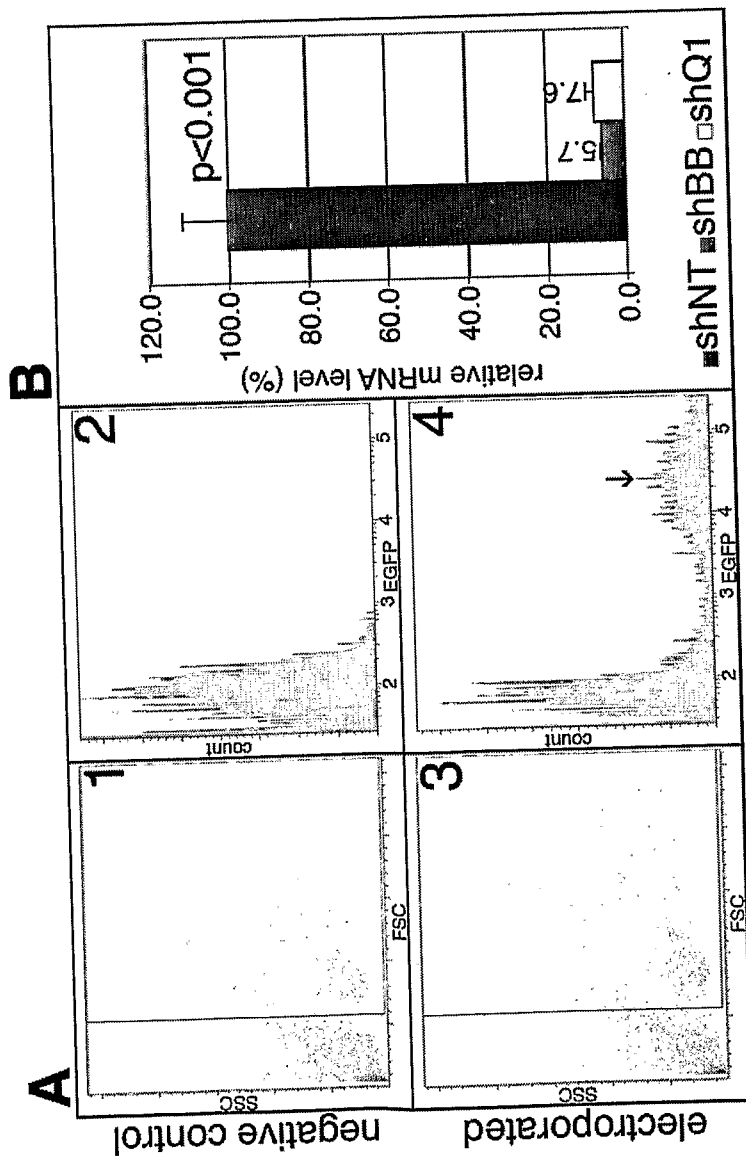


Figure 4

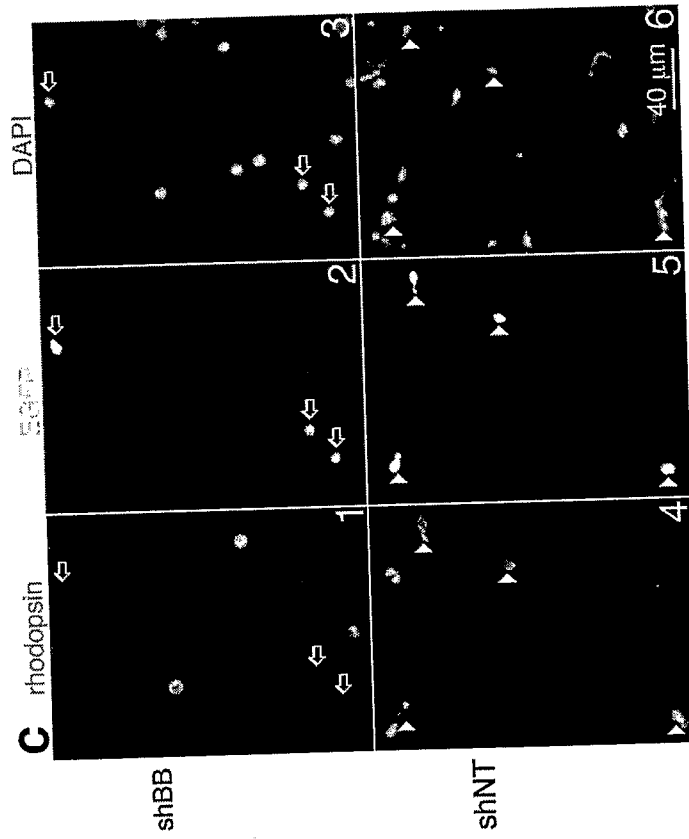
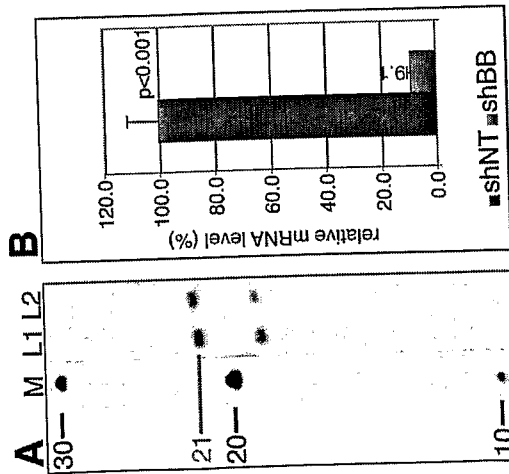


Figure 5



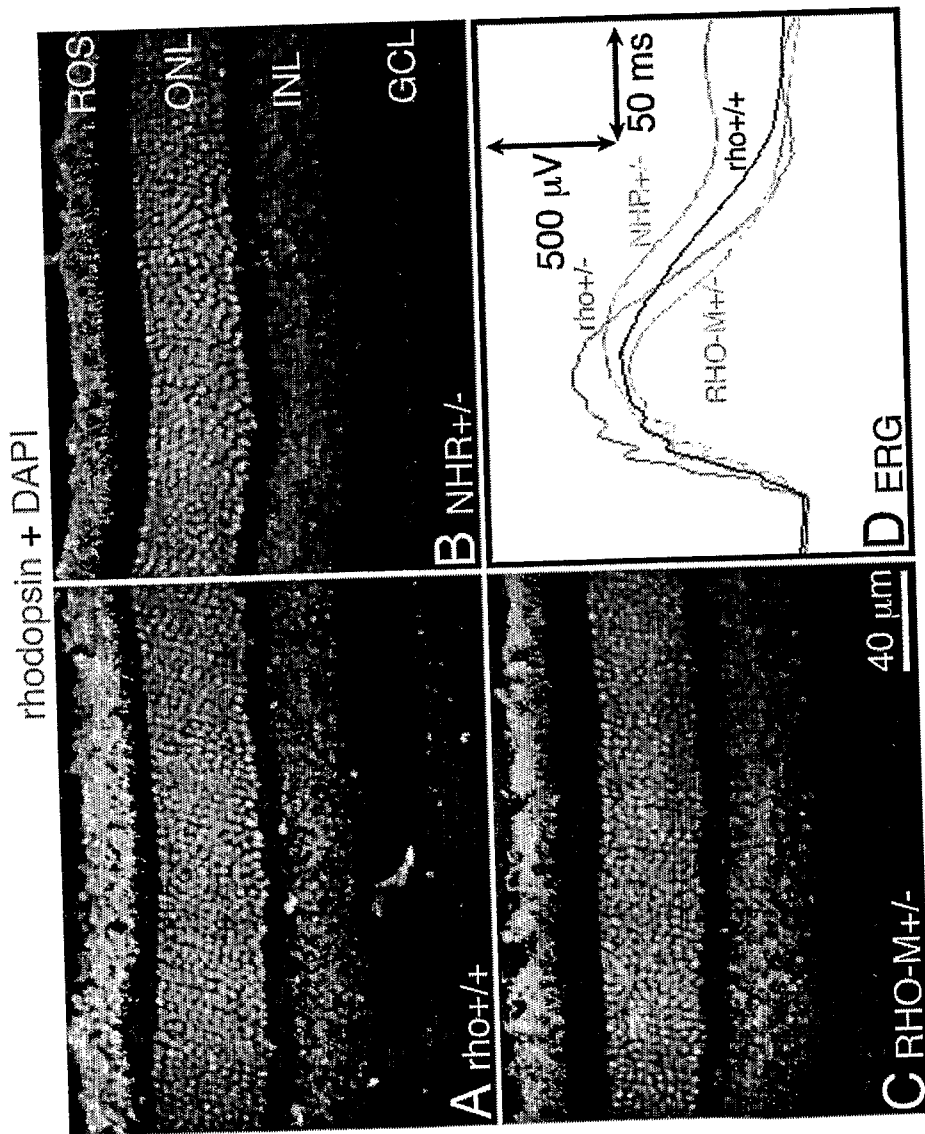


Figure 6 A-D

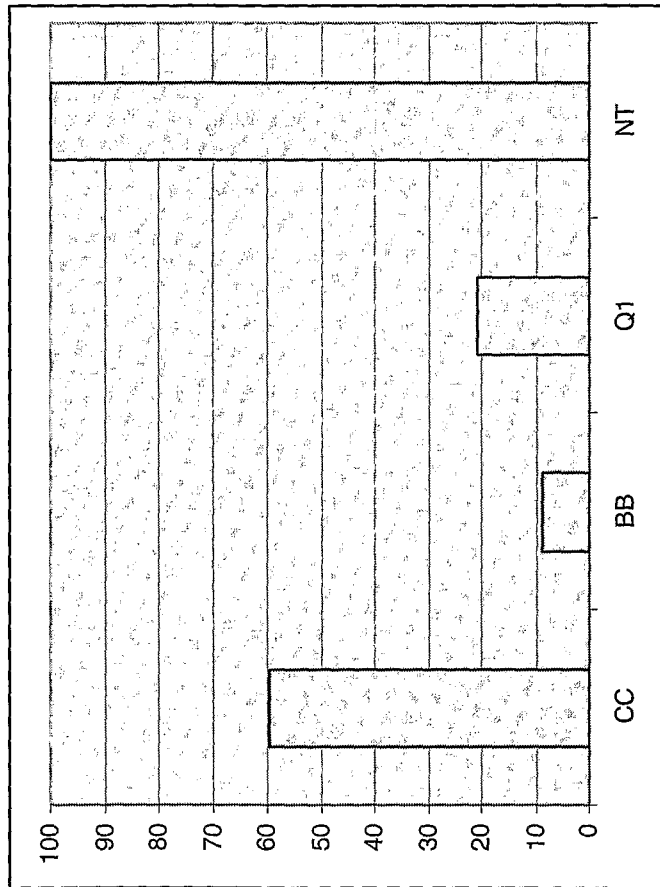
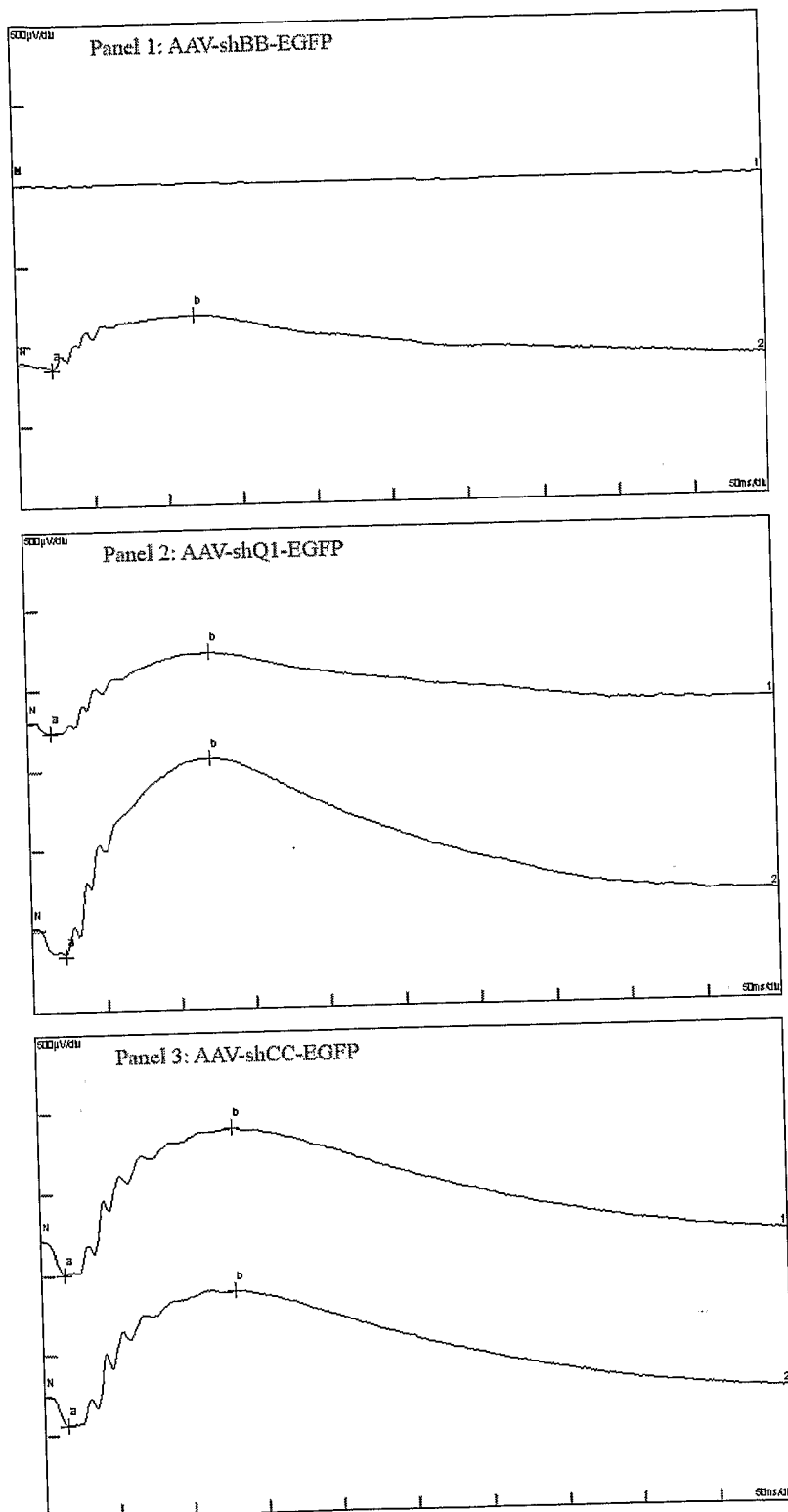
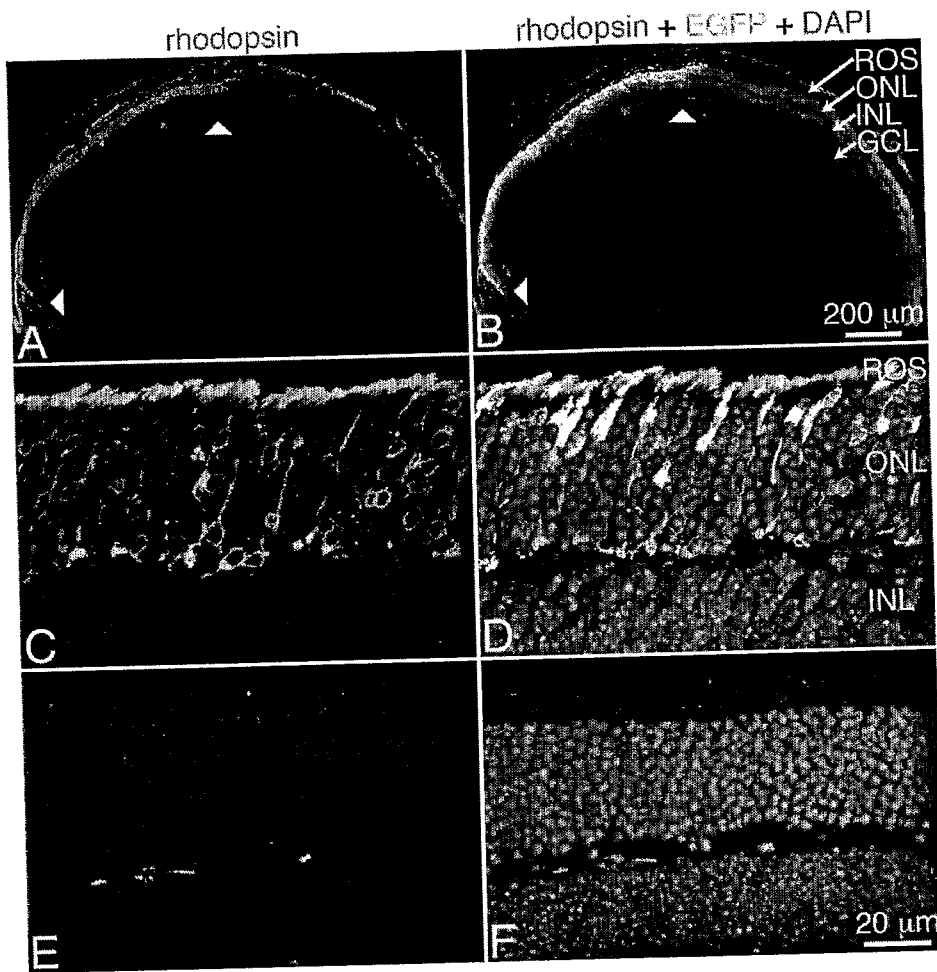


Figure 6E

**Figure 6F**

**Figure 7**

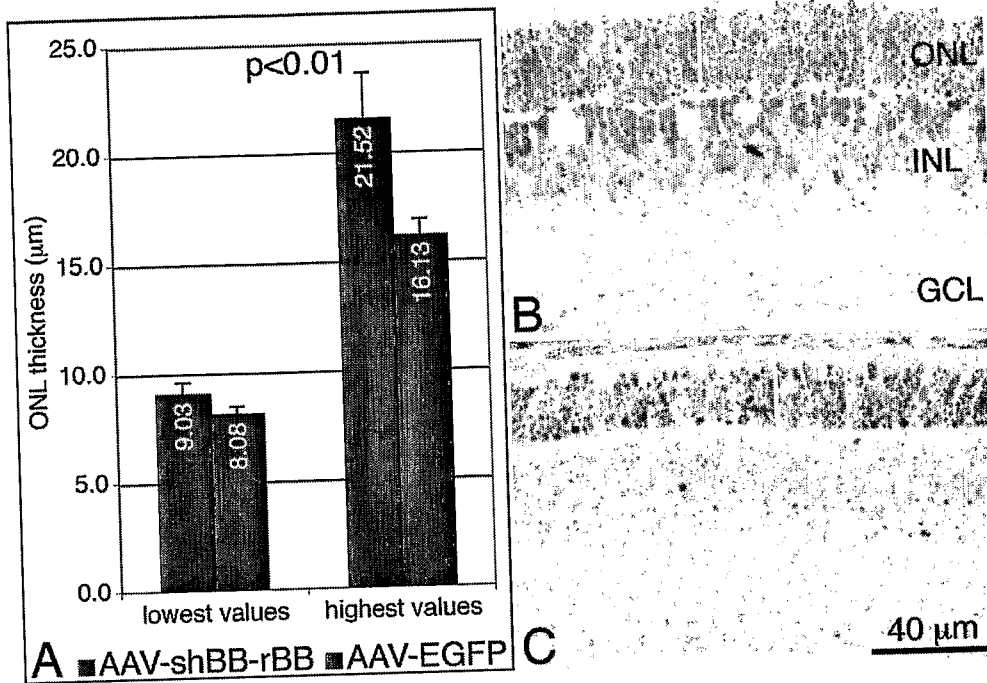
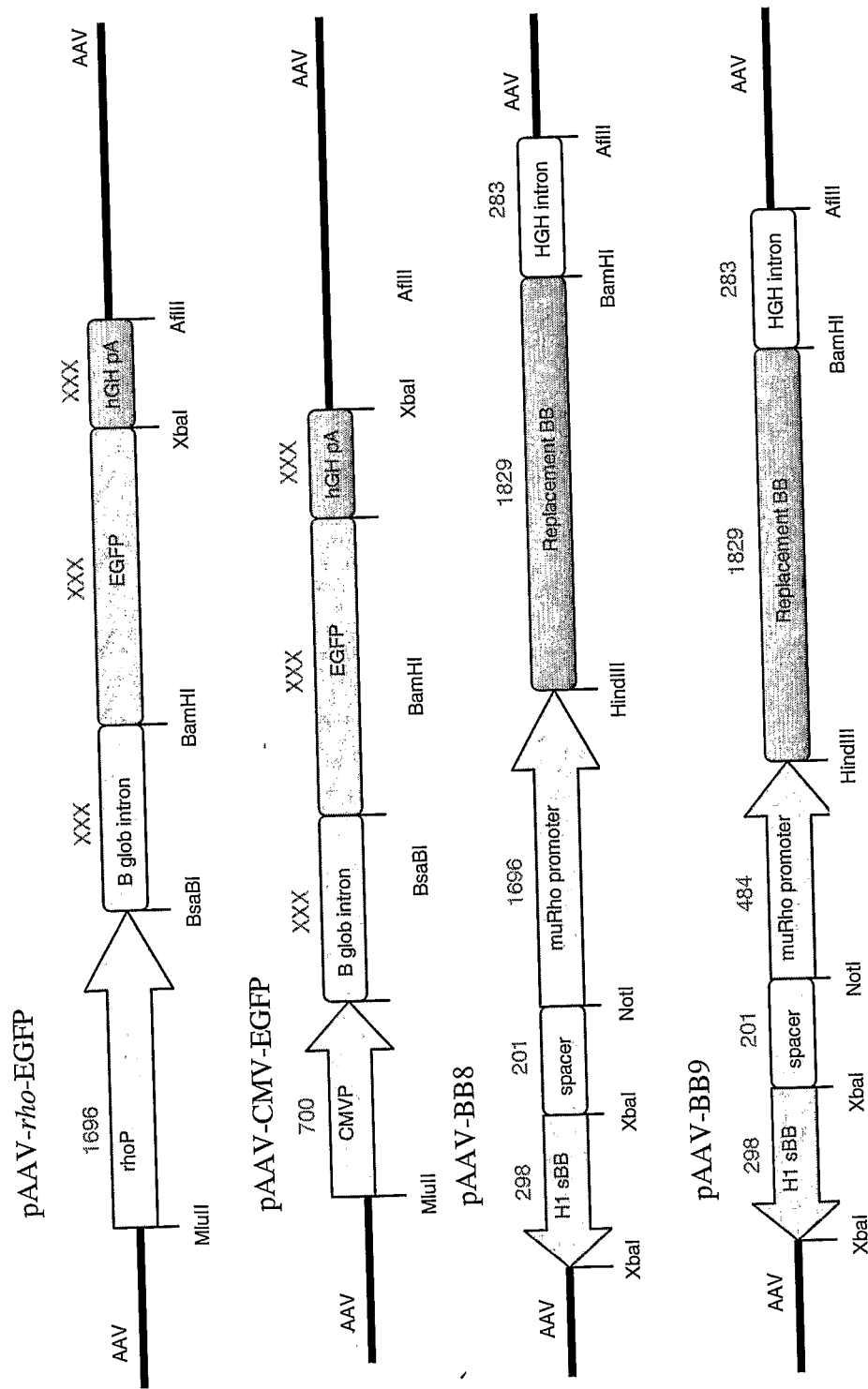


Figure 8

**Figure 9**

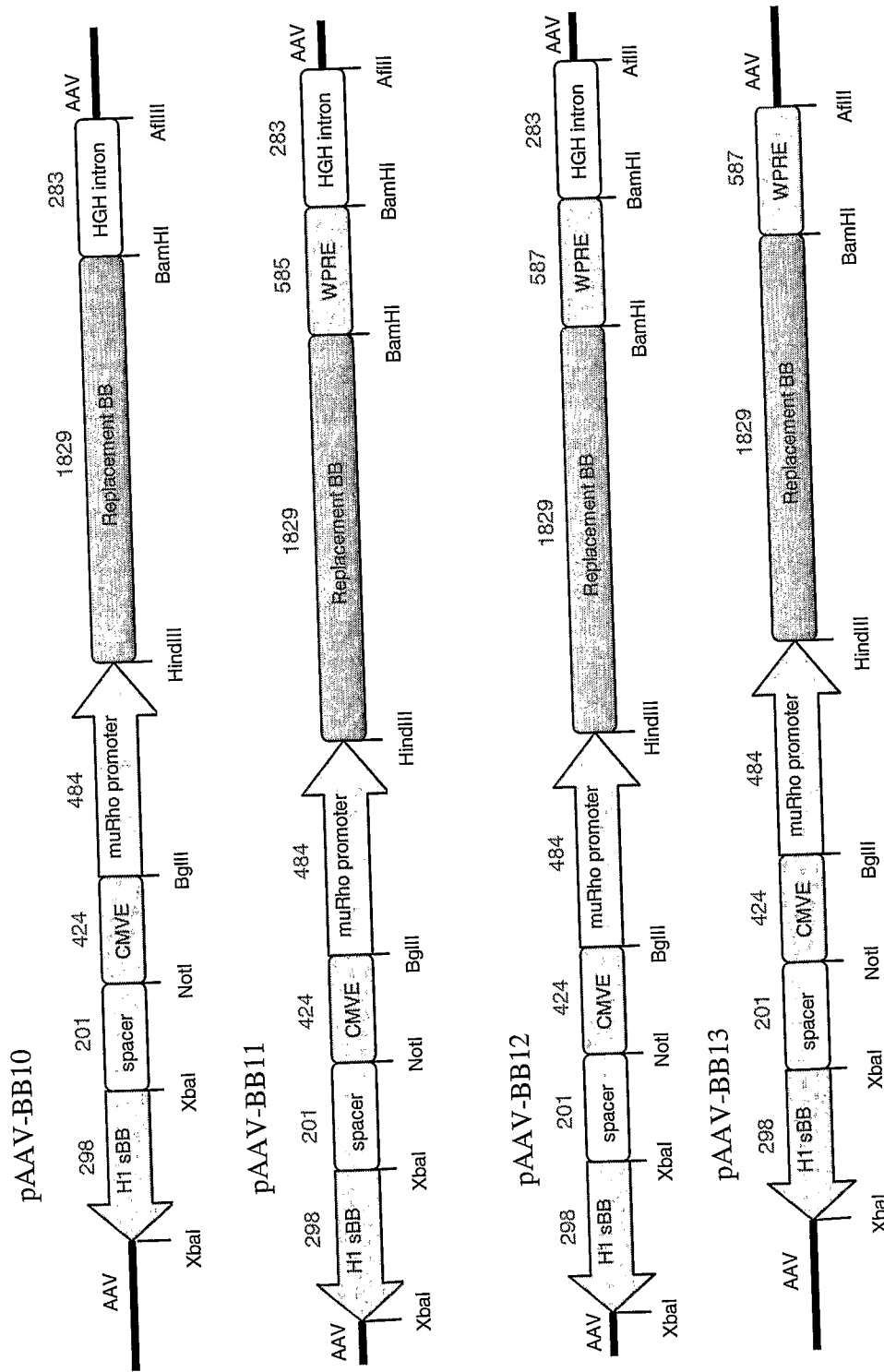


Figure 9 (Continued)

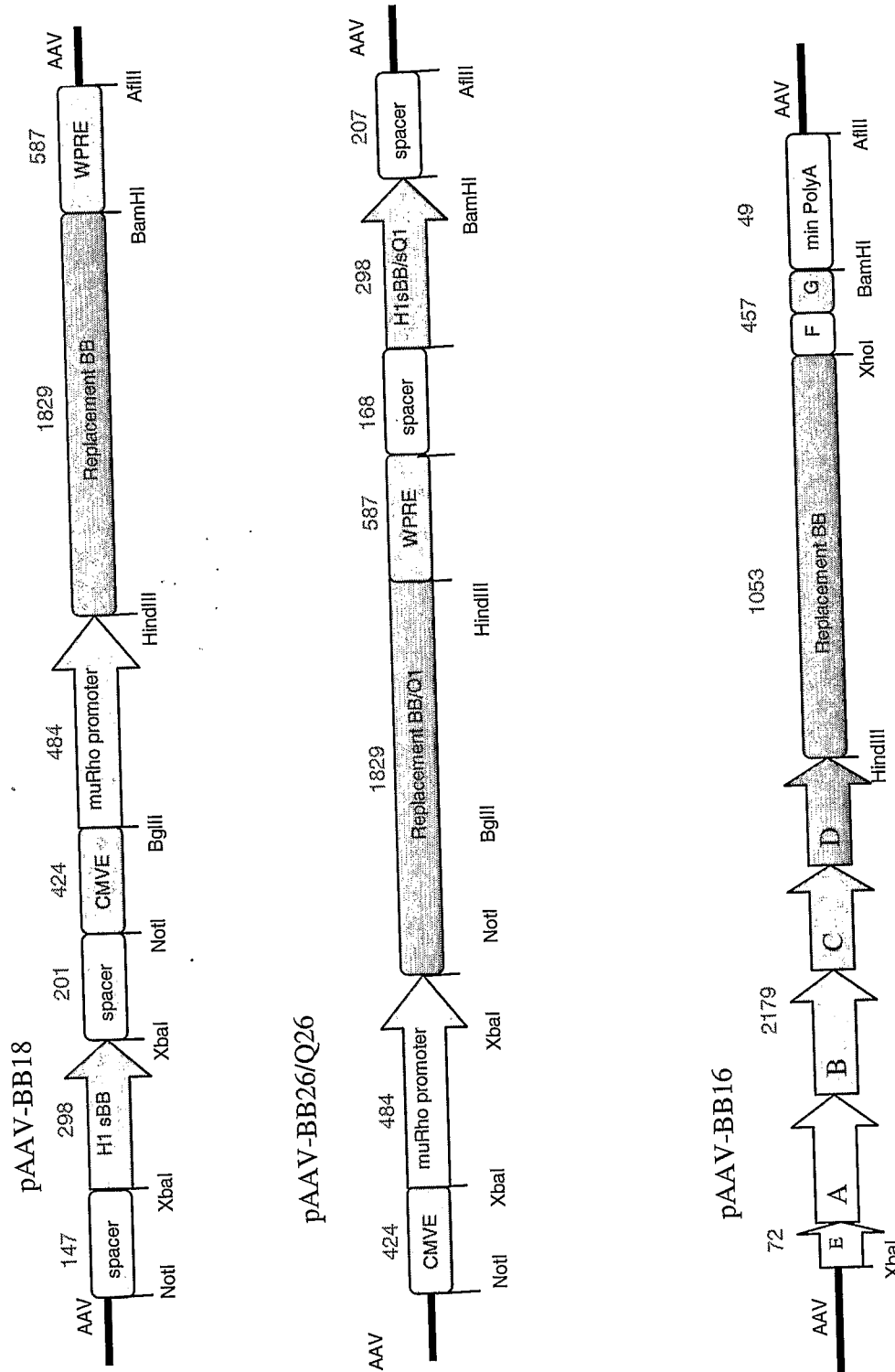
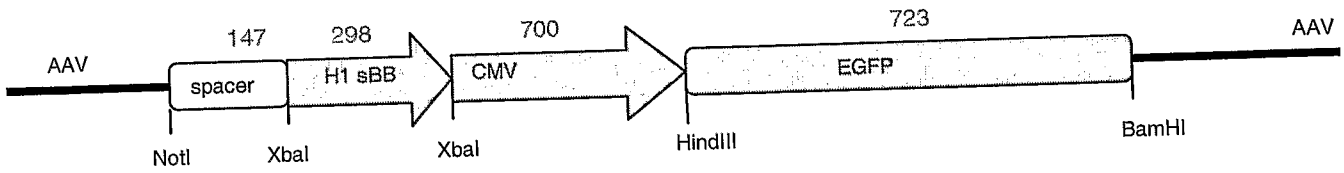


Figure 9 (Continued)

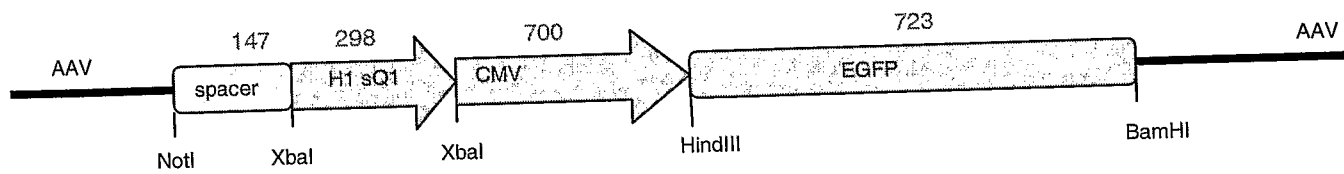


Figure 9 (Continued)

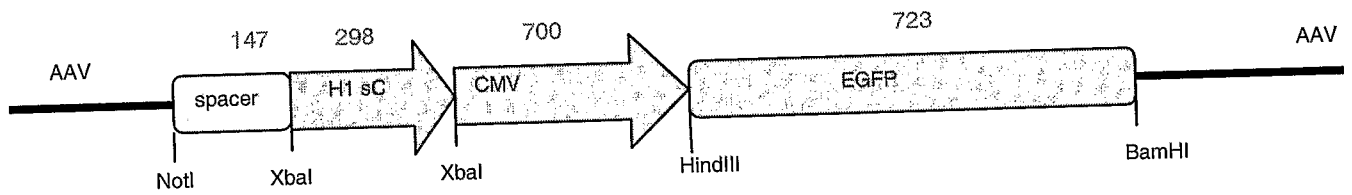
pAAV-shBB-EGFP



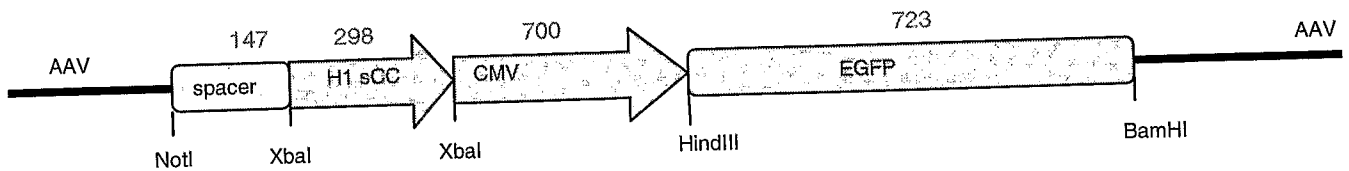
pAAV-shQ1-EGFP



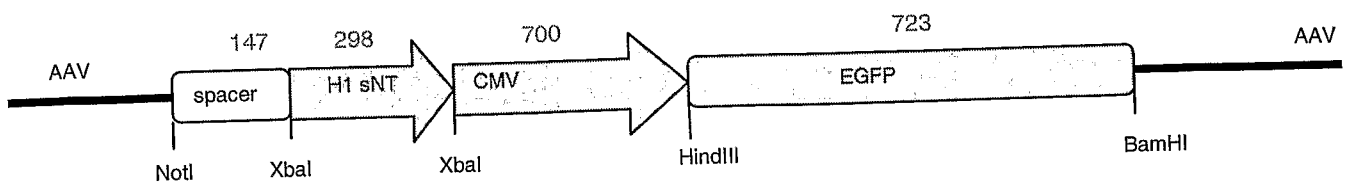
pAAV-shC-EGFP

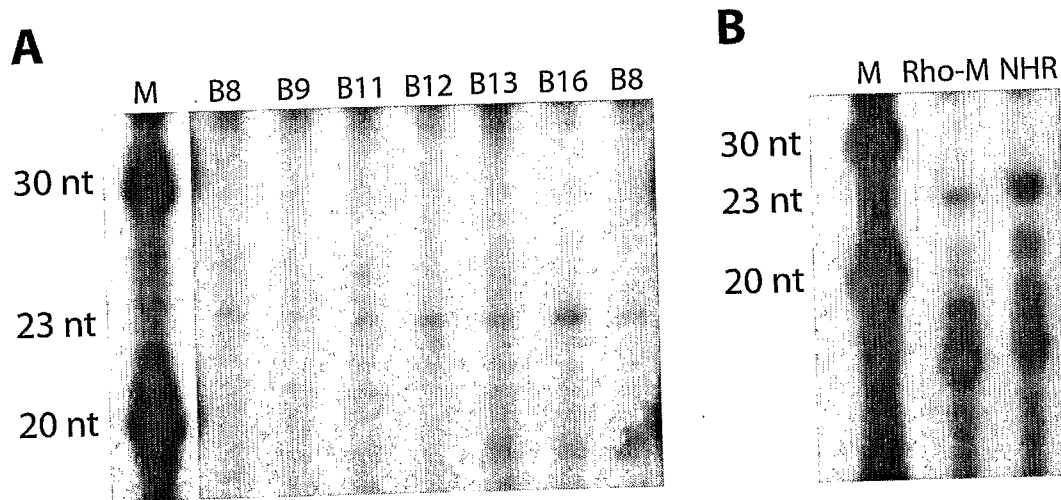


pAAV-shCC-EGFP



pAAV-shNT-EGFP



**Figure 10**

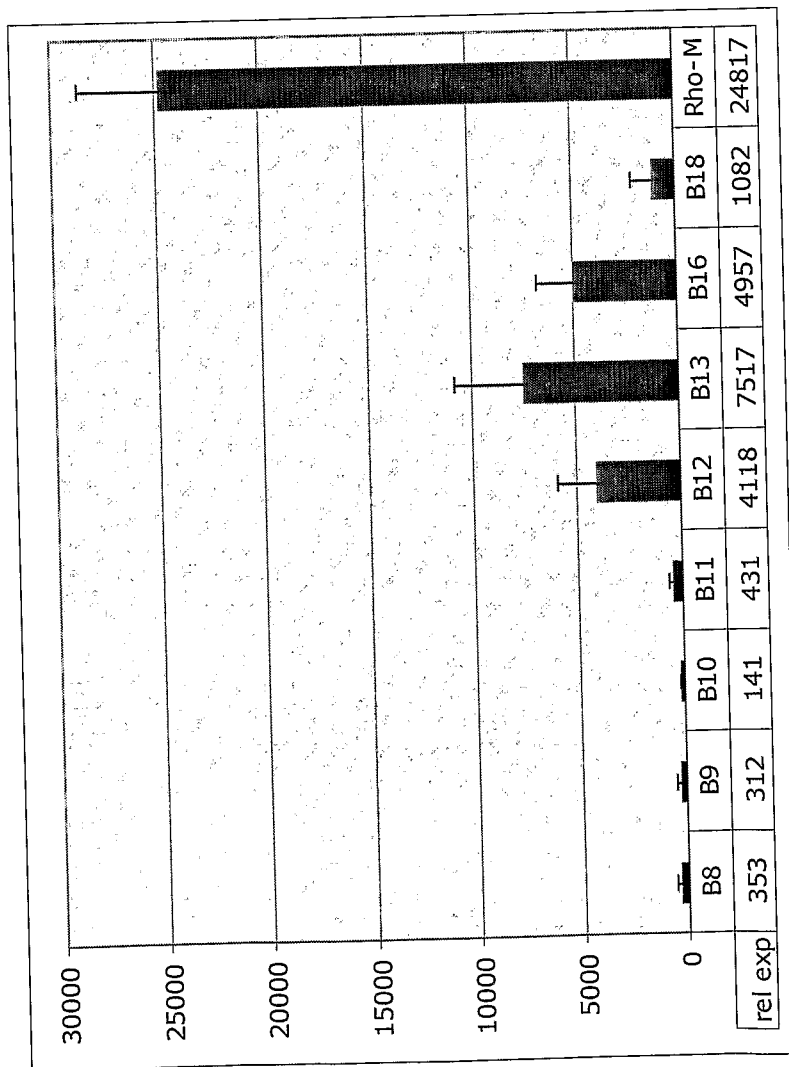


Figure 11

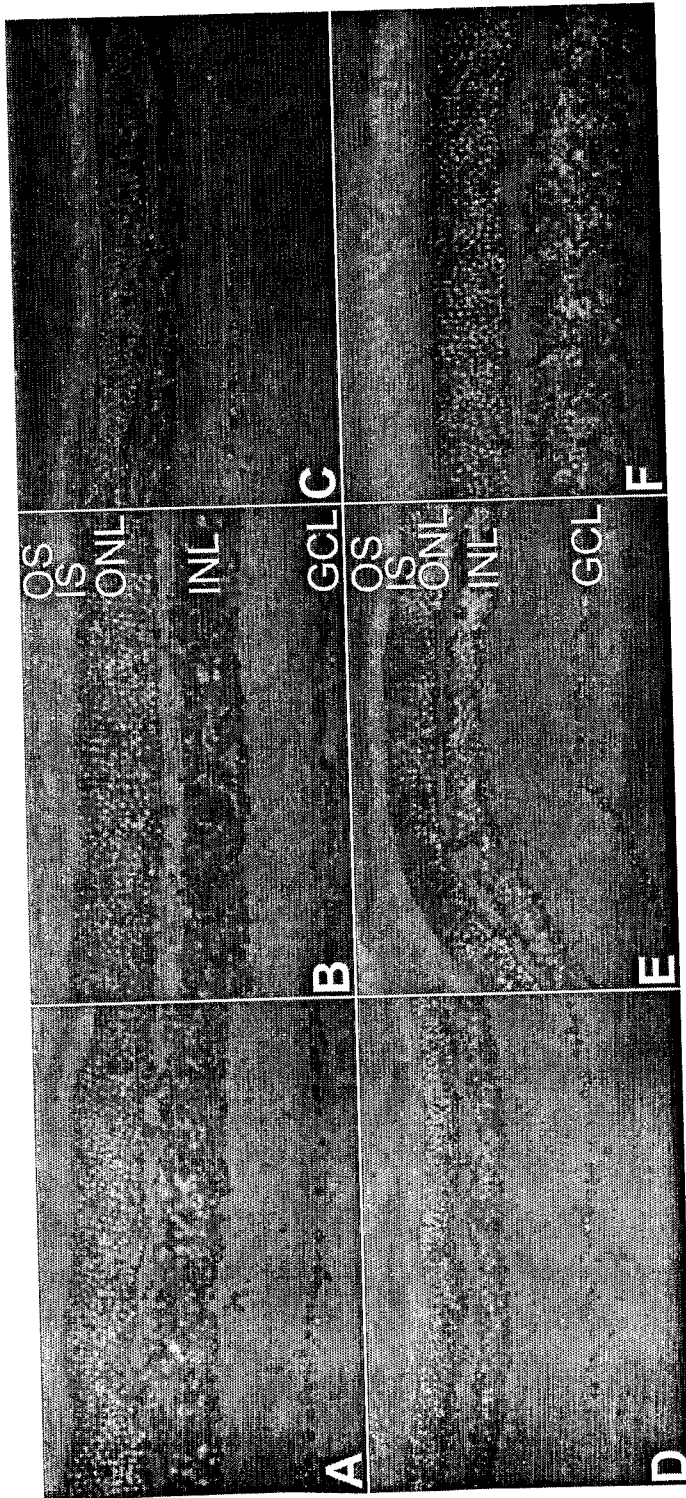


Figure 12

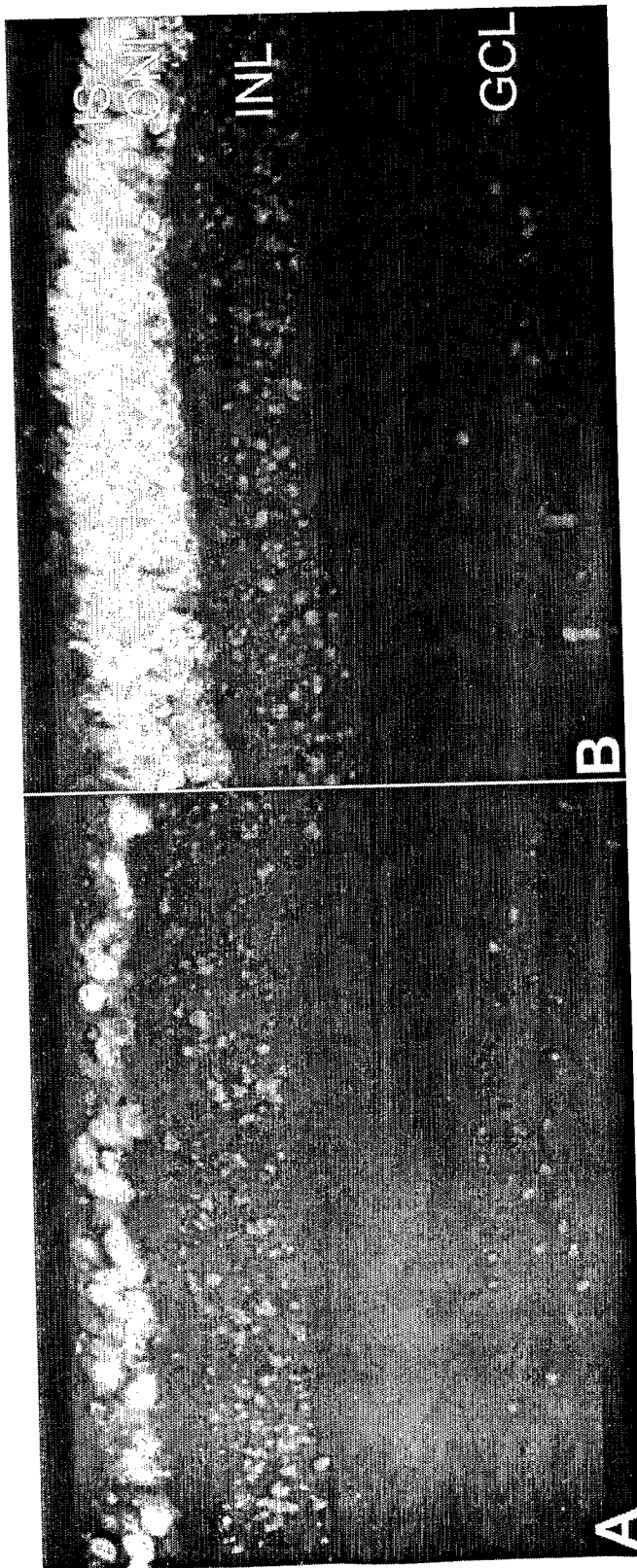


Figure 13

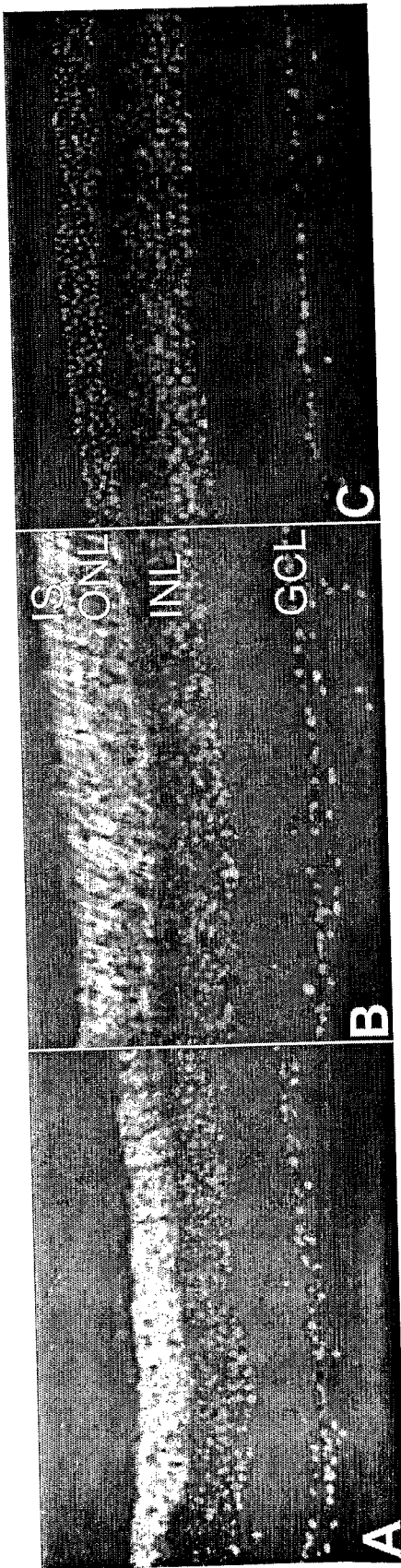


Figure 14A-C

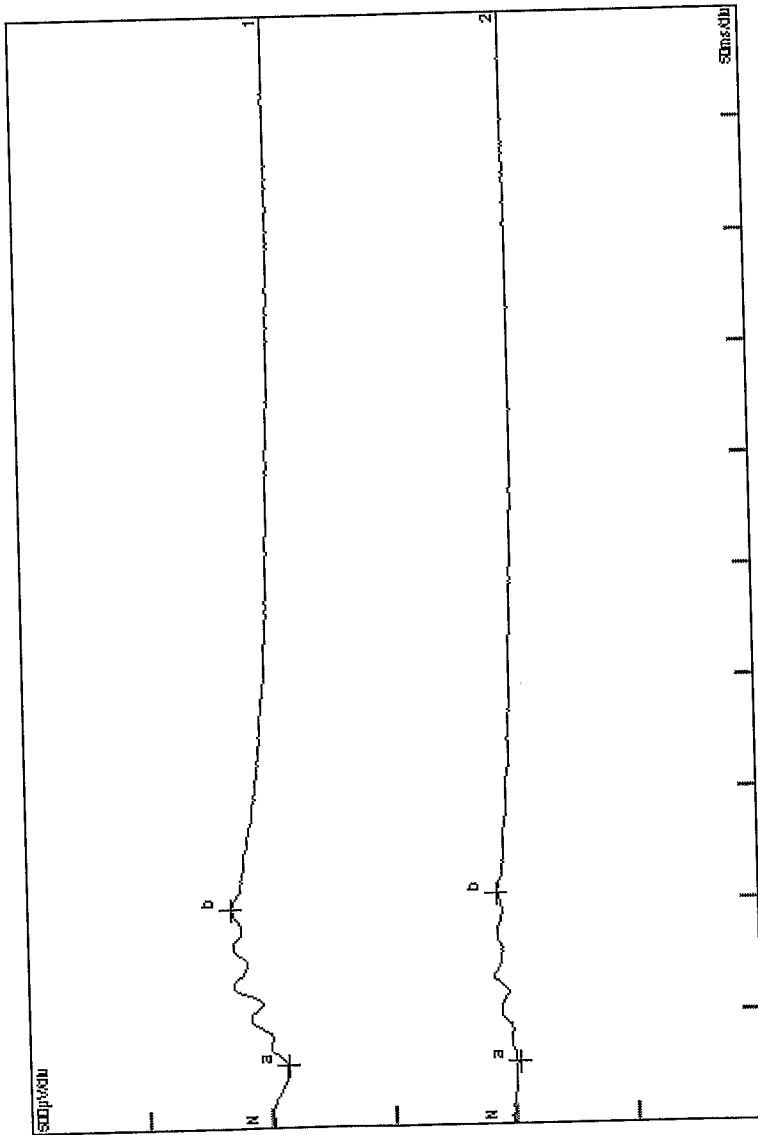


Figure 14D

Figure 15

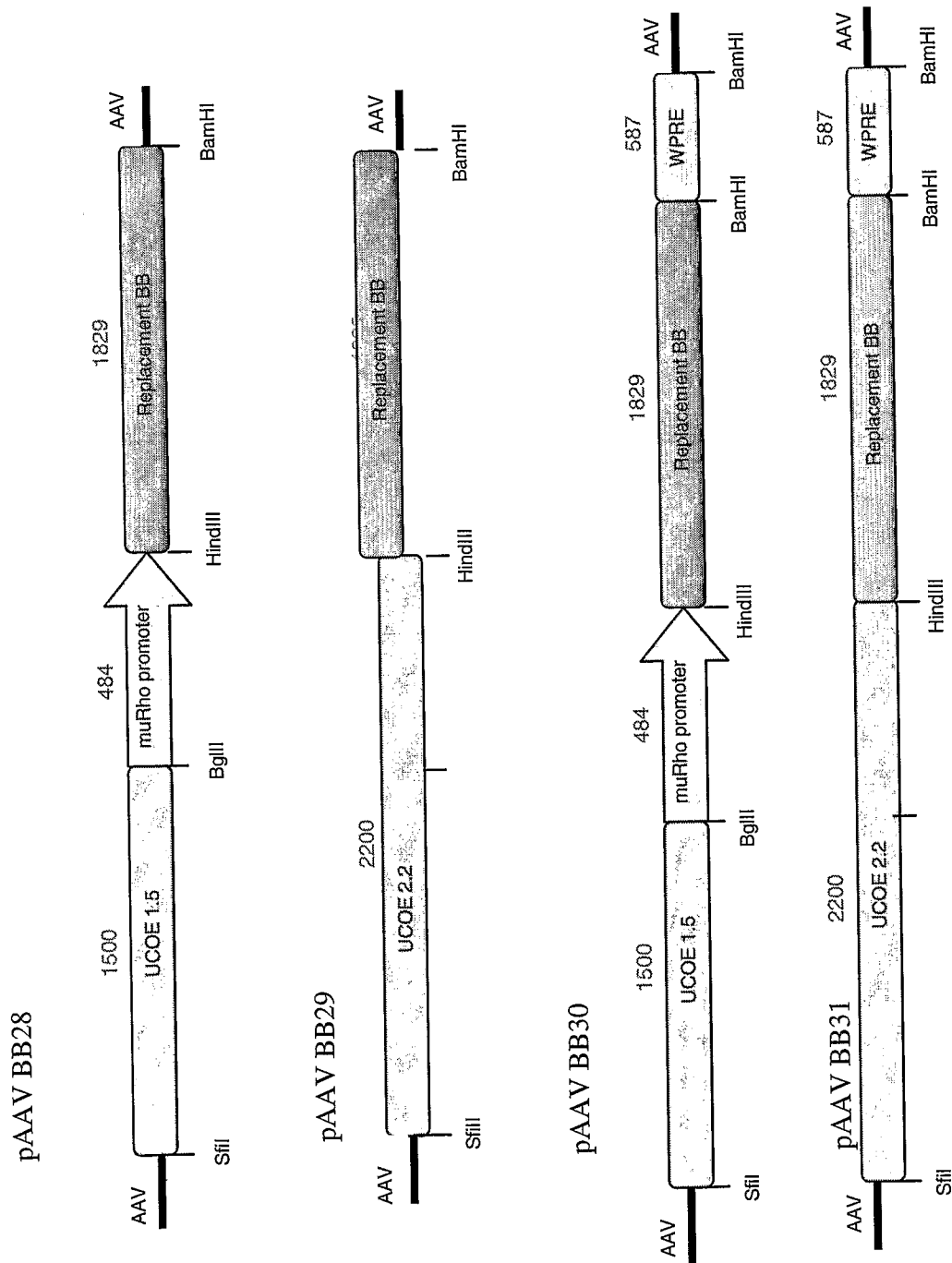


Figure 16: Sequences of exemplary elements that can facilitate modulation of chromatin structures

UCOE 1.5 (SEQ ID NO: 422)

```

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GCCGGGAGCT   GCGCGCGGGG   CCGCCGGGGG   GAGGGGTGGC   ACCGCCCACG
CCGGGCGGCC   ACGAAGGGCG   GGGCAGCGGG   CGCGCGCGCG   GCGGGGGGAG
GGGCGGGCGC   CGCGCCCGCT   GGGAATTGGG   GCCCTAGGGG   GAGGGCGGAG
GCGCCGACGA   CCGCGGCAC   TACCGTTCGC   GGCGTGGCGC   CCGGTGGTCC
CCAAGGGGAG   GGAAGGGGGA   GGCGGGGCGA   GGACAGTGAC   CGGAGTCTCC
TCAGCGGTGG   CTTTTCTGCT   TGGCAGCCTC   AGCGGCTGGC   GCCAAAACCG
GACTCCGCCC   ACTTCCTCGC   CCGCCGGTGC   GAGGGTGTGG   AATCCTCCAG
ACGCTGGGGG   AGGGGGAGTT   GGGAGCTTAA   AAAGTAGTAC   CCCTTTGGGA
CCACTTTCAG   CAGCGAACTC   TCCTGTACAC   CAGGGGTCAG   TTCCACAGAC
GCGGGCCAGG   GGTGGGTCAT   TGCGGCGTGA   ACAATAATTT   GACTAGAAGT
TGATTCCGGT   GTTTCGGGAA   GGGGCCGAGT   CAATCCGCCG   AGTTGGGGCA
CGGAAAACAA   AAAGGGAAGG   CTACTAAGAT   TTTTCTGGCG   GGGGTATCA
TTGGCGTAAC   TGCAGGGACC   ACCTCCCGGG   TTGAGGGGGC   TGGATCTCCA
GGCTGCGGAT   TAAGCCCCTC   CCGTCGGCGT   TAATTTCAAA   CTGCGCGACG
TTTCTCACCT   GCCTTCGCCA   AGGCAGGGGC   CGGGACCCTA   TTCCAAGAGG
TAGTAAC TAG   CAGGACTCTA   GCCTTCGCA   ATTCATTGAG   CGCATTTACG
GAAGTAACGT   CGGGTACTGT   CTCTGGCCGC   AAGGGTGGGA   GGAGTACGCA
TTTGGCGTAA   GGTGGGGCGT   AGAGCCTTCC   CGCCATTGGC   GGC GGATAGG
GCGTTTACGC   GACGGCCTGA   CGTAGCGGAA   GACGCGTTAG   TGGGGGGGAA
GGTTCTAGAA   AAGCGGCGGC   AGCGGCTCTA   GCGGCAGTAG   CAGCAGCGCC
GGGTCCCGTG   CGGAGGTGCT   CCTCGCAGAG   TTGTTTCTCG   AGCAGCGGCA
GTTCTCACTA   CAGCGCCAGG   ACGAGTCCGG   TTCGTGTTTC   TCCGCGGAGA
TCTCTCTCAT   CTCGCTCGGC   TGCGGGAAAT   CGGGCTGAAG   CGACTGAGTC
CGCGATGGAG   GTAACGGGT   TGAAATCAAT   GAGTTATTGA   AAAGGGCATG
GCGAGGCCGT   TGGCGCCTCA   GTGGAAGTCG   GCCAGCCGCC   TCCGTGGGAG
AGAGGCAGGA   AATCGGACCA   ATTCAGTAGC   AGTGGGGCTT   AAGGTTTATG
AACGGGGTCT   TGAGCGGAGG   CCTGAGCGTA   CAAACAGCTT   CCCCACCCTC
AGCCTCCCGG   CGCCATTTC   CTTCACTGGG   GGTGGGGGAT   GGGGAGCTTT
CACATGGCGG   ACGCTGCCCC   GCTGGGGTGA   AAGTGGGGCG   CGGAGGCGGG
AATTC TTATT   CCCTTTCTAA   AGCACGCTGC   TTCGGGGGCC   ACGGCGTCTC
C

```

UCOE 2.2 (SEQ ID NO: 423)

AAAAACAGCTT CACATGGCTT AAAATAGGGG ACCAATGTCT TTTCCAATCT
AAGTCCCATT TATAATAAAG TCCATGTTCC ATTTTAAAG GACAATCCTT
TCGGTTTAAA ACCAGGCACG ATTACCCAAA CAACTCACAA CGGTAAAGCA
CTGTGAATCT TCTCTGTTCT GCAATCCCAA CTGGTTTCT GCTCAGAAAC
CCTCCCTCTT TCCAATCGGT AATTAAATAA CAAAAGGAAA AAACCTAAGA
TGCTTCAACC TTCAACCCCG TTTCGTGACA CTTTGAAAAA AGAATCACCT
CTTGCAAACA CCCGCTCCCG ACCCCCGCCG CTGAAGCCCG GCGTCCAGAG
GCCTAAGCGC GGGTGCCAC CCCACCCCG GAGCGCGGGC CTCGTGGTCA
GCGCATCCGC GGGGAGAAAC AAAGGCCGCG GCACGGGGGC TCAAGGGCAC
TGCGCCACAC CGCACGCGCC TACCCCGCG CGGCCACGTT AACTGGCGGT
CGCCGCAGCG TCGGACAGC CGGCCGCGCG CCGCCAGGCT CGCGACGCG
GGACCCAGCG CCGCCCTCGG GGAGGCCCAA GTCTCGACCC AGCCCGCGT
GGCGCTGGGG GAGGGGGCGC CTCCGCCGGA ACGCGGGTGG GGGAGGGAG
GGGAAATGC GCTTTGTCTC GAAATGGGGC AACCCTCGCC ACAGCTCCCT
ACCCCTCGA GGGCAGAGCA GTCCCCCAC TAACTACCG GCTGGCCGCG
CGCCAGGCCA GCCGCGAGGC CACCGCCGA CCTCCACTC CTTCCCGCAG
CTCCCGCGC GGGGTCCGCG GAGAAGGGGA GGGGAGGGGA GCGGAGAACC
GGGCCCCCG GACGCGTGTG GCATCTGAAG CACCACCAGC GAGCGAGAGC
TAGAGAGAAG GAAAGCCACC GACTTCACCG CCTCCGAGCT GCTCCGGGTC
GCGGGTCTGC AGCGTCTCCG GCCCTCCGCG CTACAGCTC AAGCCACATC
CGAAGGGGGA GGGAGCCGGG AGCTGCGCG GGGGCCGCCG GGGGAGGGG
TGGCACCGCG CACGCCGGG GGCACGAAG GCGGGGCGAG CGGGCGCGCG
CGCGGGGGG GAGGGGGCG GCGCCGCGCC CGCTGGGAAT TGGGGCCCTA
GGGGGAGGGC GGAGGCGCCG ACGACCGCG CACTTACCGT TCGCGGCGTG
GCGCCCGGTG GTCCCCAAGG GGAGGGAAG GGGAGGCGGG GCGAGGACAG
TGACCGGAGT CTCCTCAGCG GTGGCTTTTC TGCTTGGCAG CCTCAGCGGC
TGGCGCCAAA ACCGGA CTCC GCCCACTTCC TCGCCCGCCG GTGCGAGGGT
GTGGAATCCT CCAGACGCTG GGGGAGGGGG AGTTGGGAGC TTAAAACTA
GTACCCCTTT GGGACCACTT TCAGCAGCGA ACTCTCCTGT ACACCAGGGG
TCAGTTCCAC AGACGCGGGC CAGGGGTGGG TCATTGCGGC GTGAACAATA
ATTTGACTAG AAGTTGATTC GGGTGT'TTCC GGAAGGGGCC GAGTCAATCC
GCCGAGTTGG GGCACGGAAC AAAAAAGGG AAGGCTACTA AGATTTTCT
GGCGGGGGT ATCATTGGCG TAACTGCAGG GACCACCTCC CGGGTTGAGG
GGGCTGGATC TCCAGGCTGC GGATTAAGCC CCTCCCGTCG GCGTTAATTT
CAAAC TGCGC GACGTTTCTC ACCTGCCTTC GCCAAGGCAG GGGCCGGGAC
CCTATTCCAA GAGGTAGTAA CTAGCAGGAC TCTAGCCTTC CGCAATTCA
TGAGCGCATT TACGGAAGTA ACGTCGGGTA CTGTCTCTGG CCGCAAGGGT
GGGAGGAGTA CGCATTTGGC GTAAGGTGGG GCGTAGAGCC TTCCCGCCAT
TGGCGGCGGA TAGGGCGTTT ACGCGACGGC CTGACGTAGC GGAAGACGCG
TTAGTGGGGG GGAAGGTTCT AGAAAAGCGG CGGCAGCGGC TCTAGCGGCA
GTAGCAGCAG CGCCGGGTCC CGTGCGGAGG TGCTCCTCGC AGAGTTGTTT
CTCGAGCAGC GGCAGTTCTC ACTACAGCGC CAGGACGAGT CCGGTTCCGT
TTCGTCCGCG GAGATCTCTC TCATCTCGCT CGGCTGCGGG AAATCGGGCT
GAAGCGACTG ATCTGCAGTC GAGGTCGACG GTATCGAT

Figure 17**GDNF Homo sapiens**

ATGAAGTTAT GGGATGTCGT GGCTGTCTGC CTGGTGCTGC TCCACACCGC GTCCGCCTTC
 CCGCTGCCCCG CCGCAAATAT GCCAGAGGAT TATCCTGATC AGTTCGATGA TGTCATGGAT
 TTTATTCAAG CCACCATTA AAGACTGAAA AGGTCACCAG ATAAACAAAT GGCAGTGCTT
 CCTAGAAGAG AGCGGAATCG GCAGGCTGCA GCTGCCAACC CAGAGAATTC CAGAGGAAAA
 GGTCCGAGAG GCCAGAGGGG CAAAACCGG GGTGTGTCT TAACTGCAAT ACATTTAAAT
 GTCACTGACT TGGGTCTGGG CTATGAAACC AAGGAGGAAC TGATTTTATG GTACTGCAGC
 GGCTCTTGCG ATGCAGCTGA GACAACGTAC GACAAAATAT TGAAAACTT ATCCAGAAAT
 AGAAGGCTGG TGAGTGACAA AGTAGGGCAG GCATGTTGCA GACCCATCGC CTTTGATGAT
 GACCTGTCGT TTTTAGATGA TAACCTGGTT TACCATATTC TAAGAAAGCA TTCCGCTAAA
 AGGTGTGGAT GTATCTGA (SEQ ID NO:424)

Translation:

MKLWDVAVC LVLLHTASAF PLPAANMPED YPDQFDDVMD FIQATIKRLK
 RSPDKQMAVL PRRERNRQAA AANPENSRGK GRRGQRGKNR GCVLTAIHLN
 VTDLGLGYET KEELIFRYCS GSCDAAETTY DKILKNLSRN RRLVSDKVGQ
 ACCRPIAFDD DLSFLDDNLV YHILRKHSK RCGCI (SEQ ID NO:425)

Homo sapiens ciliary neurotrophic factor (CNTF)**Nucleotide Sequence (603 nt):**

ATGGCTTTCA CAGAGCATTC ACCGCTGACC CCTCACCGTC GGGACCTCTG
 TAGCCGCTCT ATCTGGCTAG CAAGGAAGAT TCGTTCAGAC CTGACTGCTC
 TTACGGAATC CTATGTGAAG CATCAGGGCC TGAACAAGAA CATCAACCTG
 GACTCTGCGG ATGGGATGCC AGTGGCAAGC ACTGATCAGT GGAGTGAGCT
 GACCGAGGCA GAGCGACTCC AAGAGAACCT TCAAGCTTAT CGTACCTTCC
 ATGTTTTGTT GGCCAGGCTC TTAGAAGACC AGCAGGTGCA TTTTACCCCA
 ACCGAAGGTG ACTTCCATCA AGCTATACAT ACCCTTCTTC TCCAAGTCGC
 TGCCTTTGCA TACCAGATAG AGGAGTTAAT GATACTCCTG GAATACAAGA
 TCCCCGCAA TGAGGCTGAT GGGATGCCTA TTAATGTTGG AGATGGTGGT
 CTCTTTGAGA AGAAGCTGTG GGGCCTAAAG GTGCTGCAGG AGCTTTCACA
 GTGGACAGTA AGGTCCATCC ATGACCTTCG TTTCATTTCT TCTCATCAGA
 CTGGGATCCC AGCACGTGGG AGCCATTATA TTGCTAACAA CAAGAAAATG
 TAG (SEQ ID NO:426)

Translation:

MAFTEHSPLT PHRRDLCSRS IWLARKIRSD LTALTESYVK HQGLNKNINL
 DSADGMPVAS TDQWSELTEA ERLQENLQAY RTFHVLLARL LEDQQVHF'P
 TEGDFHQAIH TLLQVAFA YQIEELMILL EYKIPRNEAD GMPINVGDGG

LFEEKLWGLK VLQELSQWTV RSIHDLRFIS SHQTGIPARG SHYIANNKKM (SEQ
ID NO:427)

NM_170735 744 bp mRNA linear PRI
Homo sapiens brain-derived neurotrophic factor (**BDNF**)

ATGACCATCC TTTTCCTTAC TATGGTTATT TCATACTTTG GTTGCATGAA
GGCTGCCCCC ATGAAAGAAG CAAACATCCG AGGACAAGGT GGCTTGGCCT
ACCCAGGTGT GCGGACCCAT GGGACTCTGG AGAGCGTGAA TGGGCCCAAG
GCAGGTTCAA GAGGCTTGAC ATCATTGGCT GACACTTTTCG AACACGTGAT
AGAAGAGCTG TTGGATGAGG ACCAGAAAGT TCGGCCCAAT GAAGAAAACA
ATAAGGACGC AGACTTGATC ACGTCCAGGG TGATGCTCAG TAGTCAAGTG
CCTTTGGAGC CTCCTCTTCT CTTTCTGCTG GAGGAATACA AAAATTACCT
AGATGCTGCA AACATGTCCA TGAGGGTCCG GCGCCACTCT GACCCTGCCC
GCCGAGGGGA GCTGAGCGTG TGTGACAGTA TTAGTGAGTG GGTAACGGCG
GCAGACAAAA AGACTGCAGT GGACATGTCG GCGGGGACGG TCACAGTCCT
TGAAAAGGTC CCTGTATCAA AAGGCCAACT GAAGCAATAC TTCTACGAGA
CCAAGTGCAA TCCCATGGGT TACACAAAAG AAGGCTGCAG GGCATAGAC
AAAAGGCATT GGAACTCCCA GTGCCGAACCT ACCCAGTCGT ACGTGCGGGC
CCTTACCATG GATAGCAAAA AGAGAATTGG CTGGCGATTCT ATAAGGATAG
ACACTTCTTG TGTATGTACA TTGACCATTA AAAGGGGAAG ATAG (SEQ ID
NO:428)

Translation:

MTILFLTMVI SYFGCMKAAP MKEANIRGQG GLAYPGVRTH GTLESVNGPK
AGSRGLTSLA DTFEHVIEEL LDEDQKVRPN EENNKDADLY TSRVMLSSQV
PLEPPLLFL L EEYKNYLDAA NMSMRVRRHS DPARRGELSV CDSISEWVTA
ADKKTAVDMS GGTVTVLEKV PVSKGQLKQY FYETKCNPMG YTKEGCRGID
KRHWNSQCRT TQSYVRALTM DSKKRIGWRF IRIDTSCVCT LTIKRGR (SEQ ID
NO:429)

HSU78110
NM_004558 1159 bp mRNA linear PRI
Homo sapiens **neurturin (NRTN)**,

ATGCAGCGCT GGAAGGCGGC GGCCTTGGCC TCAGTGCTCT GCAGCTCCGT
GCTGTCCATC TGGATGTGTC GAGAGGGCCT GCTTCTCAGC CACCGCCTCG
GACCTGCGCT GGTCCCCCTG CACCGCCTGC CTCGAACCCT GGACGCCCGG
ATTGCCCCGC TGGCCCAGTA CCGTGCACTC CTGCAGGGGG CCCC GGATGC
GATGGAGCTG CGCGAGCTGA CGCCCTGGGC TGGGCGGCCC CCAGGTCCGC

GCCGTCGGGGC GGGGCCCCGG CGGCGGCGCG CGCGTGCGCG GTTGGGGGCG
CGGCCTTGCG GGCTGCGCGA GCTGGAGGTG CGCGTGAGCG AGCTGGGCCT
GGGCTACGCG TCCGACGAGA CGGTGCTGTT CCGCTACTGC GCAGGCGCCT
GCGAGGCTGC CGCGCGCGTC TACGACCTCG GGCTGCGACG ACTGCGCCAG
CGGCGGCGCC TGC GGCGGGA GCGGGTGCGC GCGCAGCCCT GCTGCCGCCC
GACGGCCTAC GAGGACGAGG TGTCCTTCCT GGACGCGCAC AGCCGCTACC
ACACGGTGCA CGAGCTGTCTG GCGCGCGAGT GCGCCTGCGT GTGA (SEQ ID
NO:430)

Translation:

MQRWKAAALA SVLCSSVLSI WMCREGLLLS HRLGPALVPL HRLPRTLDR
IARLAQYRAL LQGAPDAMEL RELTPWAGRP PGPRRRAGPR RRRARARLGA
RPCGLRELEV RVSELGLGYA SDETVLFRYC AGACEAAARV YDLGLRRLRQ
RRRLRRERVR AQPCCRPTAY EDEVSFDAH SRYHTVHEL S ARECACV (SEQ ID
NO:431)

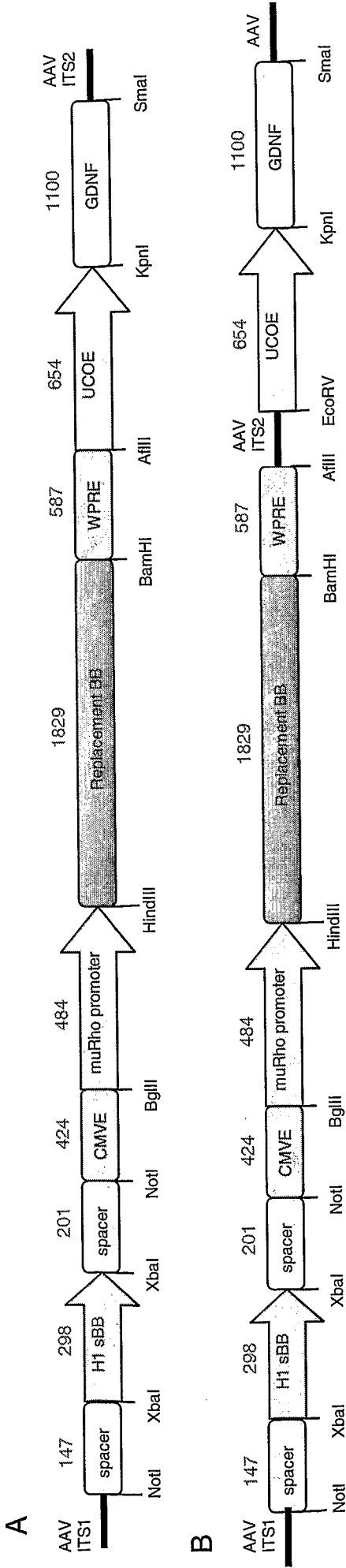


Figure 18