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(54) Title: GENE THERAPY OF USH2A-ASSOCIATED DISEASES

(57) Abstract: The disclosure describes the discovery of a dual targeting recombinant U7 RNA comprising at least two covalently linked USH2A-specific antisense sequences that act in synergy to disrupt the splicing of USH2A exon 13 and induce exon 13 skipping. Because the most prevalent pathogenic mutations causing USH2A retinitis pigmentosa reside in exon 13, for example, c.2299delG, removal of exon 13 from USH2A pre-mRNA not only deletes these mutations but also restores the open reading frame of USH2A, translation of USH2A exon13 translation and consequently the function of USH2A. Therefore, the AAV USH2A-specific recombinant U7 snRNA vector disclosed herein provides a gene therapy approach for the treatment of USH2A-associated retinal degeneration and hearing loss.



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GENE THERAPY OF USH2A-ASSOCIATED DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 63/322,083, filed March 21, 2022; and U.S. Provisional Patent Application No. 63/344,428, filed May 20, 2022; the contents of which are hereby incorporated by reference herein in their entireties.

REFERENCE TO SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on March 21, 2023, is named P1686_WO_Seq.xml and is 102,889 bytes in size.

FIELD OF DISCLOSURE

[0003] The present disclosure relates to compositions, methods, formulations for the therapeutic administration of antisense recombinant polynucleotides capable of inducing skipping of an exon in USH2A pre-mRNA, e.g., exon 13. The present disclosure further relates to methods of treating USH2A related disorders, including USH2A-associated retinopathy and hearing loss.

BACKGROUND

[0004] Usher syndrome (USH) is an autosomal recessive inherited retinal dystrophy (IRD) characterized by retinitis pigmentosa (RP), a progressive degeneration of the retinal photoreceptor cells, and a sensorineural hearing defect. USH has an estimated global prevalence of between 4 and 17 cases per 100,000 individuals, and accounts for approximately 50% of all hereditary deaf-blindness cases and 3–6% of all childhood hearing loss (HL) cases (Whatley *et al.* (2020) *Frontiers in Genetics* 11: 565216).

[0005] Usher syndrome is genetically and clinically heterogeneous with at least ten causative genes. The proteins encoded by these genes form complexes that play critical roles in the development and maintenance of cellular structures within the inner ear and retina, which have minimal capacity for repair or regeneration (Whatley *et al.* (2020)). There are three clinical

subtypes of Usher Syndrome: Usher Type I (USH1), Usher Type II (USH2), and Usher Type III (USH3) that are distinguishable based upon the presence, severity, and progression of auditory, visual, and vestibular symptoms, with Usher Type I being the severest form of the disease.

[0006] To date, over 1500 USH2A gene mutations distributed throughout the gene have been identified, of which >700 mutations are pathogenic (see LOVD database at lovd.nl website). The majority of these mutations are sporadic. Two recurrent mutations, c.2276G>T and c.2299delG, are located 22 bp from each other in exon 13. These mutations are the two most prevalent USH2A mutations and together they account for approximately one-third of the cases of USH2 and autosomal recessive Retinitis Pigmentosa (arRP). A single base deletion and frameshift at position 2299 (c.2299delG) in exon 13 creates a premature stop codon and subsequent nonsense mediated decay (NMD) of the USH2A mRNA resulting in autosomal recessive non syndromic retinitis pigmentosa (RP) and vestibular dysfunction (hearing loss). The disease typically initially manifests itself during adolescence with symptoms of night blindness that progresses to a severe constriction of the visual field (tunnel vision), decreased central visual acuity by the 4th or 5th decade, and often blindness in old age. Hearing loss may present after birth into teenage years and may be progressive.

[0007] Currently, no approved drug is available for the treatment of visual loss and deafness associated with Usher Syndrome. Because of the size constraints required for AAV gene therapy, gene replacement of the USH2A gene is not possible. Accordingly, there is an urgent, unmet medical need for innovative gene therapies that treat this otherwise incurable disease.

SUMMARY

[0008] In a first aspect, a nucleic acid engineered to express a recombinant RNA molecule in a cell containing at least a first and a second antisense ribonucleotide sequence capable of hybridizing to a human USH2A pre-mRNA is disclosed, the recombinant RNA comprising a first antisense ribonucleotide sequence having complementarity to a 3' splice site region of USH2A exon 13, and a second antisense ribonucleotide sequence with complementarity to a 5' splice site region of USH2A exon 13, wherein the second antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ

ID NO: 8, wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.

[0009] In one aspect, the second antisense ribonucleotide sequence may comprise 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0010] In one aspect, the second antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0011] In one aspect, the second antisense ribonucleotide sequence may comprise a ribonucleotide sequence of SEQ NO: 65.

[0012] In one aspect, the second antisense ribonucleotide sequence may comprise a ribonucleotide sequence of SEQ NO: 66.

[0013] In one aspect, the first antisense ribonucleotide sequence may comprise 22, 23, or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0014] In one aspect, the first antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0015] In one aspect, the first antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0016] In one aspect, the first antisense ribonucleotide sequence may comprise a ribonucleotide sequence of SEQ ID NO: 67.

[0017] In one aspect, the first antisense ribonucleotide sequence may comprise a ribonucleotide sequence of SEQ ID NO: 68.

[0018] In one aspect, the recombinant RNA molecule comprises in a 5' to 3' order: the first antisense ribonucleotide sequence, the second antisense ribonucleotide sequence, a consensus Sm-binding site (SmOpt) element, and a stem and loop sequence.

[0019] In one aspect, the first nucleotide of the first antisense ribonucleotide sequence corresponds to the first nucleotide of the recombinant RNA.

[0020] In one aspect, the first nucleotide of the second antisense ribonucleotide sequence corresponds to the first nucleotide of the recombinant RNA.

[0021] In one aspect, the recombinant RNA molecule has the ribonucleotide sequence of SEQ ID NO: 64.

[0022] In one aspect, the SmOpt element of the recombinant RNA comprises a ribonucleotide sequence of SEQ ID NO: 24.

[0023] In one aspect, the stem and loop sequence of the recombinant RNA comprises a ribonucleotide sequence of SEQ ID NO: 27.

[0024] In one aspect, the stem and loop sequences are located at about 40-80 nucleotides from the 5' end of the recombinant RNA.

[0025] In one aspect, the 3' end of the stem is located about 1-10 nucleotides from the 3' end of the recombinant RNA.

[0026] In one aspect, the expressed recombinant RNA molecule associates with one or more small nuclear ribonucleoproteins to form an snRNP complex.

[0027] In one aspect, the snRNP complex comprises spliceosomal proteins.

[0028] In one aspect, the snRNP complex comprises non-spliceosomal proteins

[0029] In one aspect, the snRNP complex is transported to a cell's nucleus.

[0030] In one aspect, the recombinant RNA molecule is not chemically modified.

[0031] In one aspect, the recombinant RNA comprises a 5' cap.

[0032] In one aspect, the recombinant RNA comprises a hypermethylated 5' cap.

[0033] In one aspect, the 5' end of the recombinant RNA 5' end comprises a 2,2,7-trimethyl guanosine (m3G) 5' cap or 7-methyl guanosine (m7G) 5' cap.

[0034] In one aspect, the recombinant RNA's first antisense ribonucleotide sequence and the second antisense ribonucleotide sequence are arranged in tandem.

[0035] In one aspect, the recombinant RNA's first antisense ribonucleotide sequence is upstream of the second antisense ribonucleotide sequence.

[0036] In one aspect, the recombinant RNA's first antisense ribonucleotide sequence and the second antisense ribonucleotide sequence are separated by a spacer sequence.

[0037] In one aspect, the spacer sequence is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11-20, 20-30, 30-40, 40-50, 50-100 or more nucleotides in length.

[0038] In one aspect, the antisense sequences capable of hybridizing to the USH2A pre-mRNA have a total length of 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 nucleotides.

[0039] In one aspect, the antisense sequences capable of hybridizing to the USH2A pre-mRNA can have a length of 47 nucleotides.

[0040] In another aspect, a nucleic acid engineered to express a recombinant RNA molecule in a cell is disclosed, the recombinant RNA molecule containing at least a first and a second antisense ribonucleotide sequence capable of hybridizing to an USH2A pre-mRNA, the first antisense ribonucleotide sequence having complementarity to the 3' splice site region of exon 13, wherein the first antisense ribonucleotide sequence may comprise 22, 23 or 24 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4 and the second antisense ribonucleotide sequence having complementarity to the 5' splice site region of USH2A exon 13, wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.

[0041] In one aspect, the second antisense ribonucleotide sequence may comprise 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8,

[0042] In one aspect, the second antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0043] In one aspect, the second antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ NO: 65.

[0044] In another aspect, the second antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ NO: 66.

[0045] In another aspect, a nucleic acid engineered to express in a cell an RNA molecule containing at least two antisense ribonucleotide sequences capable of hybridizing with 5' and 3' splice site regions of exon 13 of an USH2A pre-mRNA is disclosed, the RNA comprising a first antisense ribonucleotide sequence having complementarity to a 3' splice site region of exon 13, wherein the first antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4, and a second antisense ribonucleotide sequence with complementarity to the 5' splice site region of exon 13, wherein the first antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, and wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.

[0046] In one aspect, the second antisense ribonucleotide sequence may comprise 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8,

[0047] In one aspect, the second antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0048] In one aspect, the second antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ NO: 65.

[0049] In one aspect, the second antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ NO: 66.

[0050] In another aspect, a nucleic acid engineered to express a recombinant RNA molecule in a cell is disclosed, the RNA containing a ribonucleotide sequence comprising 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 25, wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.

[0051] In one feature of any of the previous aspects, the recombinant RNA is a modified recombinant U snRNA or dCas13d guide RNA.

[0052] In one aspect, the U snRNA can be a modified U7 snRNA or U1 snRNA.

[0053] In one feature of any of the previous aspects, the promoter is constitutive, cell-specific, or inducible.

[0054] In another aspect, the cell-specific promoter comprises a photoreceptor cell-specific promoter.

[0055] In another aspect, the constitutive promoter comprises a U snRNA promoter.

[0056] In one aspect, the constitutive promoter is a U7 snRNA promoter.

[0057] In one aspect, the constitutive promoter is a U1, U2, U4, U5 or U6 snRNA promoter.

[0058] In another aspect, the U7 snRNA promoter further comprises an enhancer element.

[0059] In another aspect, the U7 snRNA promoter further comprises a photoreceptor or cochlear cell-specific enhancer element.

[0060] In one feature of any of the previous aspects, the nucleic acid is flanked by adeno-associated virus (AAV) inverted terminal repeat sequences (ITRs).

[0061] In another aspect, a recombinant adeno-associated virus (AAV) is disclosed, comprising any one of the aforementioned nucleic acids.

[0062] In one aspect, the recombinant adeno-associated virus (AAV) comprises a capsid protein of a serotype selected from: rh10, AAV1, AAV2, AAV2.7m8, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Anc80, AV2/1, 2/2, 2/6, 2/8, 2/9 and AAV2/Anc80L6.

[0063] In another aspect, a therapeutically effective amount of a recombinant RNA is disclosed, comprising a first antisense ribonucleotide sequence capable of hybridizing with a 3' splice site region of exon 13 within an USH2A pre-mRNA, covalently linked to a second antisense ribonucleotide sequence capable of hybridizing with a 5' splice site region of exon 13 within an USH2A pre-mRNA, wherein the second antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8, the amount of recombinant RNA being effective at facilitating synergy between the covalently linked first and second ribonucleotide antisense sequences to induce skipping of USH2A's exon 13.

[0064] In one aspect, the second antisense ribonucleotide sequence may comprise 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0065] In one aspect, the second antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0066] In one aspect, the second antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ NO: 65.

[0067] In one aspect, the second antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ NO: 66.

[0068] In one aspect, the first antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0069] In one aspect, the first antisense ribonucleotide sequence may comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0070] In one aspect, the first antisense ribonucleotide sequence may comprise 22, 23, or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0071] In one aspect, the first antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ ID NO: 67.

[0072] In one aspect, the first antisense ribonucleotide sequence may comprise the ribonucleotide sequence of SEQ ID NO: 68.

[0073] In one aspect, the first antisense ribonucleotide sequence comprises a 5' cap.

[0074] In one aspect, the first antisense ribonucleotide sequence comprises a 2,2,7-trimethyl guanosine (m3G) 5' cap or 7-methyl guanosine (m7G) 5' cap.

[0075] In one aspect, the second antisense ribonucleotide sequence comprises a 5' cap.

[0076] In one aspect, the second antisense ribonucleotide sequence comprises a 2,2,7-trimethyl guanosine (m3G) 5' cap or a 7-methyl guanosine (m7G) cap.

[0077] In one aspect of any of the aforementioned aspects, exon 13 comprises a pathogenic mutation. In one aspect, the pathogenic mutation comprises c.2276G>T and/or c.2299delG.

[0078] In another aspect, a therapeutically effective amount of a RNA is disclosed containing a ribonucleotide sequence having 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 25, said amount of RNA being effective at inducing USH2A exon 13 skipping.

[0079] In one aspect, the ribonucleotide sequence of SEQ ID NO: 25 comprises a 5' cap.

[0080] In one aspect, the ribonucleotide sequence of SEQ ID NO: 25 comprises a 2,2,7-trimethyl guanosine (m3G) 5' cap or a 7-methyl guanosine (m7G) 5' cap.

[0081] In another aspect, a method for modulating the splicing of USH2A pre-mRNA in a cell is disclosed comprising expressing the aforementioned recombinant RNAs in the cell.

[0082] In another aspect, a method for treating an USH2A associated retinopathy in a human subject is disclosed comprising administering the aforementioned engineered nucleic acids to the human by injection into the eye, wherein the subject's photoreceptor cells express an USH2A pre-mRNA having a pathogenic mutation in exon 13.

[0083] In one aspect, the aforementioned engineered nucleic acids are administered by subretinal injection.

[0084] In one aspect, the engineered nucleic acids are packaged into a recombinant adeno-associated virus (rAAV).

[0085] In one aspect, the pathogenic mutation comprises c.2276G>T and/or c.2299delG.

[0086] In another aspect, a method for treating an USH2A associated hearing loss in a human subject is disclosed comprising administering the aforementioned engineered nucleic acids by injection into the subject's inner ear, wherein cochlear cells of the inner ear express an USH2A pre-mRNA having a pathogenic mutation in exon 13.

[0087] In one aspect, the aforementioned engineered nucleic acids are packaged into a recombinant adeno-associated virus (rAAV).

[0088] In another aspect, a host cell for the manufacture of a recombinant USH2A-U7 adeno-associated virus (rAAV) is disclosed comprising one of the aforementioned engineered nucleic acids.

[0089] In another aspect, a pharmaceutical composition is disclosed comprising the aforementioned recombinant adeno-associated virus (rAAV) and a pharmaceutically acceptable: excipient, diluent, or carrier.

[0090] In one aspect, the pharmaceutical composition comprises empty adeno-associated virus (rAAV) capsids at a percentage from at least about 50% cp/cp up to about 90% cp/cp.

[0091] In another aspect, a cell is disclosed comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising any one of the ribonucleotide sequences of SEQ ID Nos: 1-16 and 25.

[0092] In another aspect, a cell is disclosed comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0093] In another aspect, a cell is disclosed comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.

[0094] In one aspect, the recombinant RNA comprises a 5' cap. In another feature, the 5' cap is hypermethylated. In yet another feature, the 5' cap comprises 2,2,7-trimethyl guanosine (m3G).

[0095] In one aspect, a cell is disclosed comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25.

[0096] In another aspect, a cell is disclosed comprising an USH2A mRNA and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4, wherein the USH2A mRNA does not comprise the ribonucleotide sequence of SEQ ID NO: 55.

[0097] In another aspect, a cell is disclosed comprising an USH2A mRNA and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, wherein the USH2A mRNA does not comprise the ribonucleotide sequence of SEQ ID NO: 55.

[0098] In another aspect, a cell is disclosed comprising an USH2A mRNA and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25, wherein the USH2A mRNA does not comprise the ribonucleotide sequence of SEQ ID NO: 55.

[0099] In one aspect, the cell can be a photoreceptor cell or cochlear cell.

[0100] In another aspect, a dual targeting composition is disclosed comprising a first nucleic acid engineered to express a first antisense ribonucleotide sequence in a cell, capable of hybridizing to a 3' splice site region of USH2A exon 13 of human USH2A pre-mRNA, wherein the first antisense

ribonucleotide sequence comprises 22, 23, or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4, and a second nucleic acid engineered to express a second antisense ribonucleotide sequence in a cell capable of hybridizing to a 5' splice site region of USH2A exon 13 of human USH2A pre-mRNA, wherein the second antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.

[0101] In one aspect, a method for treating an USH2A associated disorder in a human subject is disclosed comprising administering the aforementioned dual targeting composition to the human, wherein the subject's photoreceptor and/or inner ear cells express an USH2A pre-mRNA having a pathogenic mutation in exon 13.

[0102] In one aspect, a method for treating an USH2A associated disorder in a human subject is disclosed comprising administering a recombinant USH2A-U7 adeno-associated virus (rAAV) to the human subject, wherein the USH2A-U7 adeno-associated virus expresses a therapeutically effective amount of a recombinant RNA containing a ribonucleotide sequence having 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 25, wherein the subject's photoreceptor and/or inner ear cells express an USH2A pre-mRNA having a pathogenic mutation in exon 13, and wherein said amount of recombinant RNA is effective at inducing USH2A exon 13 skipping in said cells.

[0103] In one aspect, the pathogenic mutation comprises c.2276G>T and/or c.2299delG.

[0104] In another aspect, a kit is disclosed that comprises any one of the aforementioned nucleic acids and recombinant USH2A-U7 adeno-associated virus (rAAV) compositions with instructions for their use.

[0105] In another aspect, a cell is disclosed that comprises any one or more of the aforementioned nucleic acids and recombinant USH2A-U7 adeno-associated virus (rAAV) compositions. In some aspects, the cell is suitable for manufacturing an AAV. In yet further aspects, the cell is a HEK293 cell, a HELA cell, or an insect derived Sf9 cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0106] FIG. 1 is a schematic diagram summarizing the prevalence of the USH2A pathogenic variants (reproduced from Toulbi *et al.*, (2020), Experimental Eye Research 201: 108330).

[0107] FIG. 2A shows a schematic representation of the USH2A exon – intron structure, regions of alternative splicing (horizontal bars) and protein products (reproduced from Adato *et al.* Hum Mol Genet (2005) 14(24):3921-32). Cryptic splice sites within exons 2 and 6 lead to the transcription of an mRNA lacking 950 nucleotides; the predicted protein begins at amino acid position 420, and therefore lacks the TSPN-LG and N-terminal part of LN domain. Alternative splicing of exons 20 – 22 leads to a predicted protein that lacks one FnIII module and most of the first LG domain (amino acids 1347-1377). Cryptic splice sites within exons 33 and 38 lead to a shorter mRNA, predicted to encode an isoform lacking four entire FnIII repeats (amino acids 2101-2381). Alternative splicing before exon 45 up to a cryptic splice site within exon 53 leads to a predicted protein isoform that lacks at least three FnIII repeats (amino acids 2938-3282). Finally, alternative splicing before exon 59 and within exon 64 is predicted to result in the deletion of six Fn III repeats (amino acids 3580-4121). Numbers indicate the number of exons in the longest TM usherin isoform (amino acids 1-5213). The putative protein modules are represented by symbols.

[0108] FIG. 2B shows the in-frame and out-of-frame exons of the USH2A gene together with the location of missense mutations (reproduced from Pendse *et al.*, bioRxiv 2020.02.04.934240)

[0109] FIG. 3 shows a schematic diagram of usherin localization in photoreceptors. (A) Cellular organization of a photoreceptor. The photoreceptor possesses an inner segment and an outer segment responsible for light detection. The inner segment is connected to the outer segment through the connecting cilium. The connecting cilium is wrapped in the periciliary membrane complex, where the usherin long isoform (light gray) is spatially restricted. Side view and cross section views of the periciliary membrane complex show the location of whirlin and VLGR1 (very large G protein-coupled receptor-1) with respect to usherin (reproduced from Toulbi *et al.* (2020) Exp Eye Res. (2020) 201:108330).

[0110] FIG. 4A depicts a map of an exemplary USH2A minigene plasmid.

[0111] FIG. 4B shows an exemplary schematic of the USH2A minigene including all of exon 12, exon 13, and exon 14 with approximate location of USH2A exon 13 3' and 5' splice site regions (SEQ ID Nos: 19 and 20) respectively.

[0112] FIG. 5A shows the nucleotide sequence of an exemplary USH2A exon 13 3' splice site region (SEQ ID NO: 19; nucleotides 1600-1900) including the location of exemplary antisense sequences (SEQ ID Nos: 1-4, 11- 15 and 19).

[0113] FIG. 5B shows the nucleotide sequence of an exemplary USH2A exon 13 5' splice site region (SEQ ID NO: 20; nucleotides 1900-2200) including the location of exemplary antisense sequences (SEQ ID Nos: 5-10, 21 and 22).

[0114] FIG. 5Ci-ii shows the nucleotide sequence of an USH2A exon 12 – exon 14 vector that expresses an RNA corresponding to a spliced USH2A minigene transcript after exon 13 skipping.

[0115] FIG. 5D shows the location of the AS_RNA4 antisense sequence within USH2A exon 13. An exemplary AS_RNA4 recombinant U7 is also depicted with the antisense sequence at its 5' end upstream of a consensus SmOpt sequence and a U7 stem-loop structure.

[0116] FIG. 6 shows an exemplary schematic of the USH2A minigene pre-mRNA after normal splicing (resulting in an RT-PCR product of 1022bp) or exon 13 skipping (resulting in an RT-PCR product of 297bp).

[0117] FIG. 7A shows an exemplary RT-PCR analysis of USH2A exon 13 skipping in HEK293 cells transfected with the USH2A minigene and single target AS_RNA recombinant U7 snRNAs (AS_RNA4, AS_RNA5, AS_RNA8, AS_RNA9, AS_RNA10, and AS_RNA13). GAPDH acts as an internal control. DelEx13 is a positive control for exon 12-exon 14 splicing. scU7 refers to a scrambled U7snRNA. No RT: no reverse transcriptase.

[0118] FIG. 7B shows a bar graph of the USH2A exon 13 skipping depicted in FIG. 7A normalized to DelEx13.

[0119] FIG. 7C shows an exemplary RT-PCR analysis of USH2A exon 13 skipping in HEK293 cells transfected with the USH2A minigene and combinations of AS_RNA4 with AS_RNA5, AS_RNA8, AS_RNA9, AS_RNA10, or AS_RNA13. GAPDH acts as an internal control. DelEx13 is a positive control for exon 12-exon 14 splicing. scU7 refers to a scrambled U7snRNA. NoRT: no reverse transcriptase.

[0120] FIG. 7D shows a bar graph of the USH2A exon 13 skipping depicted in FIG. 7C normalized to DelEx13.

[0121] FIG. 8Ai-iii shows an exemplary expression vector comprising a mouse U7 promoter (SEQ ID NO: 30) expressing a dual targeting AS_RNA4+8 recombinant U7 RNA upstream of a U7 3' box (SEQ ID NO: 26).

[0122] FIG. 8B shows an exemplary AS_RNA4+8 recombinant U7 snRNA comprising a consensus SmOpt sequence (SEQ ID NO: 24) and a U7 snRNA stem and loop (SEQ ID NO: 27).

[0123] FIG. 8C shows exemplary AS_RNA recombinant U7 snRNAs (AS_RNA2+6, AS_RNA2+5, AS_RNA2+4, AS_RNA2+6, AS_RNA3, AS_RNA4+6) targeting the 5' and/or 3' splicing site regions of USH2A's exon 13.

[0124] FIG. 8D shows an exemplary scrambled AS_RNA2+6 recombinant U7 snRNA (scU7)

[0125] FIG. 9A shows end point RT-PCR analysis of total RNA of HEK293 cells transfected with an USH2A minigene and 3, 1, or 0.3 mg of recombinant U7 antisense RNA expression vectors ((AS_RNA2+6, AS_RNA 4+6, AS_RNA2+4, AS_RNA3, AS_RNA2+5 and AS_RNA2+7) targeting the 5' and/or 3' splicing sites of USH2A's exon 13. Arrows point to either full-length USH2A minigene RT-PCR product (no exon skipping) or a short USH2A minigene RT-PCR product (exon skipping, Δ exon 13). GAPDH acts as an internal control.

[0126] FIG. 9B shows a bar graph of the USH2A exon 13 skipping depicted in FIG. 7A normalized to DelEx13.

[0127] FIG. 9C depicts an exemplary sequence analysis of RT-PCR products resulting from exon skipping confirming the correct splicing between exon 12 and exon 14.

[0128] FIG. 10A shows an exemplary AS_RNA4+6 gRNA dCas13d expression vector.

[0129] FIG. 10B shows an exemplary RT-PCR analysis of USH2A exon 13 skipping in HEK293 cells transfected with the USH2A minigene and 3, 1 or 0.3 μ g of AS_RNA4+6 recombinant U7 or AS_RNA4+6 gRNA dCas13d. USH2A exon 13 skipping in HEK293 cells transfected with the USH2A minigene in the presence of 200, 40, or 8nM of AS_RNA3 ASO is also depicted. GAPDH acts as an internal control. DelEx13 is a positive control for exon 12-exon 14 splicing. SCu7 refers to a scrambled U7snRNA. NoRT: no reverse transcriptase. Arrows point to either full-length

USH2A minigene RT-PCR product (no exon skipping) or a short USH2A minigene RT-PCR product (exon skipping, Δ exon 13).

[0130] FIG. 10C shows a bar graph of the USH2A exon 13 skipping depicted in FIG. 10B normalized to DelEx13.

[0131] FIG. 11A shows the location of USH2A sequences hybridized with AS_RNA4 and AS_RNA8 USH2A antisense sequences.

[0132] FIG. 11B shows a schematic diagram of exemplary recombinant U7 constructs used to evaluate exon 13 skipping by antisense sequences AS_RNAX and AS_RNAY either by (1) co-expression of two recombinant U7 RNAs constructs each targeting X or Y or (2) a single recombinant U7 RNA targeting both antisense sequences X and Y.

[0133] FIG. 11C shows an exemplary RT-PCR analysis of USH2A exon 13 skipping in HEK293 cells transfected with the USH2A minigene and (1) a combination of a AS_RNA4 U7 with a AS_RNA6 U7, (2) a dual targeting AS_RNA4+6 U7, (3) a combination of AS_RNA4 U7 and AS_RNA8 U7 or (4) a AS_RNA4+8 U7. GAPDH was an internal control. DelEx13 was a positive control for exon 12-exon 14 splicing. scU7 (a scrambled U7snRNA) was a negative control. NoRT: no reverse transcriptase.

[0134] FIG. 11D shows a bar graph of the USH2A exon 13 skipping depicted in FIG. 11C normalized to DelEx13.

[0135] FIG. 12A shows an exemplary RT-PCR analysis of USH2A exon 13 skipping in HEK293 cells transfected with the USH2A minigene and (1) a dual targeting AS_RNA4+6 U7, (2) a AS_RNA4+8 U7 of (3) an AS_RNA3 ASO. GAPDH was an internal control. DelEx13 was a positive control for exon 12-exon 14 splicing. scU7 (scrambled U7snRNA) was a negative control. NoRT: no reverse transcriptase.

[0136] FIG. 12B shows a schematic diagram of GFP+ transfected human Weri-Rb-1 retinoblastoma cells are selected using a fluorescent activated FACS Melody cell sorter (BD Biosciences).

[0137] FIG. 12C shows an exemplary RT-PCR analysis of USH2A exon 13 skipping in FACS selected GFP+ Weri-Rb-1 retinoblastoma cells transfected with the USH2A minigene, CMV-EGFP

plasmid and (1) a dual targeting AS_RNA4+6 U7, (2) a AS_RNA4+8 U7 of (3) an AS_RNA3 ASO. GAPDH was an internal control. scU7 (scrambled U7snRNA) was a negative control.

[0138] FIG. 13 shows Exon12 skipping of mouse Ush2a RNA in retinas of wt mice administered subretinally with 3×10^9 vg/eye of (1) AAV8-EF1a-dCas13d-mUsh2aEX12-7, (2) an AAV8-dCas13d-mUsh2aEX12-7 (Scrambled control) (3) AAV8-U7mUSh2aEx12-26 or (4) an AAV8-U7mUSh2aEx12-26 (scrambled control).

[0139] FIG. 14 shows an analysis of the purity and genome integrity of scAAV8-stuffer-U7hUsh2aEX13-46 and 13-48 vectors (FIG.14A) produced in-house including silver staining, alkaline gel analysis (FIG. 14B) and sequencing (FIG.14C).

[0140] FIG. 15 shows the scAAV8-stuffer-U7hUsh2aEX13-46 and 13-48 vectors transduced into wt human WeriRb-1 cells induced efficient exon 13 skipping of the endogenous USH2A pre-mRNA in a dose-dependent manner as determined by endpoint PCR (FIG. 15A) and amplicon sequencing (FIG. 15B).

[0141] FIG. 16 shows wt human retinal organoids treated with AAV2 U7hUsh2aEX13-46, AAV2 U7hUsh2aEX13-48 or AAV2/7m8 U7hUsh2aEX13-48 vectors all induced exon 13 skipping as determined by endpoint PCR (FIG. 16A) and qPCR (FIG. 16B).

[0142] FIG. 17 shows AAV8-U7hUsh2aEX13-48 vector-induced exon skipping of both human and non-human primate (NHP) USH2A minigene pre-mRNA in wt human WeriRb-1 cells. FIG. 17Ai-ii depicts a sequence alignment of NHP USH2A exon 13 with human NHP USH2A exon 13. Endpoint PCR (FIG. 17B) and qPCR (FIG. 17C) confirmed efficient exon 13 skipping of NHP USH2A minigene pre-RNA, despite one mismatch sequence in the AS RNA4 sequence between the human and NHP sequences.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0143] The disclosure describes the discovery of a dual targeting recombinant U7 RNA comprising at least two covalently linked USH2A-specific antisense sequences that act in synergy to disrupt the splicing of USH2A exon 13 and induce exon 13 skipping. Because the most prevalent pathogenic mutations causing USH2A retinitis pigmentosa reside in USH2A exon 13, for example, c.2299delG, removal of exon 13 from USH2A pre-mRNA not only deletes these mutations but also restores the USH2A open reading frame, USH2A Δ exon 13 translation and consequently

USH2A function. The AAV USH2A-specific recombinant U7 vector disclosed herein therefore provides a gene therapy approach for the treatment of USH2A associated disorders, including USH2A associated retinopathy and hearing loss.

[0144] Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the materials, methods, and examples are illustrative only and not intended to be limiting. Other features of the disclosure are apparent from the following detailed description and the claims.

[0145] Titles or subtitles may be used in the specification for the sole convenience of the reader but are not intended to influence the scope of the present disclosure or to limit any aspect of the disclosure to any subsection, subtitle, or paragraph.

I. TERMINOLOGY

[0146] As used herein, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0147] The phrase “and/or,” as used herein and in the claims, is understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one aspect, to A only (optionally including elements other than B); in another aspect, to B only (optionally including elements other than A); in yet another aspect, to both A and B (optionally including other elements); etc.

[0148] It will be further understood that the terms “comprises,” “comprising,” “includes,” and/or “including,” when used herein, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[0149] As used herein and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every

element specifically listed within the list of elements, and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one aspect, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another aspect, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another aspect, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0150] The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” are intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more items or terms, such as BB, AAA, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0151] When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below those numerical values. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20%, 10%, 5%, or 1%. In certain aspects, the term “about” is used to modify a numerical value above and below the stated value by a variance of 10%. In certain aspects, the term “about” is used to modify a numerical value above and below the stated value by a variance of 5%. In certain aspects, the term “about” is used to modify a numerical value above and below the stated value by a variance of 1%. Thus, for example, if the term “about” (or “around”) in all numerical values allows for a 5% variation, i.e., a value of about 1.25% would mean between 1.19%-1.31%.

[0152] Reference to an integer with more (greater) or less than includes any number greater or less than the reference number, respectively. Thus, for example, a reference to less than 100, includes

99, 98, 97, etc. all the way down to the number one (1); and less than 10, includes 9, 8, 7, etc. all the way down to the number one (1).

[0153] As used herein, all numerical values or numerical ranges include integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to 80% or more identity, includes 81 %, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, etc., as well as 81.1 %, 81.2%, 81.3%, 81.4%, 81.5%, etc., 82.1 %, 82.2%, 82.3%, 82.4%, 82.5%, etc., and so forth. In another example, “1-5 ng” or a range of “1 ng to 5 ng” is intended to encompass 1 ng, 2 ng, 3 ng, 4 ng, 5 ng, 1-2 ng, 1-3 ng, 1-4 ng, 1-5 ng, 2-3 ng, 2-4 ng, 2-5 ng, 3-4 ng, 3-5 ng, and 4-5 ng.

[0154] A "vector" can be any genetic element that contains a nucleic acid of interest (e.g., a transgene) that is capable of being expressed in a host cell, e.g., a nucleic acid of interest within a larger nucleic acid sequence or structure suitable for delivery to a cell, tissue, and/or organism, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc. For instance, a vector may comprise an insert (e.g., a heterologous nucleic acid comprising a transgene encoding the gene to be expressed) and one or more additional elements, e.g., a minigene cassette as described herein, and/or elements suitable for delivering or controlling expression of the insert. The vector may be capable of replication and/or expression, e.g., when associated with proper control elements, and it may be capable of transferring genetic information to a cell. In some aspects, a vector may be a replication-deficient vector suitable for episomal transgene expression in a host cell, e.g., an adeno-associated virus (AAV) vector. In some aspects, a vector may be a plasmid suitable for expression and/or replication, e.g., in a cell or bioreactor. In some aspects, vectors designed specifically for the expression of a heterologous nucleic acid sequence, e.g., non-coding RNA, e.g., recombinant U snRNA or gRNAs, in the target cell may be referred to as expression vectors, and generally have a promoter sequence that drives the expression of the transgene.

[0155] The term “recombinant” or “engineered,” as used herein, means that the vector is the product of various combinations of cloning, restriction, or ligation steps (e.g., relating to a polynucleotide comprised therein), and/or other procedures that result in a construct that is distinct from a product found in nature. A recombinant virus or vector is a viral particle comprising a recombinant polynucleotide.

[0156] As used herein, “not chemically modified” means the chemical structure of a recombinant RNA of the disclosure (including the nucleobase, backbone linkage or ribose) is not changed. The structure of the recombinant RNA is therefore the same as the native form that is transcribed in a cell.

[0157] A "fragment" of a defined nucleotide sequence is a segment of the sequence in which the 5' and/or 3' end is truncated relative to the defined nucleotide sequence. The sequence of the fragment is present within the defined nucleotide sequence as a single, contiguous nucleotide sequence. In certain aspects, an “antisense sequence” refers to a single-stranded polynucleotide fragment that is substantially complementary to a target nucleotide sequence present in USH2A pre-mRNA.

[0158] A “therapeutically effective amount” means a minimal amount of a recombinant USH2A-specific antisense RNA, which is necessary to impart a therapeutic benefit to a subject. In one example, a “therapeutically effective amount” is an amount which induces, ameliorates, stabilizes, slows down USH2A disease progression. In this instance, a therapeutically effective amount can mean the amount of a recombinant USH2A-specific antisense RNA needed to induce USH2A exon 13 skipping in an amount sufficient to reduce at least one or more symptom(s) of USH2A retinitis pigmentosa, for example, retinal degeneration. In another example, the term “therapeutically effective amount” can refer to an amount of a recombinant USH2A-specific antisense RNA that is sufficient to produce a therapeutically or prophylactically significant reduction in a symptom or clinical marker associated with USH2A disease, for example, an improvement in eyesight and/or visual acuity in a subject with USH2A retinitis pigmentosa. The effective amount will depend on a variety of factors such as, for example, species, age, weight, health of the subject, and the mode or site of administration, and may thus vary among subjects and administrations.

[0159] The terms “subject,” “host,” “individual,” and “patient” are as used interchangeably herein to refer to animals, typically mammalian animals. Any suitable mammal can be treated by the method, cell or composition described herein. A mammal can be administered a vector, an engineered polynucleotide, a precursor guide RNA, a nucleic acid, or a pharmaceutical composition, as described herein. Non-limiting examples of mammals include humans, non-human primates (e.g., apes, gibbons, chimpanzees, orangutans, monkeys, macaques, and the like), domestic animals (e.g., dogs and cats), farm animals (e.g., horses, cows, goats, sheep, pigs) and

experimental animals (e.g., mouse, rat, rabbit, guinea pig). In some aspects, a mammal is a human. A mammal can be any age or at any stage of development (e.g., an adult, teen, child, infant, or mammal *in utero*). A mammal can be male or female. A mammal can be a pregnant female. In some aspects, a subject is human. In some aspects, a subject has or is suspected of having a disease such as USH2A retinopathy. In other aspects, a subject has or can be suspected of having a disease or disorder associated with aberrant protein expression. In some cases, a human can be more than about: 1 day to about 10 months old, from about 9 months to about 24 months old, from about 1 year to about 8 years old, from about 5 years to about 25 years old, from about 20 years to about 50 years old, from about 1 year old to about 130 years old or from about 30 years to about 100 years old. Humans can be more than about: 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 years of age. Humans can be less than about: 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, or 130 years of age.

[0160] As used herein, the term “preventing” or “prevention” refers to delaying or forestalling the onset, development or progression of a condition or disease for a period of time, including weeks, months, or years.

[0161] As used herein, the terms “treat,” “treatment,” “treating” refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a disorder, e.g., USH2A retinopathy and/or hearing loss. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition, disease, or disorder, e.g., USH2A retinopathy. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disorder is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation of, or at least slowing of, the progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of the extent of disease, stabilized (i.e., not worsening) state of the disease, delay or slowing of disease progression, i.e. prevention of retinal degeneration, amelioration or palliation of the disease state, remission (whether partial or total), whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side effects of the disease (including palliative treatment).

[0162] Alleviating Usher Syndrome includes delaying the development or progression of the disease or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, “delaying” the development of a disease (such as Usher Syndrome) means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that “delays” or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces the probability of developing one or more symptoms of the disease in a given time frame and/or reduces the extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

[0163] “Development” or “progression” of a disease means initial manifestations and / or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. “Development” includes occurrence, recurrence, and onset. As used herein, “onset” or “occurrence” of Usher Syndrome includes initial onset and/or recurrence.

[0164] In various aspects, the terms “polynucleotide,” “nucleic acid” and “nucleic acid sequence” may be used interchangeably and may comprise genetic material including, but not limited to: RNA, DNA, mRNA, cDNA, etc., which may include full-length sequences, functional variants, and/or fragments thereof.

[0165] As used herein, the term “contiguous” refers to those nucleotides that are immediately adjacent to each other in a polynucleotide chain.

[0166] The term “align” refers to the process of comparing the nucleotide sequence of two or more nucleotide sequences to assess their degree of sequence identity. As used herein, a “match” refers to the alignment of two or more nucleotide sequences having 100% sequence identity.

[0167] Homology refers to sequence similarity attributed to descent from a common ancestor. Homologous biological components (genes, proteins, structures) are called homologs. Homology can be determined by comparing a position in each sequence which can be aligned for the purposes of comparison. When a position in the compared sequence can be occupied by the same base or

amino acid, then the molecules can be homologous at that position. The degree of homology between sequences can be a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, or less than 25% identity, with one of the sequences of the disclosure.

[0168] As used herein, the term “sequence identity” refers to the extent to which two (nucleotide or amino acid) sequences have the same residues at the same position in an alignment, often expressed as a percentage. Gaps are not counted. Thus, the sequence identity is not transitive, i.e., if sequences A = B and B = C then A is not necessarily equal to C.

[0169] A comparison of sequences A, B, and C is illustrative:

A: AAGGCTT
B: AAGGC
C: AAGGCAT

[0170] The sequence identity (A, B) = 100% (5 identical nucleotides A and B). The sequence identity (B, C) = 100%, but the sequence identity (A, C) = 85% (6 identical nucleotides /7). Thus, 100% identity does not necessarily mean the two sequences are the same.

[0171] A suitable computer program for carrying out an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A; Devereux *et al.*, 1984, *Nucleic Acids Research* 12:387). Examples of other software program that may perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999, *ibid.*—Chapter 18), FASTA (Atschul *et al.*, 1990, *J. Mol. Biol.* 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999, *ibid.*, pages 7-58 to 7-60).

[0172] The term “mutation” as used herein, refers to an alteration to a nucleic acid sequence encoding a protein relative to the consensus sequence of said protein. “Missense” mutations result in the substitution of one codon for another; “nonsense” mutations change a codon from one encoding a particular amino acid to a stop codon. Nonsense mutations often result in truncated translation of proteins. “Silent” mutations are those which have no effect on the resulting protein. As used herein, the term “point mutation” refers to a mutation affecting only one nucleotide in a gene sequence. “Splice site mutations” are those mutations present in pre-mRNA (prior to processing to remove introns) resulting in mistranslation and often truncation of proteins resulting

from an incorrect delineation of the splice site. A mutation can comprise a single nucleotide variation (SNV). A mutation can comprise a sequence variant, a sequence variation, a sequence alteration, or an allelic variant. The reference DNA sequence can be obtained from a reference database. A mutation can affect function. A mutation may not affect function. A mutation can occur at the DNA level in one or more nucleotides, at the ribonucleic acid (RNA) level in one or more nucleotides, at the protein level in one or more amino acids, or any combination thereof. The reference sequence can be obtained from a database such as the NCBI Reference Sequence Database (RefSeq) database. Specific changes that can constitute a mutation can include, a substitution, a deletion, an insertion, an inversion, or a conversion in one or more nucleotides or one or more amino acids. A mutation can be a point mutation.

[0173] DNA is transcribed by RNA polymerase to synthesize a pre-mRNA transcript containing sequences that are important for RNA stability and regulatory function. In the case of coding RNAs (mRNAs), pre-mRNAs are subjected to RNA processing in order to convert a pre-mRNA into mature mRNA through alternative splicing of exons. Mature mRNA is subsequently translated into a protein by the ribosome. Mutations in genomic DNA can be present, arising from mutations in the pre-mRNA transcript, affecting protein function or expression of the protein. These mutations, if present at splicing sites, can prevent proper alternative splicing by affecting the selection of affected exons. Specifically, the pre-mRNA splicing sites contain conserved splice acceptor and splice donor sites. The splice donor site is characterized by a nucleotide motif, N/GT, wherein N is any nucleotide, and “/” represents the exon-intron junction. A splice acceptor site is characterized by the motif, NAG/NN, wherein N represents any nucleotide, and “/” denotes the exon-intron junction. Mutations that remove or introduce this motif into intron and exon junctions can cause aberrant mRNA splicing leading to improper protein production.

[0174] As used herein, the term “RNA” means a molecule comprising at least one ribonucleotide residue. By “ribonucleotide” is meant a nucleotide with a hydroxyl group at the 2' position of a beta-D-ribo-furanose moiety. The terms include double-stranded RNA, partially double stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. RNAs can be synthesized in a cell by RNA polymerase I, II, or III.

[0175] The term “mRNA” refers to any RNA that is produced in a cell by RNA polymerase transcription of a gene. In one aspect, the mRNA of the disclosure is capped and polyadenylated. In one aspect, an mRNA of the disclosure encodes a recombinant USH2A-specific antisense RNA. In one aspect, an mRNA of the disclosure can refer to a 5' capped U snRNA that is not polyadenylated. In another aspect, mRNA can refer to processed or unprocessed pre-mRNA. In another aspect, the mRNA of this disclosure includes, but is not limited to, pre-mRNA, spliced mRNA, partially spliced mRNA, and alternatively spliced mRNA. In one aspect, the mRNA of the disclosure is a transcript that comprises a nonsense mutation that causes nonsense-mediated decay (NMD). In another aspect, the mRNA of the disclosure does not include USH2A exon 13.

[0176] A "pre-mRNA" is the first form of RNA created through transcription of DNA (e.g., of the nucleic acid molecule described herein) that has not yet undergone further processing, such as, for example, splicing. Thus, a pre-mRNA can include both introns and exons. Pre-mRNA molecules are further processed, e.g., through splicing, to form the "mature RNA," “spliced RNA” or "mRNA."

[0177] In eukaryotes, the 5' cap, found on the 5' end of an mRNA molecule, consists of a guanine nucleotide connected to the mRNA via an 5' to 5' triphosphate linkage. This guanosine is methylated on the 7 position directly after capping *in vivo* by a methyltransferase. It is referred to as a 7-methylguanylate 5' cap, abbreviated m7G.

[0178] Small nuclear RNAs contain hypermethylated 5'-caps, e.g., a guanosine connected to the mRNA via an 5' to 5' triphosphate linkage where the guanosine contains more than one methyl group. Sm-class snRNAs can have, for example, a 5'-trimethylguanosine cap (see, for example, Plesselet *et al.* (1994) *Molecular and Cellular Biology* 14 (6): 4160–72).

[0179] Splicing processes the primary messenger ribonucleic acid (mRNA) transcribed from deoxyribonucleic acid (DNA) before the mRNA is translated into a protein. Splicing involves removing one or more contiguous segments of mRNA and is directed, in part, by a spliceosome. The segments that are removed are often referred to as introns, but the spliceosome may remove segments that contain both introns and exons.

[0180] An “exon” can be any part of a gene that is a part of the final mature RNA produced by that gene after the introns have been removed by RNA splicing. The term “exon” refers to both the DNA sequence within a gene and to the corresponding sequence in RNA transcripts. Exons may

contain translated (e.g., protein coding region) or untranslated regions (e.g., 5' or 3' untranslated regions or UTRs).

[0181] The term “intron” refers to both the DNA sequence within a gene and the corresponding sequence in the unprocessed RNA transcript. As part of the RNA processing pathway, introns can be removed by RNA splicing either shortly after or concurrent with transcription. They can be found in a wide range of genes, including those that generate proteins, ribosomal RNA (rRNA), and transfer RNA (tRNA).

[0182] Alternative splicing is the process in which pre-mRNA is processed in order to generate mature RNA. Pre-mRNA is comprised of exons and introns, as well as other untranslated regions (3' and 5' UTR). During alternative splicing, splicing signals demarcating the exons and introns of the pre-mRNA enable the spliceosome to join multiple exons together to form a functional protein, and thereby removing the interceding introns.

[0183] In one aspect, the terms "canonical splice site" or "consensus splice site" can be used interchangeably and refer to splice sites that are conserved across species. Consensus sequences for the 5' splice site and the 3' splice site used in eukaryotic RNA splicing are well known in the art (see, e.g., Gesteland *et al.* (eds.), *The RNA World*, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, (2006), Watson *et al.*, *supra*, and Mount, *Nucleic Acid Res.*, 10: 459-472 (1982), the contents of which are incorporated by reference herein in their entirety). These consensus sequences include nearly invariant dinucleotides at each end of the intron: GT at the 5' end of the intron, and AG at the 3' end of an intron.

[0184] In one aspect, a “canonical 5' splice site” or splice donor site consensus sequence can be (for DNA) CAG/GTRAG (where A is adenosine, T is thymine, G is guanine, C is cytosine, R is purine and "/" indicates the site of cleavage).

[0185] In one aspect, the splice acceptor site consists of three separate sequence elements: the branch point or branch site, a polypyrimidine tract, and the 3' splice site consensus sequence. The branch point consensus sequence in eukaryotes is YNYTRAC (where Y is a pyrimidine, N is any nucleotide, and R is purine; the underlined A is the site of branch formation. The 3' splice site consensus sequence is YAG (where Y is a pyrimidine) (see, e.g., Griffiths *et al.*, eds., *Modern Genetic Analysis*, 2nd edition, W.H. Freeman and Company, New York (2002), the contents of which are incorporated by reference herein in their entirety).

[0186] As used herein, a “3’ splice site region” refers to a sequence up to approximately 145 nucleotides upstream and 150 nucleotides downstream of USH2A exon 13’s 3’ splice site tttag/GGCT (where A is adenosine, T is thymine, G is guanine, C is cytosine, and "/" indicates the site of cleavage). In some aspects, the “3’ splice site region” can have the nucleotide sequence of SEQ ID NO: 19 (see FIG. 5A).

[0187] As used herein, a “5’ splice site region” refers to a sequence up to approximately 485 nucleotides upstream and 110 nucleotides downstream of the USH2A exon 13 5’ splice site, having the sequence of CCAG/gtaag (where A is adenosine, T is thymine, G is guanine, C is cytosine, and "/" indicates the site of cleavage). In some aspects, the “3’ splice site region” can have the nucleotide sequence of SEQ ID NO: 20 (see FIG. 5Bi and 5Bii).

II. USH2A

[0188] Usher syndrome type II (USH2) is the most common subtype of Usher Syndrome. From 57-79% of USH2 cases harbor pathogenic mutations in the USH2A gene (OMIM: #608400), making it the predominant causative gene of non-syndromic inherited retinal degeneration (Whatley *et al.* (2020); Toualbi *et al.* (2020) Exp Eye Res. (2020) 201:108330; FIG. 1). As shown in FIG. 2A, the USH2A gene expresses two isoforms: a short, secreted protein of 1546 amino acids encoded by 21 exons and a long 600 kDa transmembrane isoform of 5202 amino acids, called usherin, that is expressed specifically in retinal photoreceptors and cochlear hair cells. In mammalian photoreceptor cells, usherin is localized to the periciliary membrane complex (PMC) where it binds to VLGR1 (USH2C) and whirlin (USH2D) at the apical inner segment recess that wraps around the connecting cilium (see FIG. 3). A c.2299delG frameshift mutation USH2A in exon 13 results in the expression of a truncated, glycosylated protein which is mislocalized to the photoreceptor inner segment. The ensuing retinal degeneration is associated with a decline in retinal function, structural abnormalities in connecting cilium and outer segment and mislocalization of the usherin interactors very long G-protein receptor 1 and whirlin (Tebbe *et al.* (2023) Nature Communications 14 (1): 972).

[0189] As used herein, “usherin,” also known as RP39, Usher Syndrome 2A (Autosomal Recessive, Mild), Usher Syndrome Type IIa Protein, Usher Syndrome Type-2A Protein, USH2, dJ1111A8.1 or US2, refers to a large 171.5 kD transmembrane protein (UniProtKB/Swiss-Prot:

O75445). Its extracellular portion contains many repeated domains, including 10 Laminin EGF-like (LE) domains and 35 Fibronectin type 3 (FN3) domains. Located on chromosome 1q41, the 800kb USH2A gene (HGNC: 12601; NCBI Entrez Gene: 7399; Ensembl: ENSG00000042781) contains up to 72 exons, 25 of which are in frame with the remaining transcript (see FIG. 2B).

[0190] Exemplary *Homo sapiens* usherin cDNA transcript variant 2 (NCBI Reference Sequence: NM_206933.4) has a nucleotide sequence of SEQ ID NO: 48 or a fragment thereof.

[0191] In certain aspects, an exemplary *Homo sapiens* usherin can refer to a polypeptide having the amino acid sequence of SEQ ID NO: 49 (NCBI Reference Sequence: NP_996816.3) or fragment thereof.

[0192] In one aspect, a *Homo sapiens* usherin cDNA comprises at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 400, 500 nucleotides of the sequence of SEQ ID NO: 48.

[0193] In another aspect, a *Homo sapiens* usherin protein comprises at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 400, 500 amino acids of the polypeptide sequence of SEQ ID NO: 49.

III. USH2A MINIGENE

[0194] As shown in FIG. 2B, removal of certain USH2A exons, e.g., exon 13, by exon skipping does not disrupt USH2A's open reading frame. Thus, splicing of exon 12 to exon 14 conserves the USH2A open reading frame (see, for example, FIG. 5Ci-ii). The diagram of FIG. 2B also points out the location of exemplary mutations within the USH2A mRNA, some of which result in premature stop codons that cause USH2A mRNA degradation by nonsense mediated decay (NMD). Thus, the skipping of exon 13 does not only remove the deleterious USH2A mutations residing in exon 13, but it also maintains the open reading after splicing of exon 12 to exon 14 enabling the translation of a functional USH2A Δ exon 13 protein, in which the amino acids encoded by exon 13 are deleted.

[0195] A “minigene,” otherwise called a “minigene cassette” or simply “cassette,” refers to a nucleic acid sequence comprising a selection of USH2A introns and exons, that when transcribed in a cell can be used to identify antisense sequences that inhibit splicing of a targeted exon, i.e., induce exon skipping.

[0196] In one aspect, the USH2A minigene may comprise any one of the “in-frame” USH2A exons identified in FIG. 2B.

[0197] In one aspect, an USH2A minigene can be constructed with those genomic sequences between exons 12 and 14 (see FIG. 4A; TABLE I). A constitutive CMV promoter/enhancer can then be placed upstream of the minigene to drive transcription in a cell line, e.g., HEK 293 cells.

[0198] For example, the minigene may comprise all of USH2A’s exon 12, a 5’ intron, exon 13, a 3’ intron, and exon 14 (see, for example, FIGs. 4A and 4B; TABLE I).

[0199] In one aspect, the USH2A exon 13’s 5’ splice site region comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 20 (FIG. 5B).

[0200] In one aspect, the USH2A exon 13’s 3’ splice site region comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 19 (FIG. 5A).

[0201] In one aspect, the USH2A minigene comprises the nucleotide sequence of SEQ ID NO: 50 (see TABLE I).

[0202] In one aspect, the USH2A minigene’s exon 12 comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 52.

[0203] In one aspect, the USH2A minigene’s exon 13 comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 55.

[0204] In one aspect, the USH2A minigene’s exon 14 comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 58.

[0205] In one aspect, the USH2A minigene’s exon 13 upstream intron comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 53.

[0206] In one aspect, the USH2A minigene’s exon 13 downstream intron comprises 10, 20, 30, 40, 50, or more nucleotides of a nucleotide sequence of SEQ ID NO: 56.

TABLE I: HUMAN USH2A MINIGENE cDNA SEQUENCE (SEQ ID NO: 50)

		Uppercase: Exons	Lower case: Introns
1	ATTGGAGGAC AGTGTAATTG TAAGAGACAC GTGCTGGCA GGCAGTGCAA TCAGTGCCAG		
61	AATGGATTCT ACAATCTACA AGAGTTGGAT CCTGATGGCT GCAGTCCCCTG TAACCTGCAAT		
121	ACCTCTGGGA CAGTGGATGG AGATATTACC TGTCAACAAA ATTCAGGCCA GTGCAAGTGC		
181	AAAGCAAACG TTATTGgtac gtgtaacgca aaagtttaca gtcacatctc tattaccatc		
241	tggaatttat tattaagtat agtcaccatg tactcagctt aaccttttat taaacatctt tagagatttc		
301	tctgtctagc tgaacctttg aaaaaaact aatttgttat atgtaattct actataattt taaatgagca		
361	ttatcttttag aaaaaaact aatttgttat aagatttggt aaagagaaaa agagctccag catatgtaac		
421	catttggttaa aatagttttt tttgcattaa gcatttttct ttgcattaa taataattaa aaatttatga		
481	agaaacaaca tttgcattaa gcatttttct taaattaaat attgtcattg aattttgaga		
541	agttcatcgc aacacagttgt atattaaagc taaattaaat attgtcattg aattttgaga		
601	gtaagattgg cccctatgg cattgcttgt gagaaaaacac tcaatatattt gtgttcgtat		
661	catctgcagt agcattgttt gtgtctcgtc tatcttgaat gaaatcattt tcccatcctc		
721	accttttaaa tatattttat ctttagggct TAGGTGTGAT CATTGCAATT TTGGATTAA		
781	ATTCTCCGA AGCTTTAATG ATGTTGGATG TGAGCCCCTGC CAGTGTAACC TCCATGGCTC		
841	AGTGAACAAA TTCTGCAATC CTCACCTCTGG GCAGTGTGAG TGCAAAAAAG AAGCCAAAGG		
901	ACTTCAGTGT GACACCTGCA GAGAAAACCTT TTATGGGTTA GATGTCACCA ATTGTAAGGC		
961	CTGTGACTGT GACACAGCTG GATCCCCTCCC TGGGACTGTC TGTAATGCTA AGACAGGGCA		
1021	GTGCATCTGC AAGCCCAATG TTGAAGGGAG ACAGTGCAAT AAATGTTTGG AGGAAAACCTT		
1081	CTACCTACGG CAAAATAAAT TCTTCCCTCTG TCTGCCCTTG CACTGTGATA AGACTGGGAC		
1141	AATAAATGGC TCTCTGCTGT GTAACAATC AACAGGACAA TGTCCTTGCA AATTAGGGGT		
1201	AACAGGTCTT CGCTGTAATC AGTGTGAGCC TCACAGGTAC AATTGACCA TTGACAAATT		
1261	TCAACACTGC CAGATGTGTG AGTGTGATTC CTTGGGGACA TTACCTGGGA CCATTGTGA		
1321	CCCAATCAGT GGCCAGTGCC TGTGTGTGCC TAATCGTCAA GGAAGAAGGT GTAATCAGTG		
1381	TCAACCCAGgt aagaaagaaa tgtattacat tttcagtgca caatgacatt ccttttgta		
1441	acttaggttaa cttctccctg tttctggttt gtggcttcta caaattttat ttccaaaatc		
1501	attactgtat ttatatcatt atccaacaca tatataacta tttaacttat tcaaaattat		
1561	ctgcataatt atgttactat tttgagagga tacttttagat aaaactcagc cgatcggatt		
1621	tatttcataa ttgagactca atttctacac ttgaagtaaa tctcctttt aacagttttt		
1681	taaaaaatcag atcaacaaga gtcaatttta ttttccagag aaaggaaaaa ttgagttgaa		
1741	tatccataca atgcccataa ttcaaatgat gaactaaatc tctgaataaa gctggctaaa		
1801	tgtttttgct gaagaggcta tatgttctag ttttatatag aaatacctag aattgtttcc		

1861 acatgccatc aaattaataa aataggccctt ggtagagtat tctgtttttc tgtgtaatca	
1921 attattatgc tttaataaaa agagtggata aaaagtatat ataaaaacttt taatttcttt	
1981 atttctaggt TTTTATATT CTCCAGGCAA TGCCACTGGC TGCCTGCCAT GCTCATGCCA	
2041 TACAACTGGT GCAGTTAATC ACATCTGTAA TAGCCTGACT GGTCACTGTG TTGCGCAAGA	
2101 TGCTTCCATT GCTGGGCAAC GTTGTGACCA ATGCAAAGAC CATTACTTTG GATTGTATCC	
2161 TCAGACTGGA AG (SEQ ID NO: 50)	

TABLE II: USH2A MINIGENE EXONS AND INTRONS	
I G G Q C N C K R H V S G R Q C N Q C Q ATTGGAGGACAGTGTAATTGTAAGAGACACGTGTCTGGCAGGCAGTGCAATCAGTGCCAG N G F Y N L Q E L D P D G C S P C N C N AATGGATTCTACAATCTACAAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAACTGCAAT T S G T V D G D I T C H Q N S G Q C K C ACCTCTGGACAGTGGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAGTGCAAGTGC K A N V I (SEQ ID NO: 51) AAAGCAAACGTTATG (SEQ ID NO: 52)	Exon 12
gtacggtgtaacgcaaaaagtttacagtcacatctctattaccatc tggaattttattattaaagtatagtcaccatgctgtacaataagagctccagaaacttattcat tctgtctagctgaaaactttgtactcagcttaaccttttatttaaacaatctttagagatttc ttatctttagaaaaaacaactaatttgttataatgtaatttctactataaattttaaatgagca catttgttaaaatagtttttaagatttgtttaaaagagaaaaaagagctccagcatatgtaac agaaaacaacatttgcattaaagcatttttctttgcattaaagtaataatataaaatttatga agttcatcgcaaacagttgtatattaaagctaaataaataattgtcatttgaatttttgaga gtaagattggccccctatggcatttgcttgtagaaaaaacactcaatatatttgtgttcgtat catctgcagtagcattgtttgtgtcgtctctatcttgaatgaaatcattttcccatcctc acctttaaatatatttttatcttttag (SEQ ID NO: 53)	Intron

L R C D H C N F G F K F L R S F N D V G GGCTTAGGTGTGATCATTGCAATTTTGGATTAAATTTCTCCGAAGCTTTAAATGATGTTGG C E P C Q C N L H G S V N K F C N P H S ATGTGAGCCCTGCCAGTGTAAACCTCCATGGCTCAGTGAACAAATTTCTGCAATCCTCACTCT G Q C E C K K E A K G L Q C D T C R E N GGCAGTGTGAGTGCAAAAAGAAGCCAAAGGACTTCAGTGTGACACCTGCAGAGAAAAC F Y G L D V T N C K A C D C D T A G S L TTTTATGGGTTAGATGTCACCAATTGTAAGGCTGTGACTGTGACACAGCTGATCCCTC P G T V C N A K T G Q C I C K P N V E G CCTGGGACTGTCTGTAATGCTAAGACAGGCGAGTGCATCTGCAAGCCCAATGTTGAAGGG R Q C N K C L E G N F Y L R Q N N S F L AGACAGTGCAATAAATGTTTGGAGGGAACCTTCTACCTACGGCAAAATAATTTCTTCCTC C L P C N C D K T G T I N G S L L C N K TGTCCTGCCCTTGCAACTGTGATAAGACTGGGACAAATAAATGGCTCTCTGCTGTGTAAACAAA S T G Q C C P C K L G V T G L R C N Q C E TCAACAGGACAAATGTCCTTGCAAATTAGGGGTAAACAGGTCTTCGCTGTAATCAGTGTGAG P H R Y N L T I D N F Q H C Q M C E C D CCTCACAGGTACAATTTGACCAATTGACAAATTTCAACACTGCCAGATGTGTGAGTGTGAT S L G T L P G T I C D P I S G Q C L C V TCCTTGGGGACATTACCTGGGACCATTGTGACCCCAATCAGTGGCCAGTGCCCTGTGTGTG P N R Q G R R C N Q C Q P (SEQ ID NO: 54) CCTAATCGTCAAGGAAGGTGTAATCAGTGTCAACCAG (SEQ ID NO: 55)	Exon 13
gtaagaaagaatgtattacatttttcagtgacacaaatgacattcccttttggtaacttaggta acttctccctgtttctggtttgtggtctctacaaaattttatttccaaaaatcattactgtat ttatatcattatccaaacacatatataactatttaactatttcaaaaattatctgcataattta tggtactattttgagaggatacttttagataaaaactcagccgatcggattttatttcataaatt gagactcaatttctacacttgaagtaaatctccttttttaacagttttttaaaaaatcagatc aacaagagtcaattttattttccagagagaaagaaaaatttgagttgaatatccatacaatgc	Intron

caaatattcaaatgatgaactaaaatctctgaataaaagctggctaataatgtttttgctgaaga ggctatatgttctagtttttatatagaaaatacctagaattgtttccacatgccatcaaat ataaaaatagcccttggtagagtattctgttttctgtgtaatacaattattatgctttaaat aaaagagtggataaaaaagtatatataataaaacttttaatttcttatttctag (SEQ ID NO: 56)	
F Y I S P G N A T G C L P C S C H T T G GTTTTATATTCTCCAGGCAATGCCACTGGCTGCCATGCTCATGCCATAACAAC TGGT A V N H I C N S L T G Q C V C Q D A S I GCAGTTAATCACATCTGTAATAGCCCTGACTGGTCAGTGTGTTGCCAAGATGCTTCCATT A G Q R C D Q C K D H Y F G F D P Q T G GCTGGCAACGTTGTGACCAATGCAAAGACCATTACTTTGGATTGTGATCCTCAGACTGGA (SEQ ID NO: 57) AG (SEQ ID NO: 58)	Exon 14

TABLE III: USH2A SPLICED MINIGENE cDNA (EXON 12-EXON 13-EXON 14)	
I G G Q C N C K R H V S G R Q C N Q C Q ATTGGAGGACAGTGTAATTGTAAGAGACACGCTGTCTGGCAGGCAGTGCAATCAGTGCCAG N G F Y N L Q E L D P D G C S P C N C N AATGGATTCTACAATCTACAAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAACTGCAAT T S G T V D G D I T C H Q N S G Q C K C ACCTCTGGACAGTGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAGTGCAAGTGC K A N V I G L R C D H C N F G F K F L R AAAGCAAACGTTATTG* *GGCTTAGGTGTGATCATTTGCAATTTTGGATTAAATTTCTCCGA S F N D V G C E P C Q C N L H G S V N K AGCTTTAATGATGTTGGATGTGAGCCCTGCCAGTGTAACCTCCATGGCTCAGTGAACAAA F C N P H S G Q C E C K K E A K G L Q C	* *: exon boundaries

TTCTGCAATCCTCACTCTGGCAGTGTGAGTGCAAAAAAGAGCCAAAGGACTTCAGTGT
D T C R E N F Y G L D V T N C K A C D C
GACACCTGCAGAGAAAACCTTTATGGGTTAGATGTCACCAATTGTAAGGCCTGTGACTGT
D T A G S L P G T V C N A K T G Q C I C
GACACAGCTGGATCCCTCCCTGGACTGTCTGTAAATGCTAAGACAGGCAGTGCATCTGC
K P N V E G R Q C N K C L E G N F Y L R
AAGCCCAATGTTGAAGGAGACAGTGCAATAAATGTTGGAGGAAACTTCTACCTACGG
Q N N S F L C L P C N C D K T G T I N G
CAAAATAATTCTTCCCTCTGTCTGCCTTGCAACTGTGATAAGACTGGGACAAATAAATGGC
S L L C N K S T G Q C P C K L G V T G L
TCTCTGCTGTGAACAAATCAACAGGACAATGTCTTGCAAATTAGGGGTAACAGGTCTT
R C N Q C E P H R Y N L T I D N F Q H C
CGCTGTAATCAGTGTGAGCCTCACAGGTACAATTTGACCATTGACAAATTTCAACACTGC
Q M C E C D S L G T L P G T I C D P I S
CAGATGTGTGAGTGTGATTCCCTTGGGGACATTACCTGGGACCATTGTGACCCCAATCAGT
G Q C L C V P N R Q G R R C N Q C Q P G
GGCCAGTGCCTGTGTGTCCTAATCGTCAAGGAAGAGGTGTAATCAGTGTCAACCAG*||*GT
F Y I S P G N A T G C L P C S C H T T G
TTTTATATTCTCCAGGCAATGCCACTGGCTGCCCTGCCATGCTCATGCCATACAACTGCT
A V N H I C N S L T G Q C V C Q D A S I
GCAGTTAATCACATCTGTAATAGCCTGACTGGTCAAGTGTGTTTGCCCAAGATGCTTCCATT
A G Q R C D Q C K D H Y F G F D P Q T G
GCTGGGCAACGTTGTGACCAATGCAAGACCATTACTTTGGATTGTGATCCTCAGACTGGA
(SEQ ID NO:59)
AG (SEQ ID NO:60)

TABLE IV: USH2A Δ13 cDNA after exon 13 skipping(Exon 12-Exon 14)	
I G G Q C N C K R H V S G R Q C N Q C Q ATTGAGGACAGTGTAAATTGTAAGAGACACAGTGTCTGGCAGGCAGTGCAATCAGTGCCAG N G F Y N L Q E L D P D G C S P C N C N AATGGATTCTACAATCTACAAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAACTGCAAT T S G T V D G D I T C H Q N S G Q C K C ACCTCTGGACAGTGGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAGTGCAAGTGC K A N V I G F Y I S P G N A T G C L P AAAGCAAACGTTATTG <u>* </u> *GT TTTTATATTCTCCAGGCAATGCCACTGGCTGCCTGCCA C S C H T T G A V N H I C N S L T G Q C TGCTCATGCCATACAACCTGGTGCAGTTAATCACATCTGTAAATAGCCCTGACTGGTCAGTGT V C Q D A S I A G Q R C D Q C K D H Y F GTTTGCCAAAGATGCTTCCATTGCTGGGCAACGTTGTGACCAATGCAAAGACCATTACTTT G F D P Q T G (SEQ ID NO:61) GGATTGATCCTCAGACTGGA (SEQ ID NO:62)	* *: exon boundaries

IV. ANTISENSE SEQUENCES

[0207] Introns are defined by a set of short “splice elements” which are conserved RNA segments that bind splicing factors required for spliceosome assembly. Thus, each intron is defined by a 5' splice site, a 3' splice site, and a branch point situated there between.

[0208] Exon-skipping prevents splicing of a target exon, which results in a mature mRNA where the targeted exon is removed from the mature transcript.

[0209] Within the context of the USH2A gene, an exon targeted for exon skipping can be any one of the “in-frame” exons shown in FIG. 2B, including, for example, exon 13, where several of the most common pathogenic USH2A mutations reside.

[0210] In one aspect, a splice element may be “blocked” using an antisense oligonucleotide (AON) having a nucleotide sequence that can hybridize with a USH2A pre-mRNA.

[0211] The term “antisense” refers generally to any approach reliant upon a single-stranded oligonucleotide, recombinant RNA or DNA, that is sufficiently complementary to a target sequence to associate with the target sequence in a sequence-specific manner (e.g., hybridize to the target sequence).

[0212] As used herein, the term “antisense sequence” (AS) refers to a nucleic acid having sufficient sequence complementarity to a single target RNA sequence (i.e., the RNA for which splice site selection is modulated) to block a region of a target RNA (e.g., pre-mRNA) in an effective manner.

[0213] As used herein, an “antisense sequence” and “antisense oligonucleotide” are used interchangeably and refer to a single strand of DNA or RNA that is complementary to a single target sequence of a pre-mRNA. The term “complementary”, as used herein, refers to a nucleic acid sequence that can form a hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base pairing or other non-traditional types of pairing (e.g., Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleosides or nucleotides.

[0214] As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be complementary to adenosine. However, when a U is denoted in the context of the present disclosure, the ability to substitute a T is implied, unless otherwise stated.

[0215] An “antisense sequence” having a “sequence sufficiently complementary to a target RNA sequence to modulate splicing of the target RNA” means that the antisense sequence has a sequence sufficient to trigger the masking of a binding site for a native protein that would otherwise modulate splicing and/or alters the three-dimensional structure of the targeted RNA.

[0216] As used herein, “targeting” means the process of design and selection of nucleic acid sequence that will hybridize to a target nucleic acid and induce a desired effect, e.g., exon skipping.

[0217] It is understood in the art that a nucleic acid molecule need not be 100% complementary to a target nucleic acid sequence to be complementary. That is, two or more nucleic acid molecules may be less than fully complementary. Complementarity is indicated by the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds with a second nucleic acid molecule. For example, if a first nucleic acid molecule has 10 nucleotides and a second nucleic acid molecule has 10 nucleotides, then the base pairing of 5, 6, 7, 8, 9, or 10 nucleotides between the first and second nucleic acid molecules represents 50%, 60%, 70%, 80%, 90%, and 100% complementarity, respectively. “Fully” complementary nucleic acid molecules mean those in which all the contiguous residues of a first nucleic acid molecule will hydrogen bond with the same number of contiguous residues in a second nucleic acid molecule, wherein the nucleic acid molecules either both have the same number of nucleotides (i.e., have the same length) or the two molecules have different lengths. One of ordinary skill in the art would recognize that the antisense sequence can have about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a nucleotide sequence that is 100% complimentary to a selected target sequence, e.g., an USH2A pre-mRNA sequence.

[0218] In one aspect, the antisense sequence (AS) has sufficient complementarity with a target RNA sequence to inhibit splicing of a specific exon without inhibiting the splicing of non-targeted exons.

[0219] In one aspect, the antisense sequence (AS) has sufficient complementarity with a target RNA sequence to inhibit splicing of a specific exon without inhibiting splicing of genes other than USH2A.

[0220] In one aspect, the antisense sequence (AS) has sufficient complementarity with a target RNA sequence to inhibit the splicing of USH2A exon 13 without inhibiting splicing of any of the other USH2A exons.

[0221] In another aspect, an antisense sequence (AS) can be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200 or more nucleotides in length.

[0222] In another aspect, an antisense sequence (AS) can have a % GC of about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, or 75%.

[0223] In another aspect, the antisense sequence can be any one of the sequences shown in FIGs. 5A and 5B, including SEQ ID Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18.

[0224] In another aspect, the antisense sequence can be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides of the nucleotide sequence of SEQ ID NO: 19 or 20.

Antisense oligonucleotides (AONs)

[0225] In certain aspects, the antisense sequences disclosed herein can be delivered to a cell as an antisense oligonucleotide (AON).

[0226] In one aspect, an AON may comprise one, two, three, or more covalently linked antisense sequences, with each antisense sequence targeting a different USH2A pre-mRNA sequence required for a target exon's splicing.

[0227] In another aspect, an AON may comprise a population of AONs, each antisense sequence targeting a single USH2A pre-mRNA sequence required for a target exon's splicing.

[0228] In one aspect, the antisense oligonucleotides may not be chemically modified.

[0229] In one aspect, the antisense oligonucleotides are single stranded.

[0230] In one aspect, an antisense oligonucleotides can be modified oligonucleotides having a length of about 5 to 50 nucleotides (or nucleotide analogs), e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides (or nucleotide analogs). In certain aspects, antisense oligonucleotides are modified oligonucleotides having a length of about 15 to 40 nucleotides (or nucleotide analogs). In certain aspects, antisense oligonucleotides are modified oligonucleotides

having a length of about 3 to 80 nucleotides (or nucleotide analogs), or for example, about 3-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80 or 80 or more nucleotides (or nucleotide analogs).

[0231] Exemplary chemical modifications at certain positions may comprise or consist of one or more (additional) modifications to the nucleobase or backbone linkage, which may or may not be present in the same monomer, for instance at the 3' and/or 5' position. A backbone modification indicates the presence of a modified version of the ribosyl moiety as naturally occurring in RNA (i.e. the pentose moiety), such as bicyclic sugars, tetrahydropyrans, hexoses, morpholinos, 2'-modified sugars, 4'-modified sugar, 5'-modified sugars and 4'-substituted sugars. Examples of suitable modifications include, but are not limited to 2'-O-modified RNA monomers, such as 2'-O-alkyl or 2'-O-(substituted)alkyl such as 2'-O-methyl, 2'-O-(2-cyanoethyl), 2'-O-(2-methoxy)ethyl (2'-MOE), 2'-O-(2-thiomethyl)ethyl, 2'-O-butyryl, 2'-O-propargyl, 2'-O-allyl, 2'-O-(2-aminopropyl), 2'-O-(2-(dimethylamino)propyl), 2'-O-(2-amino)ethyl, 2'-O-(2-(dimethylamino)ethyl); 2'-deoxy (DNA); 2'-O-(haloalkyl)methyl such as 2'-O-(2-chloroethoxy)methyl (MCEM), 2'-O-(2,2-dichloroethoxy)methyl (DCEM); 2'-O-alkoxycarbonyl such as 2'-O-[2-(methoxycarbonyl)ethyl] (MOCE), 2'-O-[2-(V-methylcarbamoyl)ethyl] (MCE), 2'-O-[2-(/V/V-dimethylcarbamoyl)ethyl] (DCME); 2'-halo e.g. 2'-F, 2'-F arabinosyl nucleic acid (FANA); 2'-O-[2-(methylamino)-2-oxoethyl] (NMA); a bicyclic or bridged nucleic acid (BNA) backbone modification such as a conformationally restricted nucleotide (CRN) monomer, a locked nucleic acid (LNA) monomer, a xylo-LNA monomer, an a-LNA monomer, an O-L-LNA monomer, a b-D-LNA monomer, a 2'-amino-LNA monomer, a 2'-(alkylamino)-LNA monomer, a 2'-(acylamino)-LNA monomer, a 2'-/V-substituted 2'-amino-LNA monomer, a 2'-thio-LNA monomer, a (2'-O,4'-C) constrained ethyl (cEt) BNA monomer, a (2'-O,4'-C) constrained methoxyethyl (cMOE) BNA monomer, a 2',4'-BNANC(NH) monomer, a 2',4'-BNANC(NMe) monomer, a 2',4'-BNANC(NBn) monomer, an ethylene-bridged nucleic acid (ENA) monomer, a carba-LNA (cLNA) monomer, a 3,4-dihydro-2/-pyran nucleic acid (DpNA) monomer, a 2'-C-bridged bicyclic nucleotide (CBBN) monomer, an oxo-CBBN monomer, a heterocyclic-bridged BNA monomer (such as triazolyl or tetrazolyl-linked), an amido-bridged BNA monomer (such as AmNA), an urea-bridged BNA monomer, a sulfonamide-bridged BNA monomer, a bicyclic carbocyclic nucleotide monomer, a TriNA monomer, an a-L-TriNA monomer, a bicyclo DNA (bcDNA) monomer, an F-bcDNA monomer, a tricyclo DNA (tcDNA) monomer, an F-tcDNA

monomer, an alpha anomeric bicyclo DNA (abcDNA) monomer, an oxetane nucleotide monomer, a locked PMO monomer derived from 2'-amino LNA, a guanidine-bridged nucleic acid (GuNA) monomer, a spirocyclopropylene-bridged nucleic acid (scpBNA) monomer, and derivatives thereof; cyclohexenyl nucleic acid (CeNA) monomer, altrial nucleic acid (ANA) monomer, hexitol nucleic acid (HNA) monomer, fluorinated HNA (F-HNA) monomer, pyranosyl-RNA (p-RNA) monomer, 3'-deoxypyranosyl DNA (p-DNA), unlocked nucleic acid UNA); an inverted version of any of the monomers above.

[0232] A “backbone modification” indicates the presence of a modified version of the ribosyl moiety, and/or the presence of a modified version of the phosphodiesteras naturally occurring in RNA (“backbone linkage modification”). Examples of internucleoside linkage modifications are phosphorothioate (PS), chirally pure phosphorothioate, Rp phosphorothioate, Sp phosphorothioate, phosphorodithioate (PS2), phosphonoacetate (PACE), thophosphonoacetate, phosphonacetamide (PACA), thiophosphonacetamide, phosphorothioate prodrug, S- alkylated phosphorothioate, H-phosphonate, methyl phosphonate, methyl phosphonothioate, methyl phosphate, methyl phosphorothioate, ethyl phosphate, ethyl phosphorothioate, boranophosphate, boranophosphorothioate, methyl boranophosphate, methyl boranophosphorothioate, methyl boranophosphonate, methyl boranophosphonothioate, phosphoryl guanidine (PGO), methylsulfonyl phosphoroamidate, phosphoramidite, , N3'- P5' phosphoramidate, N3'- P5' thiophosphoramidate, phosphorodiamidate, phosphorothiodiamidate, sulfamate, dimethylenesulfoxide, sulfonate, triazole, oxalyl, carbamate, methyleneimino (MMI), and thioacetamido (TANA); and their derivatives.

[0233] In certain aspects, AONs are a chirally enriched population of modified AONs, wherein the population is enriched for modified AONs comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration, preferably wherein the population is enriched for modified AONs comprising at least one particular phosphorothioate internucleoside linkage having the Sp configuration, or wherein the population is enriched for modified AONs comprising at least one particular phosphorothioate internucleoside linkage having the Rp configuration.

[0234] In one aspect, the nucleotide analogue or equivalent comprises a modified backbone, exemplified by morpholino backbones, carbamate backbones, siloxane backbones, sulfide,

sulfoxide and sulfone backbones, formacetyl and thioformacetyl backbones, methyleneformacetyl backbones, riboacetyl backbones, alkene containing backbones, sulfamate, sulfonate and sulfonamide backbones, methyleneimino and methylenehydrazino backbones, and amide backbones. Phosphorodiamidate morpholino oligomers are modified backbone oligonucleotides that have previously been investigated as antisense agents. Morpholino oligonucleotides have an uncharged backbone in which the deoxyribose sugar of DNA is replaced by a six-membered ring and the phosphodiester linkage is replaced by a phosphorodiamidate linkage. Morpholino oligonucleotides are resistant to enzymatic degradation and appear to function as antisense agents by arresting translation or interfering with pre-mRNA splicing rather than by activating RNase H. Morpholino oligonucleotides have been successfully delivered to tissue culture cells by methods that physically disrupt the cell membrane, and one study comparing several of these methods found that scrape loading was the most efficient method of delivery; however, because the morpholino backbone is uncharged, cationic lipids are not effective mediators of morpholino oligonucleotide uptake in cells. It is further preferred that the linkages between the residues in a backbone do not include a phosphorus atom, such as a linkage that is formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. A preferred nucleotide analogue or equivalent comprises a Peptide Nucleic Acid (PNA), having a modified polyamide backbone (Nielsen, *et al.* (1991) *Science* 254, 1497-1500). PNA-based molecules are true mimics of DNA molecules in terms of base-pair recognition. The backbone of the PNA is composed of N-(2-aminoethyl)-glycine units linked by peptide bonds, wherein the nucleobases are linked to the backbone by methylene carbonyl bonds. An alternative backbone comprises a one-carbon extended pyrrolidine PNA monomer. Since the backbone of a PNA molecule contains no charged phosphate groups, PNA-RNA hybrids are usually more stable than RNA-RNA or RNA-DNA hybrids, respectively (Egholm *et al.* (1993) *Nature* 365:566-568).

[0235] It is understood by a skilled person that it is not necessary for all positions in an AON to be modified uniformly. In addition, more than one of the analogues or equivalents may be incorporated in a single AON or even at a single position within an AON. In certain aspects, an AON of the disclosure has at least two different types of analogues or equivalents. A preferred exon skipping AON according to the disclosure comprises a 2'-O alkyl phosphorothioated antisense oligonucleotide, such as 2'-OMe modified ribose (RNA), 2'-O-ethyl modified ribose, 2'-O-propyl

modified ribose, and/or substituted derivatives of these modifications such as halogenated derivatives. An effective AON according to the disclosure comprises a 2'-OMe ribose and/or 2'-MOE ribose with a preferably fully phosphorothioated backbone.

Recombinant RNAs

[0236] In one aspect, the antisense sequences disclosed herein can be stably expressed *in vivo* as part of a recombinant RNA. Recombinant RNA refers to an engineered RNA having a plurality of antisense sequences covalently linked to an RNA moiety such as a U snRNA, e.g., a U1, U2, U4, U5, U6, or U7 snRNA. In one aspect, the antisense sequences target one or more splice junction regions of an in-frame exon to induce antisense-mediated splicing modulation of pre-mRNA, e.g., exon skipping.

[0237] As used herein, “expression” refers to the process by which a transgene encoding a recombinant RNA is transcribed into a single-stranded RNA suitable for gene therapy applications. In certain aspects, single stranded RNA may form stem and loop structures.

[0238] As used herein, the term “antisense sequence” as it pertains to a recombinant RNA refers to an oligonucleotide, preferably an oligoribonucleotide, that is substantially complementary to a section of about 10-50 nucleotides, e.g., about 15-30, 16-25, 18-23 or 19-22 nucleotides of the pre-mRNA targeted for modulation of splicing. The antisense sequence comprises a sequence sufficiently complementary to the desired target pre-mRNA sequence to direct target-specific modulation of RNA splicing (e.g., complementarity sufficient to trigger the formation of a desired target mRNA through modulation of splicing, e.g., by altering the recruitment of the splicing machinery or process).

[0239] As used herein, the “5' end”, as in the 5' end of an antisense sequence, refers to the 5' terminal nucleotides, e.g., between one and about 5 nucleotides from the 5' terminus of the antisense sequence.

[0240] As used herein, the “3' end”, as in the 3' end of an antisense sequence, refers to the 3' terminal nucleotides, e.g., between one and about 5 nucleotides, at the 3' terminus of the antisense sequence.

[0241] In one aspect, a recombinant RNA comprises one, two, three, or more covalently linked antisense sequences, each being about 20-30 nucleotides in length, wherein each antisense sequence targets a different pre-mRNA sequence required for a target exon's splicing.

[0242] In another aspect, the antisense sequence that can anneal to its target RNA sequence may have one or more mismatches comprising, for example, at least one adenine-guanine (A-G) mismatch, at least one adenine-adenine (A-A) mismatch, or at least one adenine-cytosine (A-C) mismatch.

[0243] In certain aspects, the recombinant RNA can be a modified recombinant U snRNA, e.g., a U1 or U7 snRNA, or RNA- or guide RNAs of non-catalytic RNA or DNA-targeting enzymes (e.g. CRISPR-Cas guide RNAs).

[0244] In one aspect, the modified recombinant U snRNA comprises an Sm or Sm-like protein binding domain, or variant thereof, from a spliceosomal snRNA small nuclear RNA (snRNA).

[0245] Recombinant U7 snRNAs for the expression of antisense RNA sequences were first described by Gorman *et al.* (1998) Proc. Natl. Acad. Sci. USA vol. 95, pp. 4929 – 493 and in the published U.S. Patent Publication No. 2003/0114411, the contents of which are incorporated by reference herein in their entireties. Comparable recombinant U1 snRNAs have also been reported (see, for example, for example, Martone *et al.* (2012) Methods Mol Biol. 867:239-57).

Recombinant U snRNAs

[0246] U1 snRNP recognizes the 5' splice site by base pairing to the 5' splice site exon-intron junction (at positions –3 to +6) (reviewed by Rosbash *et al.* Trends Biochem Sci. (1991) 16(5):187-90). In contrast, a U7 small nuclear ribonucleoprotein (snRNP) is an essential co-factor required for the 3' end processing of replication-dependent histone messenger pre-mRNAs (reviewed in Schümperli, D., and R. S. Pillai (2004) *Cellular and Molecular Life Sciences: CMLS* 61 (19-20): 2560–70). The 5' end of the U7 snRNA anneals to the histone downstream element (HDE) within the histone mRNA's 3' UTR. The U7 associated ribonucleoproteins then direct the 3' endonucleolytic cleavage of the histone pre-mRNA.

[0247] In one aspect, a recombinant U snRNA refers to a modified U small nuclear RNA (snRNA), e.g., a modified U7 or U1 snRNA comprising a targeting sequence that at least partially hybridizes to at least a portion of a target RNA; an Sm or Sm-like protein binding domain or variant thereof

from a spliceosomal snRNA or a non-spliceosomal small nuclear RNA (snRNA); and a hairpin from a spliceosomal snRNA or a non-spliceosomal snRNA, a variant or any combination thereof.

[0248] In one aspect, those sequences at the 5' end of a U7 snRNA that anneal to the HDE can be replaced by the antisense sequences of the present disclosure. In one aspect, the 5' end of a U7 snRNA may comprise one, two, three, or more antisense sequences that anneal to single or multiple targeted pre-mRNAs. In one aspect, an antisense sequence of a recombinant U7 RNA can be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more nucleotides in length.

[0249] In one aspect, any two antisense sequences within a recombinant RNA may be in tandem or separated by a spacer sequence. In one aspect, the spacer sequence can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 nucleotides in length.

[0250] Exemplary recombinant U7 RNAs are depicted in FIG. 5D and FIG. 8B.

[0251] In one aspect, the U7-specific Sm binding site (AAUUUGUCUAG (SEQ ID NO: 63) can be replaced with a consensus Sm sequence (AAUUUUUGGAG; SEQ ID NO: 24; SmOpt). The SmOPT modification of U7 snRNA renders the recombinant U7 particle inactive in histone pre-mRNA processing. The antisense sequence embedded in an snRNP particle is also protected from degradation and facilitates transport to the nucleus where splicing occurs.

[0252] In one aspect, a recombinant U7 RNA may be capped at its 5' end.

[0253] In one aspect, the recombinant U7 snRNA may comprise a hypermethylated 5' cap.

[0254] In one aspect, the recombinant U7 snRNA may comprise a 2,2,7-trimethyl guanosine (m3G) 5' cap (see Plessel *et al.* Molecular and Cellular Biology 14 (6): 4160–72).

[0255] In one aspect, the recombinant U7 comprises an USH2A exon 13 antisense sequence.

[0256] In another aspect, the recombinant U7 comprises any one of the antisense sequences of SEQ ID NO. 1-18.

[0257] In another aspect, the recombinant U7 comprises the USH2A antisense sequences of SEQ ID NO: 25.

[0258] In one aspect, the recombinant U snRNA is not an aptamer.

[0259] In another aspect, the recombinant U snRNA does not comprise enzymatic activity, e.g a ribozyme.

[0260] In another aspect, the recombinant U snRNA does not comprise a guide RNA for gene editing.

[0261] In another aspect, the recombinant U snRNA does not comprise a binding site for ADAR (Adenosine Deaminases that Act on RNA) RNA editing.

[0262] In another aspect, the recombinant U snRNA is not bound to a polypeptide, either covalently or noncovalently.

[0263] In another aspect, the recombinant U snRNA does not comprise a sequence configured for RNA interference (RNAi or miRNA).

[0264] In another aspect, expression of the recombinant USH2A-specific U snRNA in a photoreceptor or cochlear cell enhances the amount of USH2A Δ Exon 13 protein expression to at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 99% of the expression of wild-type USH2A.

CRISPR-Cas Guide RNAs

[0265] In one aspect, the recombinant RNA can be a CRISPR-Cas system guide nucleic acid molecule (such as a guide RNA, gRNA) comprising one or more antisense sequences that can hybridize with one or more sequences of a target exon, e.g., USH2A exon 13. The Cas13d protein forms a complex with the gRNA that then anneals to its target sequence (see the published U.S. Patent Publication 2019/0062724, the content of which is incorporated by reference herein in its entirety).

[0266] In one aspect, a Cas13d protein can include one or more HEPN domains, which is no more than 150 kD, no more than 140 kD, no more than 130 kD, no more than 120 kD, such as about 90 to 120 kD, about 100 to 120 kD or about 110 kD; includes one or more mutated HEPN domains, and can process the guide RNA, but cannot cleave or cut the one or more target RNA molecules, includes an Cas13d ortholog from a prokaryotic genome or metagenome, gut metagenome, an activated sludge metagenome, an anaerobic digester metagenome, a chicken gut metagenome, a

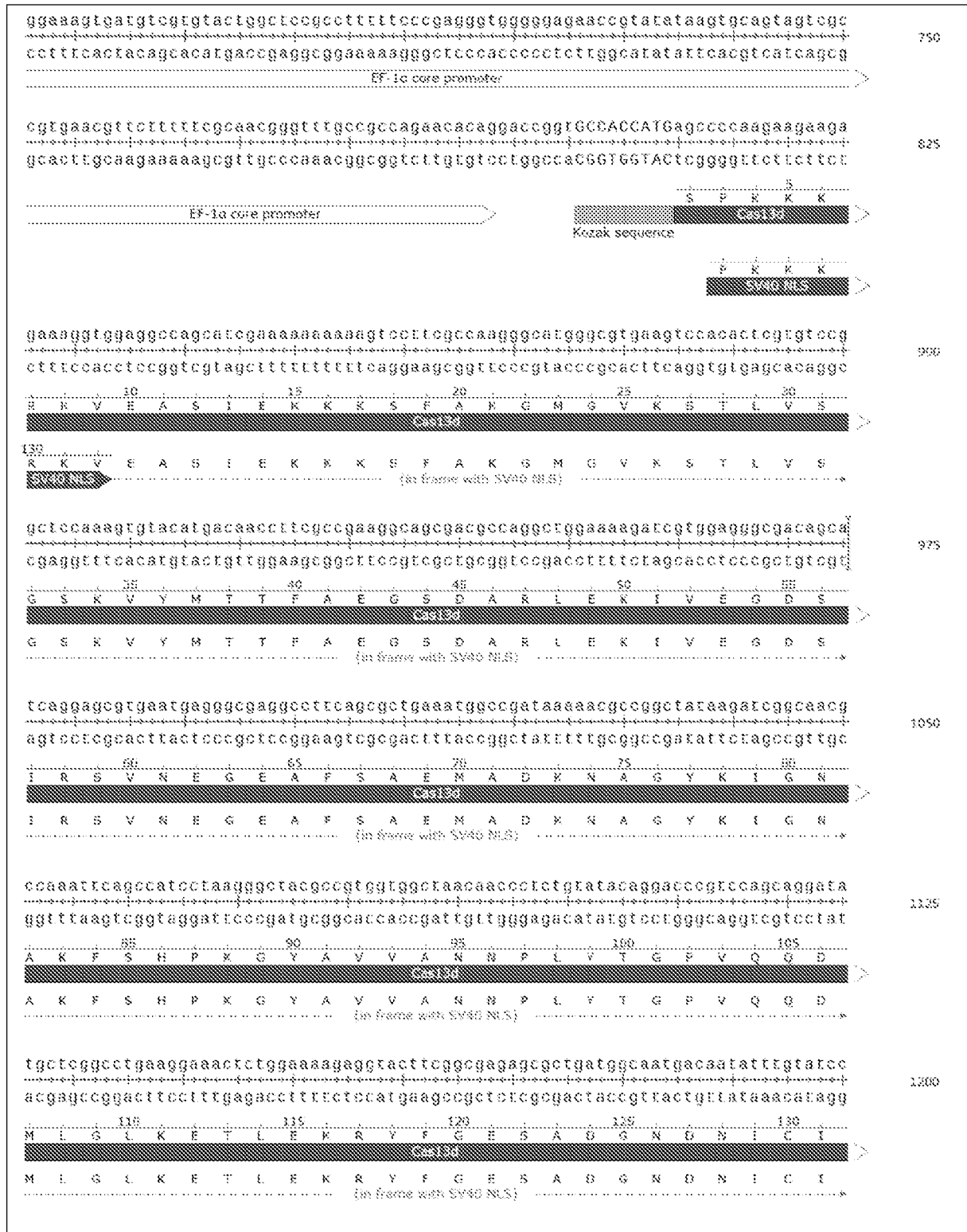
human gut metagenome, a pig gut metagenome, a bovine gut metagenome, a sheep gut metagenome, a goat gut metagenome, a capybara gut metagenome, a primate gut metagenome, a termite gut metagenome, a fecal metagenome, a genome from the Order *Clostridiales*, or the Family *Ruminococcaceae*; includes an Cas13d ortholog from *Ruminococcus albus*, *Eubacterium siraeum*, a *flavefaciens* strain XPD3002, *Ruminococcus flavefaciens* FD-1, uncultured *Eubacterium* sp TS28-c4095, uncultured *Ruminococcus* sp., *Ruminococcus bicirculans*, or *Ruminococcus* sp CAG57.

[0267] In one aspect, the Cas13d protein may comprise one or more mutations in the conserved HEPN RNase domains. The resulting dCas13d is catalytically dead but retains the ability to bind its guide RNA (see Konermann *et al.* (2018), *Cell* 173 (3): 665–76 and the published U.S. Patent Application No. 2019/0062724, the content of which is incorporated by reference herein in its entirety).

[0268] An exemplary dCas13d – recombinant gRNA expression vector is depicted in TABLE V.

TABLE V: EXEMPLARY dCAS13d – RECOMBINANT gRNA VIRAL VECTOR

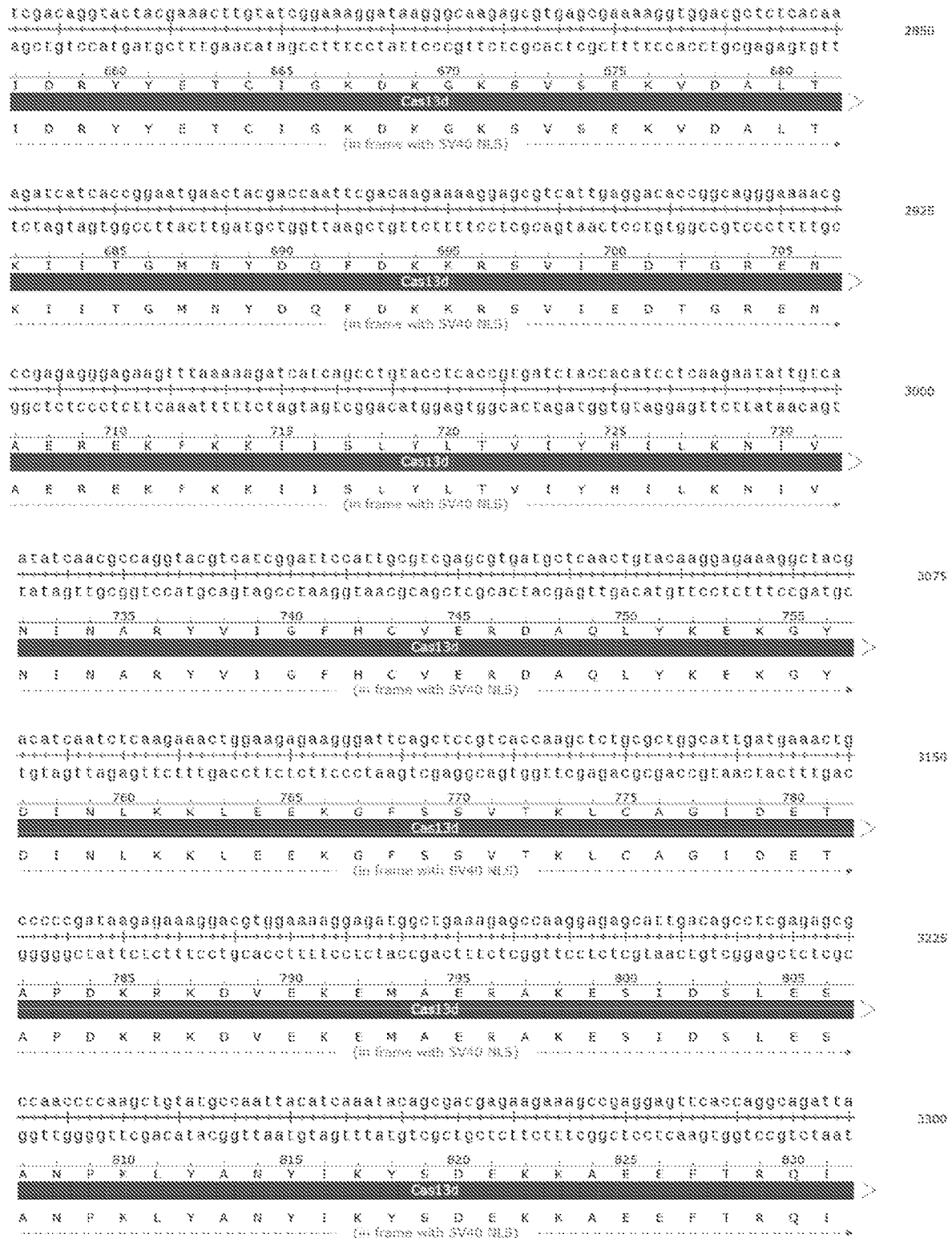
CCTGCAGGCAGCTGCGCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGCTGCCCGGGCTCA	75
GGACGTCCGTGACGCGCGAGCGAGCGAGTGAATCCGGCGGGCCCGACCCCGCTGGAAACAGCGGGCCGAGT	
AAV2 ITR (alternate)	
GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTgcgggccgcacgcgtgagggc	150
CACTCGCTCGCTGCGCGCTCTCTCCCTCACCAGTTGAGGTAGTGATCCCCAAGGAcgcccgcgtgcgcactcccg	
AAV2 ITR (alternate)	
U6 promoter	
ctatttcccatgattccctcattttgcatatagacacagggctgttagagagaaattggaattaatttgact	225
gataaagggtactaaggaagtataaacgtatatgtctgttccgacaatctctctattaaccttaattaaactga	
U6 promoter	
gtaaacacaaagatatttagtacaaaatagtgacgtagaaagtaataatttcttgggtagtttgagttttaaaa	300
catttggtgtttctataatcatgttttatgcactgcattcttcattatataaagaacccatcaaacgtcaaaat	
U6 promoter	
ttatgttttaaaatggactatcatatgcttaccgttaacttgaaagtatttcgatttcttggctttatatatcttg	375
aatacaaaattttacctgatagtatacgaatggcattgaacttccataaagctaaagaaccgaaatatatagaac	
U6 promoter	
tggaaaggacGAAACACCGCAAGTAAACCCCTACCAACTGGTCGGGGTTTGAAACAagcttcggagaaatttaa	450
acctttccctgCTTTGTGGCGTTTCATTTGGGGATGGTTGACCAGCCCCAACTTTGttcgaagcctctttaattt	
U6 promoter	
direct repeat	
pre-gRNA-h4	
Rfx-Cas13d DR30	
pre-gRNA-1	
conserved human/mouse	
tccaaaattgCAAGTAAACCCCTACCAACTGGTCGGGGTTTGAAACCTTACCTGGCTGACACTGCTCACaccttc	525
aggttttaacGTTTCATTTGGGGATGGTTGACCAGCCCCAACTTTGGAATGGACCGACTGTGACGAGTgtggaag	
pre-gRNA-h4	
direct repeat	
pre-gRNA6	
Rfx-Cas13d DR30	
exon 12 (human exon 13)	
conserved human/mouse	
tttttttggtagcagggtcttgaaaggagtggaattgggtccggtgcccgtcagtgggcagagcgcacatcgcc	600
aaaaaaaaaccatggtccagaactttctcacccttaaccgagggccacgggcagtcacccgtctcgcgtgtagcgg	
pre-gRNA6	
EF-1a core promoter	
exon 12 (human exon 13)	
cacagtccccgagaagtgtgggggaggggtcggaattgatccggtgcctagagaaggtggccgggggtaaactg	675
gtgtcaggggtcttcaacccccctcccagcgttaactaggccacggatctcttccacgcgccccatttgac	
EF-1a core promoter	



agggtgatccatcacatccctggacattgaaaaaaccctcgccgaatacattaccaacgcgcctacgccgtcaaca	1275
tccactaggtattgtaggacctgttaacttttttaggagcggttatgtaatggttgcggcggatgcggcagttgt	
Q V I H N I L D I E K I L A E Y I T N A A Y A V N	
Q V I H N I L D I E K I L A E Y I T N A A Y A V N	
(in frame with SV40 NLS)	
atatctccggcctggataaggacattattggattcggcaagttctccacagtgatatacctacgacgaattcaaag	1350
tatagaggccggacctattcctgttaataacctaaagccgtcaagagggtgcacatatggatgctgcttaagtttc	
N I S G L D Y D I I G F G K F S T V Y T Y D E F K	
N I S G L D Y D I I G F G K F S T V Y T Y D E F K	
(in frame with SV40 NLS)	
accccgagcaccatagggccggttttcaacaataacgataagctcatcaacgccatcaaggcccagtatgacgagt	1425
tggggctcgtgggtatcccgccgaaaagttgttatctgctattccagtagtttgcggtagttccgggtcatactgctca	
D P E H H R A A F N N N D K L I N A I K A Q Y D E	
D P E H H R A A F N N N D K L I N A I K A Q Y D E	
(in frame with SV40 NLS)	
tcgacaaactctctcgataaccccagactcggctattttcggccaggccittttcagcaaggaggccagaaatlaca	1500
agctgttgaaggagctatttgggtctgagccgataaagccggtccggaaaaagtcgttccctcccgctcttaatgt	
F D N F L D N P R L G Y F G Q A F F S K E G R N Y	
F D N F L D N P R L G Y F G Q A F F S K E G R N Y	
(in frame with SV40 NLS)	
tcatcaattacggcaacgaatgctatgacattcttggccctcctgagcgggactgscgactgggttggtcctcaaca	1575
agtagttaatgccgttgcctacgatactgttaagaccgggaggactcgcctgaacccgctgacccaccagcctatgt	
I I N Y G N E C Y D I L A L L S G L A H W V V A N	
I I N Y G N E C Y D I L A L L S G L A H W V V A N	
(in frame with SV40 NLS)	
dCas13d mutation area 1 of 2	
acgaagaagagttccaggatctccaggacctggctctacaacctcgataaagaacctcgacaacgaatacatctcca	1650
tgcttctctcaggctcctagaggctcctggaccgagatgttggagctattcttggagctgttgcctatgttagaggt	
N E E E S R I S R T W I Y N L D K N L D N E Y I S	
N E E E S R I S R T W I Y N L D K N L D N E Y I S	
(in frame with SV40 NLS)	
cctcaactacctctacgacaggatcaccaatgagctgacccaactccttcccaagaactccgcgcgaactgtga	1725
gggagttgatggagatgctgtcctagtggttactcgaactgggttgaggaaagaggttcttgaggcggcggttgcact	
T L N Y L Y D R I T N E L T N S F S K N S A A N V	
T L N Y L Y D R I T N E L T N S F S K N S A A N V	
(in frame with SV40 NLS)	

actatattgccgaacctctgtgggaatcaacctgcccgaattcgccgaacaatatttcagattcagcattatgaaag tgatataacggcttrigagaccttagittgggaaggcttaagcggcttggatataaagtcataagtcgtaatacttcc	1800
N Y I A E T L G I N P A E F A E Q Y F R F S I M K Cet124 (in frame with SV40 NLS)	
agcagaaaaacctcggattccaatatcaccgaagctcagggaagtgatgctggacagggaaggatargtccgagatca tcgtctcttriggaggccoraagttatagttggttcgagtcacctcactacgaacctgtctcttccatcacagggtctagt	1875
E Q K N L G F N I Y K L R E V M L D R K D M S E I Cet125 (in frame with SV40 NLS)	
ggaaaaatcataagggtgttcgaacctcaacagggaaccaaggtctacaccatgatggaccttctgtgatttatagggtatt ccttttttagtattccacaagctgaggtagtctctgggtccagatgtgggtactacctgaaacactaaataaccataa	1950
R K N H K V F D S I R T K V Y T M M D F V I Y R Y Cet126 (in frame with SV40 NLS)	
acatcgaaaggaggatgccaaagggtggtgccgcgaataagtccttccccgataatgagaagtcctctgagcgagaagg tgttagcttctctacgggtccaccgacggcggttattccagggggggtattactcttcagggaactcgctctctcc	2025
Y I E E D A K V A A A N K S L P D N E K S L S E K Cet127 (in frame with SV40 NLS)	
atatcttrigtgattaaacctgagggggtccttccaacgacgaccagaaggargccctctactacgatgaagctaata tatagaaacactaaattggactcccccaggaagttgctgctgggtcttctctacggggagatgatgctacttcgatttat	2100
D I F V I N L R G S F N D D Q K D A L Y Y D E A N Cet128 (in frame with SV40 NLS)	
gaatttriggagaaagctcgaaaaataatcatgcacaaacatcaaggaatttaggggaaacaagacaagagagatataaga cttaaacctcttccgagcttttatagttacgtgtttagtttccctaaatccccctttgtctgtctctcatattct	2175
R I W R K L E N I M H N I K E F R G N K T R E Y K Cet129 (in frame with SV40 NLS)	
agaaggacgcccctagactgcccagaatcctgcccgcctggccgtgatgtttccgccttcagcaaaactcatgtatg cttctctgcccggggtctgacgggtcttaggacgggacgaccggcactacaaaaggcgggaagtcgtttgagttacatac	2250
K K D A P R L P R I L P A G R D V S A F S K L M Y Cet130 (in frame with SV40 NLS)	

ccttgaccatgttccctggatggcaaggagatcaacgacctcctgaccaccccgatttaataaattcgataaacatcc gggactgggtacaaggacctaccgttccctctagttgctggaggactgggtgggactaattattttaagctatttgragg	2325
A L T M F L D G K E I N D L L T Y T L I N K F D N I Caa132 (in frame with SV40 NLS)	
agagcttcctgaaggatgatgcctctcatcggagtcacagcttaagttcgtggagggaatcagccctttttcaagact tctcgaaggacttccactacggagagtagccctcagttgcgattcaagcaccctccttatgcggaaaaagtcttga	2400
Q S F L K V M F L I G V N A K F V E E Y A F F K D Caa132 (in frame with SV40 NLS)	
cogccaagatcgccgatgagctgaggctgatcaagtccttcgctagaatgggagaacctattgccgatgccagga ggcggttctagcgggtactcgactccgactagtttcaggaagcgatcttaccctcttggataacggctacgggtct	2475
S A K I A D E L R L I K S F A R M G E P I A D A R Caa132 (in frame with SV40 NLS)	
gggccaatgtatatcgagcccatccgratttttaggaaccaacctgtcctatgatgagctcaaggccctcgccgaca cccggtacatatagctgcggtaggcataaaatccttgggtggacaggatactactcgagttccgggagcgggtgt	2550
R A M Y I D A I R I L G T N L S Y D E L K A L A D Caa132 (in frame with SV40 NLS)	
ccitttccctggacgagaacggaaacaagctcaagaaggcaagcacgggcargagaaatttcatttataataacg ggaaaagggaacctgtctcttgcccttggttcgagttctttccggtcgtgcgactctttaaagtaataattattgc	2625
T F S L D E N G N K L K K G X H G M R N F I I N N Caa132 (in frame with SV40 NLS)	
tgatcagcaataaaagggttccactaccctgatcagatcgggtgatccctgccacacctccatgagatcgccaaaaacg actagtcgttatatttccaagggtgatggactagtctatgccactaggacgggtggagggtactctagcgggtttttgc	2700
V I S N K R F H Y L I R Y G D P A H L H E I A K N Caa132 (in frame with SV40 NLS)	
aggccgtgggtgaggttcggtctcggcaggatcgtctgacatccagaaaaaacagggtccagaacggcagaaccaga tccggcaccacttcagacacgagccgtcctagcagctgtaggtcttttttgtcccggtcttgcggtctcttgggtct	2775
E A V V K F V L G R I A D I Q K K Q G Q N G K N Q Caa132 (in frame with SV40 NLS)	



acaggggagaaggccaaaacogccctgaacgcctacctgaggaacaccaagtgggaatgfgatcatcagggaggacc 3375
 tftccctcttccgggttttggcgaggacttgcggatggactcccttgtgggtccacettacactagtagtccctcctgg
 N R E K A K T A L N A Y L R N T K W N V I I R E D
 Cas13d
 N R E K A K T A L N A Y L R N T K W N V I I R E D
 (in frame with SV40 NLS)

tctgaggaattgacaacacagacatgtaccctgttcccaaacaggccgtcccttgggaagtggccaggtatgtcc 3450
 aggactcttaactgttgttctgtacatgggacaaagccttgggtccggcagctgggaccttcacccgggtccatcagg
 L L R I D N K Y C T L F A N K A V A L E V A R Y V
 Cas13d
 L L R I D N K Y C T L F A N K A V A L E V A R Y V
 (in frame with SV40 NLS)

dCas13d mutation area 2 of 2

acgcctatatcaacgacattgcccagggtcaattccctacttccaaactgtaccattscatcatgcagagaattatca 3525
 tgcggatatagltgctgttaacggctccagtttaaggatgaaggttgacatggtaattgtagtacgtctcttaactagt
 H A Y I N D I A E V N S Y F Q L Y H Y I M Q R I I
 Cas13d
 H A Y I N D I A E V N S Y F Q L Y H Y I M Q R I I
 (in frame with SV40 NLS)

tgaatgagaggtacgagaaaaagcagcgggaagggtgtccgaggtacttcgacgcctgtgaatgacgagagaagaagta 3600
 acttactctccatgctcttttccgtgcctttccacagggtccatgagctgcgacacttactgctcttcttccatgt
 M N E R Y E K S S G K V S E Y F D A V N D E K K Y
 Cas13d
 M N E R Y E K S S G K V S E Y F D A V N D E K K Y
 (in frame with SV40 NLS)

acgatagggtccctgaanaactgctgtgtgtgctttccggtactgtatccccagggtttaagaacctgagacacaggg 3675
 tgcctatccgaggactttgacgacacacacggaaagccgatgacataggggtcccaattcttggactcgtagctcc
 N D R L L K L L C V P F G Y C I P R F K N L S I E
 Cas13d
 N D R L L K L L C V P F G Y C I P R F K N L S I E
 (in frame with SV40 NLS)

ccctgttccgataggaacagaggccgcccaagttcgacnaaggagaaaagaggtgtccggcnaattccggatccggac 3750
 gggacaagctatccttgcctccggcgggtccagctgttccctcttttctccacaggccgttaaggccctagggtctg
 A L F D R N E A A K F D K E K K K V S G N S G S G
 Cas13d
 A L F D R N E A A K F D K E K K K V S G N S G S G
 (in frame with SV40 NLS)
 SV40 NLS

ctaagaaaaagaggaagggtggcgccgcttaccataccgatgtccagattacgccttaacGAATTCCTAGAGCTCG 3825
 gattctttttctccttccacgcggcggaatgggtatgctacaaggctcaatgcgaattCTTAAGGATCTCGAGC
 P E E E R E V A A A Y P Y D V P D Y A (SEQ ID NO: 36)
 Cas13d
 P E E E R E V A A A Y P Y D V P D Y A
 SV40 NLS

CTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATDTGTTGTTTGCCTCCTCCCTGCTGCTTCTTGACCTT	
GACTAGTCGGAGGTGACAGGGAAGATCAACGGTTCGTAGACAAACAADGGGCAAGGGGACAGGAAGGAAGTGGGA	3900
bGH poly(A) signal	
GGAAAGGTGCCACTCCCACTGTCTTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAATC	
CCTTCCACGGTGAGGGTGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAG	3975
bGH poly(A) signal	
TATTTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGAGAAATAGCAGGSCATGCTGGGCAAGT	
ATAAGACCCCAACCCCAACCCCTCTGTCGTTCCCTCTCTTAACGCTTCTCTTATCGTCCGTACGACCCCTCCA	4050
bGH poly(A) signal	
ACCTTTTTGCGGCGCAGGAACCCCTAGTATGAGGTTGGCCACTCCCTCTCTGCGCGCTCCTCTCGCTCACTGAG	
TGGAAAACGCCGCGCTCTTGGGATCACTACCTCAACCGGTGAGGGAAGAGACGCGCGAGCGAGCGAGTGAATC	4125
AAV2 ITR	
GCCGGCGGACCAAAGGTGCCCCGAGGCCCGGGCTTTGCCGGGGCGGCTCAGTGAAGGAGCGAGCGCGCACTGC	
CGGCGCGCTGGTTTCCAGCGGGCTGCGGGCCCGAAACGGGCGCGCGGAGTCACTCGCTCGCTCGCGCTCGACG	4200
AAV2 ITR	
CTGCAGGGGCGCTGATGCGGTATTTCTCCTTAGGCATCTGTGCGGTATTTACACCGGCATACGTCAAAGCAAC (SEQ ID NO: 34)	
GACGTCCCGCGGACTACGCCATAAAAGAGGAATGCCTAGACACGCCATAAAGTGTGCGGTATGCAATTTCTT	
AAV2 ITR	

V. EXPRESSION VECTORS

[0269] Nucleic acid sequences encoding recombinant RNAs can be inserted into delivery vectors and expressed from transcription units within the vectors (e.g., AAV vectors). The recombinant vectors can be DNA plasmids or viral vectors. Generation of the vector construct can be accomplished using any suitable genetic engineering techniques well known in the art, including, without limitation, the standard techniques of PCR, oligonucleotide synthesis, restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing, for example as described in Sambrook *et al.* *Molecular Cloning: A Laboratory Manual*. (1989)), Coffin *et al.* (Retroviruses. (1997)) and “RNA Viruses: A Practical Approach” (Alan J. Cann, Ed., Oxford University Press, (2000)). As will be apparent with one of ordinary skill in the art, a variety of suitable vectors are available for transferring nucleic acids of the disclosure into cells. The selection of an appropriate vector to deliver nucleic acids and optimization of the conditions for insertion of the selected expression vector into the cell are within the scope of one of ordinary skill in the art without the need for undue experimentation. Viral vectors comprise nucleotide sequences for the production of recombinant virus in a packaging cell. Viral vectors expressing nucleic acids of the disclosure can be constructed based on viral backbones including, but not limited to, retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV), pox virus or alphavirus. The recombinant vectors capable of expressing the nucleic acids of the disclosure can be delivered as described herein, and persist in target cells (e.g., stable transformants).

[0270] Nucleic acid sequences used to practice this disclosure can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Adams (1983) *J. Am. Chem. Soc.* 105:661; Belousov (1997) *Nucleic Acids Res.* 25:3440-3444; Frenkel (1995) *Free Radic. Biol. Med.* 19:373-380; Blommers (1994) *Biochemistry* 33:7886-7896; Narang (1979) *Meth. Enzymol.* 68:90; Brown (1979) *Meth. Enzymol.* 68:109; Beaucage (1981) *Tetra. Lett.* 22:1859; U.S. Pat. No. 4,458,066, the content of which is incorporated by reference herein in its entirety.

Transgene

[0271] As used herein, a transgene refers to a nucleotide sequence of a recombinant RNA as disclosed herein. The transgene can be, for example, a modified recombinant U7 or dCas13d guide

RNA comprising one, two, or three or more of the aforementioned USH2A exon 13-specific antisense sequences.

Exemplary Regulatory Sequences

[0272] The term "promoter" or "promoter sequence" as used herein is a DNA regulatory sequence capable of facilitating transcription (e.g., capable of causing detectable levels of transcription and/or increasing the detectable level of transcription over the level provided in the absence of the promoter) of an operatively linked to a downstream (3' direction) coding or non-coding sequence. In some aspects, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements needed to initiate transcription at levels detectable above background. In some aspects, a promoter sequence may comprise a transcription initiation site, as well as binding sites for transcription factors. In addition to sequences sufficient to initiate transcription, a promoter may also include sequences of other cis-acting regulatory elements that are involved in modulating transcription (e.g., enhancers, silencers, and/or insulators, such as locus control regions (LCRs) or matrix attachment regions (MARs)).

[0273] Examples of promoters known in the art that may be used in some aspects, e.g., in the viral vectors disclosed herein, include constitutive promoters, e.g., unregulated promoters that allow for the continual transcription of its associated transgenes in any cell type and/or under any conditions. Examples of constitutive promoters include, but are not limited to, a human β -actin promoter, a human elongation factor-1 α promoter (Kim, D.W., Uetsuki, T., Kaziro *et al.* (1990) *Gene* 91, 217–223), a cytomegalovirus (CMV) promoter (Thomsen *et al.* (1984) *Proc. Natl. Acad. Sci. U.S.A.* 81, 659–663), a modified CMV promoter that is resistant to gene silencing (see, for example, the published U.S. Patent Application No. 2014/0017726 and International PCT Patent Application WO2012099540, the contents of which are incorporated by reference herein in their entireties), a chicken β -actin promoter combined with cytomegalovirus early enhancer (CBA) (see, for example, U.S. Patent No. 5,770,400, the content of which is incorporated by reference herein in its entirety), a truncated chimeric CMV-chicken β -actin (smCBA) promoter (see, for example, U.S. Patent No. 8,298,818, the content of which is incorporated by reference herein in its entirety), a Cbh promoter (see, for example, Gray *et al.* (2011) *Human Gene Therapy* 22 (9): 1143–53, the content of which is incorporated by reference herein in its entirety); a CMVd2 promoter; an

shCMV promoter; a SCP3 promoter (Even *et al.* (2016) PLoS ONE 11(2): e0148918; a PGK promoter (Gilham *et al.* (2010) J. Gene Med. 12, 129–136; a UbC promoter (Gill *et al.*, Gene Ther. 8, 1539–154), an SV40 minimal promoter, an RSV promoter (Yamamoto *et al.* (1980), Cell 22, 787–797), or a murine stem cell virus (MSCV) promoter (Hawley *et al.* (1994) Gene Ther. 1, 136–138).

[0274] In addition, standard techniques are known in the art for creating functional promoters by mixing and matching known regulatory elements. Fragments of promoters, e.g., those that retain at least a minimum number of bases or elements to initiate transcription at levels detectable above the background, may also be used. In some aspects, a CMV enhancer may be combined with a tissue-specific promoter.

[0275] In some aspects, a promoter can be a synthetic promoter (see, for example, Jüttner *et al.* (2019), Nature Neuroscience 22 (8): 1345–56; Leeuw *et al.* (2016) Molecular Brain 9 (1): 52; Sanches-Medeiros *et al.* (2019) J Med Artif. Intell. 2:25; Wu *et al.* (2019) Nature Communications 10 (1): 2880, the contents of which are incorporated by reference herein in their entireties).

[0276] In some aspects, the promoter is tissue-specific such that, in a multicellular organism, the promoter drives expression only in a subset of specific cells. For example, RPE-specific promoters include, for example, the RPE-65 promoter, the tissue inhibitor of metalloproteinase 3 (Timp3) promoter, and the tyrosinase promoter. Still, other RPE-specific promoters are known to those of skill in the art. See, e.g., the promoters described in International Patent Publication No. WO 00/15822, the content of which is incorporated by reference herein in its entirety.

[0277] Examples of photoreceptor cell-specific promoters include, without limitation, the rod opsin promoter, the red-green opsin promoter, the blue opsin promoter, the inter-photoreceptor binding protein (IRBP) promoter and the cGMP- β -phosphodiesterase promoter. See, e.g., the promoters described in International Patent Publication No. WO 98/48097, the content of which is incorporated by reference herein in its entirety.

[0278] In other aspects, a promoter can be an inducible promoter (i.e., a promoter whose activity is controlled by an external stimulus, e.g., the presence of a particular temperature, compound, or protein). In some aspects, a promoter may be a temporally restricted promoter that drives expression depending on the temporal context in which the promoter is found. For example, a

temporally restricted promoter may drive expression only during specific stages of a biological process.

[0279] Prokaryotic (Gossen *et al.*, *TIBS* 18: 471-475, 1993) and insect regulatory systems (No *et al.* *Proc. Natl. Acad. Sci., USA* 93: 3346-3351, 1996) have been adapted to construct gene switches that function in mammalian cells. Since inducer molecules are not expected to have targets in mammalian cells, the possibility of interference with cellular processes is reduced. Of the prokaryotic proteins, the repressors of the lac operon (Brown, M., *et al.* *Cell* 49: 603-612, 1987; and Hu, M. C. -T. and N. Davidson *Cell* 48: 555-566, 1987), the tet operon (e.g., U.S. Patent No. 7,541,446, the content of which is incorporated by reference herein in its entirety) and the cumate operon (e.g., U.S. Patent No. 7,745,592, the content of which is incorporated by reference herein in its entirety) have been shown to function in mammalian cells. Many have been incorporated in eukaryotic inducible expression systems using different strategies to control activation and repression of expression. Activation of expression is mediated by a chimeric transactivator protein formed by the fusion of the bacterial repressor with an activation domain (Gossen, M. and H. Bujard, *Proc. Natl. Acad. Sci. USA* 89: 5547-5551, 1992, and Gossen, M., *et al.* *Science* 268: 1766-1769, 1995; U.S. Patent No. 7,745,592, the contents of which are incorporated by reference herein in their entireties). The transactivator can activate transcription when bound to its DNA recognition sequence placed upstream of the minimal promoter. The ability of the activator to bind DNA depends on the presence/absence of the inducer molecule (e.g., doxycycline or cumate depending on the inducible system being used). Repression of expression is mediated by the repressor bound to operator sites placed downstream of the minimal promoter in the absence of an inducer and repression is relieved by the addition of the inducer (Brown, M., *et al.* *Cell* 49: 603-612, 1987).

[0280] In one aspect, the promoter may have a length of less than 1 kb. In other aspects, the promoter may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800. The promoter may have a length between 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800 nucleotides.

[0281] In one aspect, the promoter can be a pol III-dependent promoter, e.g., a U6 snRNA or H1-RNA promoter.

[0282] In another aspect, the promoter can be a polymerase II U snRNA-dependent promoter, e.g., a human U1 or U7 snRNA gene promoter (see, for example, published U.S. Patent No. 7,947,823, the content of which is incorporated by reference herein in its entirety). The transcriptional regulation of human small nuclear RNA genes has been reviewed by Jawdekar *et al.*, (2008) *Biochimica et Biophysica Acta* 1779 (5): 295–305, the content of which is incorporated by reference herein in its entirety.

[0283] In another aspect, the snRNA gene promoters used herein may contain an essential PSE (proximal sequence element) and DSE (distal sequence element) unique to these genes together with a 13–16-nt-long element (a so-called U snRNA 3' box) that directs the production of a 3' end to pre-snRNA. RNA promoter-specific recognition of the 3'-box RNA processing signal is required for U snRNA transcription termination and 3' end formation (reviewed by Egloff *et al.* (2008) *Biochemical Society Transactions* 36 (Pt 4): 590–94). In certain aspects, a U snRNA promoter may be combined with a pol II enhancer element of a constitutive or tissue-specific promoter, e.g., a photoreceptor cell-specific enhancer.

[0284] In one aspect, the promoter can be a U7 promoter having the nucleotide sequence of SEQ ID NO: 30.

[0285] Use of a U7 snRNA as a carrier for gene therapy applications has been reviewed recently by Lesman *et al.*, (2021), *Human Gene Therapy*, 32(21-22):1317-1329 and by Gadgil, *et al.*, (2021) *The Journal of Gene Medicine* 23 (4): e3321, the contents of which are incorporated by reference herein in their entireties.

VI. DESIGN OF VIRAL EXPRESSION VECTORS

[0286] A recombinant adeno associated virus or rAAV is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including 145 nucleotide inverted terminal repeats (ITRs). The nucleotide sequence of the AAV serotype 2 (AAV2) genome is presented in Srivastava *et al.* *J Virol*, 45: 555-564 (1983) as corrected by Ruffing *et al.*, *J Gen Virol*, 75: 3385-3392 (1994). Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the ITRs.

Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding the rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins.

[0287] As the signals directing AAV replication, genome encapsidation, and integration are contained within the ITRs of the AAV genome, some or all these internal sequences, approximately 4.3 kb of the genome encoding replication and structural capsid proteins, rep-cap can be replaced with foreign DNA such as an expression cassette, as disclosed herein, with the rep and cap proteins provided in *trans*. The sequence located between ITRs of an AAV vector genome is referred to herein as the “payload”.

[0288] The actual capacity of any AAV particle may vary depending on the viral proteins employed. Typically, the vector genome (including ITRs) is not more than about 5 kb, e.g., not more than about 4.9 kb, 4.8 kb, or 4.7 kb.

[0289] The ITRs are each 145 bases in length. Thus, the payload is typically not more than about 4.7 kb, 4.6 kb, 4.5 kb, or 4.4 kb in length. Preferably it is not more than 4.4 kb in length. A recombinant AAV (rAAV) may therefore contain up to about 4.7 kb, 4.6 kb, 4.5 kb, or 4.4 kb of unique payload sequence.

[0290] However, following infection of a target cell, protein expression and replication of the vector requires synthesis of a complementary DNA strand to form a double-stranded genome. This second strand synthesis represents a rate-limiting step in transgene expression. The requirement for second strand synthesis can be avoided using so-called “self-complementary AAV” (scAAV) vectors in which the payload contains two copies of the same transgene payload in opposite orientations to one another, i.e., a first payload sequence followed by the reverse complement of that sequence. These scAAV genomes can adopt either a hairpin structure, in which the complementary payload sequences hybridize intramolecularly to each other, or a double-stranded

complex of two genome molecules hybridized to one another. Transgene expression by such scAAVs is much more efficient than conventional rAAVs, but the effective payload capacity of the vector genome is halved because of the need for the genome to carry two complementary copies of the payload sequence.

[0291] An scAAV vector genome may contain one or more mutations in one of the ITR sequences to inhibit resolution of one terminal repeat, and consequently increase yield in an scAAV preparation. Thus, one of the ITRs in an scAAV may be deleted at the terminal resolution site or may contain an inactivating mutation in the terminal resolution site. See, for example, Wang *et al.* Gene Therapy (2003) 10, 2105-2111 and McCarty *et al.*, Gene Therapy (2003) 10, 2112-2118. It will therefore be apparent that the two ITR sequences at either end of an AAV genome need not be identical. scAAVs are reviewed in McCarty, Molecular Therapy, 16(10), 2008, 1648-1656.

[0292] In this specification, the term “rAAV vector” is generally used to refer to vectors having only one copy of any given payload sequence (i.e., a rAAV vector is not an scAAV vector), and the term “AAV vector” is used to encompass both rAAV and scAAV vectors.

Serotypes

[0293] AAV sequences in the AAV vector genomes (e.g., ITRs) may be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes rh10, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, Anc80 and AAV PHP.B. The nucleotide sequences of the genomes of the AAV serotypes are known in the art. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava *et al.*, J. Virol., 45: 555-564 (1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_01829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_001862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao *et al.*, J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther. 13(1): 67-76 (2006); the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004); AAV PHP.B is described by Deverman *et al.*, Nature

Biotech. 34(2), 204-209 and its sequence deposited under GenBank Accession No. KU056473.1. Reviews of AAV serotypes may be found in Choi *et al* (2005) *Curr Gene Ther* 5(3); 299-310 and Wu *et al* (2006) *Molecular Therapy* 14(3), 316-327. AAV2/Anc80L6 and Anc80 capsids are described, for example, in U.S. Patent No. 10,738,087, the content of which is incorporated by reference in its entirety. Pseudotyped AAVs are described, for example, in Burger *et al.* (2004) *Molecular Therapy: The Journal of the American Society of Gene Therapy* 10 (2): 302-17). Other exemplary serotypes that may used to transduce and express an USH2A-specific recombinant RNA in a photoreceptor or cochlear cell are disclosed in U.S. Patent No. 11,603,542, the content of which is incorporated by reference herein in its entirety).

[0294] In one aspect, AAV particles may utilize or be based on a serotype selected from any of the following AAV1, AAV2, AAV2G9, AAV3, AAV3a, AAV3b, AAV3-3, AAV4, AAV4-4, AAV5, AAV6, AAV6.1, AAV6.2, AAV6.1.2, AAV7, AAV7.2, AAV8, AAV9, AAV9.11, AAV9.13, AAV9.16, AAV9.24, AAV9.45, AAV9.47, AAV9.61, AAV9.68, AAV9.84, AAV9.9, AAV100, AAV11, AAV12, AAV16.3, AAV24.1, AAV27.3, AAV42.12, AAV42-1b, AAV42-2, AAV42-3a, AAV42-3b, AAV42-4, AAV42-5a, AAV42-5b, AAV42-6b, AAV42-8, AAV42-10, AAV42-11, AAV42-12, AAV42-13, AAV42-15, AAV42-aa, AAV43-1, AAV43-12, AAV43-20, AAV43-21, AAV43-23, AAV43-25, AAV43-5, AAV44.1, AAV44.2, AAV44.5, AAV223.1, AAV223.2, AAV223.4, AAV223.5, AAV223.6, AAV223.7, AAV1-7/rh.48, AAV1-8/rh.49, AAV2-15/rh.62, AAV2-3/rh.61, AAV2-4/rh.50, AAV2-5/rh.51, AAV3.1/hu.6, AAV3.1/hu.9, AAV3-9/rh.52, AAV3-11/rh.53, AAV4-8/rh.64, AAV4-9/rh.54, AAV4-19/rh.55, AAV5-3/rh.57, AAV5-22/rh.58, AAV7.3/hu.7, AAV16.8/hu.10, AAV16.12/hu.11, AAV29.3/bb.1, AAV29.5/bb.2, AAV106, 1/hu.37, AAV114.3/hu.40, AAV127.2/hu.41, AAV127.5/hu.42, AAV128.3/hu.44, AAV130.4/hu.48, AAV45.1/hu.53, AAV145.5/hu.54, AAV145.6/hu.55, AAV161.10/hu.60, AAV161.6/hu.61, AAV33.12/hu.17, AAV33.4/hu.15, AAV33.8/hu.16, AAV52/hu.19, AAV52.1/hu.20, AAV58.2/hu.25, AAVA3.3, AAVA3.4, AAVA3.5, AAVA3.7, AAVC1, AAVC2, AAVC5, AAV-DJ, AAV-DJ8, AAVF3, AAVF5, AAVH2, AAVrh.72, AAVhu.8, AAVrh.68, AAVrh.70, AAVpi.1, AAVpi.3, AAVpi.2, AAVrh.60, AAVrh.44, AAVrh.65, AAVrh.55, AAVrh.47, AAVrh.69, AAVrh.45, AAVrh.59, AAVhu.12, AAVH6, AAVLK03, AAVH-1/hu.1, AAVH-5/hu.3, AAVLG-10/rh.40, AAVLG-4/rh.38, AAVLG-9/hu.39, AAVN721-8/rh.43, AAVCh.5, AAVCh.5R1, AAVcy.2, AAVcy.3, AAVcy.4, AAVcy.5, AAVCy.5R1, AAVCy.5R2, AAVCy.5R3, AAVCy.5R4, AAVcy.6, AAVhu.1, AAVhu.2, AAVhu.3, AAVhu.4, AAVhu.5, AAVhu.6, AAVhu.7,

AAVhu.9, AAVhu.10, AAVhu.11, AAVhu.13, AAVhu.15, AAVhu.16, AAVhu.17, AAVhu.18, AAVhu.20, AAVhu.21, AAVhu.22, AAVhu.23.2, AAVhu.24, AAVhu.25, AAVhu.27, AAVhu.28, AAVhu.29, AAVhu.29R, AAVhu.31, AAVhu.32, AAVhu.34, AAVhu.35, AAVhu.37, AAVhu.39, AAVhu.40, AAVhu.41, AAVhu.42, AAVhu.43, AAVhu.44, AAVhu.44R1, AAVhu.44R2, AAVhu.44R3, AAVhu.45, AAVhu.46, AAVhu.47, AAVhu.48, AAVhu.48R1, AAVhu.48R2, AAVhu.48R3, AAVhu.49, AAVhu.51, AAVhu.52, AAVhu.54, AAVhu.55, AAVhu.56, AAVhu.57, AAVhu.58, AAVhu.60, AAVhu.61, AAVhu.63, AAVhu.64, AAVhu.66, AAVhu.67, AAVhu.14/9, AAVhu.t 19, AAVrh.2, AAVrh.2R, AAVrh.8, AAVrh.8R, AAVrh.10, AAVrh.12, AAVrh.13, AAVrh.13R, AAVrh.14, AAVrh.17, AAVrh.18, AAVrh.19, AAVrh.20, AAVrh.21, AAVrh.22, AAVrh.23, AAVrh.24, AAVrh.25, AAVrh.31, AAVrh.32, AAVrh.33, AAVrh.34, AAVrh.35, AAVrh.36, AAVrh.37, AAVrh.37R2, AAVrh.38, AAVrh.39, AAVrh.40, AAVrh.46, AAVrh.48, AAVrh.48.1, AAVrh.48.1.2, AAVrh.48.2, AAVrh.49, AAVrh.51, AAVrh.52, AAVrh.53, AAVrh.54, AAVrh.56, AAVrh.57, AAVrh.58, AAVrh.61, AAVrh.64, AAVrh.64R1, AAVrh.64R2, AAVrh.67, AAVrh.73, AAVrh.74, AAVrh8R, AAVrh8R A586R mutant, AAVrh8R R533A mutant, AAV, BAAV, caprine AAV, bovine AAV, AAVhE1.1, AAVhEr1.5, AAVhER1.14, AAVhEr1.8, AAVhEr1.16, AAVhEr1.18, AAVhEr1.35, AAVhEr1.7, AAVhEr1.36, AAVhEr2.29, AAVhEr2.4, AAVhEr2.16, AAVhEr2.30, AAVhEr2.31, AAVhEr2.36, AAVhER1.23, AAVhEr3.1, AAV2.5T, AAV-PAEC, AAV-LK01, AAV-LK02, AAV-LK03, AAV-LK04, AAV-LK05, AAV-LK06, AAV-LK07, AAV-LK08, AAV-LK09, AAV-LK10, AAV-LK11, AAV-LK12, AAV-LK13, AAV-LK14, AAV-LK15, AAV-LK16, AAV-LK17, AAV-LK18, AAV-LK19, AAV-PAEC2, AAV-PAEC4, AAV-PAEC6, AAV-PAEC7, AAV-PAEC8, AAV-PAEC11, AAV-PAEC12, AAV-2-pre-miRNA-101, AAV-8h, AAV-8b, AAV-h, AAV-b, AAV SM 10-2, AAV Shuffle 100-1, AAV Shuffle 100-3, AAV Shuffle 100-7, AAV Shuffle 10-2, AAV Shuffle 10-6, AAV Shuffle 10-8, AAV Shuffle 100-2, AAV SM 10-1, AAV SM 10-8, AAV SM 100-3, AAV SM 100-10, BNP61 AAV, BNP62 AAV, BNP63 AAV, AAVrh.50, AAVrh.43, AAVrh.62, AAVrh.48, AAVhu.19, AAVhu.11, AAVhu.53, AAV4-8/rh.64, AAVLG-9/hu.39, AAV54.5/hu.23, AAV54.2/hu.22, AAV54, 7/hu.24, AAV54, 1/hu.21, AAV54.4R/hu.27, AAV46.2/hu.28, AAV46.6/hu.29, AAV128, 1/hu.43, true type AAV (ttAAV), UPENN AAV10, Japanese AAV10 serotypes, AAV CBr-7.1, AAV CBr-7.10, AAV CBr-7.2, AAV CBr-7.3, AAV CBr-7.4, AAV CBr-7.5, AAV CBr-7.7, AAV CBr-7.8, AAV CBr-B7.3, AAV CBr-B7.4, AAV CBr-E1, AAV CBr-E2, AAV CBr-E3, AAV CBr-E4, AAV CBr-E5, AAV CBr-e5, AAV CBr-E6, AAV CBr-E7, AAV CBr-E8, AAV CHt-1, AAV CHt-2, AAV CHt-3, AAV CHt-6.1, AAV

CHt-6.10, AAV CHt-6.5, AAV CHt-6.6, AAV CHt-6.7, AAV CHt-6.8, AAV CHt-P1, AAV CHt-P2, AAV CHt-P5, AAV CHt-P6, AAV CHt-P8, AAV CHt-P9, AAV CKd-1, AAV CKd-10, AAV CKd-2, AAV CKd-3, AAV CKd-4, AAV CKd-6, AAV CKd-7, AAV CKd-8, AAV CKd-B1, AAV CKd-B2, AAV CKd-B3, AAV CKd-B4, AAV CKd-B5, AAV CKd-B6, AAV CKd-B7, AAV CKd-B8, AAV CKd-H1, AAV CKd-H2, AAV CKd-H3, AAV CKd-H4, AAV CKd-H5, AAV CKd-H6, AAV CKd-N3, AAV CKd-N4, AAV CKd-N9, AAV CLg-F1, AAV CLg-F2, AAV CLg-F3, AAV CLg-F4, AAV CLg-F5, AAV CLg-F6, AAV CLg-F7, AAV CLg-F8, AAV CLv-1, AAV CLv1-1, AAV CLv1-10, AAV CLv1-2, AAV CLv-12, AAV CLv1-3, AAV CLv-13, AAV CLv1-4, AAV CLv1-7, AAV CLv1-8, AAV CLv1-9, AAV CLv-2, AAV CLv-3, AAV CLv-4, AAV CLv-6, AAV CLv-8, AAV CLv-D1, AAV CLv-D2, AAV CLv-D3, AAV CLv-D4, AAV CLv-D5, AAV CLv-D6, AAV CLv-D7, AAV CLv-D8, AAV CLv-E1, AAV CLv-K1, AAV CLv-K3, AAV CLv-K6, AAV CLv-L4, AAV CLv-L5, AAV CLv-L6, AAV CLv-M1, AAV CLv-M11, AAV CLv-M2, AAV CLv-M5, AAV CLv-M6, AAV CLv-M7, AAV CLv-M8, AAV CLv-M9, AAV CLv-R1, AAV CLv-R2, AAV CLv-R3, AAV CLv-R4, AAV CLv-R5, AAV CLv-R6, AAV CLv-R7, AAV CLv-R8, AAV CLv-R9, AAV CSp-1, AAV CSp-10, AAV CSp-11, AAV CSp-2, AAV CSp-3, AAV CSp-4, AAV CSp-6, AAV CSp-7, AAV CSp-8, AAV CSp-8.10, AAV CSp-8.2, AAV CSp-8.4, AAV CSp-8.5, AAV CSp-8.6, AAV CSp-8.7, AAV CSp-8.8, AAV CSp-8.9, AAV CSp-9, AAV.hu.48R3, AAV.VR-355, AAV3B, AAV4, AAV5, AAVF1/HSC1, AAVF11/HSC11, AAVF12/HSC12, AAVF13/HSC13, AAVF14/HSC14, AAVF15/HSC15, AAVF16/HSC16, AAVF17/HSC17, AAVF2/HSC2, AAVF3/HSC3, AAVF4/HSC4, AAVF5/HSC5, AAVF6/HSC6, AAVF7/HSC7, AAVF8/HSC8, AAVF9/HSC9, AAV-PHP.B (PHP.B), AAV-PHP.A (PHP.A), G2B-26, G2B-13, TH1.1-32, TH1.1-35, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B-EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B-SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B-STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A15/G2A3, AAVG2B4, AAVG2B5 and variants thereof.

[0295] The skilled person can select an appropriate serotype, Glade, clone, or isolate of AAV for use in the present vector on the basis of their common general knowledge. It should be understood, however, that also encompassed herein is the use of an AAV genome of other serotypes that may

not yet have been identified or characterized. The AAV serotype can assist in determining the tissue specificity of infection (or tropism) of an AAV virus. Accordingly, preferred AAV serotypes for use in the AAV-AS_RNA4+8 recombinant U7 to be administered to patients, as described herein, are those which have a natural tropism for or a high efficiency of infection of target cells within the eye. Depending on the mode of administration, it may also be advantageous for the AAV serotype to be AAV8 or AAV2.

Packaging virions

[0296] Virion particles comprising vector genomes are generated by packaging cells capable of replicating viral genomes, expressing viral proteins (e.g., rep and cap proteins), and assembling virion particles. Packaging cells may also require helper virus functions, e.g., from adenovirus, El-deleted adenovirus, or herpesvirus. Techniques to produce AAV vector particles in packaging cells are standard in the art. Production of pseudotyped AAV is disclosed in, for example, WO 01/83692, the content of which is incorporated by reference herein in its entirety. In various aspects, AAV capsid proteins may be modified to enhance delivery of the recombinant vector. Modifications to capsid proteins are generally known in the art (see, for example, the published U.S. Patent Application Nos. US 2005/0053922 and US 2009/0202490, the contents of which are incorporated by reference herein in their entireties).

[0297] One method of generating a packaging host cell is to create a cell line that stably expresses all necessary components for AAV particle production. For example, a plasmid (or multiple plasmids) comprising an AAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the AAV genome, and a selectable marker, such as a neomycin resistance gene, are integrated into the genome of a cell. AAV genomes have been introduced into bacterial plasmids by procedures such as GC tailing (Samulski *et al.*, 1982, Proc. Natl. Acad. S6. USA, 79:2077-2081), addition of synthetic linkers containing restriction endonuclease cleavage sites (Laughlin *et al.*, 1983, Gene, 23:65-73) or by direct, blunt-end ligation (Senapathy & Carter, 1984, J. Biol. Chem., 259:4661-4666). The packaging cell line is then infected with a helper virus such as adenovirus. The advantages of this method are that the cells are selectable and are suitable for large-scale production of AAV. Other examples of suitable methods employ adenovirus or baculovirus rather than plasmids to introduce AAV genomes and/or rep and cap genes into packaging cells.

[0298] Alternatively, a packaging cell can be generated by simply transforming a suitable cell with one or more plasmids encoding an AAV genome, AAV proteins, and any required helper virus functions. The so-called "triple transfection" method utilizes three plasmids, each carrying one of these sets of genes. See Grieger *et al.*, Nature Protocols 1(3), 1412-128 (2006), and references cited therein. General principles of AAV production are reviewed in, for example, Carter, 1992, Current Opinions in Biotechnology, 1533-539; and Muzyczka, 1992, Curr. Topics in Microbiol and Immunol., 158:97-129). Various approaches are described in Ratschin *et al.*, Mol. Cell. Biol. 4:2072 (1984); Hermonat *et al.*, Proc. Natl. Acad. Sci. USA, 81:6466 (1984); Tratschin *et al.*, Mol. Cell. Biol. 5:3251 (1985); McLaughlin *et al.*, J. Virol., 62:1963 (1988); and Lebkowski *et al.*, 1988 Mol. Cell. Biol., 7:349 (1988). Samulski *et al.* (1989, J. Virol., 63:3822-3828); U.S. Pat. No. 5,173,414; WO 95/13365 and corresponding U.S. Pat. No. 5,658,776; WO 95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US96/14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin *et al.* (1995) Vaccine 13:1244-1250; Paul *et al.* (1993) Human Gene Therapy 4:609-615; Clark *et al.* (1996) Gene Therapy 3:1124-1132; U.S. Pat. Nos. 5,786,211; 5,871,982; and 6,258,595, the contents of each of which are incorporated by reference herein in their entireties.

[0299] Techniques for scAAV production are described, for example, in Grieger *et al.*, Molecular Therapy 24(2), 287-297, 2016, the content of which is incorporated by reference herein in its entirety.

[0300] In one aspect, packaging cells may be stably transformed cell lines such as HeLa cells, HEK 293 cells, and PerC.6 cells (a cognate 293 line). In another aspect, packaging cells are cells that are not transformed cells such as low passage HEK 293 cells (human fetal kidney cells transformed with E1A of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells).

[0301] The term "recombinant virus," as used herein, is intended to refer to a non-wild-type and/or artificially produced recombinant virus (e.g., parvovirus, adenovirus, lentivirus or adeno-associated virus etc.) that comprises a transgene or other heterologous nucleic acid. The recombinant virus may comprise a recombinant viral genome (e.g., comprising cis-acting

regulatory sequences as described herein and a transgene) packaged within a viral (e.g., AAV) capsid.

[0302] In various aspects, the disclosure provides a AAV viral particle as described herein comprising any one or more of the aforementioned nucleic acids.

CpG content

[0303] In one aspect, a rAAV vector, including an rAAV vector genome as described herein, comprises at least one synthetic AAV ITR, wherein one or more CpG islands (a cytosine base followed immediately by a guanine base (CpG) in which the cytosines tend to be methylated) that typically occur at, or near the transcription start site in an ITR are deleted and/or substituted. In one aspect, deletion or reduction in the number of CpG islands can reduce the immunogenicity of the rAAV vector. This results in a reduction or complete inhibition in TLR-9 binding to the rAAV vector DNA sequence, which occurs at CpG islands. It is also well known that methylation of CpG motifs results in transcriptional silencing. Removal of CpG motifs in the ITR is expected to result in decreased TLR-9 recognition and/or decreased methylation and therefore decreased transgene silencing. In some aspects, it is the minimal functional ITR in which one or more CpG islands are deleted and/or substituted. In one aspect, AAV ITR2 is known to contain 16 CpG islands of which one or more, or all 16 can be deleted.

[0304] In some aspects, at least 1 CpG motif is deleted and/or substituted, e.g., at least 4 or more or 8 or more CpG motifs, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 CpG motifs. The phrase “deleted and/or substituted” as used herein means that one or both nucleotides in the CpG motif are deleted, substituted with a different nucleotide, or any combination of deletions and substitutions. Examples of CpG modified recombinant adeno-associated viral (AAV) vector is disclosed, for example, in U.S. Patent Publication No. 2022/0154208, the content of which is incorporated by reference herein in its entirety).

[0305] In certain aspects, the transgene nucleic acid sequence can also be optimized to enhance expression *in vivo* and/or to reduce the number of CpG islands and avoid an innate immune response to the vector.

VII. PHARMACEUTICAL COMPOSITIONS AND MODES OF EXPRESSION VECTOR ADMINISTRATION *IN VIVO*

[0306] As is well known in the art, a vector is a tool that allows or facilitates the transfer of an entity from one environment to another. By way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell. Optionally, once within the target cell, the vector may then serve to maintain the heterologous DNA within the cell. Examples of vectors used in recombinant DNA techniques include plasmids, chromosomes, artificial chromosomes, or viruses.

[0307] Non-viral delivery systems include but are not limited to DNA transfection methods. Here, transfection includes a process using a non-viral vector to deliver a gene to a target mammalian cell. Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection, liposomes, immunoliposomes, exosomes, lipofectin, cationic agent-mediated, cationic facial amphiphiles (CFAs) (Nature Biotechnology 1996 14; 556), and combinations thereof.

[0308] Viral delivery systems include, but are not limited to, adenovirus vectors, adeno-associated viral (AAV) vectors, herpes viral vectors, retroviral vectors, lentiviral vectors, or baculoviral vectors. Other examples of vectors include *ex vivo* delivery systems, which include, but are not limited to, DNA transfection methods, such as, for example, electroporation, DNA biolistics, lipid-mediated transfection, or compacted DNA-mediated transfection.

[0309] For gene therapy applications, the expression vectors disclosed herein can be incorporated into a non-replicating viral vector, for example, an adeno-associated virus (AAV). In certain aspects, the rAAV vectors and/or rAAV genome as disclosed herein can be formulated in a solvent, emulsion, or other diluent in an amount sufficient to obtain a desired titer of an rAAV vector disclosed herein.

[0310] In certain aspects, the rAAV vectors and/or rAAV genome as disclosed herein may be formulated in a solvent an emulsion or a diluent in an amount of, e.g., less than about 90% (v/v), less than about 80% (v/v), less than about 70% (v/v), less than about 65% (v/v), less than about 60% (v/v), less than about 55% (v/v), less than about 50% (v/v), less than about 45% (v/v), less than about 40% (v/v), less than about 35% (v/v), less than about 30% (v/v), less than about 25% (v/v), less than about 20% (v/v), less than about 15% (v/v), less than about 10% (v/v), less than about 5% (v/v), or less than about 1% (v/v). In other aspects, the rAAV vectors and/or rAAV

genome as disclosed herein can be disclosed herein may comprise a solvent, emulsion or other diluent in an amount in a range of, e.g., about 1% (v/v) to 90% (v/v), about 1% (v/v) to 70% (v/v), about 1% (v/v) to 60% (v/v), about 1% (v/v) to 50% (v/v), about 1% (v/v) to 40% (v/v), about 1% (v/v) to 30% (v/v), about 1% (v/v) to 20% (v/v), about 1% (v/v) to 10% (v/v), about 2% (v/v) to 50% (v/v), about 2% (v/v) to 40% (v/v), about 2% (v/v) to 30% (v/v), about 2% (v/v) to 20% (v/v), about 2% (v/v) to 10% (v/v), about 4% (v/v) to 50% (v/v), about 4% (v/v) to 40% (v/v), about 4% (v/v) to 30% (v/v), about 4% (v/v) to 20% (v/v), about 4% (v/v) to 10% (v/v), about 6% (v/v) to 50% (v/v), about 6% (v/v) to 40% (v/v), about 6% (v/v) to 30% (v/v), about 6% (v/v) to 20% (v/v), about 6% (v/v) to 10% (v/v), about 8% (v/v) to 50% (v/v), about 8% (v/v) to 40% (v/v), about 8% (v/v) to 30% (v/v), about 8% (v/v) to 20% (v/v), about 8% (v/v) to 15% (v/v), or about 8% (v/v) to 12% (v/v).

[0311] The recombinant AAV containing the desired transgene and U snRNA promoter for use in the target ocular cell, as detailed above, is preferably assessed for contamination by conventional methods and then formulated into a pharmaceutical composition intended for subretinal injection. Such formulation involves the use of a pharmaceutically and/or physiologically acceptable vehicle or carrier, particularly one suitable for subretinal injection, such as buffered saline or other buffers, e.g., HEPES, to maintain pH at appropriate physiological levels. A variety of such known carriers are provided in, for example, U.S. Patent No. 7,629,322, the content of which is incorporated by reference herein in its entirety. If the virus is to be stored long-term, it may be frozen in the presence of glycerol or Tween 20.

[0312] As used herein, the phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0313] In certain aspects, the pharmaceutically acceptable excipient is 1×PBS (e.g., 0.154M NaCl, 0.056M Na₂HPO₄, and 0.0106 M KH₂PO₄) or DPBS (e.g., 0.337M NaCl, 0.27 M KCl, 0.015M Na₂HPO₄, and 0.0015M KH₂PO₄).

[0314] In certain aspects of the methods described herein, the pharmaceutical composition described above is administered to the subject by subretinal injection. In other aspects, the

pharmaceutical composition is administered by intravitreal, suprachoroidal or orbital injection. Other forms of administration that may be useful in the methods described herein include, but are not limited to, direct delivery to a desired organ (e.g., the eye), oral, inhalation, intranasal, intratracheal, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

[0315] Furthermore, in certain aspects it is desirable to perform non-invasive retinal imaging and functional studies to identify areas of specific ocular cells to be targeted for therapy. In these aspects, clinical diagnostic tests are employed to determine the precise location(s) for one or more subretinal injections). These tests may include ophthalmoscopy, electroretinography (ERG) (particularly the b-wave measurement), perimetry, topographical mapping of the layers of the retina and measurement of the thickness of its layers by means of confocal scanning laser ophthalmoscopy (cSLO) and optical coherence tomography (OCT), topographical mapping of cone density via adaptive optics (AO), functional eye exam, etc. These, and other desirable tests, are described in U.S. Patent No. 9,770,491, the content of which is incorporated by reference herein in its entirety). In view of the imaging and functional studies, in some aspects, one or more injections are performed in the same eye in order to target different areas of retained bipolar cells. The volume and viral titer of each injection are determined individually, as further described below, and may be the same or different from other injections performed in the same, or contralateral, eye. In another aspect, a single, larger volume injection is made in order to treat the entire eye. In one aspect, the volume and concentration of the rAAV composition is selected so that only a specific region of ocular cells is impacted. In another aspect, the volume and/or concentration of the rAAV composition is a greater amount, in order to reach larger portions of the eye, including non-damaged ocular cells.

[0316] In various aspects, the composition can be delivered in a volume of from about 0.1 pL to about 1 mL, including all numbers within the range, depending on the size of the area to be treated, the viral titer used, the route of administration, and the desired effect of the method. In one aspect, the volume is about 50 pL. In another aspect, the volume is about 70 pL. In another aspect, the volume is about 100 pL. In another aspect, the volume is about 125 pL. In another aspect, the volume is about 150 pL. In another aspect, the volume is about 175 pL. In yet another aspect, the volume is about 200 pL. In another aspect, the volume is about 250 pL. In another aspect, the volume is about 300 pL. In another aspect, the volume is about 450 pL. In another aspect, the

volume is about 500 pL. In another aspect, the volume is about 600 pL. In another aspect, the volume is about 750 pL. In another aspect, the volume is about 850 pL. In another aspect, the volume is about 1000 pL.

[0317] In certain aspects, the viral vector is present at a concentration of 2.5×10^1 vg/ml, 7.5×10^{11} vg/ml, or 2.5×10^{12} vg/ml.

[0318] In certain aspects, the pH of the composition is 6.5 to 7.5; 7.0 to 7.5; 6.8 to 7.2. In some aspects, the pH of the composition is 7.0 or 7.4. In one aspect, a pharmaceutical formulation is disclosed comprising: (a) an rAAV8 USH2A-U7 vector, and (b) 1×PBS. The pharmaceutical formulation optionally further comprises: (c) about 200 mM NaCl. The pharmaceutical formulation optionally further comprises rAAV8 USH2A-U7 vector at a concentration of about 5.7×10^{11} vg/ml. Particular note is made that the pharmaceutical formulation optionally further comprises: (d) empty capsids. Particular note is made that the pharmaceutical formulation optionally comprises empty capsids at a percentage of at least about 0.1% cp/cp.

[0319] In another aspect, compositions and methods for increasing rAAV8 USH2A-U7 gene therapy transduction are disclosed. In one aspect, the disclosure provides a pharmaceutical formulation comprising an rAAV8-USH2A-U7 vector and empty capsids. Optionally, the empty capsids may be present in a percentage of at least about 0.1% cp/cp, at least about 10% cp/cp, at least about 50% cp/cp, at least about 75% cp/cp, or at least about 90% cp/cp.

[0320] Optionally, the empty capsids may be present in a percentage ranging from about 0.1% to about 90% cp/cp, from about 1% to about 90% cp/cp, from about 10% to about 80% cp/cp, from about 20% to about 70% cp/cp, from about 40% to about 60% cp/cp, from about 10% to about 50% cp/cp, from about 10% to about 25% cp/cp, or from about 25% to about 75% cp/cp. Preferably the empty capsids may be present in at about 10% vg/vg, about 20% vg/vg about 30% cp/cp, about 40% cp/cp, about 50% cp/cp, about 60% cp/cp, about 70% cp/cp, about 80% cp/cp, or about 90% cp/cp. In one aspect, the percentage of empty capsids may be at least about 50% cp/cp. In another particular aspect, the percentage of empty capsids may be at least about 88% cp/cp. In another particular aspect, the percentage of empty capsids may be about 88% cp/cp. In another particular aspect, the pharmaceutical formulation may comprise about 1.76×10^{12} cp empty capsids and about 2.4×10^{11} vg rAAV8 USH2A-U7 vector.

[0321] Optionally, the empty capsids may be present in a ratio of empty capsids to rAAV8 USH2A-U7 vectors of at least about 9 to about 1, at least about 1 to about 1, or at least about 1 to about 9. Optionally, the pharmaceutical formulation may comprise empty capsids that are present in an excess over rAAV8 USH2A-U7 vectors. In one aspect, the pharmaceutical formulation may comprise empty capsids that are present in at least about a $10\times$ excess over rAAV8 USH2A-U7 vectors.

[0322] In one aspect, a pharmaceutical formulation may comprise: (a) rAAV8 USH2A-U7 vectors, and (b) empty capsids. Optional aspects include, but are not limited to, pharmaceutical formulations, wherein the percentage of empty capsids is at least about 0.1% cp/cp, at least about 10% cp/cp, at least about 50% cp/cp, at least about 75% cp/cp, or at least about 90% cp/cp. Another optional aspect includes pharmaceutical formulations, wherein the percentage of empty capsids ranges from about 10% to about 90%. In one particular aspect, the percentage of empty capsids is at least about 88% cp/cp. In another particular aspect, the pharmaceutical formulation comprises about 1.76×10^{12} cp empty capsids, and about 2.4×10^{11} vg rAAV8 USH2A-U7 vector.

[0323] Other optional aspects include, but are not limited to, pharmaceutical formulations, wherein the ratio of empty capsids to rAAV8 USH2A-U7 vectors is at least about 9:1, is at least about 1:1, is at least about 1:9. Other optional aspects include, but are not limited to, pharmaceutical formulations, wherein the ratio of empty capsids to rAAV8 USH2A-U7 vectors is any ratio from about 1:1, to about 1:100,000. Other optional aspects include, but are not limited to, pharmaceutical formulations, wherein the ratio of empty capsids to rAAV8 USH2A-U7 vectors is about 1:10 to 1:100, 1:100 to 1:1000, 1:1000 to 1:10,000, 1:10,000 to 1:100,000, or 1:100,000 to $1:>100,000$.

[0324] The term “empty capsid” shall mean a virus protein coat that does not contain a vector genome. An empty capsid can be a virus-like particle in that it reacts with one or more antibodies that react with intact (e.g., vector genome carrying) virus (e.g., adeno-associated virus, AAV). In a non-limiting example, an empty AAV8 capsid retains the ability to react with one or more antibodies that bind to an AAV, such as an AAV8 or another AAV serotype. For example, an empty AAV2 capsid retains the ability to react with one or more antibodies that bind to AAV8.

[0325] Empty capsids may sometimes be naturally found in AAV vector preparations. Such preparations can be used in accordance with this disclosure. Optionally, such preparations may be

manipulated to increase or decrease the number of empty capsids. For example, the amount of empty capsid can be adjusted to an amount that would be expected to reduce the inhibitory effect of antibodies. Empty capsids can also be produced independently of vector preparations, and optionally (i) added to vector preparations, or (ii) administered separately to a subject. See F. Mingozzi et al., U.S. Patent Application Publication No. 2014/0336245 “Virus vectors for highly efficient transgene delivery,” the content of which is incorporated by reference herein in its entirety).

[0326] The term “mutant empty capsid” shall mean an empty capsid comprising a mutation that disrupts virus receptor binding. In one aspect, a mutant empty capsid is a non-infective mutant capsid. In another aspect, an empty capsid can absorb an antibody but cannot enter a target cell. In another aspect, an empty capsid can absorb a neutralizing antibody. See C. J. Aalbers, et al., “Empty Capsids and Macrophage Inhibition/Depletion Increase rAAV Transgene Expression in Joints of Both Healthy and Arthritic Mice,” *Human Gene Therapy*, 2017 February; 28(2):168-1781; and Ayuso E, et al. “High AAV vector purity results in serotype- and tissue independent enhancement of transduction efficiency.” *Gene Ther* 2010; 17:503-510, the contents of which are incorporated by reference herein in their entireties.

[0327] The term “capsid particle” or “cp” shall be broadly understood to encompass any capsid. For convenience, the capsids may be full (e.g., encapsulating a gene insert) or empty. Capsid particles include, but not limited to, capsids carrying vector genomes (e.g., AAV viruses, and rAAV vectors), empty capsids, modified capsids, content-modified capsids, and mutant empty capsids.

[0328] For quantitative purposes, “cp” is calculated as a count of the total number of combined capsids carrying vector genomes (e.g., AAV viruses, and rAAV vectors), empty capsids, modified capsids, content-modified capsids, and mutant empty capsids. In one example, “1 cp” shall mean one empty capsid, while about 1.76×10^{12} cp shall mean about 1.76×10^{12} empty capsids. In another example, a pharmaceutical formulation comprising 88% cp/cp empty capsids comprises 88 empty capsid particles per 100 total capsid particles (full and empty). In another example, a pharmaceutical formulation can comprise a total of about 2.0×10^{12} cp capsid particles, wherein the pharmaceutical formulation comprises about 2.4×10^{11} vg rAAV8 USH2A-U7 vectors and about 1.76×10^{12} cp empty capsids.

[0329] The term “decoy,” “decoy particle” or “viral decoy” shall mean a particle or other composition that mimics a virus. The decoy is preferably devoid of virulent activity. Without being bound by theory, a decoy can mimic a native virus in size, shape, structure, or composition thereby causing, and thereby can be consumed by macrophages (e.g., through phagocytosis), leaving functional vectors free to transduce cells. Examples of decoy particles include, but are not limited to, empty capsids, modified capsids, and mutant empty capsids.

[0330] In one particular aspect, decoy particles are administered to a subject as part of the disclosed method. A non-limiting example of a decoy particle is an empty capsid. In one particular aspect, empty AAV capsids are administered to a subject as part of the disclosed method.

[0331] Much effort in gene-therapy research has been devoted to minimizing the presence of empty capsids. Some researchers believe, counterintuitively, that the presence of empty capsids is advantageous for gene transduction. See J. F. Wright, “AAV Empty Capsids: For Better or Worse?” *Molecular Therapy* 2014 January; 22(1):1-2; D. Grimm, et al. “Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2” *Gene Therapy*, 1999, Volume 6, Number 7, Pages 1322-1330. Without being bound by theory, literature reports that empty capsids act as decoys and therefore increase the chances that full virus particles will be able to reach target cells. (see C. J. Aalbers, et al., “Empty Capsids and Macrophage Inhibition/Depletion Increase rAAV Transgene Expression in Joints of Both Healthy and Arthritic Mice,” *Human Gene Therapy*, 2017 February; 28(2):168-178; F. Mingozzi et al., “Overcoming Preexisting Humoral Immunity to AAV Using Capsid Decoys,” *Sci. Transl. Med.* 2013 Jul. 17; 5(194)). Without being bound by theory, literature reports disclose using viral decoys that are incapable of infectious behavior while at the same time being fully capable of effecting an immune response and otherwise being antigenically bioreactive. See N. Kossovsky et al., U.S. Pat. No. 5,334,394 “A Human immunodeficiency virus decoy,” and F. Mingozzi et al., U.S. Patent Application Publication No. 2014/0336245 “Virus vectors for highly efficient transgene delivery.”

[0332] The count of full and empty capsids can be, in one aspect, accomplished using transmission electron microscopy and chromatography. One chromatographic method, which uses a linear gradient elution on CIM QA disk, assesses charge differences between full and empty capsids (see BIA Separations 2015, M. Lock, et al.: “Analysis of Particle Content of Recombinant Adeno-

Associated Virus Serotype 8 Vectors by Ion-Exchange Chromatography.” Human Gene Therapy Methods: Part B 23:56-64 (2012)).

[0333] Other counting techniques include, but are not limited to, (i) CsCl or iodixanol gradients, and (ii) electron microscope (EM) assay, total particle assay (ELISA) combined with genome copy titration (qPCR). Reference is also made to J. M. Sommer, “Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement” Molecular Therapy 2003; 7(1):122-128, and D. Grimm, et al. “Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2” Gene Therapy, 1999, Volume 6, Number 7, Pages 1322-1330. Methods for separating empty capsids from full capsids, include but are not limited to, those provided in U.S. Pat. No. 8,137,948, “Methods for Producing Preparations of Recombinant AAV Virions Substantially Free of Empty Capsids”, the contents of which are incorporated by reference herein in their entireties).

[0334] In one aspect, a pharmaceutical formulation comprises: (a) rAAV8 USH2A-U7 vectors, and (b) empty capsids. Optional aspects include but are not limited to, pharmaceutical formulations, wherein the percentage of empty capsids is at least about 0.1% cp/cp, at least about 10% cp/cp, at least about 50% cp/cp, at least about 75% cp/cp, or at least about 90% cp/cp. Another optional aspect includes pharmaceutical formulations, wherein the percentage of empty capsids ranges from about 10% to about 90%. In other aspects, the percentage of empty capsids ranges from about 10%-90%, 20%-90%, 30%-90%, 40%-90%, 50%-90%, 60% to 90%, 70% to 90%, or 80%-90% cp/cp. In yet more aspects, the percentage of empty capsids ranges from about 10% to 20%, 10% to 30%, 10% to 40%, 10% to 50%, 10% to 60%, 10% to 70%, or 10% to 80% cp/cp. In yet more aspects, the percentage of empty capsids ranges from about 50% to 60%, 50% to 70%, 50% to 80%, or 50% to 90% cp/cp. In one particular aspect, the percentage of empty capsids is at least about 88% cp/cp. In another particular aspect, the pharmaceutical formulation comprises about 1.76×10^{12} cp empty capsids, and about 2.4×10^{11} vg rAAV8 USH2A-U7 vector.

[0335] Other optional aspects include, but are not limited to, pharmaceutical formulations, wherein the ratio of empty capsids to rAAV8 USH2A-U7 vectors is at least about 9:1, is at least about 1:1, is at least about 1:9. Other optional aspects include, but are not limited to, pharmaceutical formulations, wherein the ratio of empty capsids to rAAV8 USH2A-U7 vectors is any ratio from about 1:1, to about 1:100,000. Other optional aspects include, but are not limited

to, pharmaceutical formulations, wherein the ratio of empty capsids to rAAV8 USH2A-U7 vectors is about 1:10 to 1:100, 1:100 to 1:1000, 1:1000 to 1:10,000, 1:10,000 to 1:100,000, or 1:100,000 to 1:>100,000.

[0336] Another particular aspect includes a pharmaceutical formulation, wherein the empty capsids are present in an excess over rAAV8 USH2A-U7 vectors. Another particular aspect, it is useful for empty capsids to be present in any number from about a $10\times$ excess over rAAV8 USH2A-U7 vectors to about $1,000,000\times$ excess over rAAV8 USH2A-U7 vectors. Other particular aspects include a pharmaceutical formulation, wherein the empty capsids are present in at least about a $10\times$ excess, $100\times$ excess, or $1,000\times$ excess over rAAV8 USH2A-U7 vectors.

[0337] In another example, a pharmaceutical formulation can comprise a total of about 2.0×10^{12} cp capsid particles, wherein the pharmaceutical formulation comprises about 2.4×10^{11} vg rAAV8 USH2A-U7 vector and about 1.76×10^{12} cp empty capsids.

[0338] (i) an rAAV8 USH2A-U7 vector, and (ii) an empty capsid; and (b) delivering the pharmaceutical formulation to the eye of the subject.

[0339] In another particular aspect, an rAAV8 USH2A-U7 vector, is also administered along with an immunosuppressive agent to a subject as part of the disclosed method. A non-limiting example of a method for increasing rAAV8 USH2A-U7 gene therapy transduction comprises the steps of: (a) providing a pharmaceutical formulation comprising: (i) an rAAV8 USH2A-U7 vector, and (ii) an immunosuppressive agent; and (b) delivering the pharmaceutical formulation to the eye of the subject. Another non-limiting example of a method for increasing rAAV8 USH2A-U7 gene therapy transduction comprises the steps of: (a) providing a pharmaceutical formulation comprising: (i) an rAAV8 USH2A-U7 vector, (ii) an empty capsid, and (iii) an immunosuppressive agent; and (b) delivering the pharmaceutical formulation to the eye of the subject.

[0340] The composition may further comprise empty capsids at a percentage of about 95% cp/cp, 90% cp/cp, 85% cp/cp, 80% cp/cp, 75% cp/cp, 70% cp/cp, 65% cp/cp, 60% cp/cp, 55% cp/cp, 50% cp/cp, 45% cp/cp, 40% cp/cp, 35% cp/cp, 30% cp/cp, 25% cp/cp, 20% cp/cp, 15% cp/cp, 10% cp/cp, 5% cp/cp or less.

[0341] In certain aspects, human subjects receive a one-time treatment of a subretinally delivered vector (e.g., AAV8 or AAV2) comprising AS_RNA4+6 recombinant U7 in an amount of about 1×10^{13} GC to about 10×10^{13} GC over a period of about 24 hours.

[0342] For example, a conventional syringe and needle can be used to inject a rAAV virion suspension into the retina of a subject. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain agents for a pharmaceutical formulation, such as suspending, stabilizing and/or dispersing agents or enough salts or monosaccharides to make the solution isotonic with blood. Alternatively, the rAAV vectors and/or rAAV genome as disclosed herein can be in powder form (e.g., lyophilized) for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

[0343] In some aspects, the dose is an amount of about 1×10^{11} vg, 2×10^{11} vg, 3×10^{11} vg, 4×10^{11} vg, 5×10^{11} vg, 6×10^{11} vg, 7×10^{11} vg, 8×10^{11} vg, 9×10^{11} vg, 1×10^{12} vg, 2×10^{12} vg, 3×10^{12} vg, 4×10^{12} vg, 5×10^{12} vg, 6×10^{12} vg, 7×10^{12} vg, 8×10^{12} vg, 9×10^{12} vg, 1×10^{13} vg, 2×10^{13} vg, 3×10^{13} vg, 4×10^{13} vg, 5×10^{13} vg, 6×10^{13} vg, 7×10^{13} vg, 8×10^{13} vg, 9×10^{13} vg, 1×10^{14} vg.

[0344] According to the method of this disclosure for treating an ocular disorder such as USH2A associated retinitis pigmentosa in the ocular cells of a human or animal subject, the pharmaceutical composition described above is administered to the subject having such a disease by subretinal injection. The use of subretinal injection as the route of delivery is a critical component of this method, as intravitreal administration may not enable the same therapeutic effects. The vector and carrier may not diffuse across multiple cell layers in the retina to reach the RPE, when intravitreal injection is used. Similarly, intravenous delivery is unacceptable because the material may not penetrate the blood-brain (blood-retinal) barrier. Because the virus does not diffuse well, topical administration of eye drops is similarly not preferred for this method.

[0345] An effective amount of a recombinant adeno-associated virus carrying a nucleic acid sequence encoding the desired transgene under the control of the U7 snRNA promoter sequence can have a range between about 1×10^9 to 2×10^{12} rAAV infectious units in a volume of between about 150 to about 800 μ l. The rAAV infectious units are measured as described in S. K. McLaughlin *et al*, 1988 *J. Virol.*, 62:1963. More desirably, an effective amount is between about

1×10^{10} to 2×10^{11} rAAV infectious units in a volume of between about 250 to about 500 μL . Still, other dosages in these ranges may be selected by the attending physician, considering the physical state of the subject, preferably human being treated, the age of the subject, the particular ocular disorder and the degree to which the disorder, if progressive, has developed.

[0346] It may also be desirable to administer multiple “booster” dosages of the pharmaceutical composition of this disclosure. For example, depending upon the duration of the transgene within the ocular target cell, one may deliver booster dosages at 6 month intervals, or yearly following the first administration. The fact that AAV-neutralizing antibodies may not be generated by administration of the rAAV vector to the eye should allow additional booster administration(s) as needed.

[0347] Such booster dosages and the need therefore can be monitored by the attending physicians, using, for example, retinal and visual function tests and visual behavior tests. Other similar tests may be used to determine the status of the treated subject over time. Selection of the appropriate tests may be made by the attending physician. Still, alternatively, the method of this disclosure may also involve the injection of a larger volume of virus-containing solution in a single or multiple injections to allow levels of visual function close to those found in wild-type retinas.

[0348] Methods of delivering viral particles containing a transgene to inner ear cells *in vivo* are known in the art (see, for example, the published U.S. Patent Application Nos. 2019/0351072 and 2019/0038778, the contents of which are incorporated by reference herein in their entireties).

[0349] For ocular or auditory canal administration, about 10^8 to about 10^{12} viral particles can be administered to a subject, and the virus can be suspended within a suitable volume (e.g., 10 μL , 50 μL , 100 μL , 500 μL , or 1000 μL) of, for example, artificial perilymph solution.

[0350] A virus can be delivered to inner ear cells (e.g., cells in the cochlea) using methods that are well known in the art. For example, a therapeutically effective amount of a composition including virus particles can be injected through the round window or the oval window, typically in a relatively simple (e.g., outpatient) procedure. In addition, delivery vehicles (e.g., polymers) are available that facilitate the transfer of agents across the tympanic membrane and/or through the round window, and any such delivery vehicles can be used to deliver the viral particles described herein. See, for example, Arnold *et al.*, 2005, *Audiol. Neurotol.*, 10:53-63.

[0351] The methods described herein enable the highly efficient delivery of AAV8 USH2A-U7 to inner ear cells, e.g., cochlear cells. For example, the compositions and methods described herein enable the delivery to, and expression of, a transgene in at least 80% (e.g., at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%) of inner hair cells or delivery to, and expression in, at least 80% (e.g., at least 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99) of outer hair cells.

[0352] In certain aspects, an AAV vector capable of inducing USH2A exon 13 skipping, e.g., AAV8 USH2A-U7, may be delivered to cochlear cells or retinal as an AAV USH2A-U7 exosome as disclosed in the published U.S. Patent Application No. 2019/0038778, the content of which is incorporated by reference herein in its entirety.

[0353] In various aspects, the pharmaceutical composition is formulated for administration to the eye or ear. When used for ocular applications, the pharmaceutical composition can be an ophthalmic composition. The ophthalmic composition can comprise one or more carriers suitable for administration to the eye. For example, the ophthalmic composition can be formulated as an eye drop or a ointment. The ophthalmic composition can be formulated at a pH suitable for administration to the eye. The ophthalmic composition can be, in various examples, formulated to a pH of 4.7 to 7.40, 4.7 to 5.5, 6.0 to 6.8, 7.2 to 7.4, or 6.5 to 7.4. The ophthalmic composition can be formulated as an oil in water emulsion or a water in oil emulsion. The ophthalmic composition can be formulated as a semisolid. The ophthalmic composition can be formulated as an ocular drug delivery system, which can include liposomes and other nanotechnology-based formulations configured for administration to the eye.

[0354] In various aspects, the pharmaceutical composition can also be formulated for administration to the ear. For example, the composition can be formulated as an ear drop.

VIII. USH2A GENE THERAPY

[0355] At least 10 genes have been implicated in the etiology of Usher syndrome (USH), a condition that leads to progressive vision loss and hearing loss. Usher syndrome type 2 (Usher 2) is the most common form of the disorder, representing over half of all cases, 80% of which are initiated by mutations in the USH2A gene (Toms *et al.* (2020) *Therapeutic Adv Ophthalmol.* 2020, 12: 1–19). The most common USH2A mutation, c.2299delG, is a single base pair (bp) deletion and frameshift mutation in USH2A's exon 13, which is predicted to result in a severely truncated

nonfunctional protein and/or subject the transcribed mRNA to nonsense mediated decay. The subsequent depletion of USH2A protein ultimately leads to a degeneration of light-sensitive photoreceptor cells in the retina and auditory hair cells in the cochlea of the inner ear (Dinculescu *et al.* (2021) *International Ophthalmology Clinics* 61 (4): 109–24).

[0356] The size of the USH2A coding sequence (15.6kb) exceeds the packaging capacity of viral vectors and thus precludes any traditional gene replacement approach. However, the USH2A protein contains multiple repetitive domains (see FIG. 2A). Recent CRISPR/cas9 gene editing reports indicate the removal of exon 12 of murine *Ush2a* (homolog to human exon 13) not only reconstitutes the reading frame but restores the production of a shortened, yet adequately functional USH2A protein. Consistent with these observations, the expression of *Ush2a*-ΔEx12 in *Ush2a* null mice was also able to rescue deficits in vision (Pendse *et al.* (2020). *bioRxiv* 2020.02.04.934240; Pendse *et al.*, *Adv Exp Med Biol.* 2019;1185:91-96 and Thein Thuzar (2020) *Investigation of Base-Editing-Mediated Exon Skipping as a Potential Gene Therapy for Usher Syndrome*. Doctoral dissertation, Harvard Medical School).

[0357] Therefore, methods to induce targeted exon skipping in USH2A transcripts were investigated as a potential therapy of USH2A-related retinopathy and congenital bilateral sensorineural hearing loss.

[0358] U snRNAs can be modified into a versatile tool for splicing modulation. For example, U7 snRNA complexed with at least two U7-specific proteins (Lsm proteins) and eight common Sm proteins forms a ribonucleoprotein particle (U7 snRNP) that is normally involved in processing the 3' end of histone pre-mRNAs. The 5' end of the U7 snRNA includes a short antisense sequence that can anneal to the HDE (histone downstream element), a conserved purine-rich region located 15 nucleotides downstream the histone mRNA cleavage site. The HDE-specific antisense sequence can, however, be replaced with any antisense sequence targeting pre-mRNA splicing regulatory sequences (Gorman *et al.* (1998) 'Stable Alteration of Pre-mRNA Splicing Patterns by Modified U7 Small Nuclear RNAs. *Proc. Natl. Acad. Sci. USA* 95 (9): 4929–34). The composition of U7 snRNP can be further modified by introducing a mutation into the U7 Sm binding site that allows U7 snRNA to bind to the spliceosomal proteins D1 and D2 (Schümperli, D., and R. S. Pillai. 2004. *Cellular and Molecular Life Sciences: CMLS* 61 (19-20): 2560–70). Incorporation of recombinant

U7snRNA into modified U7 snRNPs protects the snRNA from degradation and drives the accumulation of the engineered U7snRNA in the nucleus where splicing occurs.

[0359] The highly prevalent mutations (c.2299delG and c.2276G > T) located in exon 13, account for more than 30% of USH2A-related retinopathy. Antisense sequences targeting the 5' and 3' exon 13 splice site regions of USH2A pre-mRNA were tested for induction of USH2A exon 13 skipping using a human USH2A minigene-derived transcript. AAV vectors expressing recombinant USH2A U7 small nuclear RNAs (snRNAs) were then engineered to deliver antisense sequences to the targeted mutant USH2A transcripts expressed in the retina or in the cochlear and vestibular cells of the inner ear.

[0360] In various aspects, the disclosure provides use of 1) a recombinant RNA of the present disclosure; 2) a polynucleotide encoding the recombinant RNA of the present disclosure; 3) a vector of the present disclosure; or 4) a pharmaceutical composition of the present disclosure for treating a disorder or symptom associated with USH2A.

[0361] In various aspects, the disclosure also provides 1) a recombinant RNA of the present disclosure; 2) a polynucleotide encoding the recombinant RNA of the present disclosure; 3) a vector of the present disclosure; or 4) a pharmaceutical composition of the present disclosure for use in treating a disorder or symptom associated with USH2A.

[0362] In various aspects, the disclosure yet further provides a method of manufacturing a medicament comprising 1) a recombinant RNA of the present disclosure; 2) a polynucleotide encoding the recombinant RNA of the present disclosure; 3) a vector of the present disclosure; or 4) a pharmaceutical composition of the present disclosure. In various aspects, the medicament is for treating a disorder or symptom associated with USH2A.

In various aspects, treating a disorder or symptom associated with USH2A can involve assessing how a patient's disease state has progressed relative to a baseline or earlier assessment of the patient. In various aspects, changes in a disease state (e.g., vision loss) can be determined by assessing one or more of best corrected visual acuity (BCVA), low luminance visual acuity (LLVA), ellipsoid zone (EZ) area/width by spectral domain optical coherence tomography (SD-OCT), static perimetry, microperimetry, optical coherence tomography (OCT) thickness of the retinal nerve fiber layer, optic nerve and/or macula, or any combination thereof. Moreover, in

various aspects, changes in vision can also be assessed using an eye chart, such as a Snellen Eye Chart.

IX. KITS

[0363] The term “kit” as used herein refers to a packaged product or article of manufacture comprising components. The kit preferably comprises a box or container that holds the components of the kit. The box or container is affixed with a label, or a Food and Drug Administration approved protocol. The box or container holds components of the disclosure which are preferably contained within plastic, polyethylene, polypropylene, ethylene, or propylene vessels. The vessels can be capped tubes or bottles. The kit can also include instructions for the use of the reagents.

[0364] Provided herein is a kit, comprising 1) the recombinant RNA of the present disclosure; 2) a polynucleotide encoding the recombinant RNA of the present disclosure; 3) the vector of the present disclosure; or 4) the pharmaceutical composition of the present disclosure aliquoted into separate containers.

[0365] In another aspect, provided herein are kits comprising, in a container, AAV-AS_RNA4+6 virions as described herein, and instructions for use. In some aspects, the kits further comprise a negative control, such as phosphate buffered saline.

[0366] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or a portion thereof, that is said to be incorporated by reference herein, but which conflicts with the existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

EXAMPLES

[0367] Examples are set forth below for the purpose of illustration and to describe certain specific aspects of the disclosure. However, the scope of the claims is not to be in any way limited by the examples set forth herein. Various changes and modifications to the disclosed aspects will be

apparent to those skilled in the art and such changes and modifications may be made without departing from the spirit of the disclosure and the scope of the appended claims.

[0266] The practice of the present disclosure employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics, and recombinant DNA, which are within the skill of the art. See, e.g., Bailey, J. E. and Ollis, D. F., *Biochemical Engineering Fundamentals*, McGraw-Hill Book Company, NY, 1986; *Current Protocols in Immunology*, John Wiley & Sons, Inc., NY, N.Y. (1991-2015), including all supplements; Green and Sambrook, (Molecular Cloning: A Laboratory Manual. 4th, ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2014); *Current Protocols In Molecular Biology* (F. M. Ausubel, *et al.* eds., (2017)) including all supplements; *Short Protocols in Molecular Biology*, (Ausubel *et al.*, 1999)) including all supplements; the series *Methods In Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), *Antibodies, A Laboratory Manual*, Second Edition (Harlow and Lane, eds. (2014) and *Culture Of Animal Cells: A Manual Of Basic Technique*, 7th Edition (R. I. Freshney, ed. (2016)), all contents of which are incorporated by reference herein in their entireties.

EXAMPLE I: USH2A MINIGENE CONSTRUCT

[0368] A human USH2A minigene was constructed to evaluate exon 13 skipping in a human USH2A pre-mRNA. The minigene, hUSH2AEX12-14 consists of three exons (exon 12 to exon 14) and two shortened introns. The minigene includes short intron sequences flanking exon 13. A human cytomegalovirus (CMV) enhancer and promoter in pcDNA3.1 expression vector was placed upstream of the minigene to drive transcription of USH2A minigene pre-mRNA (see Figs. 4A and 4B). The hUSH2AEX12-14 minigene was then co-transfected into HEK293 cells together with various combinations of recombinant U7 or gRNA dCas13d constructs comprising one or more antisense sequences capable of hybridizing to the 5' or 3' splice site regions of an USH2A pre-mRNA. Exon 13 skipping was then evaluated by RT-PCR and qPCR analysis.

EXAMPLE II: ANALYSIS OF MINIGENE SPLICING/EXON SKIPPING

Transfection protocol

[0369] The indicated amounts of U7snRNA constructs or dCas13d constructs were co-transfected into HEK239 with 0.3 µg minigene using Lipofectamine LTX (Life Technologies) according to the manufacturer's instructions. Combined DNAs were diluted in 250 µl Opti-MEM (Life Technologies) followed by addition of 3.3 µl PLUS reagent. 6 µl of lipofectamine LTX (Life Technologies) was separately diluted in 250 µl Opti-MEM (Life Technologies). The diluted DNA and reagent were mixed and incubated for 20 minutes at room temperature, then added to each well of a 6-well culture plate.

RNA purification

[0370] 24 hours or 48 hours after transfection, total RNA was extracted using RNeasy mini kit (Qiagen) according to the manufacturer's instructions. Transfected cells in 6-well culture plates were homogenized in 350 µl RLT Plus Lysis buffer supplemented with 40 mM DTT. Further purification used a QIAcube automatic extraction system (Qiagen) as per the standardized protocol. The purity and quantity of all RNA samples were measured using the spectrophotometric method on Nanodrop 8000 (ThermoFisher Scientific)..

RT-PCR analysis

[0371] Reverse transcription reaction was carried out with 3.75 ng (per PCR rxn) using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Samples were incubated at 46°C for 20 min, followed by 1 min at 95°C. Transcripts were amplified with Platinum Taq DNA polymerase (Life Technologies) using synthesized cDNA and the primer set for hUSH2A or hGAPDH. Thermal cycling conditions for hUSH2A were 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, preceded by 3 min at 98°C, and followed by a final elongation step at 72°C for 10 min. Thermal cycling conditions for hGAPDH were 25 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, preceded by 3 min at 98°C, and followed by a final elongation step at 72°C for 10 min. The PCR products were resolved by Mother E-Base electrophoresis system (ThermoFisher Scientific) through 1% agarose gel.

RT-qPCR analysis

[0372] qPCR was performed with qScript XLT One-Step RT-qPCR ToughMix (Quantabio, Beverly, MA) following the manufacturer's instructions. For the reaction, 50 ng RNA, hUSH2A primers/probe (FAM/MGB) and hGAPDH primers/probe (VIC/MGB, ThermoFisher Scientific)

were added to the reaction mixture. Thermal cycling conditions using CFX384 Touch Real-Time PCR Detection System (Bio-Rad) were 48°C for 15 min, 95 °C for 10 min, 39 cycles of 95 °C for 15 s, and 60°C for 1 min.

Primers

[0373] The primer sequences used for endpoint PCR specific to human USH2A and human GAPDH were as follows: hUSH2A-F: 5'-CAATCAGTGCCAGAATGGAT-3' (SEQ ID NO: 32) and hUSH2A-R: 5'- TTGCATTGGTCACAACGTTG-3' (SEQ ID NO: 33); hGAPDH-F: 5'-ATGACCCCTTCATTGACCTCA-3' (SEQ ID NO: 37) and hGAPDH-R: 5'-TGATCTTGAGGCTGTTGTCATACTT-3' (SEQ ID NO: 38).

[0374] The primer and probe sequences for qPCR specific to the junction between exon12 and exon14 are as follows: the forward primer: 5'-GCAAAGCAAACGTTATTGGTT-3' (SEQ ID NO: 44), the reverse primer: 5'-CCAGTTGTATGGCATGAGCA-3' (SEQ ID NO: 45) and the TaqMan probe (FAM/MGB): 5'- ATTTCTCCAGGCAATGCCACTGG-3' (SEQ ID NO: 46).

Sequence analysis

[0375] The PCR products were resolved in a 1% agarose gel and the PCR products corresponding to the exon skipped band (297 bp) were excised. The DNAs in the gel slices were purified using QIAquick Gel Extraction Kit (Qiagen) following the manufacturer's instructions. Sanger sequencing of purified DNA was performed using hUSH2A-F and hUSH2A-R primers at Genewiz (South Plainfield, NJ).

EXAMPLE III: EXON SKIPPING ACTIVITY OF RECOMBINANT USH2A AS_RNA U7 AND USH2A AS_RNA gRNA-dCas13d CONSTRUCTS

Single targeting USH2A AS-U7 constructs

[0376] USH2A AS-U7 constructs were engineered to screen for novel targeting sequences of approximately 24 nucleotides in length within the 3' splice site and the 5' splice site regions (FIG. 5A and Fig. 5B). Antisense sequences capable of hybridizing to USH2A pre-mRNA were cloned upstream of a recombinant U7 snRNA comprising an SmOpt sequence and a stem loop (see FIG. 5D). As an initial step, these new constructs were tested individually for exon 13 skipping of the hUSH2AEX12-14 minigene pre-mRNA. 1 µg or 3 µg of the USH2A AS_RNA4, AS_RNA5,

AS_RNA8, AS_RNA9, and AS_RNA10 U7 constructs were co-transfected into HEK293 cells with 0.3 µg of the hUSH2AEX12-14 minigene using Lipofectamine LTX (Life Technologies, Carlsbad, CA). A DelEx13 minigene construct expressing Exon 12 fused to Exon 14 was used as a positive control for exon 13 skipping (see FIGs. 5Ci-ii). After 48 hours, total RNA was extracted with RNeasy mini kit (Qiagen, Hilden, Germany). First-strand cDNA synthesis was carried out using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The resulting cDNA was amplified by PCR using USH2A-specific primer set and a GAPDH primer set. RT-PCR products were then visualized by gel electrophoresis and 297 bp RT-PCR fragments corresponding to exon 13 skipping were identified and quantified (see FIG. 6). After normalization to GAPDH signals, the fold change was calculated relative to DelEx13.

[0377] As shown in FIGs. 7A and 7B, only the AS_RNA4 U7 recombinant RNA construct induced exon 13 skipping that was approximately 20% relative to the splicing of the delEx13 control.

Co-expression of single targeting USH2A AS-U7 constructs

[0378] To evaluate if exon skipping could be enhanced by co-expressing different AS RNA U7 constructs, the single targeting USH2A AS RNA5, AS RNA8, AS RNA9, and AS RNA10 U7 constructs were co-transfected into HEK293 cells with the USH2A AS- RNA4 construct and 0.3 µg of the hUSH2AEX12-14 minigene using Lipofectamine LTX (Life Technologies). The combined amount of the two transfected USH2A AS-U7 constructs was either 1 µg or 3 µg. A DelEx13 minigene construct expressing Exon 12 fused to Exon 14 was used as a positive control for exon 13 skipping (see FIGs. 5Ci-ii). After 48 hours, total RNA was extracted with RNeasy mini kit (Qiagen, Hilden, Germany). First-strand cDNA synthesis was carried out using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The resulting cDNA was amplified by PCR using USH2A-specific primer set and GAPDH-specific primers. RT-PCR products were then visualized by gel electrophoresis and 297 bp RT-PCR fragments corresponding to exon 13 skipping were identified and quantified (see FIG. 6). After normalization to GAPDH signals, the fold change was calculated relative to DelEx13. As shown in FIGs. 7C and 7D, the combination of USH2A AS4-U7 with USH2A AS8-U7 led to an 8 fold increase in exon 13 skipping relative to the DelEx13 control.

Dual targeting USH2A AS-U7 constructs

[0379] A new dual targeting USH2A AS-U7 expression vector (AS RNA4+8) was constructed containing a mouse U7 promoter (SEQ ID NO: 30), two USH2A antisense sequences: AS_RNA4

(SEQ ID NO: 4) in tandem with AS_RNA8 (SEQ ID NO: 8), an SmOPT sequence (SEQ ID NO: 24) and a U7 snRNA stem and loop (SEQ ID NO: 27) (see FIG. 8Ai-iii). An expression vector expressing a scrambled recombinant U7 RNA sequence (scU7) acted as a negative control. The exon skipping efficiency of the AS RNA4+8 RNA (FIG. 8C) was compared with that of other dual AS_RNA2+6, AS_RNA4+6, AS_RNA2+4, AS_RNA2+5, AS_RNA2+7, AS_RNA2+6, U7 constructs targeting antisense USH2A sequences (see FIG. 8D).

[0380] Dual targeting AS_RNA2+6, AS_RNA4+6, AS_RNA2+4, AS_RNA2+5, AS_RNA2+7, and AS_RNA2+6 U7 constructs were co-transfected into HEK293 cells with the hUSH2AEX12-14 minigene using Lipofectamine LTX (Life Technologies). The combined amount of constructs was either 3 µg, 1 µg, or 0.3 µg. A DelEx13 minigene construct expressing Exon 12 fused to Exon 14 was used as a positive control for exon 13 skipping (see FIGs. 5Ci-ii). After 48 hours, total RNA was extracted with RNeasy mini kit (Qiagen, Hilden, Germany). First-strand cDNA synthesis was carried out using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The resulting cDNA was amplified by PCR using USH2A-specific primer set and GAPDH-specific primers. RT-PCR products were then visualized by gel electrophoresis and 297 bp RT-PCR fragments corresponding to exon 13 skipping were identified and quantified (see FIG. 6). After normalization to GAPDH signals, the fold change was calculated relative to DelEx13.

[0381] As shown in FIGs. 9A and 9B, AS_RNA4+6 induced the highest level of exon skipping relative to DelEx13. Sequence analysis of the RT-PCR fragment corresponding to the skipping of exon 13 confirmed that exon 12 was correctly joined to exon14 (see FIGs. 9Ci and 9Cii).

EXAMPLE IV: COMPARISON OF DUAL TARGETING USH2A-dCAS13d AND USH2A-U7 CONSTRUCTS

[0382] An AS_gRNA4+6 dCas13d targeting construct was generated containing a U6 promoter driving the expression of a gRNA array and a human elongation factor-1 alpha (EF-1 alpha) promoter driving the expression of a dCas13d transgene (FIG. 10A). The two gRNAs targeted the same USH2A sequences as the U7 AS_RNA4+6. The dCas13d transgene expressed a catalytically deactivated Cas13 protein with mutations in the catalytic HEPN domain. These mutations abolished the catalytic cleavage activity but not Cas13d RNA binding to target sequences.

[0383] The exon 13 skipping activity of recombinant U7snRNA or dCas13d constructs having the same dual targeting antisense sequences (AS RNA 4+6) was then tested in parallel. 0.03, 0.1, and 0.3 nM of AS_RNA4+6 U7snRNA or AS_gRNA4+6 dCas13d construct were co-transfected into HEK293 cells with 0.3 µg hUSH2AEX12-14 minigene. After 48 hours post-transfection, total RNA was purified, and the amount of exon 13 skipping was determined by end-point RT-PCR and RT-qPCR.

[0384] As shown in FIG. 10B and 10C, AS_RNA4+6 U7 was significantly more effective at inducing exon skipping than AS_gRNA4+6 dCas13d.

EXAMPLE V: EXON 13 SKIPPING ACTIVITY OF SINGLE AND DUAL TARGETING USH2A U7 CONSTRUCTS

[0385] The preceding experiments indicated that optimal exon 13 skipping was observed when USH2A AS_RNA4 was combined with either USH2A AS_RNA6, or AS_RNA8 antisense sequences (FIG. 11A). To determine which expression vector was most effective at inducing USH2A exon 13 skipping, the combination of USH2A AS_RNA4 with USH2A AS_RNA6 or USH2A AS_RNA8 was tested either in a dual targeting recombinant U7 RNA configuration or by co-expression of two single target recombinant U7 RNAs each targeting a different antisense sequence (see, e.g., FIG. 11B).

[0386] The recombinant AS_RNA4+6 U7 RNA expression vector or a combination of recombinant USH2A AS_RNA4 U7 and USH2A AS_RNA6 U7 expression vectors was co-transfected into HEK293 cells with 0.3 µg hUSH2AEX12-14 minigene using Lipofectamine LTX (Life Technologies). In parallel, the recombinant AS_RNA4+8 U7 expression vector or a combination of recombinant USH2A AS_RNA4 U7, and USH2A AS_RNA8 U7 expression vectors was co-transfected into HEK293 cells with 0.3 µg hUSH2AEX12-14 minigene using Lipofectamine LTX (Life Technologies). The combined amount of constructs was either 1 µg or 0.3 µg. A DelEx13 minigene construct expressing Exon 12 fused to Exon 14 was used as a positive control for exon 13 skipping (see FIGs. 5Ci-ii). An expression vector expressing a scrambled recombinant U7 RNA sequence (scU7) acted as a negative control. After 48 hours, total RNA was extracted with RNeasy mini kit (Qiagen, Hilden, Germany) and the level of exon 13 skipping was

evaluated by RT-PCR as described above. After normalization to GAPDH signals, the fold change was calculated relative to DelEx13.

[0387] As shown in FIG. 11C and 11D, the dual targeting recombinant AS_RNA4+8 U7 expression vector (see FIG. 8Ai-iii and 8C) exhibited a 7 fold increase in exon 13 skipping relative to DelEx13.

EXAMPLE VI: USH2A-U7 INDUCED EXON 13 SKIPPING OF ENDOGENOUS USH2A PRE-mRNA IN WERI-Rb CELLS

[0388] The dual targeting recombinant AS_RNA4+8 and AS_RNA4+6 U7 RNA expression vectors were then tested in a USH2A expressing Weri-Rb-1 human retinoblastoma cell line (HTB-169™, ATCC, Manassas, VA) to determine if the U7 constructs could induce exon 13 skipping in the endogenous USH2A pre-mRNA.

[0389] One million Weri-Rb-1 cells in 900 µl of Opti-MEM (Life Technologies) were seeded into each well of a 6 well plate and incubated at 37°C for 1 hour in RPMI-1640 medium (Gibco, Waltham, Massachusetts) with 10% fetal bovine serum (Gibco) to allow the cells to adhere to the plate. The indicated molarities of U7snRNA constructs or AS_RNA3 ASO were transfected into the adherent Weri-Rb-1 cells using Lipofectamine 2000 (Life Technologies). The indicated molarities of the U7snRNA constructs or the AS_RNA3 ASO were diluted in 50 µl Opti-MEM (Life Technologies) and 1.25 µl of lipofectamine 2000 was separately diluted in 50 µl Opti-MEM (Life Technologies). The diluted ASO or DNA constructs and reagent were mixed and incubated for 20 minutes at room temperature, then added to each well of the 6-well culture plate and cultured for an additional 48 hours at 37°C. To evaluate exon 13 skipping of endogenous USH2A pre-mRNA, total RNA was purified from the transfected cells using the RNeasy mini kit (Qiagen) and 37.5 ng (per PCR rxn) of purified RNA was used for cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad). PCR was performed using Platinum Taq DNA polymerase (Life Technologies) and the primer sets for hUSH2A or hGAPDH described previously. The number of thermal cycles for hUSH2A and hGAPDH was 40 and 25, respectively.

[0390] As shown in FIG. 12A, the dual targeting recombinant AS_RNA4+8 and AS_RNA4+6 U7 RNA expression vectors induced exon 13 skipping of the endogenous USHA2A pre-mRNA.

[0391] The results suggest the efficiency of exon 13 skipping was suboptimal because only a small fraction of the Weri-Rb cells were transfected. To compensate for this possibility, the recombinant AS_RNA U7 constructs and the AS_RNA3 ASO were co-transfected with 0.5 µg CMV-EGFP plasmid using Lipofectamine 2000 (Life Technologies). After 48 hours post-transfection, 3 million cells per sample were pooled from 3 wells of a 6 well plate and resuspended in FACS buffer (BD Biosciences, Franklin Lakes, NJ). The suspended cells passed through a fluorescent activated FACS Melody cell sorter (BD Biosciences; FIG. 12B) to separate and collect GFP-positive fractions corresponding to those cells that were transfected. To minimize RNA degradation and material loss during sorting, the sorted cells were directly collected into RLT Plus lysis buffer supplemented with 40 mM DTT. Between 20,000 and 30,000 GFP-positive cells were collected for each sample. Total RNA was purified from the sorted GFP-positive cells using the RNeasy micro kit (Qiagen) according to the manufacturer's instructions. 5 ng (per PCR rxn) of purified RNA was used for cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad). PCR was performed using Platinum Taq DNA polymerase (Life Technologies) and the primer set for hUSH2A or hGAPDH described previously. The number of thermal cycles for hUSH2A and hGAPDH was 40 and 25, respectively. As shown in FIG. 12C, the recombinant AS_RNA4+8 U7 RNA expression vector induced efficient exon 13 skipping in the endogenous USH2A pre-mRNA that was at least comparable to the exon skipping induced with the AS_RNA3 ASO.

EXAMPLE VII: GENERATION OF AAV8 USH2A-U7 VECTORS

[0392] Two self-complementary AAV8 vectors for two selected U7snRNAs (13-46 and 13-48) were generated in-house (FIG. 14A). The silver-stained gel demonstrated the high purity of viral capsids (FIG. 14B), and the alkaline gel and sequencing results confirmed the high integrity and purity of the viral genome with no detectable truncation of the viral genome (FIG. 14C).

EXAMPLE VIII: AAV8 USH2A-U7 INDUCED EXON SKIPPING OF ENDOGENOUS USH2A PRE-mRNA IN WERI-Rb CELLS

[0393] The potency of the two AAV8 USH2A-U7 vectors for inducing USH2A exon skipping of endogenous USH2A RNA in transduced WeriRb-1 cells was evaluated. As shown in FIG. 15A, the AAV8 USH2A-U7 vector induced efficient exon13 skipping of endogenous USH2A RNA in a

dose-dependent manner. Correct exon skipping was confirmed through amplicon sequencing (FIG. 15B).

EXAMPLE IX: AAV8 USH2A-U7 INDUCED EXON SKIPPING IN RETINAL ORGANOIDs GENERATED FROM USH2A PATIENT HUMAN iPSCs

[0394] Six independent human induced pluripotent stem cells (hiPSCs) are generated from fibroblasts taken from three USH2A patients having the c.2299delG mutation in USH2A exon 13 and three healthy control donors.

[0395] HiPSC-derived retinal organoids (ROs) are differentiated as previously described (Achberger K et al. Stem Cell Rep., 2021. 16: p. 2242-56; Achberger et al. eLife 2019;8:e46188). Briefly, for embryoid body (EB) formation, 2.88×10^6 hiPSCs are detached on day 0 using TrypLE (ThermoFisher Scientific, USA) and dissociated to single cells. Cells are then mixed with PeproGrow (PeproTech, USA) medium, 10 mM Y-27632 (ROCK-inhibitor, Ascent Scientific, USA) and 10 mM blebbistatin (Sigma-Aldrich, USA) and aliquoted to 96 untreated V-shaped 96-wells (Sarstedt, Germany). For re-aggregation, the plate is centrifuged at 400 g for 4 min. On day 1, 80% of the medium is removed and replaced with N2 medium (DMEM/F12 (1:1)+Glutamax supplement (ThermoFisher Scientific, USA), 24 nM sodium selenite (Sigma-Aldrich, USA), 16 nM progesterone (Sigma-Aldrich, USA), 80 mg/ml human holotransferrin (Serologicals, USA), 20 mg/ml human recombinant insulin (Sigma-Aldrich), 88 mM putrescine (Sigma-Aldrich, USA), 1x minimum essential media-non essential amino acids (NEAA, ThermoFisher Scientific, USA), 1x antibiotics-antimycotics (AA, ThermoFisher Scientific, USA)). Medium is changed again on day 4. On day 7, EBs are plated on Growth-Factor-Reduced Matrigel (BD Biosciences, USA)-coated six well plates at a density of 32 EBs/well and medium is changed daily. On day 16, medium is switched to a B27-based Retinal differentiation medium (BRDM) (DMEM/F12 (3:1) with 2% B27 (w/o vitamin A, ThermoFisher Scientific, USA), 1x NEAA and 1x AA). On day 24, eye fields are detached using 10 ml tips and collected in 10 cm bacterial petri dishes (Greiner Bio One, Germany) with BRDM, adding 10 mM ROCK-Inhibitor Y-27632 for one day. After completed formation, ROs are selected and if necessary, detached from non-retinal spheres using microscissors. From day 40 onwards, ROs in BRDM are supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, USA) and 100 mM taurine (Sigma-Aldrich, USA). From

day 70–100, BRDM with FBS and taurine is further supplemented with 1 mM retinoic acid (Sigma-Aldrich, USA), which is reduced to 0.5 mM during days 100–190 and removed afterwards.

[0396] The recombinant AAV2 and the engineered AAV2 7m8 AS_RNA4+8 U7 RNA or AAV8 AS_RNA4+8 U7 RNA expression vectors are generated as described previously (see, for example, U.S. Patent Nos. 7,282,199, 10,590,435, 8,962,332 and 9,193,956 , the contents of which are incorporated by reference herein in their entireties). After 120 or 150 days of differentiation, organoids are transferred to the wells of 96-well plate and incubated with from 1 to 3 10^{11} viral genomes (vg) of AAV8-AS_RNA4+8 U7 RNA vectors.

[0397] After 180 or 210 days of differentiation, AAV treated- and untreated-patient-derived retinal organoids are compared with healthy control organoids.

[0398] First, USHA2A induced exon 13 skipping of USH2A pre-mRNA is analyzed in the AAV treated- and untreated-patient-derived retinal organoids.

[0399] Second, the effect of USH2A exon 13 skipping on disease-related parameters in patient-derived retinal organoids is determined, including changes in the outer segment of photoreceptor cells, changes in the thickness of the outer nuclear layer (ONL), as well as changes in rhodopsin and cone opsin localization.

EXAMPLE X: AAV2 USH2A-U7 INDUCED EXON SKIPPING IN WT HUMAN RETINAL ORGANIDS

[0400] Fully differentiated WT human retinal organoids were generated as described above and transduced with AAV2-13-48, AAV2-13-46 or AAV2 7m8-23-48 vectors at DD 200. USH2A exon 13 skipping was then evaluated using endpoint PCR and qPCR. As shown in FIG. 16A, highly efficient exon skipping was detected in all transduced retinal organoids. The full-length band resulting from normal splicing was significantly reduced and a smaller band corresponding to transcripts without exon 13 (Δ exon 13) was readily detected. The AAV2 7m8 vector induced the highest exon skipping efficiency (FIG. 16B), which was confirmed through endpoint PCR and qPCR.

EXAMPLE XI: USH2A-U7 INDUCED EXON SKIPPING IN RETINA OF WT MICE AFTER SUBRETINAL INJECTION OF AAV8-U7mUSH2aEx12-26

[0401] C57BL/6 mice were subretinally injected with 3×10^9 vg/eye AAV8 vectors according to the protocol depicted in TABLE VI. After 2 months, whole retina were collected from the treated C57BL/6 WT mice, immediately frozen on dry ice, and stored at -80°C . Total RNA was then extracted from the whole retina using AllPrep DNA/RNA kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed with 5.2 ng (per PCR rxn) using an iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Samples were incubated at 46°C for 20 min, followed by 1 min at 95°C .

[0402] Synthesized cDNA was amplified with Platinum Taq DNA polymerase (Life Technologies) using the primer set for mouse Ush2a (mUsh2a-F: 5'-CCTTCAGTGCCAGGATGGAT-3' (SEQ ID NO: 69) and mUsh2a-R: 5'-TGACACTGGTGACAGCTACG-3' (SEQ ID NO: 70)) or mouse GAPDH (mGAPDH-F: 5'-ACTCCACTCACGGCAAATTC-3' (SEQ ID NO: 17) and mGAPDH-R: 5'-TCTCCATGGTGGTGAAGACA-3' (SEQ ID NO: 18)). Thermal cycling conditions for mouse Ush2a were 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, preceded by 3 min at 98°C , and followed by a final elongation step at 72°C for 10 min. Thermal cycling conditions for mouse GAPDH were 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, preceded by 3 min at 98°C , and followed by a final elongation step at 72°C for 10 min. The PCR products were resolved by Mother E-Base electrophoresis system (ThermoFisher Scientific) using a 1% agarose gel.

[0403] As shown in FIG. 13, subretinal injection of AAV8-Stuffer U7mUsh2aEX12-26 in wild type C57 Black mice resulted in mUsh2a exon 12 skipping within the endogenous wt mUsh2a mRNAs. Neither the AAV8-EF1a-dCas13d-mUsh2aEX12-11 vector, nor the AAV8-Stuffer U7mUsh2aEX12-26 (scrambled control) vectors induced exon 12 skipping.

TABLE VI

GROUP	VECTOR	DOSE (VG/EYE)	NUMBER OF C57BL/6 MICE	DURATION	ANALYSIS OF GFP SORTED PR/RETINAS
CAS13D MEDIATED EXON SKIPPING					

GROUP	VECTOR	DOSE (VG/EYE)	NUMBER OF C57BL/6 MICE	DURATION	ANALYSIS OF GFP SORTED PR/RETINAS
Cas13d control (Scrambled)	AAV8-EF1a- dCas13d- mUsh2aEX12-11 (Scrambled) + AAV8-CMV-GFP	3e9 vg	12	8 weeks	PCR for exon skipping
Cas13d targeting vector	AAV8-EF1a- dCas13d- mUsh2aEX12-7 + AAV8-CMV-GFP	3e9 vg	12		qPCR for Cas9/ vector genome
U7 MEDIATED EXON SKIPPING					
U7 control (Scrambled)	AAV8-Stuffer U7mUsh2aEX12-26 (Scrambled) + AAV8-CMV-GFP	3e9 vg	12	8 weeks	PCR for exon skipping
U7 targeting vector	AAV8-Stuffer U7mUsh2aEX12-26 + AAV8-CMV-GFP	3e9 vg	12		qPCR for vector genome

EXAMPLE XII: EXON 13 SKIPPING OF NON-HUMAN PRIMATE (NHP) USH2A MINIGENE RNA BY HUMAN USH2A-TARGETING U7 snRNA

[0404] The human USH2A-targeting U7 snRNA (13-48) does not induce exon skipping of the mouse USH2A minigene pre-RNA (data not shown). Therefore, human USH2A-targeting U7 snRNA was tested on a nonhuman primate (NHP) USH2A minigene RNA to determine if it could induce exon skipping despite differences in sequence (FIG. 17A). Endpoint PCR and qPCR confirmed that human USH2A-targeting U7 snRNA (13-48) was able to induce efficient exon 13 skipping in NHP USH2A minigene RNA, despite a mismatch sequence in the AS RNA4 sequence between the human and NHP sequences (FIG. 17B and 17C).

EXAMPLE XIII: USH2A-U7 INDUCED EXON SKIPPING IN COCHLEAR CELLS AFTER INJECTION OF AAV8-U7mUSH2aEx12-26 INTO THE INNER EAR OF Ush2a c.2290delG KNOCK-IN MOUSE

Animal Surgery

[0405] An usherin (USH2A) knock-in mouse is generated, which carries the common human c.2299delG frameshift mutation (corresponding to Ush2A c.2290delG in mice) is generated for inner ear gene therapy with the AAV8 USH2A-U7 virus (Tebbe *et al.* (2023). *Nature Communications* 14 (1): 972). Mouse pups (P0 to P2) or AAV2.7m8 USH2A-U7 virus (see, for example, Isgrig *et al.* (2019) “AAV2.7m8 Is a Powerful Viral Vector for Inner Ear Gene Therapy.” *Nature Communications* 10 (1): 427) are injected via the round window membrane (RWM) using beveled glass microinjection pipettes. The pipettes are pulled from capillary glass (WPI) with a P-2000 pipette puller (Sutter Instrument, Novato, Calif.) and beveled (~20 µm tip diameter at a 28 ° angle) using a micropipette beveler (Sutter Instrument, Novato, Calif.). EMLA cream (lidocaine 2.5% and prilocaine 2.5%) is applied externally for analgesia using sterile swabs to cover the surgical site (left mastoid prominence). Body temperature is maintained on a 38 ° C heating pad prior to surgery. Pups are anesthetized by rapid induction of hypothermia in ice / water for 2-3 minutes until loss of consciousness, and this state is maintained on a cooling platform for 5-10 minutes during surgery. The surgical site is disinfected by scrubbing with betadine and wiping with 70% Ethanol three times. A post-auricular incision is made to expose the transparent otic bulla, a micropipette is advanced manually through the bulla and overlying fascia, and the RWM is penetrated by the tip of the micropipette. Approximately 1 µL of virus is injected unilaterally within 1 min in the left ear manually. Injections are performed per group in a nonblind fashion. Occasionally, the injection needle is inserted too deep, too shallow, or at the wrong angle. If there is visible damage to the structures of the middle or inner ear, the samples are excluded from further analysis. Injection success rates range from <50% to <80% depending on the injector's level of experience. After the injection, the skin incision is closed using a 6-0 black monofilament suture (Surgical Specialties Corporation, Wyomissing, Pa., now Corza Medical). The pups are subsequently returned to the 38 ° C. warming pad for 5-10 min and their mother for breeding.

Auditory Brainstem Response Measurements

[0406] Auditory brainstem response testing is used to evaluate hearing sensitivity. Testing is carried out in all animals at P30 (normal control mice, untreated control mice, and treated mice that received AAV8 USH2A-U7 gene therapy). In mice treated with AAV8 USH2A-U7 therapy that show hearing recovery at P30, a repeat test is performed at P120. The animals are anaesthetised with ketamine (100 mg/kg) and dexmedetomidine (0.5 mg/mL) via intraperitoneal injections and

placed on a warming pad inside a sound booth (ETS-Lindgren Acoustic Systems). The animal's temperature is maintained using a closed feedback loop and monitored using a rectal probe (ATC-1000; World Precision Instruments). Subdermal needle electrodes are inserted at the vertex (+) and test the ear mastoid () with a ground electrode under the contralateral ear. Stimulus generation and ABR recordings are completed using Tucker Davis Technologies hardware (RZ6 Multi I/O Processor) and software (BioSigRx, version 5.1). ABR thresholds are measured at 4, 8, 16, and 32 kHz using 3-ms Blackman-gated tone pips presented at 29.9/s with alternating stimulus polarity. At each stimulus level, 512–1,024 responses are averaged. Thresholds are determined by visual inspection of the waveforms and are defined as the lowest stimulus level at which any wave could be reliably detected. The maximal stimulus level tested is at 90 dB SPL. A minimum of two waveforms are obtained at the threshold level to ensure the repeatability of the response. Physiological results are analyzed for individual frequencies and then averaged for each of these frequencies from 4 to 32 kHz.

[0407] The treated mice show hearing recovery as measured by auditory brainstem response (ABR) threshold testing. None of the untreated control mice show measurable ABR thresholds (0 of 15, $p = 0.024$, Fisher's exact test). Improvement in hearing is seen at all four tested frequencies (4, 8, 16, and 32 kHz), with most of the hearing improvement at 8 kHz, where the recorded ABR thresholds are as low as 60 dB sound pressure level (SPL). Examination of cochleas from the treated mice reveals robust AAV8 USH2A-U7 transduction of both inner and outer hair cells.

RNA preparation and Endpoint PCR

[0408] Four Ush2a c.2290delG knock-in mice aged 6 weeks are sacrificed by decapitation under deep anesthesia induced by an intraperitoneal injection containing 75 mg / kg of Ketamine (Daiichi Sankyo, Tokyo, Japan) and 32.4 mg / kg of pentobarbital sodium (Kyoritsu, Tokyo, Japan). Inner ears are rapidly extracted from the temporal bone and transferred to a RNA-later solution (Ambion, Austin, TX, USA). After removing the otic capsule, the cochlea including the lateral wall comprising the stria vascularis, spiral ligament, and spiral prominence, the organ of Corti, and the spiral ganglion neurons are dissected and separated into the apical, middle and basal turns. All of these dissections are performed in RNA-later solution to prevent RNA degradation. Total RNA is extracted using the QIAGEN RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.

[0409] Reverse transcription is performed with 5.2 ng (per PCR rxn) using an iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Samples are incubated at 46 ° C for 20 min, followed by 1 min at 95 ° C.

[0410] Synthesized cDNA is amplified with Platinum Taq DNA polymerase (Life Technologies) using the primer set for mouse Ush2a (mUsh2a-F: 5'-CCTTCAGTGCCAGGATGGAT-3 (SEQ ID NO: 69) and mUsh2a-R: 5'- TGACACTGGTGACAGCTACG-3 (SEQ ID NO: 70)) or mouse GAPDH (mGAPDH-F: 5'-ACTCCACTCACGGCAAATTC-3 (SEQ ID NO: 17) and mGAPDH-R: 5'- TCTCCATGGTGGTGAAGACA-3 (SEQ ID NO: 18)). Thermal cycling conditions for mouse Ush2a are 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, preceded by 3 min at 98°C, and followed by a final elongation step at 72°C for 10 min. Thermal cycling conditions for mouse GAPDH are 30 cycles of 94 ° C for 30 s, 58°C for 30 s, and 72°C for 60 s, preceded by 3 min at 98°C, and followed by a final elongation step at 72°C for 10 min. The PCR products are resolved by the Mother E-Base electrophoresis system (ThermoFisher Scientific) using a 1% agarose gel.

[0338] Cochlear injection of AAV8-Stuffer U7mUsh2aEX12-26 into knock-in mice of Ush2a c.2290delG knock-in mice results in the skipping of mUsh2a exon 12 within endogenous wt mUsh2a mRNAs expressed in the inner ear. Control AAV8-Stuffer U7 having scrambled antisense sequences (scrambled control) vectors fail to induce exon 12 skipping.

TABLE OF SEQUENCES

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
1	CACCUAAGCCCUAAAGAUAAAAU	AS_RNA_1	FIG. 5A
2	AAUGAUCACACACCUAAGCCCUAAA	AS_RNA2	FIG. 5A
3	UGAUCACACCUAAGCCCUAAA	AS_RNA_3	FIG. 5A
4	AAGCUUCGGAGAAAUUAAAUCCA	AS_RNA4	FIG. 5A
5	CA AUGGUCAAA UUGUACCU GUGA	AS_RNA5	FIG. 5Bi
6	CUUACCUUGUUGACACUGAUUAC	AS_RNA6	FIG. 5Bi
7	GCAGAUGCACUGCCCUUGUCUAG	AS_RNA7	FIG. 5Bi
8	CUUGACGAUUAAGGCACACACAGG	AS_RNA8	FIG. 5Bi
9	GCACACACAGGCACUGGCCACUGA	AS_RNA9	FIG. 5Bi
10	UCACACAUCUGGCAGUGUUGAAA	AS_RNA10	FIG. 5Bi
11	AAGCUUCGGAGAAAUUAAAUCCAAAUUGCAAUGAUCACACCUAAGCCCUAAA	AS_RNA_11	FIG. 5A
12	AGCUUCGGAGAAAUUAAAUC	AS_RNA12	FIG. 5A
13	GGCUCACAUCCAACAUCAU	AS_RNA13	FIG. 5A
14	GAGCCAUUGGAGGUUACACUGGCAG	AS_RNA14	FIG. 5A
15	UCACACUGCCCAAGAGUGAG	AS_RNA15	FIG. 5A
16	ACACAGGCACUGGCCACUGAUU	AS_RNA16	FIG. 5Bi
17	ACTCCACTCAGGCGAAATTC	mGAPDH-F primer	p.73
18	TCTCCATGGTGGTGAAGACA	mGAPDH-F primer	p.73
19	GAAATCATTT TCCCATCCTC ACCTTTTAAA TATATTTTAT CTTTAGGGCT TAGGTGTGAT CATTGCAATT TTGGATTAA ATTTCTCCGA AGCTTTAATG ATGTTGGATG TGAGCCCTGC CAGTGTACC TCCATGGCTC AGTGAACAAA TTCTGCAATC CTCACCTCTGG GCAGTGTGAG TGCAAAAAAG AAGCCAAAGG		FIG. 5A
20	ACTTCAGTGT GACACCTGCA GAGAAAACCT TTATGGGTTA GATGTCACCA ATTGTAAGGC CTGTGACTGT GACACAGCTG GATCCCTCCC TGGGACTGTC TGTAATGCTA AGACAGGGCA GTGCATCTGC AAGCCCAATG TTGAAGGGAG ACAGTGCAAT AAATGTTTGG AGGAAAACCT CTACCTACGG CAAAAATAAT		FIG. 5Bi-5Bi

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	CTTTCCTCTG TCTGCCCTTG AACTGTGATA AGACTGGGAC AATAAATGGC TCTCTGCTGT GTAACAAATC AACAGGACAA TGTCTTTGCA AATTAGGGGT AACAGGTCTT CGCTGTAATC AGTGTGAGCC TCACAGGTAC AATTTGACCA TTGACAAATTT TCAACACTGC CAGATGTGTG AGTGTGATTC CTTGGGGACA TTACCTGGGA CCATTGTGA CCCAATCAGT GGCCAGTGCC TGTGTGTGCC TAATCGTCAA GGAAGAAGGT GTAATCAGTG TCAACCAGGT AAGAAAGAAA TGTATTACAT TTTTCAGTGCA CAATGACATT CCTTTTGTTA ACTTAGGTAA CTTCTCCCTG TTTCTGGTTT GTGGCTTCTA CAAATTTTAT TTCCAAAATC		
21	ACCUGGUUGACACUGAUJACACC	ASss5E+2	FIG. 5Bii
22	AAUGUAAUACAUUUUCUUUA	ASss51I2-24	FIG. 5Bii
23	GCAAAAGUUAUUGGUUUUUUAUUAUU	EXON12-EXON14	FIG. 9C
24	AAUUUUUGGAG	SmOPT consensus	FIG. 8Aii
25	AAGCUUGGAGAAUUUAAUCCACUUGACGAUUAUAGGCACACACAGG	AS RNA4+8	
26	GTCTACAATGAAGC	U7 3' box	FIG. 8Aiii
27	CAGGUUUUCUGACUUGGUCGAAUAAACCCCU	U7 snRNA stem-loop	FIG. 8Aiii
28	ATTGCGATA	DSE	FIG. 8Aii
29	CTCACCCCTCATCGAAAGTG	PSE	FIG. 8Aii
30	TTAACAAACATAGGAGCTGTGATTGGCTGTTTTTCAGCCCAATCAGCACTGACTCATTTG CATAGCCCTTTACAAGCGGTACAAACTCAAGAAACGAGCGGTTTTTAATAGTCTTTTA GAATATTGTTTATCGAAACCGAAATAAGGAACTGTGCTTTGTGATTCACATATCAGTGG AGGGGTGTGGAATGGCACCTTGATCTCACCCCTCATCGAAAGTGAGTTGATGTCTT TCCCTGGCTCGCTACAGCGCACTTCCG	Mouse U7 snRNA PROMOTER	FIG. 8Aii
31	GCCGACGCGTTAACAAACATAGGAGCTGTGATTGGCTGTTTTTCAGCCCAATCAGCACT GACTCATTTGCATAGCCCTTTACAAGCGGTACAAACTCAAGAAACGAGCGGTTTTAA TAGTCTTTTAGAATAATTGTTTATCGAACCGAAATAAGGAACTGTGCTTTGTGATTCAC ATATCAGTGGAGGGGTGTGAAATGGCACCTTGATCTCACCCCTCATCGAAAGTGAG TTGATGTCCTTCCCTGGCTCGCTACAGACGCACTTCCGAAGCTTCGGAGAAATTTAA ATCCACTTGACGATTAGGCACACACAGGAGAAATTTTGGAGCAGGTTTCTGACTTC GGTCGGAAAAACCCCTCCCAATTTCACCTGGTCTACAATGAAAGCAAAACAGTTCTCTT	U7 expression vector	FIG. 8 Ai-iii

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	CCCCGCTCCCCGGTGTGTGAGAGGGGCTTTGATCCTTCTCTGGTTTCCTAGGAAACGCGTATGTGGGTACCTTTTTCGGCCCGCACTAGT		
32	CAATCAGTGCCAGAAATGGAT	hUSH2A-F primer	p. 66
33	TTGCAATTGGTCACAACGTTG	hUSH2A-R primer	p. 66
34	CCTGCAGGCAGCTGCGGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCTGGCGGACC TTTGGTCGCCCCGGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCC ATCACTAGGGGTTCTCTGCGGCCGCACGCGTGAGGGCCTATTCCCATGATTCCCTTCA TATTGCAATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAAATTGACTGTA AACACAAAGATAATTAGTACAAAAATACGTGACGTAGAAAAGTAATAATTCTTTGGGTAG TTTGCAAGTTTAAAAATTATGTTTAAAAATGGACTATCATATGCTTACCGTAACCTGA AAGTATTTTCGATTTCTTGGCTTTATATATCTTTGTGAAAGGACGAAACACCGCAAGT AAACCCCTACCAACTGGTCGGGTTTGAACAAGCTTCGGAGAAATTTAAATCCAAA ATTGCAAGTAAACCCCTACCAACTGGTCGGGTTTGAACCTTACCTGGCTGACACT GCTCACACCTTCTTTTGTGTTACCAAGTCTTGAAGAGAGTGGAAATTGGCTCCGG TGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAGTGGGGGAGG GGTCCGCAATTGATCCGGTGCCTAGAGAAAGTGGCGGGGTAACTGGGAAAAGTGA TGTCTGTACTGGCTCCGCCCTTTTCCGAGGTGGGGAGAACCGTATATAAGTGC AGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTTCGCCCGCAGAACACAGGACCG GTGCCACCATGAGCCCCAAGAAAGAGAAAGGTGGAGGCCAGCATCGAAAAAATAA AGTCTTCGCCAAGGGCATGGGCGTGAAGTCCACACTCGTGTCCGGCTCCAAAGTGT ACATGACAACCTTCGCCGAAGGCAGCGACGCCAGGCTGGAAAGATCGTGGAGGGCG ACAGCATCAGGAGCGTGAATGAGGGCGAGGCCCTTCAGCGCTGAAATGGCCGATAAAA ACGCCGGCTATAAGATCGGCAACGCCAAAATTCAGCCATCCTAAGGGCTACGCCGTGG TGGCTAACAAACCCCTCTGTATACAGGACCCGTCCAGCAGGATATGCTCGGCCCTGAAGG AAACCTCTGGAAAAGAGGTACTTCGGCGAGAGCGCTGATGGCAATGACAAATATTGTA TCCAGGTGATCCATAACATCCTGGACATTGAAAAATCCTCGCCGAAATACATTACCA ACGCCGCCCTACGCCGTCAACAAATATCTCCGGCTGGATAAGGACATTAATTGGATTTCG GCAAGTTCCTCACAGTGTATACCTACGACGAATTCAAAGACCCCGAGCACCATAGGG CCGCTTTCACAAATAACGATAAGCTCATCAACGCCATCAAGGCCCATGACGAGT TCGACAACTTCTCTCGATAACCCCGACACTCGGCTATTTCGGCCAGGCCCTTTTTCAGCA	AS_RNA4+6 dCAS13d - RECOMBINANT gRNA VIRAL VECTOR	TABLE V

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	AGGAGGGCAGAAAATTACATCATCAATTACGGCAACGAATGCTATGACATTCTGGCCCC TCCTGAGCGGACTGGCGCACTGGGTGGTCTGCTAACAAACGAAGAGTCCAGGATCT CCAGGACCTGGCTCTACAACCTCGATAAGAACCCTCGACAACGAATACATCTCCACCC TCAACTACCTCTACGACAGGATCACCAATGAGCTGACCAACTCCTTCTCCAAAGAACT CGCCGCCAACGTGAACATAATTGCCGAACTCTGGGAATCAACCTGCCGAAATTCG CCGAACAATAATTCAGATTTCAGCATTAAGAAAGAGCAGAAAAACCTCGGATTCAATA TCACCAAGCTCAGGGAAGTGCTGGACAGGAAGGATATGTCCGAGATCAGGAAAA ATCATAAAGGTGTTTCGACTCCATCAGGACCAAGGTCTACACCATGATGGACTTTGTGA TTTATAGGTATTACATCGAAGAGGATGCCAAGGTGGCTGCCGCCAATAAGTCCCTCC CCGATAATGAGAAATCCCTGAGCGAGAGGATATCTTTGTGATTAACTTAACCTGAGGGGCT CCTTCAACGACGACAGAAAGGATGCCCTCTACTACGATGAAGCTAATAGAAATTTGGA GAAAGCTCGAAAAATATCATGCACAACATCAAGGAAATTTAGGGGAAAAACAAGACAAGAG AGTATAAGAAAGAGGACGCCCCTAGACTGCCCAGAAATCCTGCCCGTGGCCGTGATG TTTCCGCCCTTCAGCAAACTCATGTATGCCCTGACCATGTTCTCTGGATGGCAAGGAGA TCAACGACCTCTGACCACTCTGATTAAATAATTCGATAACATCCAGAGCTTCTCTGA AGGTGATGCCCTCTCATCGGAGTCAACGCTAAGTTCTGTGGAGGAATACGCCCTTTTCA AAGACTCCGCCAAGATCGCCGATGAGCTGAGGCTGATCAAGTCTTTCGCTAGAAATGG GAGAACCTATTGCCGATGCCAGGAGGCCATGTATATCGACGCCATCCGTATTTAG GAACCAACCTGTCTCTATGATGAGCTCAAGGCCCTCGCCGACACCTTTTCCCTGGACG AGAACGGAAACAAGCTCAAGAAAGGCAAGCAGGCAATGAGAAATTTCAATTATAATA ACGTGATCAGCAATAAAAGGTTCCACTACCTGATCAGATACGGTGATCCTGCCACC TCCATGAGATCGCCAAAACGAGGCCGTGGTGAAGTTCTGCTCGGCAGGATCGCTG ACATCCAGAAAAACAGGGCCAGAACGGCAAGAACAGATCGACAGGTACTACGAAA CTTGATTCGGAAAGGATAAGGGCAAGAGCTGAGCGAAAAGGTGGACGCTCTCACAA AGATCATCACCGGAATGAACCTACGACCAATTCGACAAGAAAAAGGAGCGTCATTGAGG ACACCGCAGGGAACCGCCGAGAGGGAGAGTTTAAAAAGATCATCAGCCTGTACC TCACCGTGATCTACCACTCTCAAGAAATATTGTCAATAATCAACGCCAGGTACGTCA TCGGATTCCATTGGCTCGAGCGTGATGCTCAACTGTACAAGGAGAAAGGCTACGACA TCAATCTCAAGAACTGGAAGAGAGGGAATTCAGCTCCGTCAACCAAGCTCTGCCGTG GCATTGATGAAACTGCCCCCGATAAGAGAAAGGACGTGGAAGAGGAGATGGCTGAAA GAGCCAAAGGAGAGCATTGACAGCCTCGAGAGCGCCAAACCCCAAGCTGTATGCCAATT ACATCAAAATACAGCGACGAGAAAGAAAGCCGAGGAGTTTCACCGGCAGATTAAACAGGG		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	AGAAGGCCAAAACCGCCCTGAACGCCCTACCTGAGGAACACCAAGTGGAATGTGATCA TCAGGAGGACCTCCTGAGAAATTGACAACAAGACATGTACCTGTTGCGAAACAAGG CCGTCGCCCTGGAAGTGCCAGGTATGTCCACGCCATATCAACGACATTGCCGAGG TCAATTCTTACCTCCAACTGTACCATTACATCATGCAGAGAAATTATCATGAATGAGA GGTACGAGAAAAGCAGCGGAAAGGTGTCGAGTACTTCGACGCTGTGAATGACGAGA AGAAGTACAACGATAGGCTCCTGAAACTGCTGTGTGCTGCTTTCGGCTACTGTATCC CCAGGTTTAAGAACCTGAGCATCGAGGCCCTGTTTCGATAGGAACGAGGCCGCCAAGT TCGACAAGGAGAAAAGAGGTGTCGGGCAATTCCGGATCCGGACCTAAGAAAAAGA GGAAGGTGGCGCCGCTTACCCATACGATGTTCCAGATTACGCTTAAGAATTCCTAG AGCTCGCTGATCAGCCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTTGTTTGCCC CTCGCCGCTGCTTCTTGACCTGGAAGTGCCACTCCCACTGTCTTCTCCTAATA AAATGAGGAAAATTGCATCGCATGCTCTGAGTAGGTGTCTATCTGCGGGGTGG GGTGGGCAGGACAGCAAGGGGAGGATTGGGAAGAGAAATAGCAGGCATGCTGGGA GGTACCTTTTGGCGCCGAGGAACCCCTAGTGTGAGTTGGCCACTCCCTCTCTG CGGCTCGCTCGCTCACTGAGCGCGGCGACCAAGGTCGCCGACGCCCGGGCTTT GCCCGGGCGCTCAGTGAGCGAGCGAGCGCGCTGCTGTCAGG		
35	SPKKRKVEASIEKKKSFAGMGVKSTLVSGSKVYMTTFAEGSDARLEKIVEGDSIR SVNEGEAFSAEMADKNAGYKIGNAKFSHPKGYAVVANNPLYTGPVQQDMLGLKETLE KRYFGESADGNDNICIQVIHNILDIEKILAEYITNAAYAVNNISGLDKDIIIGFKFS TVYTYDEFKDPEDHRAAFNNNDKLINAIKAQYDEFDNFLDNPRLYFGQAFFSKEGR NYIINYGNECYDILALLSGLAHVVVANNEEESRISRTWLYNLDKNLDNEYISTLNYL YDRI TNELTNSFSKNSAANVNYIAETLGINPAEFQYFRFSIMKEQKNLGFNITKL REVMLDRKDMSEIRKNHKVFDSEIRTKVYTMDFVIYRYIIEEDAKVAAANKSLPDNE KSLSEKDI FVINLRGSFNDDQKDALYDEANRIWRKLENIMHNIKEFRGNKTREYKK KDAPRLPRI LPAGRDVSASFSLMYALTMFLDGKEINDLLTTLINKFDNIQSFLKVMP LIGVNAKFVEEYAFFKDSAKIADELRLIKSFARMGEPIDARRAMYIDAIRILGTNL SYDELKALADTFSLDENGNLKKGKHGMRNFIINNVI SNKRPHYLI RYGDPAHLHEI AKNEAVVKFVLGRIADI QKKQGQNGKNQIDRYETCIGKDKGKSVSEKVDALTKIIT GMNYDQFDKKRSVIEDTGRENAEREKFKKIIISLYLTVIYHILKNIVNINARYVIGFH CVERDAQLYKEKGYDINLKKLEEKGFSSVTKLCAGIDE TAPDKRKDVKEKEMAEAKE SIDSLE SANPKLYANYIKYSDEKKAEEFTRQINREKAKTALNAYLRNTKWNVI IRED LLRIDNKTCTLFANKAVALAVARYVHAYINDIAEVNSYFQLYHYIMQRI IMNERYEK	dCas13d amino acid sequence	TABLE V

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	SSGKVSSEYFDAVNDEKKYNDRLLLKLLCVPFYCYIPRFKNLSIEALFDRNEAAKFDKE KKKVSNGSGSGPKKKRKKVAAAYPYDVPDYA		
37	ATGACCCCTTCATTGACCTCA	GAPDH-F primer	
38	TGATCTTGAGGCTGTTGTCACTT	GAPDH-R primer	
39	ACGACCACCTTGGCGTCAAGGTAT	Scrambled gRNA2	FIG. 8D
40	GCCTAACCTGACCACCTCCTGTGT	Scrambled gRNA6	FIG. 8D
41	TTAACAAACATAGGAGCTGTGATTGGCTGTTTTTCAGCCCAATCAGCAC TGACTCATTTG CATAGCCCTTTACAAGCGGTACAAACTCAAGAAACGAGCGGTTTAAATAGTCTTTTA GAATAATTGTTTATCGAACCGGAAATAGGAACTGTGCTTTGTGATTCACATATCAGTGG AGGGGTGTGGAAATGGCACCTTGATCTCACCTCATCGAAAGTGAGTTGATGTCTCT TCCCTGGCTCGCTACAGACGCACCTCCGACGACCACTTGGCGTCAAGGTATGCCTAA CCTGACCACCTCCTGTGTAGAAATTTTGGAGCAGGTTTCTGACTTCGGTCGGAAAC CCCTCCCAATTTCACTGGTCTACAAATGAAAGCAAAACAGTTCTCTTCCCCGCTCCCC GGTGTGTGAGAGGGCTTTGATCTCTCTGTTTCTCCTAGGAAACGCGTATGTGGGT ACCTTTTTCGGCGCCAC TAGT	scU7 expression vector	FIG. 8D
42	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCTATA GCCCCATATATGGAGTTCGGCTTACATAAATTACGGTAAATGGCCCCGCTGGCTGAC CGCCCAACGACCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC CAATAGGGACTTTCCCATTGACGTCAATGGGTGGACTATTACGGTAAACTGCCCACT TGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTTATTGACGTCAATGACG GTAATGGCCCCGCTGGCATTA TGCCCAAGTACATGACCTTATGGGACTTTCCTACTTT GGCAGTACATCTACGTATTAGTCAATCGCTATTACCATGGTGTATGCGGTTTGGCAGT ACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCCA TTGACGTCAATGGGAGTTTGTTTTGGCACCAAAAATCAACGGGACTTTCCAAAAATGTC GTAACAACTCCGCCCCCATTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCT ATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAA TTAATACGACTCACA TAGGGAGACCCCAAGCTGGCTAGCATTTGGAGGACAGTGTAAAT TGTAAGAGACACGTGTCTGGCAGGCGAGTGCAATCAGTGCCAGAAATGGATTCTACAAT	Delex13 expression vector	FIG. 5 Ci-ii

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	CTACAAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAACTGCAATACCTCTGGGACA GTGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAGTGCAAGTGCAAGCAAAAC GTTATTGGTTTATTATATTCTCCAGGCAATGCCACTGGCTGCCATGCTCATGTC CATACAACGTGGTGCAGTTAATCACATCTGTAATAGCCTGACTGGTCAAGTGTGTTGC CAAGATGCTTCCATTGCTGGGCAACGTTGTGACCAATGCAAGAGACCAATTACTTTGGA TTTGATCCTCAGACTGGAAGGGCGGCTCGAGTCTAGAGGGCCCGTTTAAACCCGC TGATCAGCCTCGACTGTGCCCTTC TAGTTGCCAGCCATCTGTGTTGTTGCCCTCCCCC GTGCCCTTCCCTTGACCCCTGGAAGGTGCCACTCCCACTGTCCCTTTCCTAATAAAAATGAG GAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGTGGG CAGGACAGCAAGGGGAGGATTGGGAAGACAAATAGCAGGCAATGCTGGGGATGCGGTG GGCTCTATGG		
43	ATTGGAGGACAGTGTAAATTGTAAGAGACACGCTGTCTGGCAGGCGAGTGCAATCAGTGC CAGAAATGGATTCTACAAATCTACAAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAAC TGCAATACCTCTGGGACAGTGGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAG TGCAAGTGCAAGCAAAACGTTATTGGTTTTTATATTTCTCCAGGCAATGCCACTGGC TGCCGTGCCATGCTCATGCCATACAACTGGTGCAGTTAATCACATCTGTAATAGCCTG ACTGGTCAGTGTGTTTGCCCAAGATGCTTCCATTGCTGGGCAACGTTGTGACCAATGC AAAGACCAATTACTTTGGATTGTATCCTCAGACTGGAAG	EXON 12 - EXON14	FIG. 5 Ci-ii
44	GCAAAAGCAACGTTATTGGTT	qPCR-F	[0278]
45	CCAGTTGTATGGCATGAGCA	qPCR-R	[0278]
46	ATTTCTCCAGGCAATGCCACTGG	Taqman probe	[0278]
47			
48	AGTTCCAAGAGGGCCACCAAGCAGACACGCTCTGAGCTTCAGGTAACCAAGTGTTT GCTCTGCAGAAATACTTTACCTGGGCACCCCAAGTCTTCCTTCCAGCATTCCTGTGCT ACAGCCCTATTGCTGAGTAACCAAGGGTTACAGCAGCGTTGCCAGGCAACGAGGGAC AGCGGTCCCTGTTGAAGAGCCATTGTGCACACTGAGGGGACTGGTTGAAATGCAATAA AGAAATGATACCAAGCAGCTACTCATGTCTTCGCCATTGCTAAGAACGTCGTTGGTAT TACCTTACTCTGAGAACGTGTCTGCAGTTTCCAGAAATGGAGTATCGCAACATCAC TTAAAGTACCCCTGCTTCAAAGTATTGCTGGCAAGTGGCGTGGCCCTGATTATTATT TAGAAATGCTTTATCAGGAGGAGAAATGCTTTTGTAAACATGAATGCCCCAGTTCT TTCATTTGGGCTCTGGCTTCTTGTGTTTCAGGTTCATTGAAATGTTGATCTTTGCCCTATTT	Homo sapiens usherin cDNA transcript variant 2 (NCBI Reference Sequence: NM_206933.4)	

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	TGCTTCAATATCCTTGACTGAGTCACGAGGTCCTTTCCCAAGGCTGGAGAACGTGGGAGCTTCAAGAAAGTTTCCATCGTGCCAAACCAAGCAGTATGTGGACTCCCAGACCGAAGCACTTTTGTTCACAGCTCTGCTGCTGCTGAAAGTATTACAGTTCTGTACCCAGCGGTTTGTGATTACAGGATTGCCCATACAGATCTTACACCCCTACCTACACTGCCCTTTTCTCAGCAGGCCCTCAGTAGCTGCATCACACCAGACAGAAGAAATGATCTGCATCCTAAACGCCATAGCAATTCTGCAAGTTTATTTTTGGAAATCACAAAGAGCTGCTTTCTCTCTCCTCCTTCTCCAAAGCTGATGGCATCATTTACCTTAGCTGTATGGCTGAAACCTGAGCAACAAGGTGTAAATGTGTATTAGAAAAGACAGTAGATGGGCAGATTGTGTTCAAACCTACAAATATCTGAGAAAAGAGACCATTGTTTATTATTCGCACAGTAAATGGTTTGCACACCTCCAAATAAAAAGTAATGACACTGGGGAGAAATCTTGTGAAGAAATGGATTTCATCTTAGTGCAGGTGCATCAGACAAAATCAGCTTCTTTATCAATGGCGTGGAGAAGGATCATACACCTTCAATGCAAGAACTCTAAGTGGTTCAAATTACAGATTTTGCATCTGGTACTGTGCAAAATAGGACAGAGTTTAAATGGTTTAGAGCAGTTTGTGCGAAAGAAATGCAAGAATTTTCGATTATACCAAGTGGCACTTACAAAACAGAGAGATTCTGGAAGTCTTCTCTGGAGATCTTCTCAGATTGCAATGCCCAATCACATTTGCCGTTGCCCTGGCAGCCACCCGCGGGTCCACCTTTTGGCACAGCGGTACTGCAATTCCTAAATGATGCAGGAGACACAGCTGATAATAGAGTGTACGGTTGAATCCTGAAGCCCCATCCTCTCTCTTTTGTCAATGATAATGATGTTGGTACTTCAATGGGTTTCAAAATGTGTTTACAAAACATTACACAGCTTAAATCAAGAGTGACTATTTTCAGTTGATTGGAAAAATGGACAGTATCAGGTGTTTATATATATCATTCAGTTCTTTAGTCCACAACCAACGGAATAAAGGATTCAAAGGAAGAAGGAAAAATAGTTTAGATTGGAGGACTGGCAATATTTTGCCAGGAATTGTGGTGTCTTTTGGAAATGAAAAACAATGGAGATTGGAAAAACCTGATTCTGTCAACTGTCATTACAGCTTTCCTCAATTTTACTCCATATTTCCCGTGGCAATGTCACATTTAGCATCCTTGACACCTGGACCCAAATTAATCGTCTGGATACAAATAACTTCTATAATACCCCATCTCTTCAAGAGTTCTGTAAAAGCCACGCAAAATAAGGTTTTCATTTTTCATGGGCAGTACTATACAACCTGAGACTGCTGTAAACCTCAGACACAGATATTATGCAGTGGACGAAAATCACCATTAGTGGGAGATGTCAATGCGCATGGTCAATGCGCATGCGACACAACAAAGCCAGCCATATAGATGCCCTCTGTCCCAGGAGAGCTTCACTGAAGGACTTCAATTTGTGATCGCTGCTTGCCCTCTTTATAATGACAAAGCCTTTCCGCCCAAGGTGATCAAGTTTACGCTTTTCAATTTGTAACCTTGTCAATGCAACACAGCCATTCCAAAAGCTGCCATTACAACATCTCTGTAGACCCCATTTCCCTTTTGAGCACTTCAGAGGGGAGGAGGAGTTTGTGATGATTTGTGAGCATAAACACTACAGGAAGAACTGTGAGCTGTGCAAGGATTACTTTTTCCGACAAGTTGGTGGCAGATCCTTC		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
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SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	CCCTCCCCAAAGACTAAGTCCACCTAAGATGCAGAAAAATCAGTTCTACAGAACTTCA TGTAAGATGGTCTCCACAGCGGAACATAAATGGAAATAATTAAGATAATGAACATA CATGAGAAGACTGAGATCTACTAAAGAAACACATCTGAGGAAAGTCGAGTTTTCAT GAGCAGTGGTTGGCTCAGTCCATCTTCAATTTGTAGAAATCGGCCAATGAAAAATGCATT AAAACTCTCAAAACAATGACAACCATCAGTGGCTTGGAGCCATACACCAAGTATGA GTTACAGAGTCTTAGCTGTGAATAATGGCTGGAAGTGTCTTCTGCCCTGGGTCTCAGA AAGAAAGGAGAAATCAGCACCTGTATTCATGATCCCTCCTTCAGTCTTTCCCCCTCTC TTCCGTACTCTCTCAATATCTCC TGGGAGAGCCAGCAGATAATGTTACAAGAGGAAA AGTTGTGGGTATGACATCAATATGCTTTCTGAACAATCACCTCAACAGTCTATTCC CATGGCGTTTTCACAGCTGTTGCACACTGCTAAAATCCCAAGAACTATCTTACACTGT AGAAGACTGAACCTTATAGGATAATATGAGTTTACTATTAATCTCTGCAATTCAGT TGGTTGTGTGACCCAGTGTCTCGGAGCAGGACAAAATTTAGCAGCAGCACCAGCACAA ACTGAGGCCACCTCTGGTTAAAAGGAATCAACAGCACAAACATCCATCTTAGGTGGTT TCCACCTGAAGAACTGAATGGACCCCTCTCCTATATATCAGCTGGAAGGAGAGAGTC ATCTCTACAGCTGTGATGACCACGATGATGAAGGAATCCGTTTTCATAGGAAATGG GTATTGTAAAATTTCCAGCTCCACTCACCCAGTCAATACAGACTTCACCTGGCAATTA GGCCAGCTTTTCGAACAAAAGTGCCCTGAAGGTTTGATTGCTTTTGCAGCATCACCTGG CAATCAGGAAGAGTATTTTGCACTTCAGTTGAAGAGGACGCTTTTATTTTCTTTT TGATCTCAGGGGTACCCAGTGGAAAGTAATACAACTAATGATCATGGCAAAACAATA TAGTGATGGAATAATGGCATGAAATAATTGCTATTAGGCATCAGGCTTTTGGCCAAAT CACCTCGGATGGGATATATACAGGTTCCCTCTGGAGGGCTCCCGGAAAGTTATACCATCCTCAG TGGAGATAACACAGGAGTCTTTCTGGAGGGCTCCCGGAAAGTTATACCATCCTCAG GAAGGATCCTTGAGATAATCCAAAAGGTTTGTGGGCTGTCTCAAGGATGTACATTT TATGAAGAAATTACAAATCCGTGAGCTATTTGGGAACCTCTGGATTGGCAGAGTTCTGA AGAACAATAACAGTGTAACAGCTGGGAGGGAATGTCCTCCGCTTCATTAATGAGGG AGCTCAGTTCCTAGGAGCAGGTTCCCTGGAACCTTCATCCATATATGTTTCATGGTGG AATGAACCTTTGAGATTTCCCTTTAAGTTCAGAACTGACCAATTAATGGAATGCTTCT TTTTCGTTTATAACAAAGATGGACCTGATTTTCTTGCTATGGAGCTGAAAAGTGGAAAT ATTGACCTTCCGGTTAAATACCACTCTTGCCCTTTACACAAGTGGATCTATTGCTGGG GCTATCCTATTTGTAATGGAAGTGAATAAAGTCAATTAATAAAGGAAGGCTCTTT CATATCAGCAAGTGTGAATGGACTGATGAAGCATGCATCGGAGTCCGGAGACCCAGCC ACTGGTGGTGAAATTCACCAGTTTATGTGGGAGGAAATCCACAGGAACTGCTGAACCTC		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
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SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
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SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	AATGAATGTGCCCTGGGTCGTTTATTCTTGAGAGACCTGTCTCCCTTCACTATCTATGA CATTCAGGTTGAAGTCTGCACAAATATATGCCCTGCGTGAAAGCAATGGAACCCAAAT TACCACGTGGAAGACACTCCAAGTGATATACCAACACCCACAAATTCGTGGCATCAC TTCAAGATCTCTTCAAAATTGATTGGGTCTCCACGGAAGCCAAATGGCATCATTTCT TGGATATGATCTCCTATGGAACACATGGTATCCATGCCGTAAAACTCAAAAGTTAGT GCAGGATCAGAGTGATGAGCTCTGCAAGGCAGTGAGGTGTCAAAAACCTGAAATCTAT CTGTGGACACATTTGCTATTCTTCTGAAGCTAAGGTTTGTGTAAACGGAGTGTCTTA TAACCCCAAGCCTGGACATCGCTGTTGTGAAGAAAAGTATATCCCGTTTGTCTTGAA TTCTACTGGAGTTTGTGTGGTGGCCGAATACAGGAGGCACAAACCAATCATCAGTG CTGCTCTGGGTATTACGCTAGAAATCTACCAGGTGAAGTATGCTGTCCAGATGAACA GCACAAATCGGGTTCTGTGGCAATGGTGATTCCTGCTGTGGCAGAAATGCCGTACTC CACCTCAGGAAACCCAGATTTGCTGTGCTGGAGGCTTCATGATGGCCATGGCCAGAA GTGCTGTGGCAGACAGATTTGTGAGCAACGATTTAGAGTGTGTGGTGGAGAAGAAGG AGTGGTGACAAATCGCCCTCCAGGTATGTTCTGTGTGGGCAGGATTAATGTGAATAT GTCAGATACCAATATGCTGCTCAGCTTCAGTGGAGAGTCTAAAGCACATATTAAAAA GAATGACCCGGTGCCAGTAAAAATGCTGTGAGACTGAACCTTATTCCAAAGAGCCAGAA ATGCTGTAATGGAGTTGGATATAATCCTTTGAAAATATGTTGCTCTGACAAGATTTC AACTGGAATGATGATGAAGGAAACCAAGAGTGCAGGATCCTCTGCCCGCATCTAT GGAAGCCACAGAACATTTGTGGCAGGTGTGACTTCAACTTTACCAGCCACATTTGCAC TGTGATAAGAGGGTCTCACAAATCCACAGGGAAGGCATCAATTGAAGAAATGTGTTT ATCTGCCGAAGAAACCAATTCATACAGGGAGTGTAAACACGTAATCTTACACAGATGT GAACCTCAAGCCCTACATGACATATGAGTACAGGATTTCTGCCCTGGAACAGCTATGG GCGAGGACTCAGCAAGCTGTGAGAGCCAGAACAAAGAAAGATGTGCCCTCAAGGAGT GAGTCCCCCTACGTGGACCAAAATAGACAACTTTGAAGATACAAATGTCTTTAAACTG GAGAAAACCTATACAAATCAAAATGGTCCCTATTATTACTACATCCTTCTTCGAAAATGG AATTGAACGTTTTTCGGGGAACATCACTGAGCTTCTCTGATAAAGAGGGAATTCACCC ATTTCAGGAAATATTCATATCAGCTGAAAGCTTGACCGTTGCTGGCTGTGCCACCAG TAGCAAGGTAGTTGCAGCTACTACCCAAAGGAGTTCCGGAGAGCATCCGTGCCACCAAG CATCACAGCCCTAAGTGCAGTGGCTCTGCATCTGAGCTGGAGTGTCCCTGAGAAATC AAACGGCGTCAATTAAGAGTACAGATCAGGCAGGTTGGGAAAGGTCATCATCCACAC TGACACCCACTGACAGGAGACAGCATACGGTCAAGGTCACAGGCTCCAGCCATACACCACTA CAGCTTCACTCTTACAGCTTGTACATCTGCTGGGTGCACCTTCAAGCGAGCCTTTTCTT		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
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SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	GCTGATTTCCCTGGATCCCACCAGAACAGTCTAATGGTATTATCCAGTCCCTATAGGCT TCAAAGGAATGAAATGCTCTATCCTTTTAGCTTTGATCCTGTGACTTTCAATTACAC TGATGAAGAGCTTCTCCTTTTCCACCTATAGCTATGCATCCAAAGCCTGCACGAG TGGAGGATGCTCCACCAGCAAAACCACCAGCATCACAACTCTGGAGGCTGCTCCATC AGAAATCAGCCCTCCAGATCTTTGGGCCGTGAGTCCACTCAAAATGAATGTATGTTG GTCACCGCCACAGTGCAAAATGGAAGAATTACTAAATATTAGTTAGATATGATAA TAAAGAGTCCCCTTGTGTGCCAGGGCCTGTGCCCTGCTGGTTTCCCACCTGCAGCCTTA CTCTCAGTATAACTTCTCCCCTTGTAAGCTGCACGAATGGAGTTGCACAGCTAGTGT GTCAAAATCTGCCCTGGACAAATGGAGGCCCTGCCAGAGAACATGGACTCTCCAACATT GCAAGTCACAGGCTCAGAAATCAATAGAAATCACCTGGAAACCTCCAAGAAACCCAAA TGGCCAGATCAGAAGTTATGAACTTAGGAGGATGGAACCAATTGTATATACAGGCTT GGAAACACGCTATCGTGATTTTACTCTCACCCACAGGTGTGGAGTATAGCTACACAGT AACTGCCAGCAACAGCCAAAGGGGTATTTTGAGTCCCTCTTGTCAAAAGATCGAAACCAG CCCCTCAGCACCCCTCAGGGATGGAACCTCCAAAATTGCAGGCCAGGGTCTCTCAGGA GATCTTAGTGAACTGGGACCTCCAGTGAGAACAAATGGTGATATCATCAATATATAC CCTCTTCATCCGTGAACATTTGAAAGAGAAACTAAAATCATACACATAAACACAAC TCATAATTTCTTTGGTATGCAGTCATATATAGTAAACCAGCTGAAGCCATTTCACAG GTATGAAATACGAATTCAGCGTGCACCCACCTGGGATGTGCATCAAGTGACTGGAC ATTATACAGACCCCTGAGATTGCACCTTTGATGCAACCCCTCCACATCTGGAGGT ACAAATGGCTCCAGGAGATTCCAGCCAACTGTTTCTCTTTTGTGGACAGGACCGCT GCAGCCAAATGGAAAAGTTTGTATTACGAATTATACAGAAAGACAAAATAGCAACTCA GCCTAGAAAAATCCAAATCCAGTCCCTAATCTATAACGGAAGCTCAACATCTTTTATAGA TTCCGAACTATTGCCCTTTCACAGAGTATGAGTATCAGGTCTGGGCAGTGAATTCTGC AGGAAAGCCCCCAGTAGCTGGACATGGTGACAGAACCGGGCCAGCCCCACAGAAAG TCTCAGAGCCCCCAGTTCCATGTGATCTCTTCTACCCAAAGCAGTGGTCAACATCAG TGCCCCCTGGGAAGCCCAACGGGATCGTCAGTCTCTACAGGCTGTTCTCCAGCAGCGC CCATGGGGCTGAGACAGTGTATCCGAAGGCATGGCCACCCAGCAGACTCTCCATGG CCTTCAAGCCTTCACATACTACTCTATTGGAGTAGAGGCCCTGCACCTGCTTCAACTG TTGCAGCAAAAGACCGACAGCTGAACTGAGAACCCATCTGCCCCACCTCAGGACT GTCCCTCTCCACAAATCGGACGCTGGCCCTCAAGGACGGCCCTCCTTCCGGTGGAGTCC CCCCATGTTCCCCCAATGGTGTCAATTCACAGCTATGAACCTCCAATTCACAGTGGCTTG CCCTCCTGACTCAGCCCCCTCCCCCTGTACTCCCCAGCCAAATAGAAAACAAAGTACACGGG		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	GCTGGGGCAGAAAGCCAGCCTTGGGGGTCTCCAGGGCTACACCACATACAAGCTGAG AGTGTGGCACACAACGAGGTGGCAGTACGGCTTCCGAGTGGATCAGTTTCACCCAC CCAAAAGAAATTGCCCTCAGTACCGAGCCCCATTTTCGGTGGACAGCAATTGTCTGT GGTGTGTGAACCTGGAGTGACACCTTCCCTCCTGAACGGCCAACTGAAGGAGTACGT GTTAACCGACGGAGGGCAGCGGTGACAGCGGTGGACACCACTCTACATACC GAGAACGGCGGACAAAACCTTCTTTTCCAGGTCACTGCACGACTGACGAAAGGAG TGTTAAGACGCCGTTGATCCAAATATGATACCTCTACTGGACTTGGCTTGGTCCTAAC AACTCCTGGGAAAAGAGGATCGCGGAGCAAAAAGCACAGAGTTCTACAGCGAGCT GTGGTTCAATAGTTAATGGCGATGCTGGGCTTGATCTTGTGGCCATTTTCTGTCTC CCTGATACATAAAGAAAAATCCACAAAGAGCCATATATCAGAGAAAGACCTCCCTT GGTACCTCTTCAGAAAGAGGATGTCCTCAATTGAATGTTTACCCACCGGGGAAAAACCA TATGGGGTTAGCCGATACCAAAAATTCCTCCGGTCTGGGACACCTGTGAGTATCCGCAG CAACCGGAGTGATGTCTCGCATCCCGAGTCAAAAACCAACAGCCCTAACCTA CTCCAGGGTCTCTTCACCGCAGCGTCAGCCAGCTCATGGACATTCAGACACAAGAA AGTCTTGATGGACAACCTCACTGTGGGAAGCCATCATGGGCCACAACAGTGGACTGTA TGTGGATGAAGAGGACCCTGATGAACGCCATCAAGGATTTTCAGCTCAGTGACTAAGGA ACGCACCACATTCACAGACACCCACCTGTAAAGGATGAAACCCAGAGACGTAACC CTGGAATGCAAGGCTGCACCCCATTTCTCTCGGTATCACTCACACATCATAAAT GCTGAAAAGCCATTGTTTATTAATCCTATAATCTTTAAAGAAATGATGACTGTTTTT GAAAGTGTTCCTTCCATAAGAGGCTAAAGAAATGATATTTTCTCATCTTAAATGA GAGAGAAATATCATATGAAAATACCTTGATTTGCTCTTATTTGTAGAAAGACAAAAGAA GTATGTAATGTCACTTGTTCTGTTTGGCAGTGATGCTCCTGGTTAACTGAATAAT CAGTGGCAATTTCAAGATGGCTCACAGTTGTAGAAAGTAGTAAGTTAGTTACTGGCT CAAAAATGATCTGTGTGAAGGATGTCACTGCTGTTCATTTCTATCTGCCATTTCTG TCAGGGTTGACACAAATCCTGCAAGAAATAGTTATTCATAATGATCACAGCTGCTAAATG AATCCCAAACCTTTGCACCAAGTGCACAAAACCTTTCTGAAGGTTCTATTTATTTACCA TACATAGGGTTACTTACCAAACCTTTTGGACAAAGGCAACACATAACTTACACATAAAT TACATAGGGTTACTTACCAAACCTTTTGGACAAAGGCAACACATAACTTACACATAAAT GTCTGTGTTCTTGCAATTTATGAAATTTCCAAAAATCTAAGGAGTAAACAGCTTATTT ATACATTTTGAGGAGAAAACAAAAGTGTTCACCTAGGAACACCTCTACTTGAACCAAT GTTTTTATTTCAATATTTTATAGTTTGTGAACCTAGTTTCTCATAAAAATCTGTCAA TTCACTGAATATCAGAGAAATACTGACATCTTCAACCTAGCACATTTTCAAAATGGAAAC		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	TACTGTTCTATTTGCAATATTAGGCTGCGTGAAAATTTTAAAGGAAAAATGTATCTGTTCCCTTAGCATTAACATATAATACATGTAGAGACAAGACTATACCTATGTGTATATATGTATATCATGTATATATTAATCTCTGCACTATATCCCTTCCTTTTGGAGAACTAGCCATTAATTTAGCCACAGAAATCAGTAAGAACAGATGATATGCAACAGTACCAATTACGGTTCAAAAAATGCTGTCACCTGCTCTAGTTGGATTACAAAGTCATTGGTGAAAGTCCTATGGCAAGAAAAATTTTCTTGCAAAATCATCCACATAAAATCAGATATTTAAATTTGTTCTTCAATGGAAAAACAGAGTAAGAAAAACCTCTTGCTCTTCCCTTCAATCTTAAAGGCTCTTGTGACCCAGGAAAAATATTGACTCTGTCTAACACACAAATAGTCACAATACTTTTGTGAATCTACAACAGAGACAGGCAAAAACCTGTAAAGTAAGGATAGTCTTACTTTATCTGCCCTGAAAAACAATGTATTACCCCAAGGCCCCAACAGTAAAGATTGTGGACTTTTGGGTAATTGAGATTTCACTCTAGCTCTGTGAGAGCAGCTCCTCAGACTGACCAACTCCTAGACAAAAGTTTGCCAACCATAAAGTGTCAAAAAGCACAGGCCAGTATTAAAGCAGAAGTTCTACCACTTATTAGAACTGTATAAAACAAAAGCATCTGAAAATAATTTGTGCAATCTGGCAGTGACTGTAGAAAAATACGAAATATATATTTCTCGCCAAAGTTTATATACTTTCTGAAAATGAAAACATAGGATTGACTAGTTTACTGGTTTTTATTTCCCATATGCCCCATTCTGGGACAAATAAAGTTGTTTAAAGCTGGCACAAATAAGCATTAACCAAGGCTGTGTCCACCTTCTGTGAGCTACTTAAGGTATATAGGAAGGAGTGGTCACAAAACTTGCAATCCTAAATCCTTGGTGACTCTTCTAAGAAATACAGTTTGTAGTCACAAAGAAATAGTCTACAAAATATGCTTTGCTAGGTTTCAGAAAGATTGAGTTTATCCTGATTTTGGAAAAATTAACCAAGGTATCTTTATCACTGTGTATTTTCCAAAGCACAGTATAAAAATTTTAAACAACGCACAAAAAAATACAGAACTGCAGGGGATTTTATCTTTGGATCATTAATCCATTTAATCATCTAATTAGACATGAATCAGTTAGCTGAATCATTTACATTTTGACTCCATAGCTTTAGGCAGACAGAAAGCTGTATGGCTTCTGCCCAGAACTCTGTCCCCTGCTACATGCTTAAGTTTACTTGTAATTTATTCAGAGAAGAACTCTAAGATGTTGCTTTGCTACTTTAAGTGGTATTGCGTGCCAAAGCCTCTATTATACAAAACCATGCAGACTCGCCCTCTAGAGATTCTGATTCGGTTGATCTGGGGTGTGTGGCTGAGGCATCAGTACTTTTAAAGCTTTCAGGTGTTCTAATGTTGAGACCCACTGATGTTCCACAATCTGGAAGAAATCATGTACAGGAATAATATGCTATGCACAGGGACTATGCTCCTTGGCTCACCCCCCTCTCCCCCTTAAACAAATGAGCAGTTCTTGATGAACCTCTTTAAATTTAAATCTCCTGACTCACATTTTACCAATGTACATGCCACATCTCAGCTTACGAACCTACCAATGTTTGTATATCTTAATCAACTGTTTGGTAAGAGTACAGTTGTTTTTATACACTCTAAGAAATGTGTTTATAATCTACTGTAAATTTCCACTAAATGGAACCCCAAAATATAATGTTTATGGTACCATAT		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
49	ACTGATGTAATAATCATGCTGGCATCCATGAACACACCGGTAAATAAACATAGTCC AAGTGAAGAAATTCATTAAAGGAACCTTTAAATATGTACAAATGAATAGTTGGT TTCCAATGCACAAATATCATGTAAACTAAATCTAAAGATGGTTTGTCTTAATAAATATT TGAATGTGACCTAA MNCPVLSLGS GFLFQVLEML IFAYFASISL TESRGLFPRL ENVGAFKKVS IVPTQAVCGL PDRSTFCHSS AAAESIQFCT QRFCIQDCPY RSSHPTYTAL FSAGLSSCIT PDKNDLHPNA HSNSASFIFG NHKSCFSSPP SPKLMASFTL AVWLKPEQQG VMCVIEKTVQ GQIVFKLTIS EKETMFYVRT VNGLQPPIKV MTLGRILVKK WIHLSVQVHQ TKISFFINGV EKDHTPFNAR TILSGSITDFA SGTVQIGQSL NGLQFVGRM QDFRLYQVAL TNREILEVFS GDLLRLHAQS HCRCPGSHPR VHPLAQRYCI PNDAGDTADN RVSRLNPEAH PLSFVNDNDV GTSWVSNVFT NITQLNQGVV ISVDLENGQY QVFYIIQFF SPQPTAIRIQ RKKENS LDWE DWQYFARNCG AFGMKNNGLD EKPDSVNCLQ LSNFTPYSRG NVTF SILTPG PNYRPGYNNF YNTPSLQEFV KATQIRFHFH GQYTTETAV NLRHRYAYVD EITISGRQCQ HGHADNCDTT SQPYRCLCSQ ESFTEGLHCD RCLPLYNDKP FRQGDQVYAF NCKPCQCNSH SKSCHYNISV DPFPFEHFRG GGGVCD DCEH NTTGRNCELK KDYFFRQVGA DPSAIDVCKP CDCDITVGTNR GSILCDQIGG QCNCRRHVSQ RQCNQCQNGF YNLQELDPDG CSPCNCNTSG TVDGDITCHQ NSGQCKCKAN VIGLRCDHCN FGKFLRSFN DVGCEPCQCN LHGSVNKFCN PHSGQCECKK EAKGLQCDTC RENFYGLDVT NCKACDCDTA GSLPGTVCNA KTGQCICKPN VEGRQCCKL EGNFYLRQNN SFLCLPCNCD KTGTINGSLI CNKSTGQCPC KLGVTGLRCN QCEPHRYNLT IDNFQHCQMC ECDSLGLTLPG TICDPISGQC LCVPNRQRR CNQCQPGFYI SPGNATGCLP CSCHTTGAVN HICNSLTGQC VCQDASIAQO RCDQCKDHYF GFDPQTGRQO PCNCHLSGAL NETCHLVTGQ CFCKQFVTS KCDACVPSAS HLDVNNLLGC SKTPFQQPPP RGQVQSSSAI NLSWSPPDSP NAHWLTYSLI RDGFEIYTE DQYPYSIQYF LDTDLLPYTK YSYIETTNV HGSTRSAVT YKTKPGVPEG NLTLSYIPII GSDSVTLTWT TILSNQSGPIE KYILSCAPLA GGQPCVSYEG HETSATIWNL VPFAKYDFSV QACTSGGCLH SLPIITVTTAQ APPQRLSPPK MQKISSSTELH VEWSPPAEIN GIIIRYELYM RRLRSTKETT SEESRVFQSS GWLSPHSFVE SANENALKPP QMTTITITGLE PYTKYEFRL AVNMAGSVSS AWVSERTGES APVFMIPPSV FPLSSYSINI SWEKPADNVT RGKVVGVDIN	Homo sapiens usherin polypeptide having the amino acid sequence of SEQ ID NO: 49 (NCBI Reference Sequence: NP_996816.3)	

SEQ ID NO.	SEQUENCE (5'→3')					ALIAS	LOCATION
	MLSEQSPQOS	IPMAFSQLLH	TAKSQELSYT	VEGLKPYRIY	EFTITLCNSV		
	GCVTSASGAG	QTLAAAPAOQL	RPPLVKGINS	TTIHLRWFPF	EELNGPSPYI		
	QLERRESSLP	ALMTTMMKGI	RFIGNGYCKF	PSSTHPVNTD	FTGIKASFRT		
	KVPEGLIVFA	ASPGNQEEYF	ALQLKKGRLY	FLFDPQGSVP	EVTITNDHGK		
	QYSDCKWHEI	IAIRHQAFGQ	ITLDGIYTG	SAILNGSTVI	GDNTGVFLGG		
	LPRSYYTILRK	DPEIIQKGFV	GCLKDVHEMK	NYNPSAIWEP	LDWQSSEEQI		
	NVYNSWEGCP	ASLNEGAQFL	GAGFLELHPY	MFHGGMNFEI	SFKFRTDQLN		
	GLLLFVYNKD	GPDFLAMELK	SGILTFRLNT	SLAFTQVDLL	LGLSYCNGKW		
	NKVIIKKEGS	FISASVNGLM	KHASESGDQP	LVVNSPVYVG	GIPQELINSY		
	QHLCLEQGF	GCMKDVKFTR	GAVVNLASVS	SGAVRVNLDG	CLSTDSAVNC		
	RGNDSILVYQ	GKEQSVYEGG	LQPFTEYLRY	VIASHEGGSV	YSDWSRGRTT		
	GAAPQSVPTP	SRVRSNLNGYS	IEVTWDEPVV	RGVIEKYILK	AYSEDSTRPP		
	RMPASASAEFV	NTSNLTGILT	GLLPFKNYAV	TLTACTLAGC	TESSHALNIS		
	TPQEAQOEVO	PPVAKSLPSS	LLLSWNPPKK	ANGIITQYCL	YMDGRLLIYSG		
	SEENYIVTDL	AVFTPHQFLL	SACTHVGCNT	SSWVLLYTAQ	LPPEHVDSPV		
	LTVLDSTRTH	IQWKQPRKIS	GILERYVLYM	SNHTHDFTIW	SVIYNSTELF		
	QDHMLQYVLP	GNKYLIKLAG	CTGGGCTVSE	ASEALTDEDI	PEGVPAPKAH		
	SYPDSFNVS	WTEPEYPNGV	ITSYGLYLDG	ILIHNSSELS	YRAYGFAPWS		
	LHSFRVQACT	AKGCALGPLV	ENRTLEAPPE	GTNVFVKTKQ	GSRKAHVRWE		
	APFRPNGLLT	HSVLFYGIFY	VDPVGNNYTL	LNVTKMYSYG	EETNLWLVID		
	GLVPFTNYTV	QVNISNSQGS	LITDPIITIAM	PPGAPDGVLP	PRLSSATPTS		
	LQVWVSTPAR	NNAPGSPRYQ	LQMRSGDSTH	GFELEFSNPS	ASLSYEVSDDL		
	QPYTEYMERL	VASNGFGSAH	SSWIPFMTAE	DKPGPVVPPI	LLDVKSRMML		
	VTWQHPRKSN	GVITHYNIYL	HGRLYLRTPG	NVTNCTVMHL	HPYTAYKFQV		
	EACTSKGCSL	SPESTQVWTL	PGAPEGIPSP	ELFSDTPTS	IIISWQPPTHP		
	NGLVENFTIE	RRVKGKEEVT	TLVTLPRSHS	MRFIDKTSAL	SPWTKYEYRV		
	LMSTLHGGTN	SSAWVEVTR	PSRPAGVQPP	VVTVLEPDAV	QVTWKPPPLIQ		
	NGDILSYEIH	MPDPHITLTN	VTSAVLSQKV	THLIPFTNYS	VTIVACSGGN		
	GYLGGCTESL	PTYVTTHPTV	PQNVGPLSVI	PLSESYVVVIS	WQPPSKPNGP		
	NLRYELLRRK	IQQPLASNPP	EDLNRWHNIY	SGTQWLIEDK	GLSRFTTYEY		
	MLFVHNSVGF	TPSREVTVTT	LAGLPERGAN	LTASVLNHTA	IDVRWAKPTV		
	QDLQGEVEYY	TLFWSSATSN	DSLKILPDVN	SHVIGHLKPN	TEYWIFISVF		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	NGVHSINSAG LHATTCDGEP QGMLPPEVVI INSTAVRVIW TSPSNPNGVV TEYSIYVNNK LYKTGMNVPG SFILRDLSPF TIYDIQVEVC TIYACVKSNG TQITTVEDTP SDIPTPTIRG ITRSRLQIDW VSPRKPNGII LGYDLLWKTW YPCAKTQKLK QDQSDDELCKA VRCQKPESIC GHICYSSEAK VCCNGVLYNP KPGHRCCEEK YIPFVLNSTG VCCGGRIQEA QPNHQCCSGY YARILPGEVC CPDEQHNRVS VGIGDSCCGR MPYSTSGNQI CCAGRLHDGH GQKCCGRQIV SNDLECCGGE EGVVYNRLPG MFCGQDQVYN MSDTICCSAS SGESKAHIKK NDPVPVKCE TELIPKSQKC CNGVGYNPLK YVCSDKISTG MMMKETKECR ILCPASMEAT EHCGRCDNF TSHICTVIRG SHNSTGKASI EEMCSSAEET IHTGSVNTYS YTDVNLKPYM TYEYRISAWN SYGRGLSKAV RARTKEDVPQ GVSPPTWTKI DNLEDITVLN WRKPIQSNGP IYYILLRNG IERFRGTSL FSDKEGIGQPF QEYSYQLKAC TVAGCATSSK VVAATTQGVV ESILPPSITA LSAVALHLSW SVPEKSNQVI KEYQIRQVVK GLIHTDITDR RQHTVTGLQP YTNYSFLLTA CTSAGCTSE PFLGQTLQAA PEGVWVTPRH IINSTIVEL YWSLPEKPNG LVSQYQLSRN GNLLFLGGSE EQNFTDKNLE PNSRYTYKLE VKTGGGSSAS DDYIVQTPMS TPEEIIYPPYN ITVIGPYSIF VAWIPPGILI PEIPVEYNVL LNDGSVTPLA FSVGHHQSTL LENLTPFTQY EIRIQACQNG SCGVSSRMFV KTEPAAPMDL NSPVLKALGS ACIEIKWMP EKPNGIINI FIYRRPAGIE EESVLFVWSE GALEFMDEGD TLRPFTLYEY RVRACNSKGS VESLWSLTQT LEAPPQDFPA PWAQATSAHS VLLNWTKPES PNGIISHYRV VYQERPDPT FNSPTVHAFT VKGTSHQHL YGLEPFTTYR IGVVAANHAG EILSPWTLIQ TLESSPSGLR NFIVEQKENG RALLQWSEP MRTNGVIKTY NIFSDGFLEY SGLNRQFLER RLDPFTLYTL TLEACTRAGC AHSAPQPLWT DEAPDSQLA PTVHVKSTS VELSWSEPVN PNGKIIRYEV IRRCFEGKAW GNQTIQADEK IVFTEYNTER NTFMYNDTGL QPWTQCEYKI YTWNSAGHTC SSWNVVRTLQ APPEGLSPPV ISYVSMNPQK LLISWIPPEQ SNGIIQSYRL QRNEMLYPFS FDPVTFNYTD FELLPFSTYS YALQACTSGG CSTSKPTSIT TLEAAPSEVS PPDLWAVSAT QMNVCSPPPT VQNGKITKYL VRYDNKESLA GQGLCLLVSH LQYYSQYNFS LVACTNGGCT ASVSKSAWMT EALPENMDSP TLQVTGSESI EITWKPPRNP NGQIRSYELR RDGTIVYTGL ETRYRDFTLT PGVEYSYTVT ASNSQGGILS PLVKDRTPS APSGMEPPKL QARGPQEIIV NWDPPVRTNG DIINYTLFIR ELFERETKII HINTTHNSFG MQSYIVNQLK		

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	PFHRYEIRIQ ACTTLCASS DWTFIQTPEI APLMQPPPHL EVQMAPGGFQ PTVSLWTGP LQNGKVLYY ELYRRQIATQ PRKSNPVLII NGSSTSFIDS ELLPFTEYEQVWAVNSAGK APSSWTWCRTPAPPEGLRA PTFHVISSIQ AVVNISAPGK PNGIVSLYRL FSSSAHGAET VLSEGMATQQ TLHGLQAFN YSIGVEACTC FNCCSKGPTA ELRTHPAPPS GLSSPQIGTL ASRTASFRWS PPMFPNGVIH SYELQFHVAC PPDSALPCTP SQIETKYTGL GQKASLGGLQ PYTTYKLRVV AHNEVGSTAS EWISFTTQKE LPQYRAPFSV DSNLSVVCVN WSDTFLNGQ LKEYVLTGG RRVYSGLDTT LYIPRTADKT FFFQVICITD EGSVKTPLIQ YDTSTGLGLV LTPGKKKGS RSKSTEFYSE LWFIVLMAML GLILLAIFLS LILQKIHKE PYIRERPPLV PLQKRMSPLN VYPPGENHMG LADTKIPRSQ TPVSIRSQRS ACVLRIPSON QTSITYSQGS LHRVSQQLMD IQDKKVLMDN SLWEAIMGHN SGLYVDEEDL MNAIKDFSSV TKERTTFTDT HL		
50	ATTGGAGGACAGTGTAATTGTAAGAGACACGCTGTCTGGCAGGCAGTGCAATCAGTGC CAGAAATGGATTCTACAACTACAAGAGTTGGATCCTGTATGGCTGCAGTCCCTGTAAAC TGCAATACCTCTGGACAGTGGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAG TGCAAGTGCAAAAGCAAAACGTTATTGGTACGTGTAACGCAAAAGTTTACAGTCACATC TCTATTACCATCTGGAATTTATTATTAAAGTATAGTACCATGCTGTACAATAGAGCT CCAGAACTTATTCACTCTGTCTAGCTGAAACTTTGTACTCAGCTTAACCTTTTATTA AACATCTTTAGAGATTCTTATCTTTAGAAAACAACATAATTTGTTATATGTAATTC TACTATAATTTTAAATGAGCACATTTGTTAAAAATAGTTTTTAAAGATTGTTAAAGAG AAAAAGAGCTCCAGCATAATGTAACAGAAAACAACATTTGCAATTAAGCATTTTCTTTTG CATTAAAGTAAATAATTAATAATTTATGAAGTTTCATCGCAAAACAGTTGTATATTAAGC TAAATTAATAATTGTCAATTGAAATTTTGAGAGTAAGATTGGCCCCCTATGGCATTTGCT TGTGAGAAAACACTCAATAATTTGTGTTCTGTATCATCTGCAGTAGCATTTGTTGTGT CTCGTCTATCTTGAATGAAATCAATTTTCCCATCCTCACCTTTTAAATAATATTTATC TTTAGGGCTTAGGTGTGATCATTGCAATTTTGATTTAAATTTCTCCGAAGCTTTAA TGATGTTGGATGTGAGCCCTGCCAGTGAACCTCCATGGCTCAGTGAACAAATCTG CAATCCTCACTCTGGGCAAGTGTAGTGCAAAAAGAACCCAAAGGACTTCAGTGTGA CACCTGCAGAGAAAACTTTATGGGTTAGATGTCAACCAATTTGAAGGCCCTGTGACTG TGACACAGCTGGATCCCTCCCTGGGACTGTCTGTAAATGCTAAGACAGGCGAGTGCAT CTGCAAGCCCCAATGTTGAAGGGAGACAGTGCAATAAATGTTTGGAGGGAAACTTCTA CCTACGGGCAAAATAATCTTTTCCCTCTGTCTGCCTTGCAACTGTGATAAGACTGGGAC	Minigene cDNA	TABLE I, p.26

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	AATAAATGGCTCTCTGCTGTGTAACAAAATCAACAGGACAAATGTCTTGC AAAATTAGG GGTAACAGGTCTTTCGCTGTAATCAGTGTGAGCCTCACAGGTACAATTGACCAATTGA CAATTTCAACACTGCCAGATGTGTGAGTGTGATTCCTTGGGACATTAACCTGGGAC CATTTGTGACCCCAATCAGTGGCCAGTGCCTGTGTGTCCTAATCGTCAAGGAAGAAG GTGTAATCAGTGTCAACCCAGGTAAAGAAAAGAAATGTATTACATTTTCAGTGCACAATG ACATTCCTTTTGTAACTTAGGTAACCTCTCCCTGTTTCTGCTGTTTGTGGCTTCTACA AATTTTATTTCCAAAAATCATTACTGTATTATATCATTAATCCAACACATATATAACT ATTTAACTTATTCAAAAATTATCTGCATATTTTATGTACTATTTTGAGAGGATACCTT AGATAAAACTCAGCCGATCGGATTTATTTTATAAATGAGACTCAATTTCTACACTTG AAGTAAATCTCCTTTTAAACAGTTTTTAAAAAATCAGATCAACAAGAGTCAATTTTA TTTTCCAGAGAAAGGAAAATTTGAGTTGAATATCCATACAAATGCCAAAATATTCAAAAT GATGAACATAAATCTCTGAATAAAAGCTGGCTAAATGTTTTTCCACATGCCATCAAAATTAATAAA TTCTAGTTTTTATATAGAAATACCTAGAAATGTTTTCCACATGCCATCAAAATTAATAAA ATAGGCCCTTGGTAGAGTATCTGTTTTTCTGTGTAAATCAATTAATTATGCTTTAAATA AAAGAGTGGATAAAAAGTATATAAAAACCTTTTAAATTTCTTTATTTCTAGGTTTTTA TATTTCTCCAGGCAATGCCACTGGCTGCCATGCCATGCTCATGCCATACAACGTGGTGC AGTTAATCACATCTGTAATAGCCTGACTGGTCAGTGTGTTTGCCAAAGATGCTTCCAT TGCTGGGCAACGTTGTGACCAATGCAAAAGACCATTACTTTGGATTTGATCCTCAGAC TGGAAAG		
51	IGGQCNCNRH VSGRQCNCQ NGFYNLQELD PDGCSPCNCN TSGTVDGDIT CHQNSGQCKC KANVI	Exon 12 amino acid sequence	TABLE II, p.27
52	ATTGGAGGACAGTGTAAATTGTAAGAGACACGCTGTCTGGCAGGCAGTGCAATCAGTGC CAGAAATGGATTCTACAAATCTACAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAAAC TGCAATACCTCTGGGACAGTGGATGGAGATATTACCTGTCAACCAAAAATTCAGGCCAG TGCAAGTGCAAAAGCAAAACGTTATTG	Exon 12 nucleotide sequence	TABLE II, p.27
53	GTACGTGTAACGCAAAAAGTTTACAGTCACATCTCTATTACCATCTGGAATTTATTAT TAAGTATAGTCACCATGCTGTACAAATAGAGCTCCAGAACTTATTCATTCTGTCTAGC TGAAAATTTGTACTCAGCTTAACCTTTTATTAACATCTTTAGAGATTTCTTATCTTT TAGAAAAACAACATAATTGTTATATGTAAATTTCTACTATAAATTTTAAATGAGCACATT TGTTAAAAATAGTTTTTAAGATTTGTTAAAGAGAAAAGAGCTCCAGCATATGTAACA GAAACAACATTTGCAATTAAGCATTTTCTTTTGCATTAAGTAATAATAAAAAATTTAT GAAGTTCATCGCAAAACAGTTGTATATTAAAGCTAAATTAATAATTGTCATTGAATTT	5' intron	TABLE II, p.27

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
	TGAGAGTAAGATTGGCCCCCTATGGCATTGCTTGTGAGAAAAACACTCAATATTTTGTGTTTCGTATCATCTGCAGTAGCATTTGTTGTGTCGTCTATCTTGAATGAAATCATTTTCCCATCCTCACCTTTTAAATATATTTATCTTTAG		
54	LRCDHCFGE KFLRSFNDVG CEPQCQNLHG SVNKFCNPHS GQCECKKEAK GLQCDTCREN FYGLDVTNCK ACDCDTAGSL PGTVCNKATG QCICKPNVEG RQCNKCLEGN FYLRQNNNSFL CLPCNCDKGT TINGSLILCNK STGQCPCKLG VTGLRCNQCE PHRYNLTIDN FQHCQMCECD SLGTLPGTIC DPISGQCCLCV PNRQGRRCNQ CQP	Exon 13 amino acid sequence	TABLE II, P.28
55	GGCTTAGGTGTGATCATTTGCAATTTTGGATTTTAAATTTCTCCGAAGCTTTAATGATGTTGGATGTGAGCCCTGCCAGTGTAACTCCATGGCTCAGTGAGCAAAATTTCTGCAATCCTCACCTCTGGCAGTGTGAGTGCAAAAAAGAGCCAAAGGACTTCAGTGTGACACCTGCAGAGAAAACTTTTATGGTTAGATGTCACCAATTTGTAAGGCCCTGTGACTGTGACA CAGCTGGATCCCTCCCTGGGACTGTCTGTAATGCTAAGACAGGGCAGTGCACTCTGCAAGCCCAATGTTGAAGGGAGACAGTGCAATAAATGTTTGGAGGAACTTCTACCTACGGCAAAATAATCTTTCTCTGTCTGCCCTTGCAACTGTGATAAGACTGGGACAAATAAATGGCTCTCTGCTGTGTAAACAAATCAACAGGACAAATGTCCTTGCAAAATTAGGGGTAA CAGGTCTTCGCTGTAAATCAGTGTGAGCCTCACAGGTACAATTTGACCATTTGACAATTTTCAACACTGCCAGATGTGTGAGTGTGATTCCTTGGGGACATTAACCTGGGACCATTTGTGACCCCAATCAGTGGCCAGTGCCTGTGTGTCCTAATCGTCAAGGAAGAGGTGTAATCAGTGTCAACCAG	Exon 13 nucleotide sequence	TABLE II, P.28
56	GTAAGAAAGAAATGTATTACATTTTTCAGTGCACAAATGACATTCCTTTTGTAACTTAGTAACCTTCTCCCTGTTTCTGGTTTGTGGCTTCTACAAATTTTATTTCCAAAAATCAT TACTGTATTTATATCATATCCACACATATATACTATTTAACTTATTCAAAAATTA TCTGCATATTTATGTACTATTTTGAGAGGATACCTTTAGATAAAACTCAGCCGATCG GATTTATTTCAATAATGAGACTCAATTTCTACACTTGAAGTAAATCTCCTTTTAAAC AGTTTTTTAAAAATCAGATCAACAAGAGTCAATTTTATTTTCCAGAGAAAGGAAAT TTGAGTTGAATATCCATACAATGCCAAATATTCAAATGATGAACATACTCTGGAAT AAAGCTGGCTAAATGTTTTTGTGAGAGGCTATATGTTCTAGTTTTTATATAGAAAT ACCTAGAAATGTTTCCACATGCCATCAAAATTAATAAAATAGGCCCTTGGTAGAGTATTCTGTTTTTCTGTGTAATCAATTAATATGCTTTAAATAAAAGAGTGATAAAAAGTAT ATATAAAACTTTTAAATTTCTTTTATTTCTAG	3' intron	TABLE II, P.28-29

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
57	FYISPGNATG CLPCSCHTTG AVNHICNSLT GQVCQDASI AGQRCDQCKD HYFGEDPQTG	Exon 14 amino acid sequence	TABLE II, P.29
58	GTTTTATATTCTCCAGGCAATGCCACTGGCTGCCTGCCATGCTCATGCCATACAA CTGGTGAGTTAATCACATCTGTAATAGCCTGACTGGTCAAGTGTGTTGCCAAGATG CTTCCATTGCTGGGCAACGTTGTGACCAATGCAAGACCATTAATTGGATTGTATC CTCAGACTGGAAG	Exon 14 nucleotide sequence	TABLE II, P.29
59	IGGQCNCKRH VSGRQCNQCQ NGFYNLQELD PDGSPCNCN TSGTVDGDIT CHQNSGQCKC KANVIGLRCD HCNFGFKFLR SFNDVGCEPC QCNLHGSVVK FCNPHSGQCE CKKEAKGLQC DTCRENFYGL DVTNCKACDC DTAGSLPGTV CNAKTGQCIC KPNVEGRQCN KCLEGNFYLR QNNSFLCLPC NCDKTGTING SLLCNKSTGQ CPCKLGVTL RNCQCEPHRY NLTIDNFQHC QMCECDSLGT LPGTICDPIS GQCLCVPNRQ GRRNCQCQPG FYISPGNATG CLPCSCHTTG AVNHICNSLT GQVCQDASI AGQRCDQCKD HYFGEDPQTG	Exon 12-Exon 13-Exon 14 amino acid sequence	TABLE III, P.29-30
60	ATTGGAGGACAGTGTAATTGTAAGAGACACGCTGTCTGGCAGGCGAGTCAATCAGTGC CAGAAATGGATTCTACAAATCTACAAGAGTTGGATCCCTGATGGCTGCAGTCCCTGTAAC TGCAATACCTCTGGGACAGTGGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAG TGCAAGTGCAAGCAACGTTATTGGCTTAGGTGTGATCATTGCAATTTTGGATTT AAATTTCTCCGAAGCTTAAATGATGTTGGATGTAGCCCTGCCAGTGTAAACCTCCAT GGCTCAGTGAACAAATCTGCAATCTCTCACTCTGGGCGAGTGTGAGTGCAAAAAAGAA GCCAAAGGACTTCAGTGTGACACCTGCAGAGAAAACTTTATGGGTTAGATGTCACC AATTGTAAGGCCTGTGACTGTGACACAGCTGGATCCCTCCCTGGGACTGCTGTAAT GCTAAGACAGGCGAGTGCATCTGCAAGCCCAATGTTGAAGGGAGACAGTGCAATAAA TGTTTGGAGGGAACTTCTACCTACCGGCAAAATAATCTTTCTCTGTCTGCCCTTGC AACTGTGATAAAGACTGGGACAAATAAATGGCTCTCTGCTGTGTAACAAATCAACAGGA CAATGTCTTGCAAAATTAGGGTAACAGGCTCTCGCTGTAATCAGTGTGAGCCTCAC AGGTACAAATTGACCAATGACAAATTTCAACACTGCCAGATGTGTGAGTGTGATCC TTGGGGACATTACCTGGGACCAATTTGTGACCCCAATCAGTGGCCAGTGCCTGTGTG CCTAATCGTCAAGGAAGAGTGAATCAGTGTCAACAGGTTTTTATATTTCTCCA GGCAATGCCACTGGCTGCCATGCTCATGCCATACAACTGGTGCAGTTAATCAC ATCTGTAATAGCCTGACTGGTCAAGTGTGTTTGCCAAAGATGCTTCCATTGCTGGGCAA CGTTGTGACCAATGCAAGACCATTAATTGGATTGTGATCCTCAGACTGGAAG	Exon 12-Exon 13-Exon 14 nucleotide sequence	TABLE III, P.29-30

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
61	IGGQCNCRRH VSGRQCNCQ NGFYNLQELD PDGCSPCNCN TSGTVDGDI'T CHQNSGQCKC KANVIGFYIS PGNATGCLPC SCHTTGAVNH ICNSLTGQCV CQDASIAGQR CDQCKDHYFG FDPQTG	Exon 12- Exon14 amino acid sequence	TABLE IV, p.31
62	ATTGGAGGACAGTGTAATTGTAAGAGACACCGTGTCTGGCAGGCAGTGCAATCAGTGC CAGAAATGGATTCTACAAATCTACAAGAGTTGGATCCTGATGGCTGCAGTCCCTGTAAAC TGCAATACCTCTGGGACAGTGGATGGAGATATTACCTGTCAACCAAAATTCAGGCCAG TGCAAGTGCAAAAGCAAAACGTTATTGGTTTTTATATTCTCCAGGCAATGCCACTGGC TGCC'TGCCATGCTCATGCCATACAACTGGTGCAGTTAATCACATCTGTAATAGCCTG ACTGGTCAGTGTGTTTGCCCAAGATGCTTCCATTGCTGGGCAACGTTGTGACCAATGC AAAGACCATTTACTTTGGATTTGATCCTCAGACTGGAAG	Exon 12- Exon14 nucleotide sequence	TABLE IV, p.31
63	AAUUUGUCUAG	U7-specific Sm binding site	[0204]
64	AAGCUUCGGAGAAAUUUAAUCCACUUGACGAUUAGGCACACACAGGAG AAUUUUUGGAGCAGGUUUUCUGACUUCGGUCGAAAAACCCCU	AS_RNA4+8 U7	FIG.8B
65	ACACAGGC		[0008], [0036], [0041], [0065], Claims 3 & 11
66	CACACACAGG		[0009], Claims 3 & 11
67	AAGCUUCGGAGAAAUUUAAUCC		Claim 5, [0014], [0062]
68	AGCUUCGGAGAAAUUUAAUCC		Claim 5, [0014], [0062]
69	CCTTCAGTGCCAGGATGGAT	mUsh2a-F primer	Example VII, [301]

SEQ ID NO.	SEQUENCE (5'→3')	ALIAS	LOCATION
70	TGACACTGGTGACAGCTACG	mUsh2a-R primer	
71	GCAAAACGTTATTGGTTTATATTT	EXON12-EXON14	FIG. 15B
72	TAAATATATTTTATCTTTAGGGCTTAGGTGTGATCGTTGCAATTTTGGATTAAATT TCTCCGAAGCATTAATGATGATGGATGTGAGCCCTGCCAGTGTAACCTCCATGGCTC AGTGAACAAAATTC TGCAATCCTCTGTCTGGCAGTGTGAGTGCAAAAAGAAAGCTAA AGGACTTCAGTGTGACACCTGCAGAGAAAACCTTTATGGGTTAGATGTCACCAATTG TAAGGCCCTGTGACTGTGACACAGCTGGATCCCTCCCTGGGACTGCTGTAATGCTAA GACAGGGCAGTGCAATCTGTAAGCCCAATGTTGAAGGGAGACAGTGCAATAAAATGTTT GGAGGGACACTTCTACTTACGGCAAAAATAATCTTTCCTCTGCCCTGCCCTTGCAACTG TGATAAGGCTGGGACAAATAAATGGCTCTCTGTTGTGTGACAAATCAACAGGACAATG TCCTTGCAAAATTAGGGGTAACAGGCTTCGCTGTAAATCAGTGTGAGCCTCACAGGTA CAATTTGACCAATTGACAAATTTCAAGGCTGCCAGATGTGTGAGTGTGACTCCTTGGG GACATTACCTGGGACCAATTTGTGACCCCAATCAGTGGCCAGTGCCCTATGTGTGCCCTAA TCGTCAAGGAAGAGGTGTAATCAGTGTCAACCCAGGTAAGAAAGAAAATGTGTTAC	Cyonomoligus exon 13	FIG. 17
73	TAAATATATTTTATCTTTAGGGCTTAGGTGTGATCAATGCAATTTTGGATTAAATT TCTCCGAAGCTTTAAATGATGTTGGATGTGAGCCCTGCCAGTGTAACCTCCATGGCTC AGTGAACAAAATTC TGCAATCCTCTACTCTGGCAGTGTGAGTGCAAAAAGAAAGCCAA AGGACTTCAGTGTGACACCTGCAGAGAAAACCTTTATGGGTTAGATGTCACCAATTG TAAGGCCCTGTGACTGTGACACAGCTGGATCCCTCCCTGGGACTGCTGTAATGCTAA GACAGGGCAGTGCAATCTGCAAGCCCAATGTTGAAGGGAGACAGTGCAATAAAATGTTT GGAGGGAAACTTCTACCTACGGCAAAAATAATCTTTCCTCTGTCTGCCCTTGCAACTG TGATAAGACTGGGACAAATAAATGGCTCTCTGCTGTGTAAATCAAGAGGACAATG TCCTTGCAAAATTAGGGGTAACAGGCTTCGCTGTAAATCAGTGTGAGCCTCACAGGTA CAATTTGACCAATTGACAAATTTCAACACTGCCAGATGTGTGAGTGTGATTCCTTGGG GACATTACCTGGGACCAATTTGTGACCCCAATCAGTGGCCAGTGCCCTGTGTGTGCCCTAA TCGTCAAGGAAGAGGTGTAATCAGTGTCAACCCAGGTAAGAAAGAAAATGTATATAC	Human exon 13	FIG. 17

CLAIMS

1. A nucleic acid engineered to express a recombinant RNA molecule in a cell containing at least a first and a second antisense ribonucleotide sequence capable of hybridizing to a human USH2A pre-mRNA, said recombinant RNA comprising: a first antisense ribonucleotide sequence having complementarity to a 3' splice site region of USH2A exon 13, and a second antisense ribonucleotide sequence with complementarity to a 5' splice site region of USH2A exon 13, wherein the second antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8, and wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.
2. The nucleic acid of claim 1, wherein the second antisense ribonucleotide sequence comprises 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.
3. The nucleic acid of claim 1, wherein the second antisense ribonucleotide sequence comprises a ribonucleotide sequence of SEQ NO: 65 or 66.
4. The nucleic acid of claim 1, wherein the first antisense ribonucleotide sequence may comprise 22, 23, or 24 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.
5. The nucleic acid of claim 1, wherein the first antisense ribonucleotide sequence comprises a ribonucleotide sequence of SEQ NO: 67 or 68.
6. The nucleic acid of claim 1, wherein the recombinant RNA molecule comprises in a 5' to 3' order: the first antisense ribonucleotide sequence, the second antisense ribonucleotide sequence, an SmOpt element, and a stem and loop sequence.
7. The nucleic acid of claim 6, wherein the SmOpt element comprises a ribonucleotide sequence of SEQ ID NO: 24.

8. The nucleic acid of claim 6, wherein the stem and loop sequence comprises a ribonucleotide sequence that has at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% sequence identity to the ribonucleotide sequence of SEQ ID NO: 27.
9. The nucleic acid of claim 6, wherein the 5' end of the recombinant RNA comprises a hypermethylated cap.
10. The nucleic acid of claim 6, wherein the 5' end of the recombinant RNA comprises a 2,2,7-trimethyl guanosine (m3G) 5' cap.
11. A nucleic acid engineered to express a recombinant RNA molecule in a cell, said recombinant RNA molecule containing at least a first and a second antisense ribonucleotide sequence capable of hybridizing to a human USH2A pre-mRNA, the first antisense ribonucleotide sequence having complementarity to a 3' splice site region of exon 13, wherein the first antisense ribonucleotide sequence comprises 22, 23 or 24 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4, and the second antisense ribonucleotide sequence with complementarity to a 5' splice site region of exon 13, wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.
12. The nucleic acid of claim 11, wherein the second antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8.
13. The nucleic acid of claim 11, wherein the second antisense ribonucleotide sequence comprises a ribonucleotide sequence of SEQ NO: 65 or 66.
14. A nucleic acid engineered to express in a cell a recombinant RNA molecule containing at least two antisense ribonucleotide sequences capable of hybridizing to 5' and 3' splice site regions of exon 13 of a human USH2A pre-mRNA, said recombinant RNA comprising: a first antisense ribonucleotide sequence having complementarity to a 3' splice site region of exon 13, wherein the first antisense ribonucleotide sequence comprises at least 10 contiguous nucleotides of a ribonucleotide sequence of SEQ ID NO: 4, and a second antisense ribonucleotide sequence with complementarity to the 5' splice site region of exon 13, wherein the second antisense ribonucleotide sequence comprises at least 10 contiguous nucleotides of a ribonucleotide sequence

of SEQ ID NO: 8, and wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.

15. A nucleic acid engineered to express a recombinant RNA molecule in a cell, said recombinant RNA containing a ribonucleotide sequence comprising at least 25 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25 or a ribonucleotide having from 80%, 85%, 90%, or 95%, sequence identity with the ribonucleotide sequence of SEQ ID NO: 25, wherein the nucleic acid encoding the recombinant RNA is operably linked to a promoter.

16. The nucleic acid of any one of claims 1, 11, 14, and 15, wherein the recombinant RNA is a modified recombinant U snRNA or a dCas13d guide RNA.

17. The nucleic acid of claim 16, wherein the modified recombinant U snRNA is a modified U7 or U1 snRNA.

18. The nucleic acid of any one of claims 1, 11, 14, and 15, wherein the promoter is a constitutive, cell-specific, or inducible promoter.

19. The nucleic acid of claim 18, wherein the cell-specific promoter comprises a photoreceptor cell-specific promoter.

20. The nucleic acid of claim 18, wherein the constitutive promoter comprises a U snRNA promoter.

21. The nucleic acid of claim 20, wherein the U snRNA promoter is a U7 snRNA promoter.

22. The nucleic acid of claim 21, further comprising a U snRNA 3'-box RNA processing signal.

23. The nucleic acid of any one of claims 1, 11, 14, and 15, wherein the nucleic acid is flanked by adeno-associated virus (AAV) inverted terminal repeat sequences (ITRs).

24. A recombinant adeno-associated virus (rAAV) comprising the nucleic acid of claim 23.

25. The recombinant adeno-associated virus of claim 24, further comprising a capsid protein of a serotype selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Anc80, AV2/1, 2/2, 2/6, 2/8, 2/9 and AAV2/Anc80L6.

26. A therapeutically effective amount of a recombinant RNA comprising a first antisense ribonucleotide sequence capable of hybridizing with a 3' splice site region of human USH2A exon 13, covalently linked to a second antisense ribonucleotide sequence capable of hybridizing with an exon's 5' splice site region of human USH2A exon 13, wherein the second antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8 or a ribonucleotide sequence having 80%, 85%, 90%, or 95% sequence identity with a ribonucleotide sequence of SEQ ID NO: 8, said amount of recombinant RNA being effective at facilitating synergy between the covalently linked first and second ribonucleotide antisense sequences to induce skipping of USH2A's exon 13.
27. The recombinant RNA of claim 26, wherein the first antisense ribonucleotide sequence comprises at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4 or a ribonucleotide sequence having 80%, 85%, 90%, or 95% sequence identity with a ribonucleotide sequence of SEQ ID NO: 4.
28. The recombinant RNA of claim 26, wherein the recombinant RNA comprises a 5' cap.
29. The recombinant RNA of claim 28, wherein the first antisense ribonucleotide sequence comprises a 5' cap.
30. The recombinant RNA of claim 26, wherein the second antisense ribonucleotide sequence does not comprise a 5' cap.
31. The recombinant RNA of claim 23, wherein USH2A's exon 13 comprises a pathogenic mutation.
32. The recombinant RNA of claim 31, wherein the pathogenic mutation comprises a c.2299delG or a c.2276G>T mutation.
33. A therapeutically effective amount of a 5'-capped recombinant RNA containing a ribonucleotide sequence having at least 20 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25, or a ribonucleotide sequence having 80%, 85%, 90%, or 95% sequence identity with a ribonucleotide sequence of SEQ ID NO: 25, said amount of recombinant RNA being effective at inducing human USH2A exon 13 skipping.

34. The recombinant RNA of claim 33, wherein the recombinant RNA comprises a hypermethylated 5' cap.
35. The recombinant RNA of claim 33, wherein the recombinant RNA comprises a 2,2,7-trimethyl guanosine (m3G) 5' cap.
36. A method for modulating splicing of USH2A pre-mRNA in a cell comprising expressing the recombinant RNA of claim 26 or 33 in the cell.
37. A method for treating an USH2A associated retinopathy in a human patient, said method comprising administering the recombinant adeno-associated virus (rAAV) of claim 24 to the patient's eye by subretinal injection, wherein the subject's photoreceptor cells express an USH2A pre-mRNA having a pathogenic mutation in exon 13.
38. A method for treating an USH2A associated hearing loss in a human patient, said method comprising administering the recombinant adeno-associated virus (rAAV) of claim 24 by injection into the patient's inner ear, wherein cochlear cells of the inner ear express an USH2A pre-mRNA having a pathogenic mutation in exon 13.
39. A method for preventing Usher Syndrome in a human patient, comprising administering the recombinant adeno-associated virus (rAAV) of claim 24 to the patient's eye and inner ear, wherein the patient's photoreceptor cells in the retina and cochlear cells in the inner ear express an USH2A pre-mRNA having a pathogenic mutation in exon 13.
40. A method for treating an USH2A associated disorder in a human subject comprising administering a recombinant USH2A-U7 adeno-associated virus (rAAV) to the human, wherein the USH2A-U7 adeno-associated virus expresses a therapeutically effective amount of a recombinant RNA containing a ribonucleotide sequence having 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25 or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 25, wherein the subject's photoreceptor and/or inner ear cells express an USH2A pre-mRNA having a pathogenic mutation in exon 13, and wherein said amount of recombinant RNA is effective at inducing USH2A exon 13 skipping in said cells.

41. The method of any one of claims 37-40, wherein the pathogenic mutation comprises c.2276G>T and/or c.2299delG.
42. A host cell for the manufacture of a recombinant USH2A-U7 adeno-associated virus (rAAV) comprising the nucleic acid of claim 23.
43. A pharmaceutical composition in unit dose form comprising the recombinant adeno-associated virus (rAAV) of claim 24 and a pharmaceutically acceptable excipient, diluent, or carrier.
44. The pharmaceutical composition of claim 43, wherein said formulation further comprises empty adeno-associated virus (rAAV) capsids at a percentage from at least about 50% cp/cp up to about 90% cp/cp.
45. A cell comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising any one of the ribonucleotide sequences of SEQ ID Nos: 1-16.
46. A cell comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 8.
47. A cell comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4, or a ribonucleotide sequence having about 80%, 85%, 90%, or 95% sequence identity with the ribonucleotide sequence of SEQ ID NO: 4.
48. A cell comprising an USH2A mRNA comprising a nucleotide sequence of SEQ ID NO: 43, and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 25.
49. A cell comprising an USH2A mRNA and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 4, wherein the USH2A mRNA does not comprise the ribonucleotide sequence of SEQ ID NO: 55.

50. A cell comprising an USH2A mRNA and a recombinant RNA comprising at least 10 nucleotides of a ribonucleotide sequence of SEQ ID NO: 8, wherein the USH2A mRNA does not comprise the ribonucleotide sequence of SEQ ID NO: 55.

51. The cell of any one of claims 45-50, wherein the cell is a photoreceptor cell or cochlear cell.

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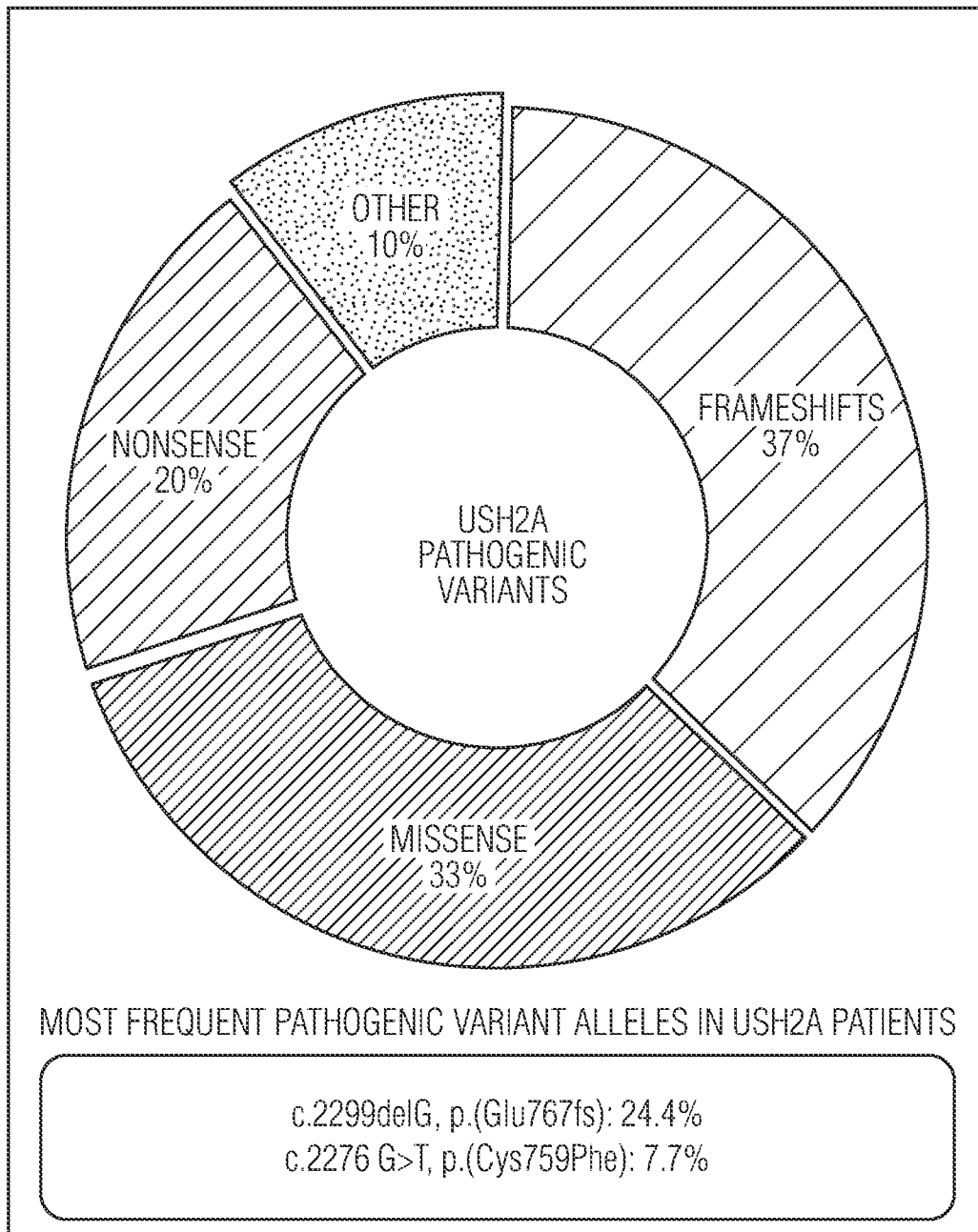


FIG. 1

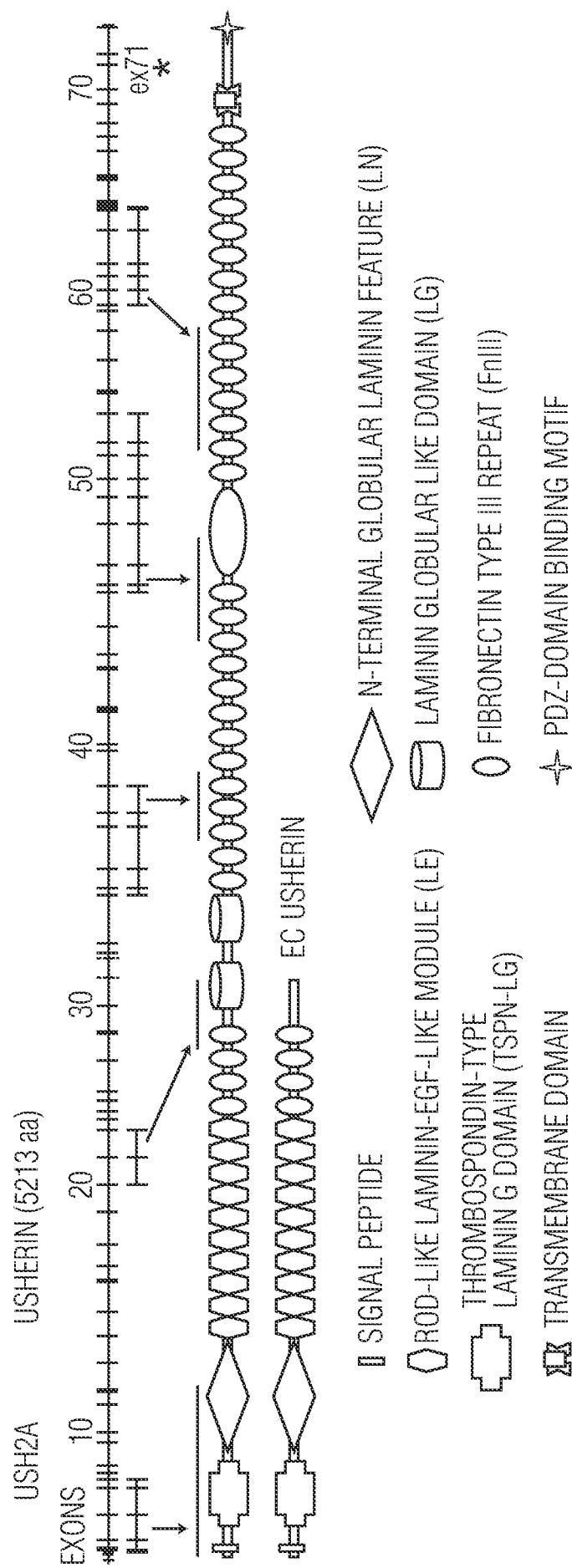


FIG. 2A

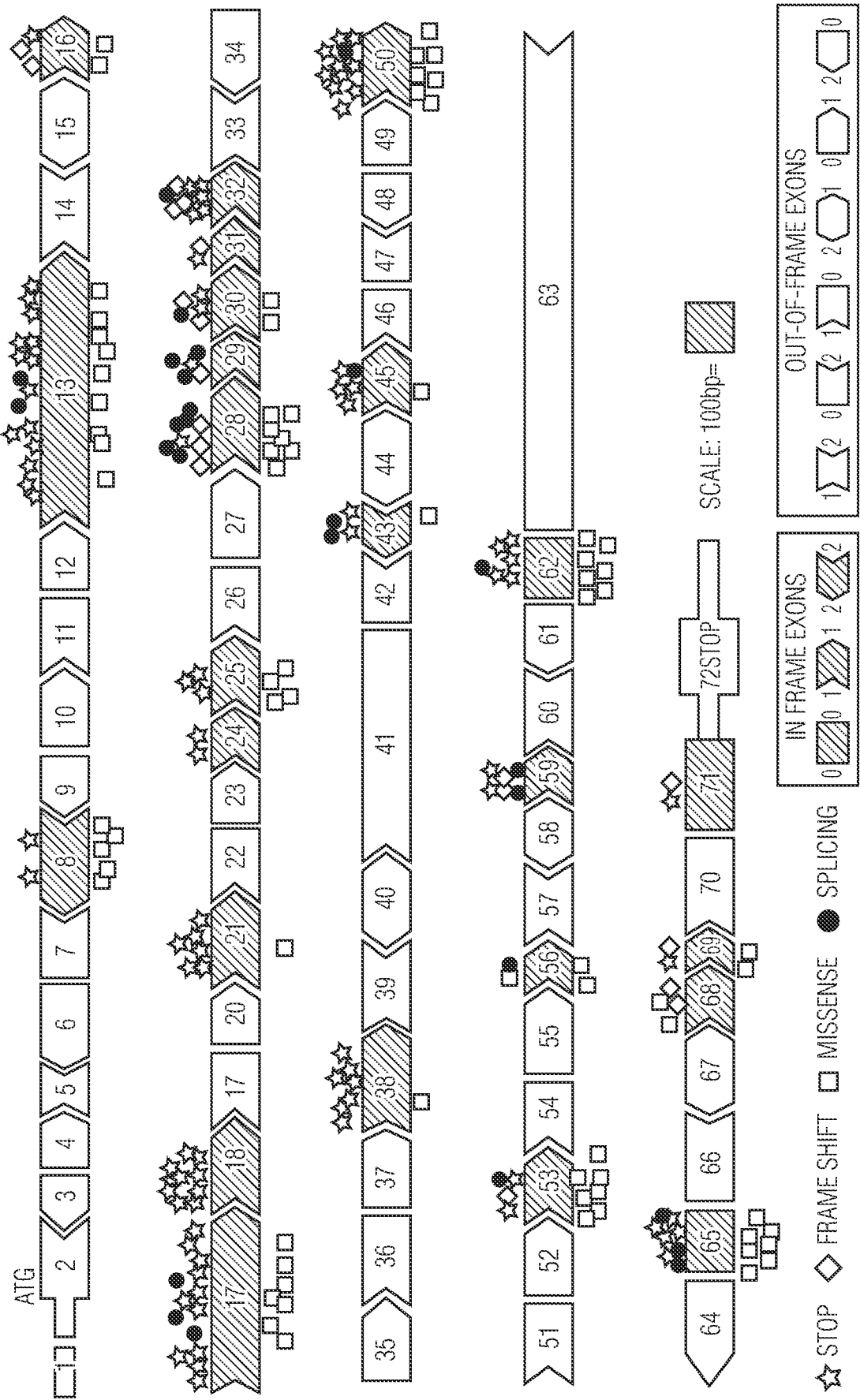


FIG. 2B

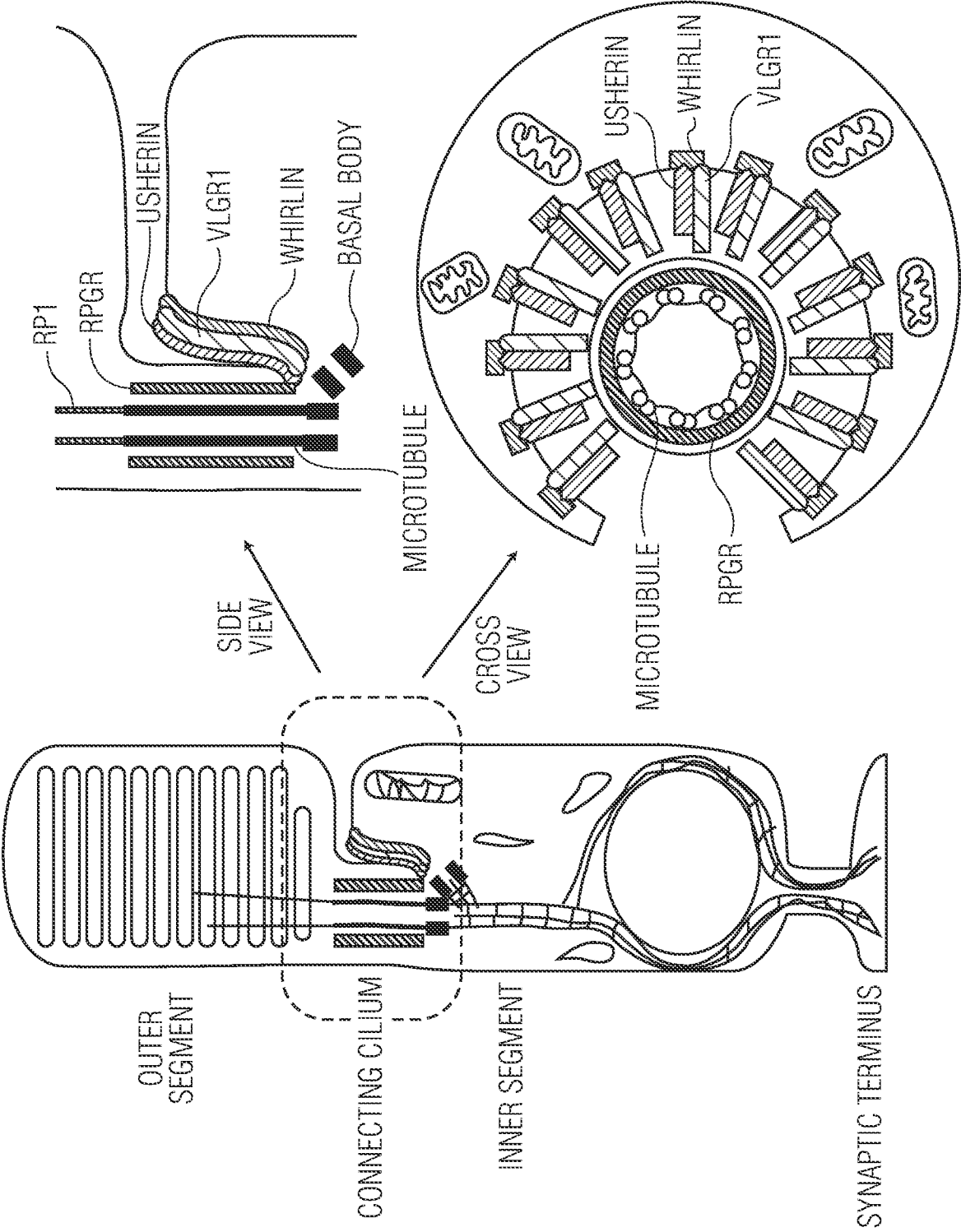


FIG. 3

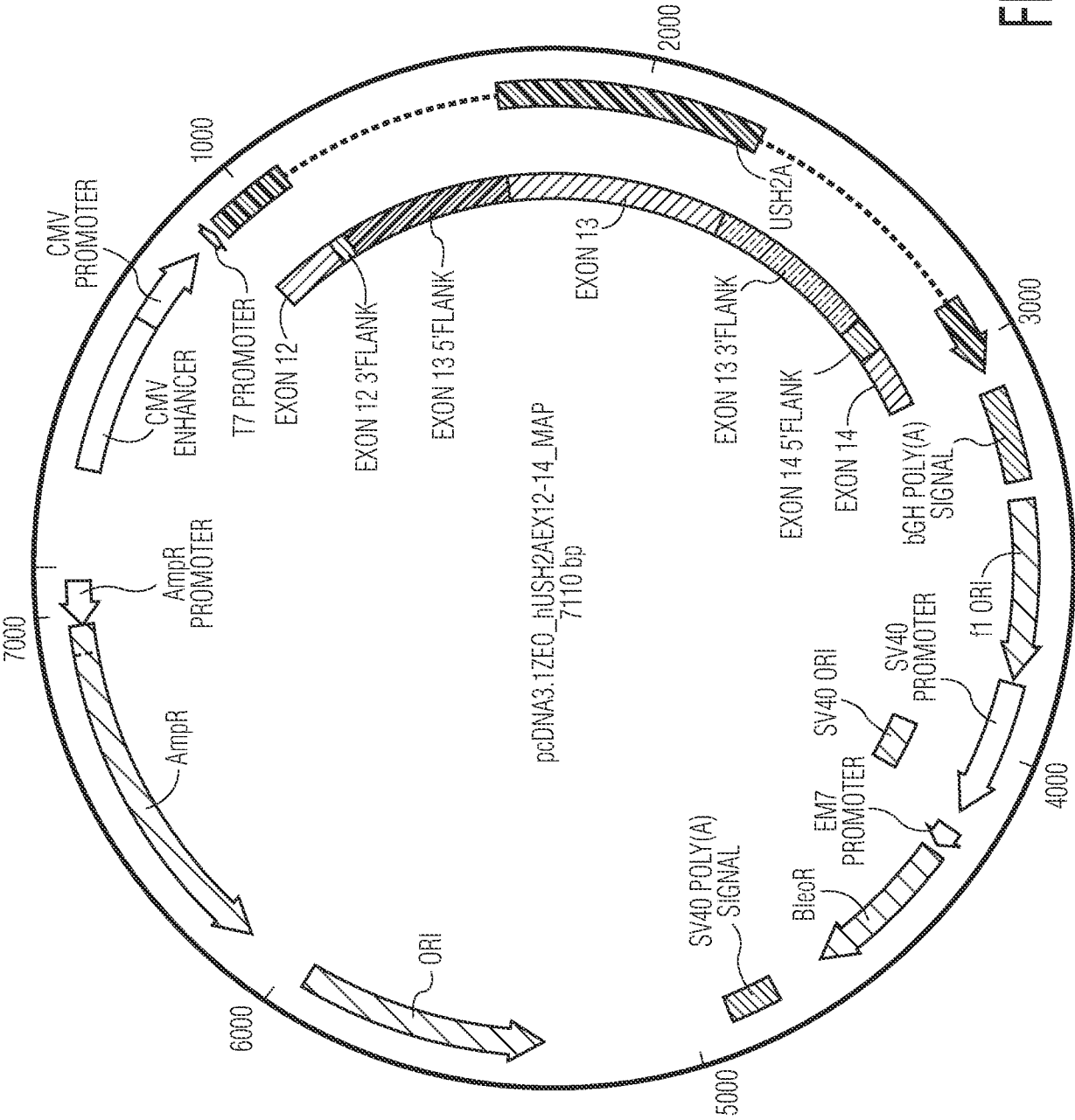


FIG. 4A

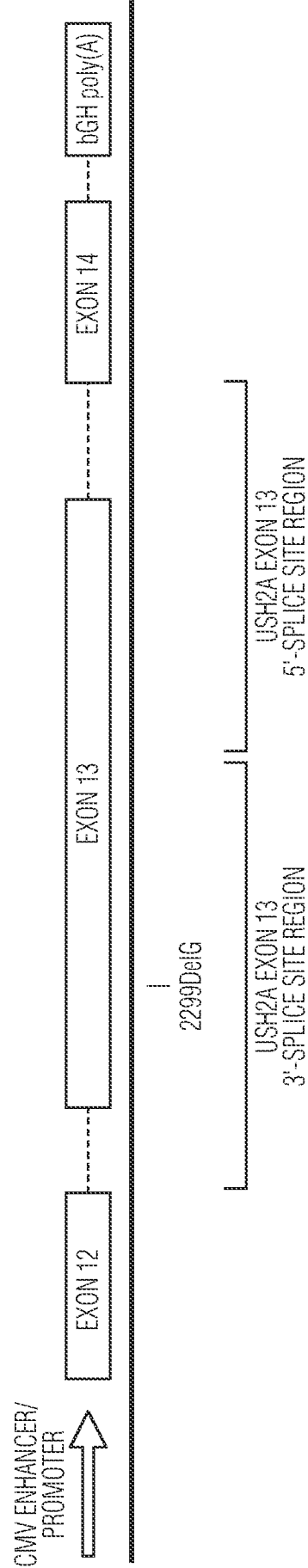
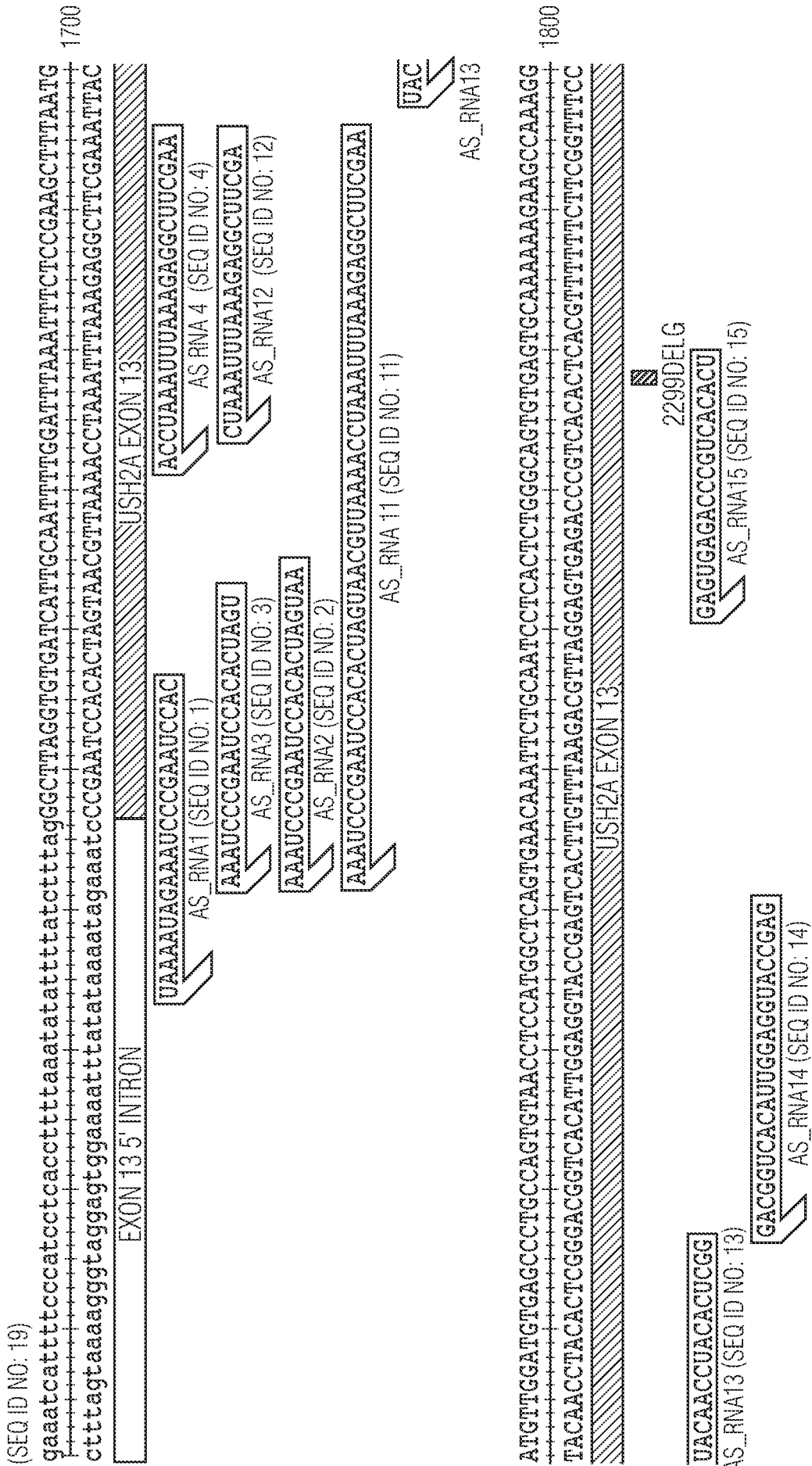


FIG. 4B



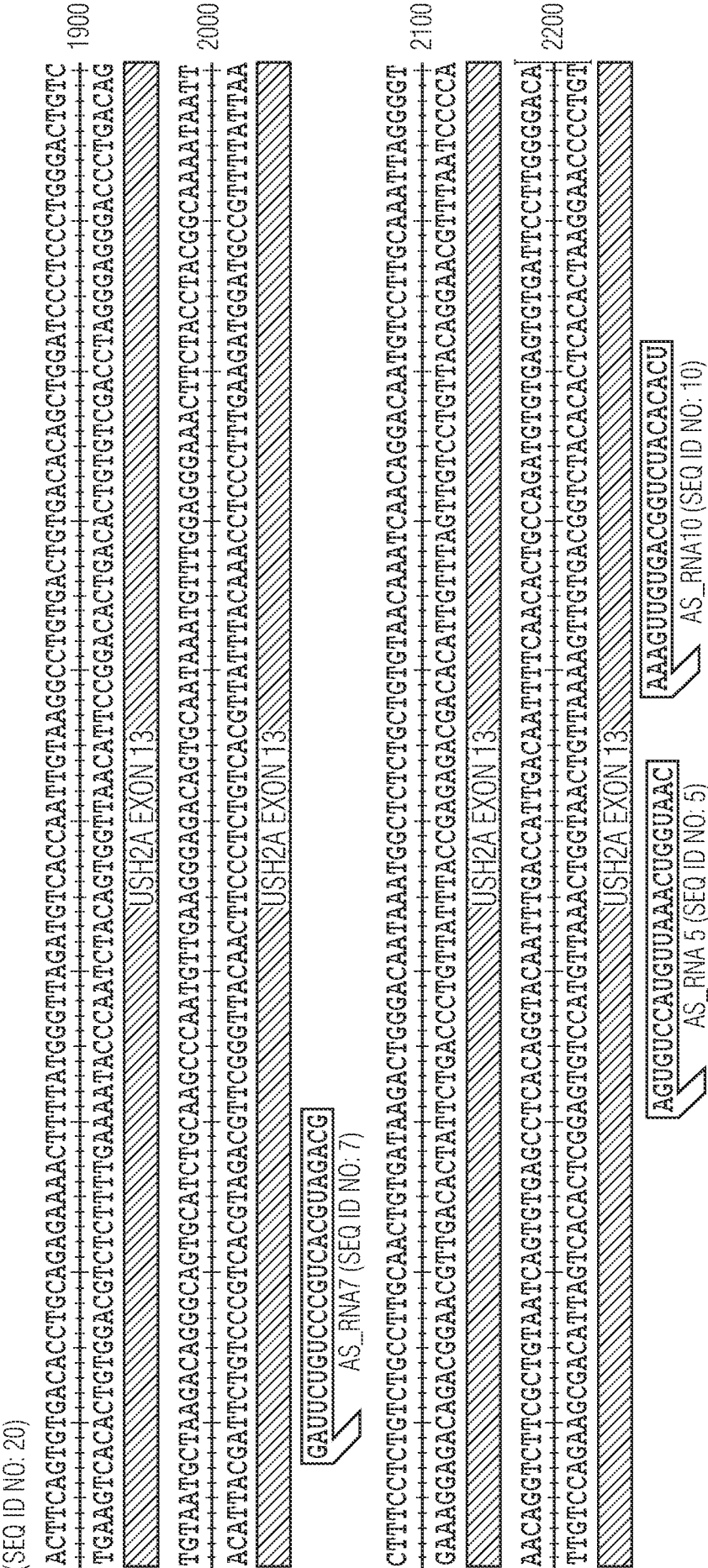


FIG. 5Bi

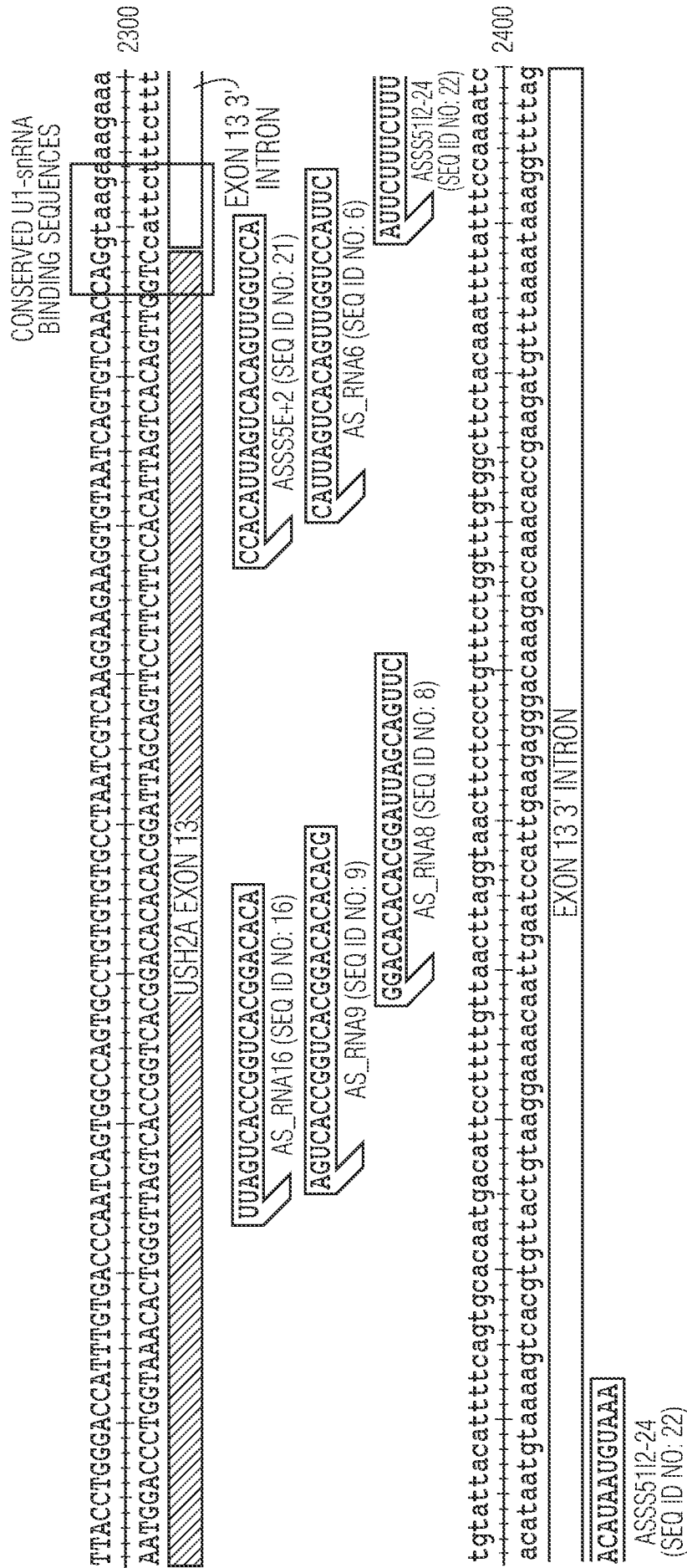


FIG. 5Bii

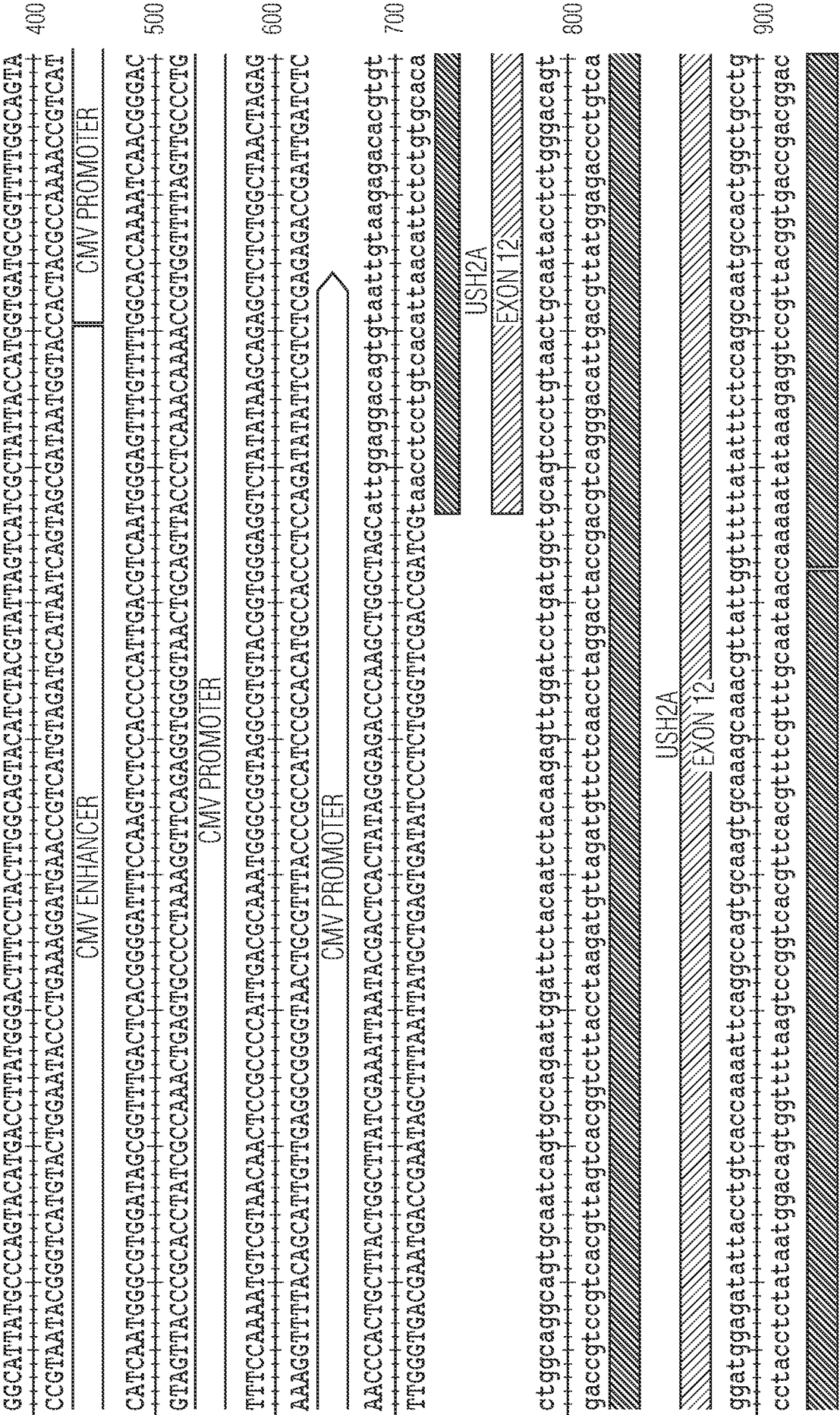


FIG. 50i

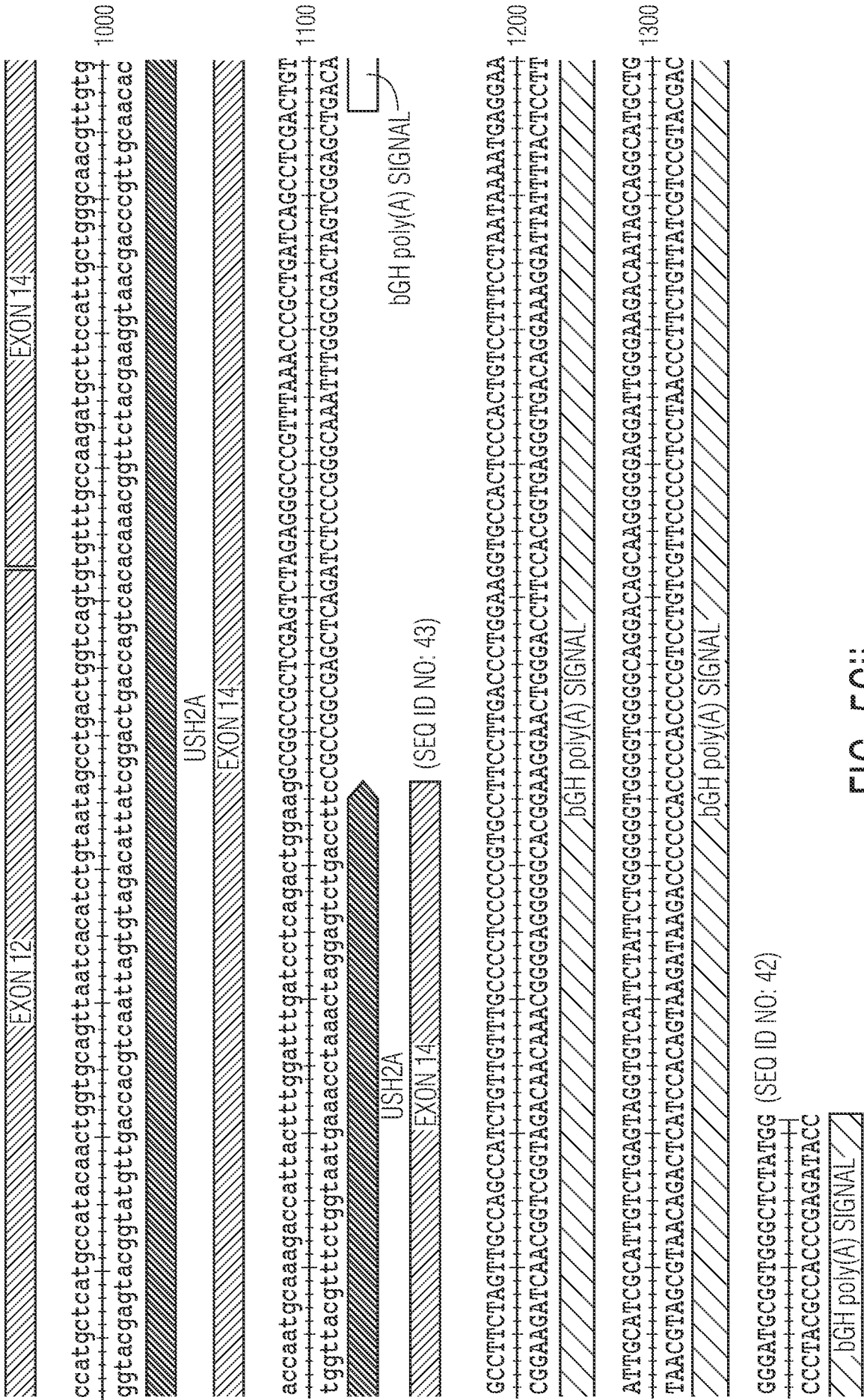


FIG. 50ii

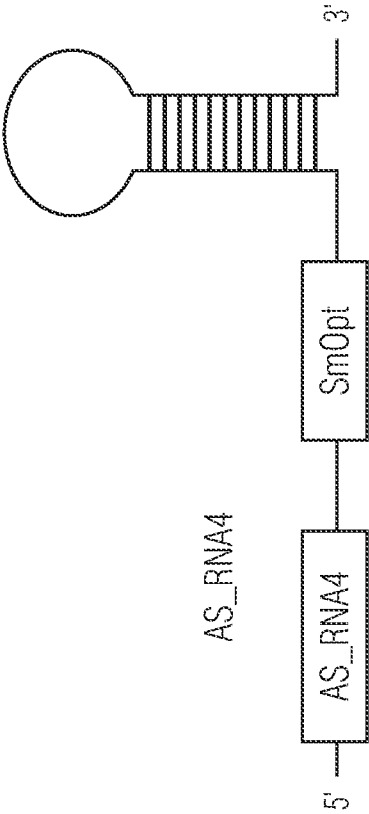
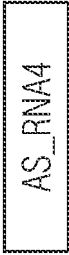


FIG. 5D

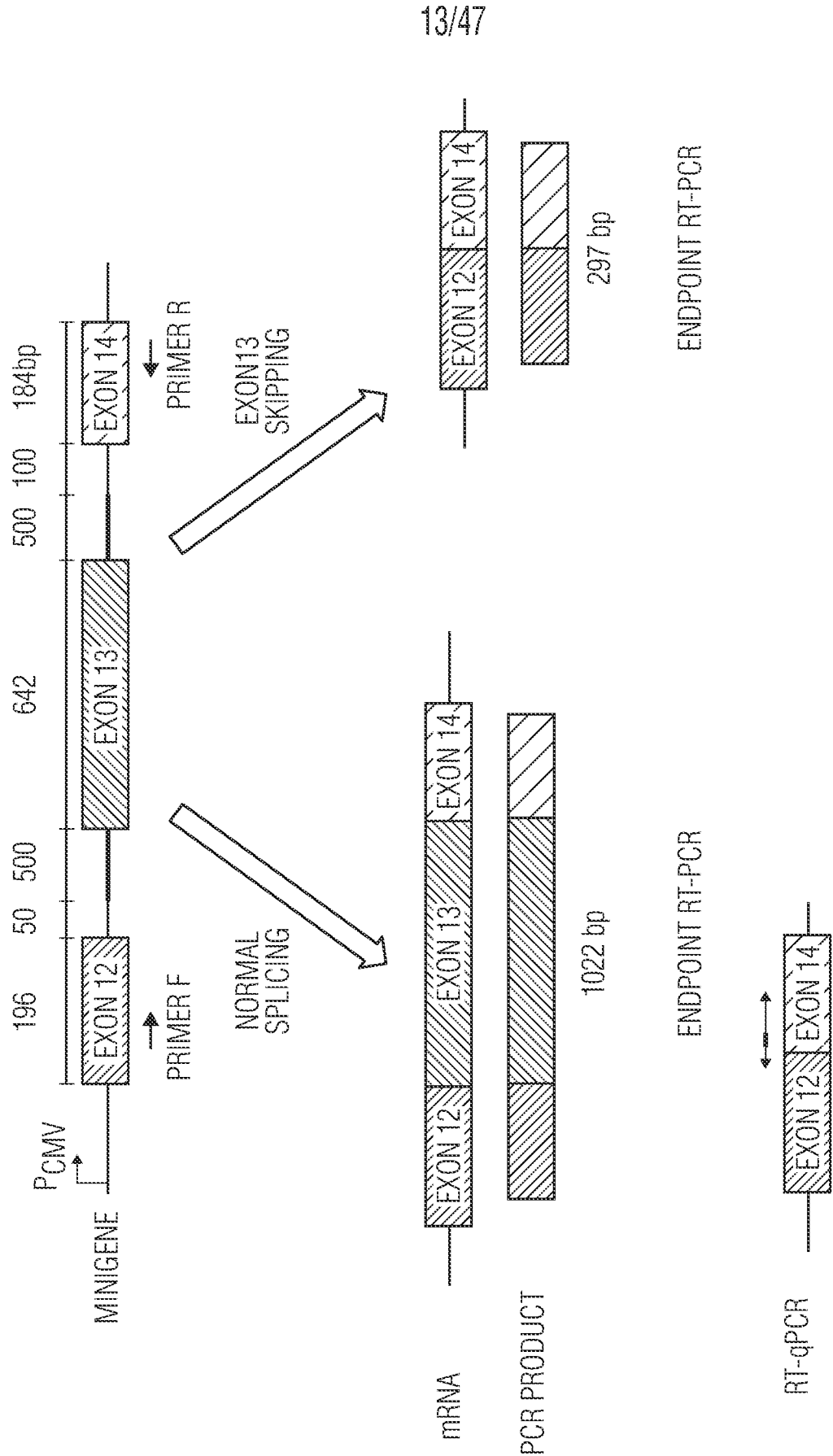


FIG. 6

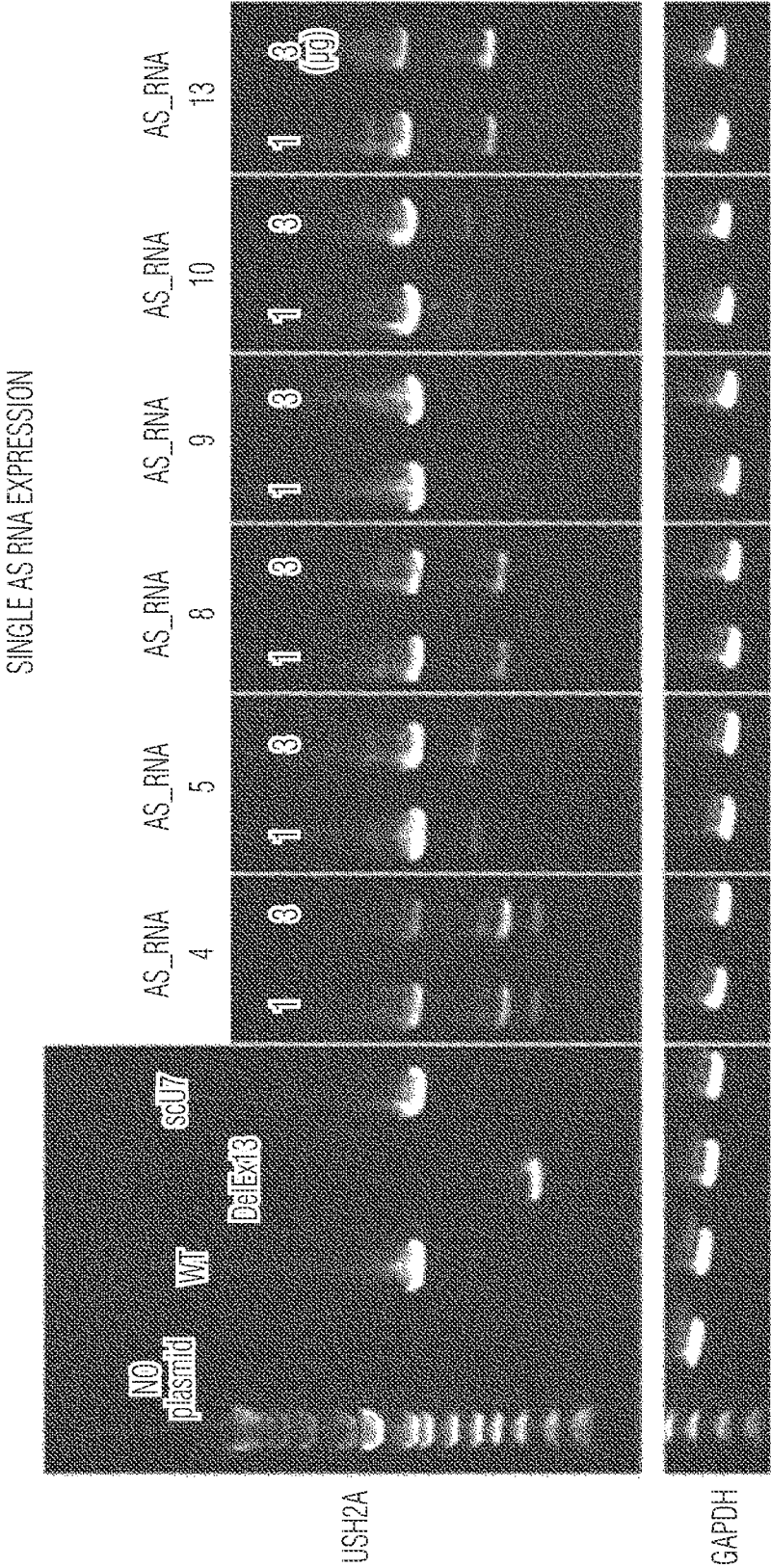


FIG. 7A

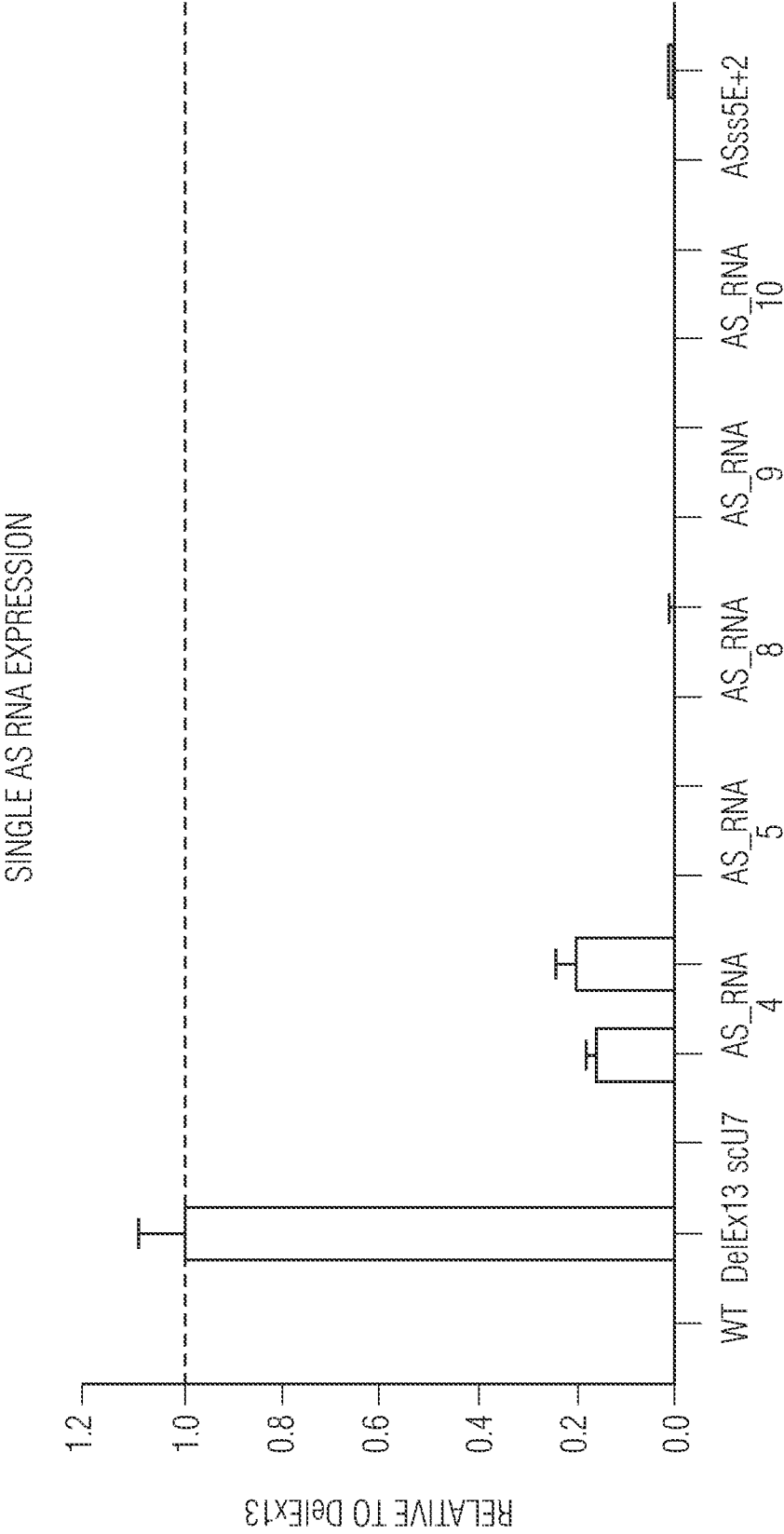


FIG. 7B

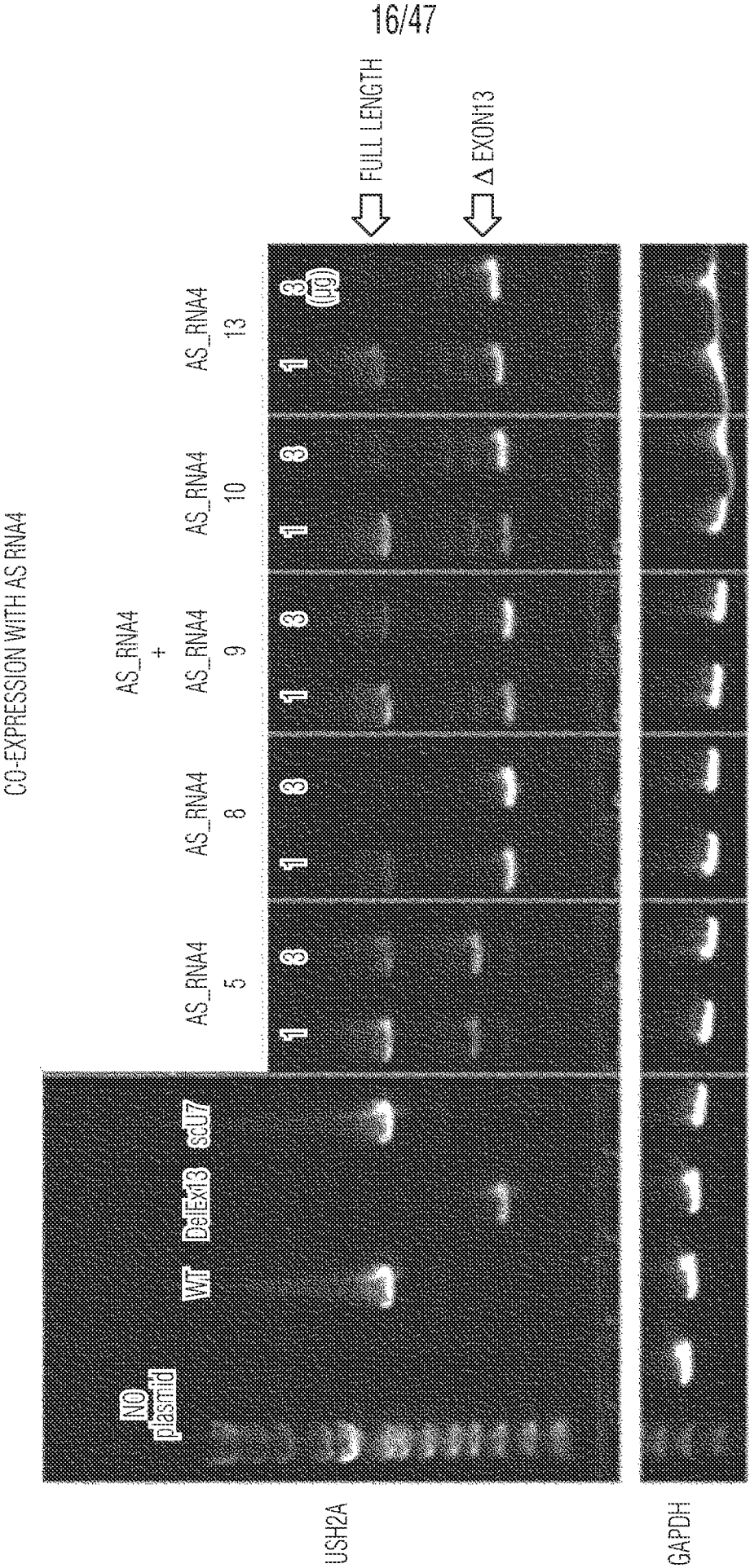


FIG. 7C

CO-EXPRESSION WITH AS RNA4

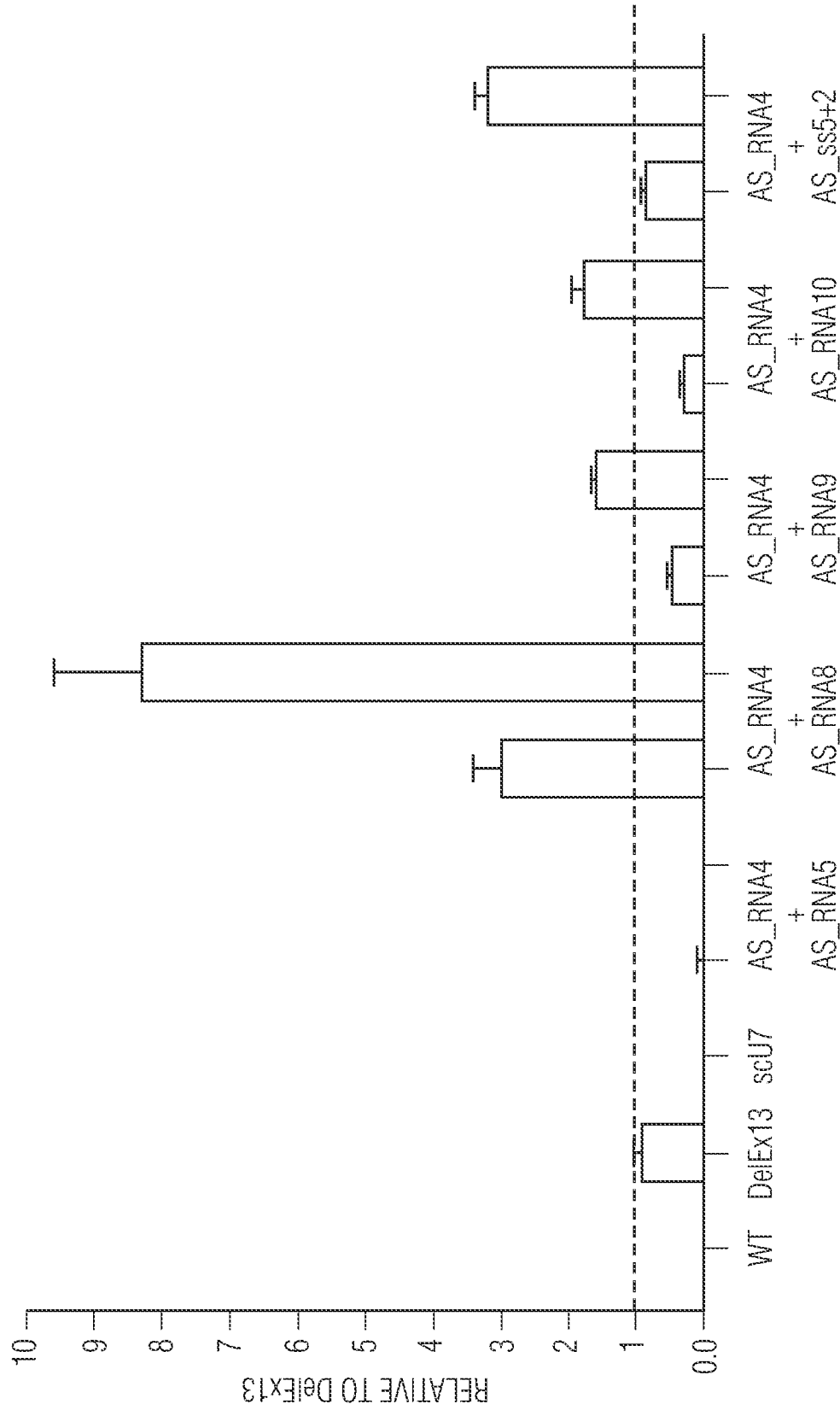


FIG. 7D

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GGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCGGGCGACCAAAGGTGCCCCGACGCCCGGGCTTTGC
CCGGTGAGGGAGAGACGCGCGAGCGAGCGAGTGACTCCGGCCCCGCTGGTTTCCAGCGGGCTGCGGGCCCCGAAACG 75
LEFT ITR
CCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGGAGGGG 150
GGCCCCGCGGAGTCACTCGCTCGCTCGCGCGTCTCTCCCTCACCGGTTGAGGTAGTGATCCCCAAGGACCTCCCC
LEFT ITR
TGGAGTCGTGACCTAGGGCGGGAGAAGATCAAGGGTTAGAGGAGTTGGGAGCAGACCCAGGGAAGGGCTTTTAGG 225
ACCTCAGCACTGGATCCCGCCCTCTTCTAGTTCCCAATCTCCTCAACCCTCGTCTGGGTCCCTTCCCCAAATCC
LEFT ITR
ACAGGGTTTAGAGACAGCTAACTTCAAGGGTTTACACTTTTACAAGGATACTCAGGCAGACAACCTTACAGAGA 300
TGTCCTCAATCTCTGTGCAATTTGAAGTTCCCAAAATGTGAAAATGTTCCATAGAGTCCGTCTGTTGGAATGTCTCT
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FIG. 8Ai

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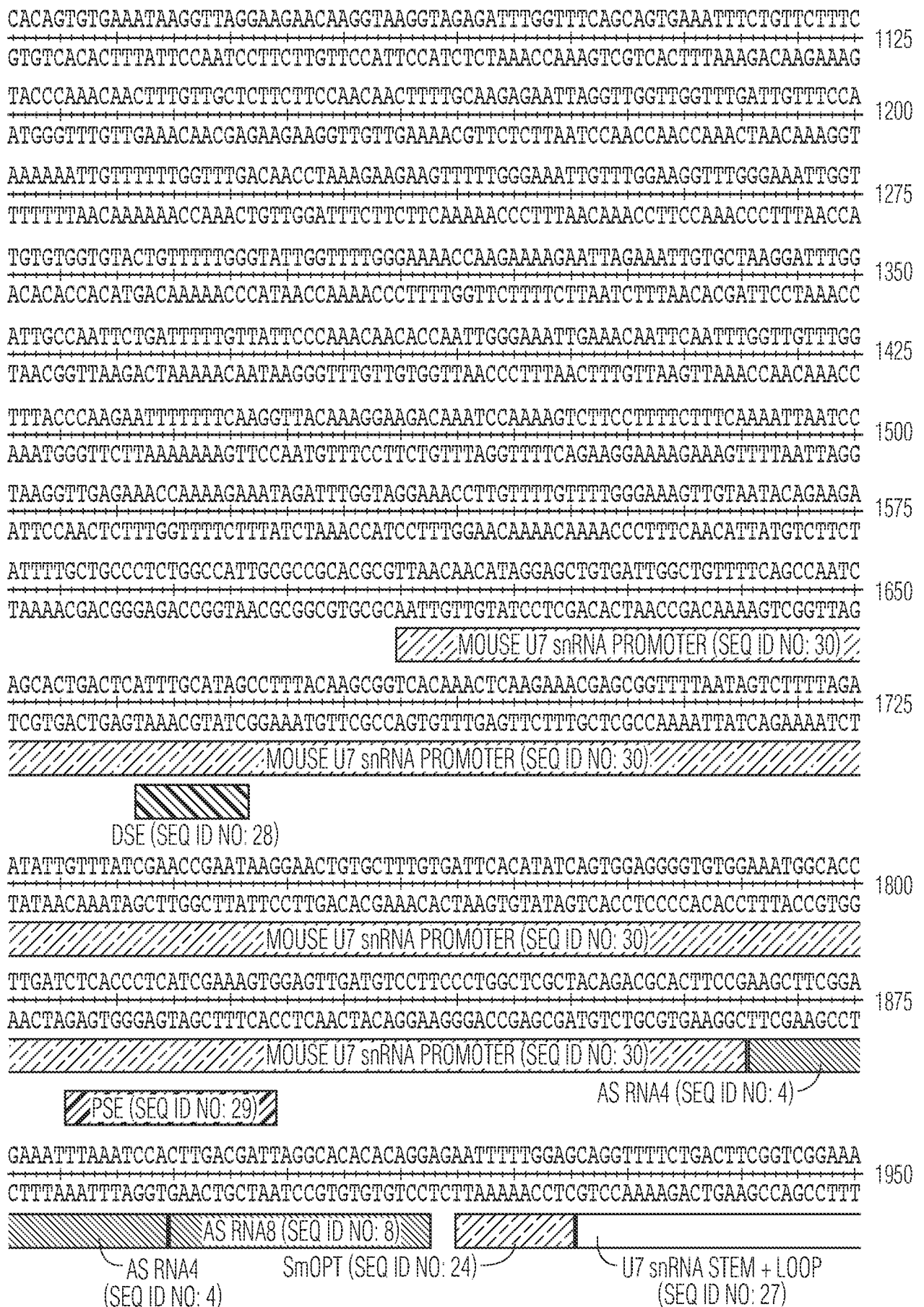


FIG. 8Aii

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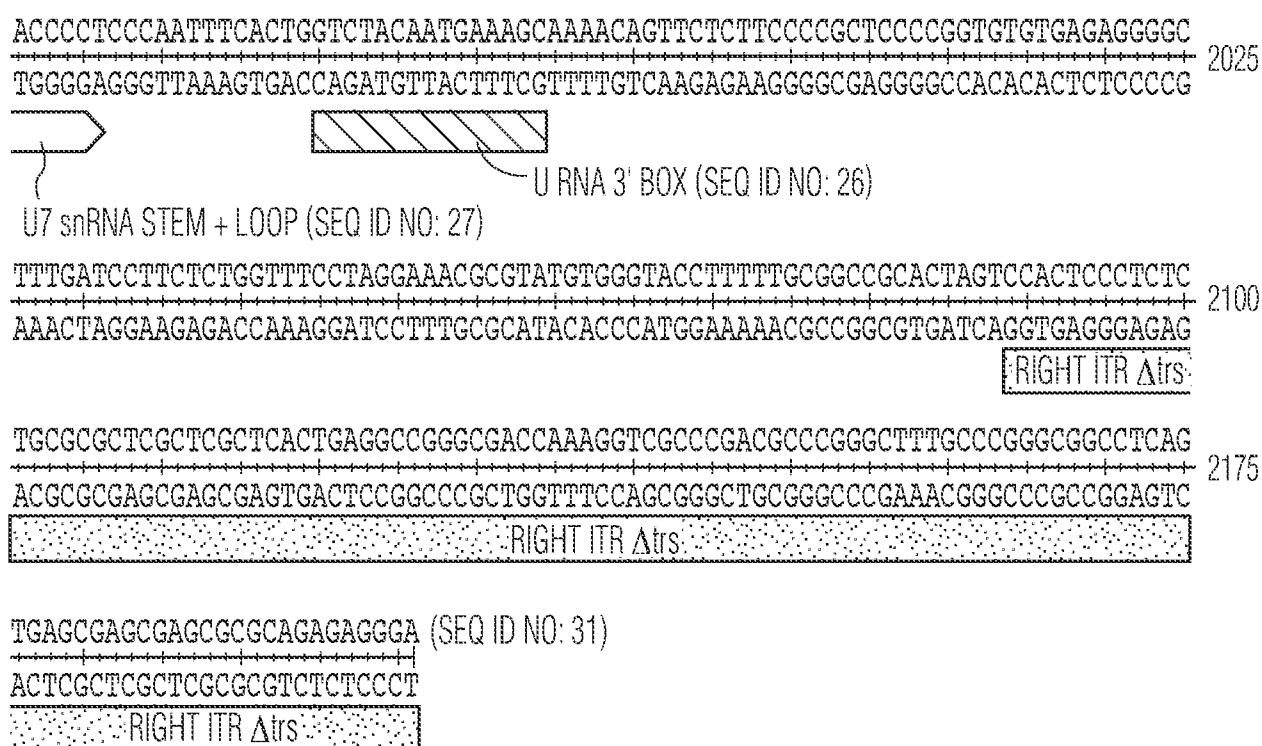


FIG. 8Aiii

AS_RNA4+8

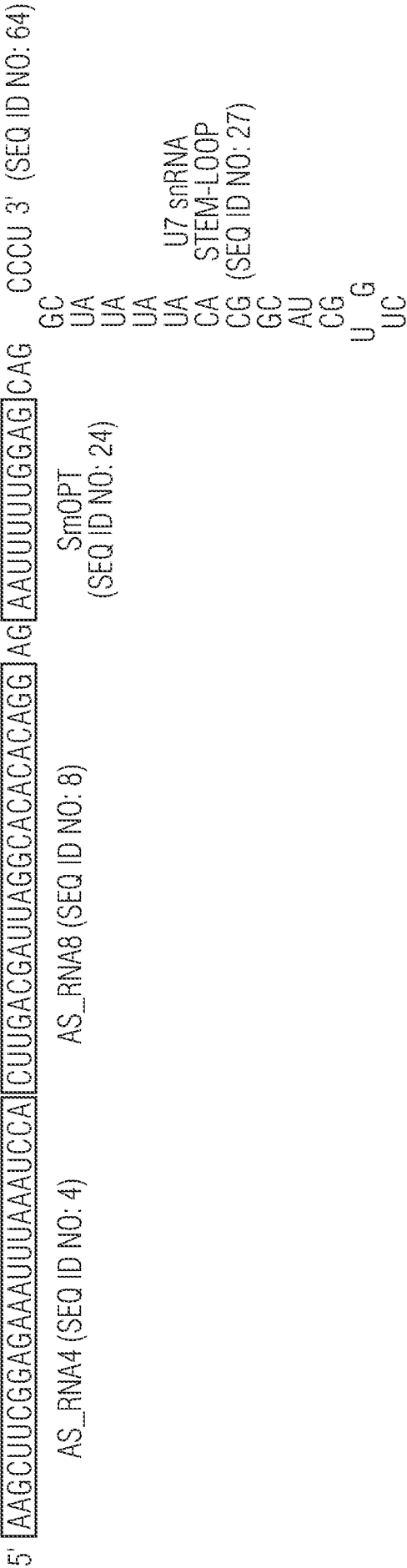


FIG. 8B

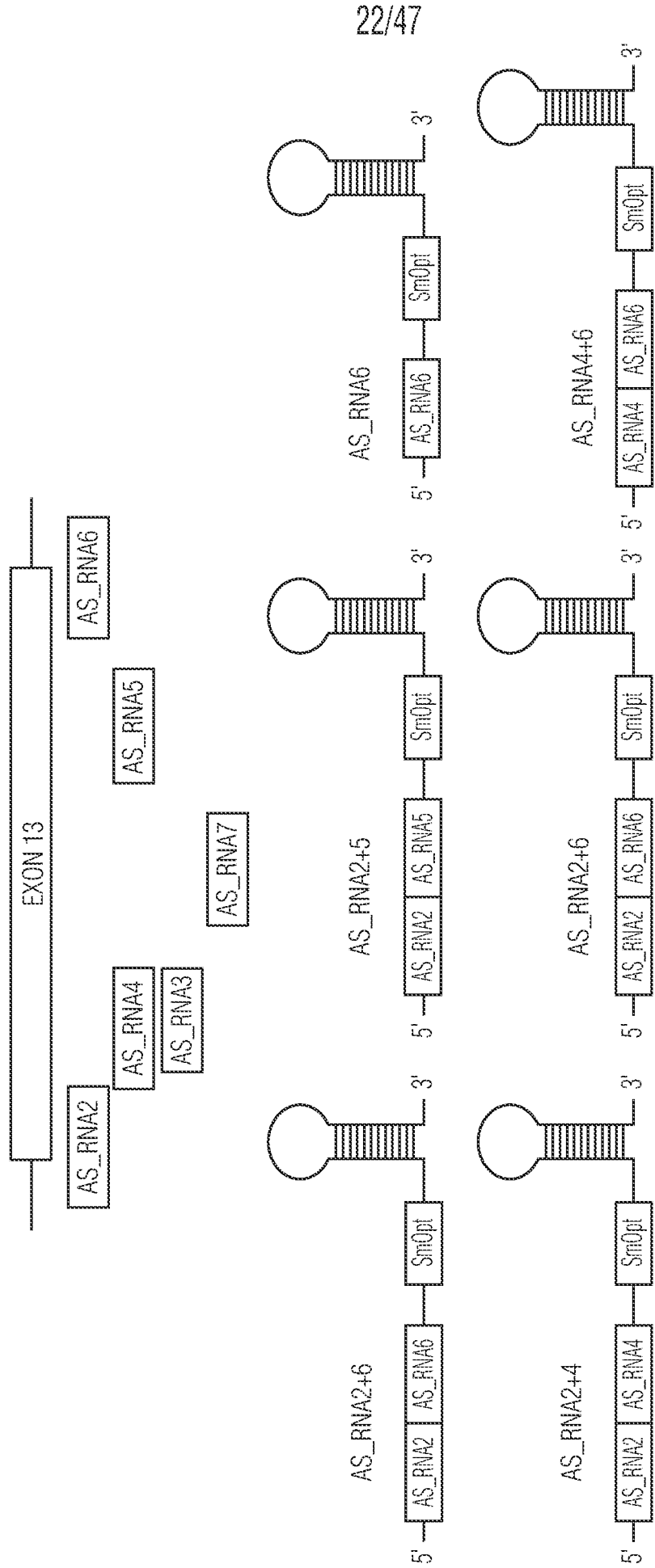


FIG. 8C

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FIG. 8D

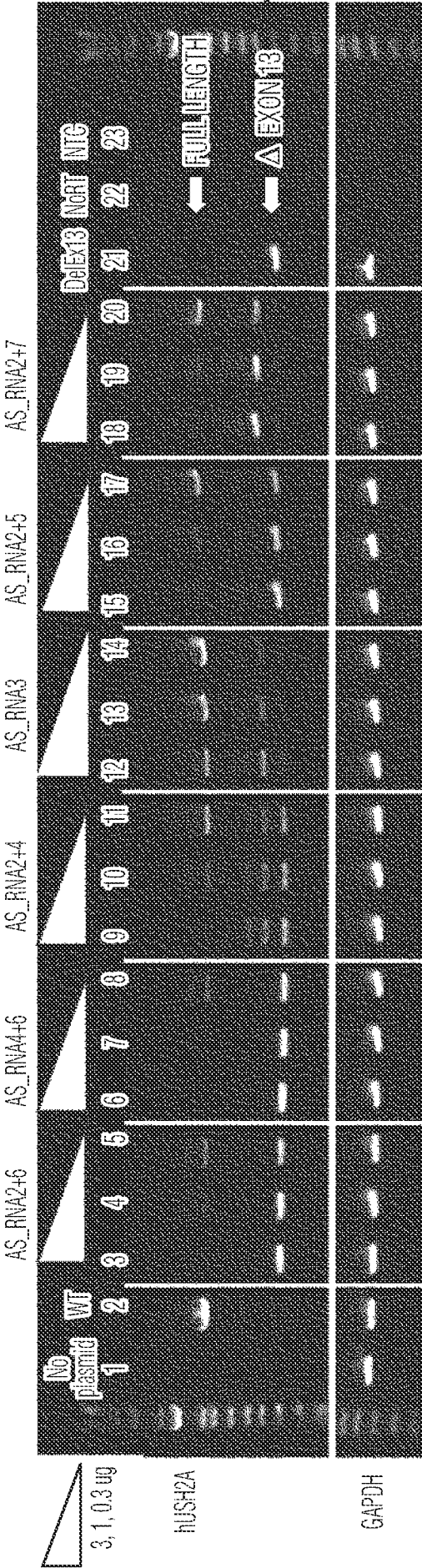


FIG. 9A

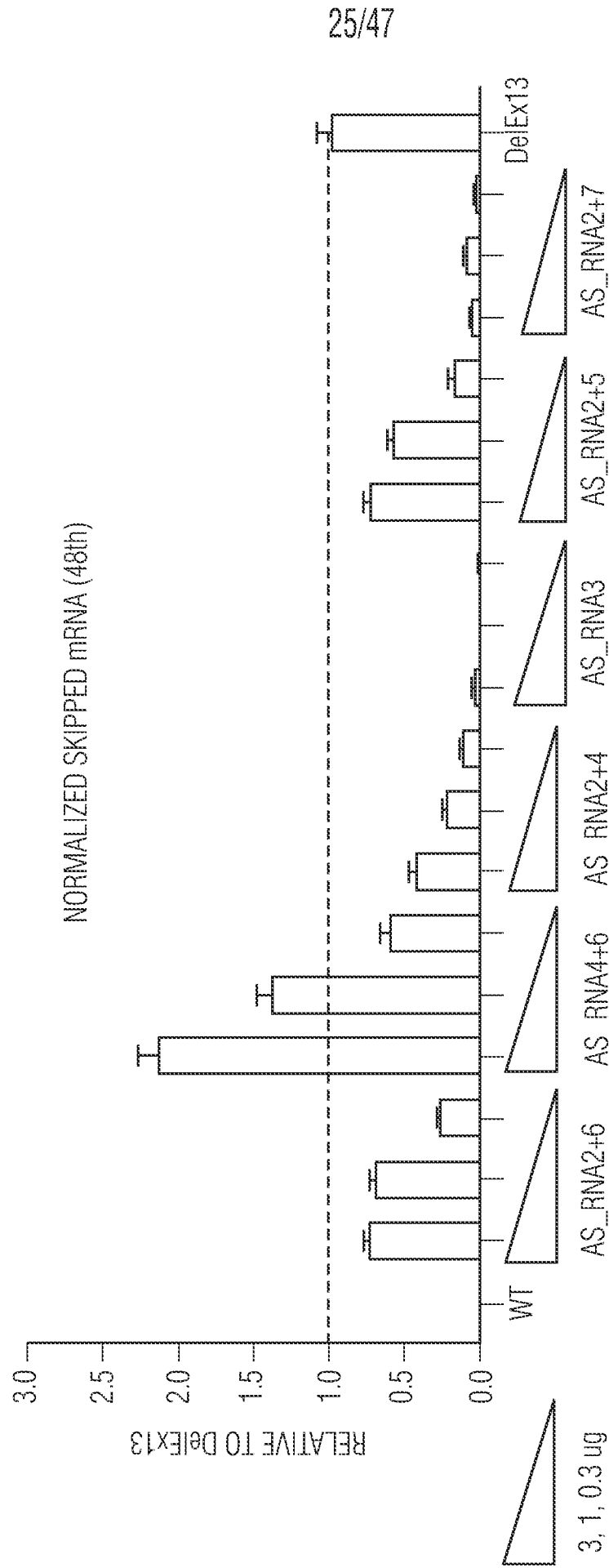


FIG. 9B

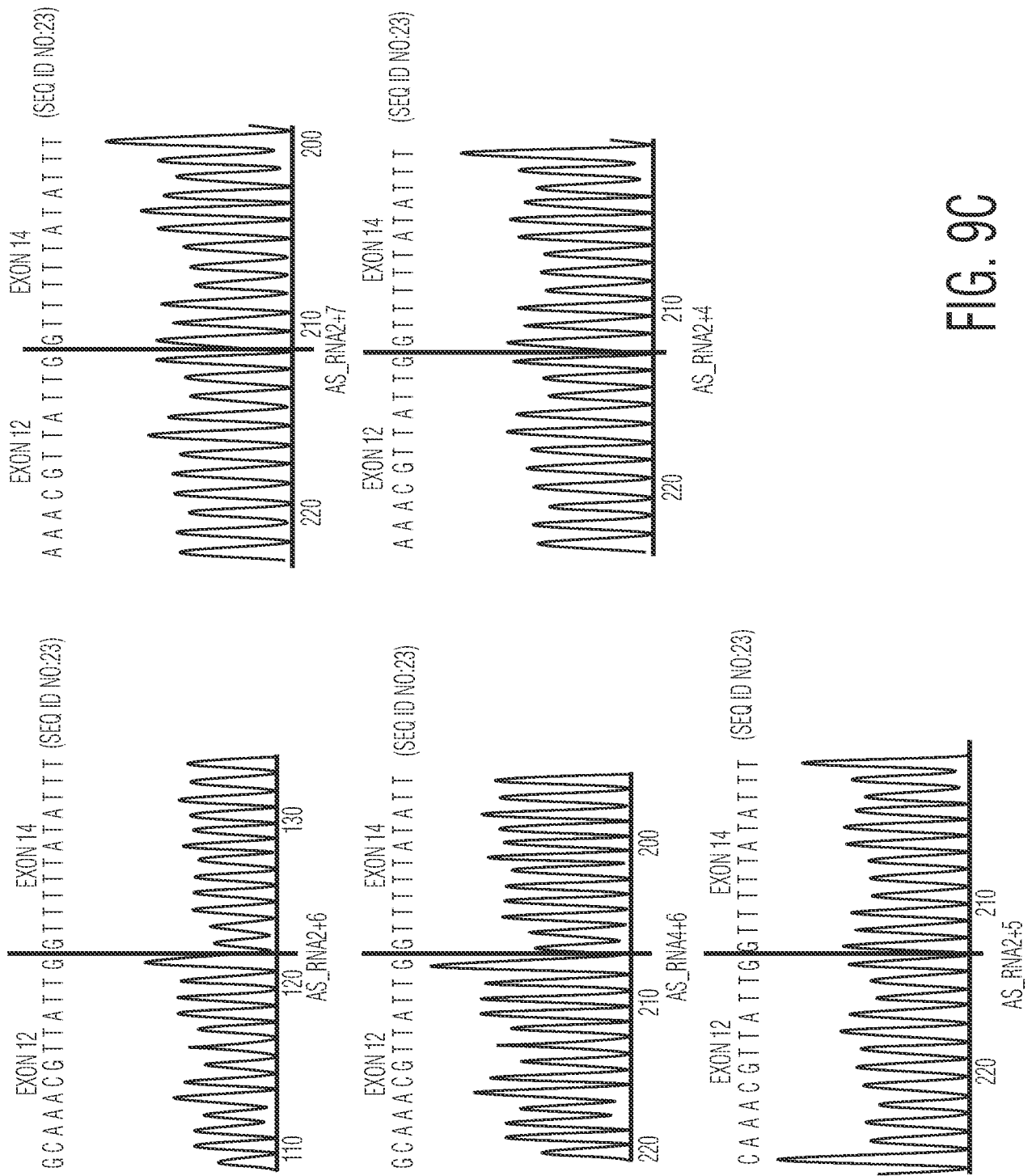


FIG. 9C

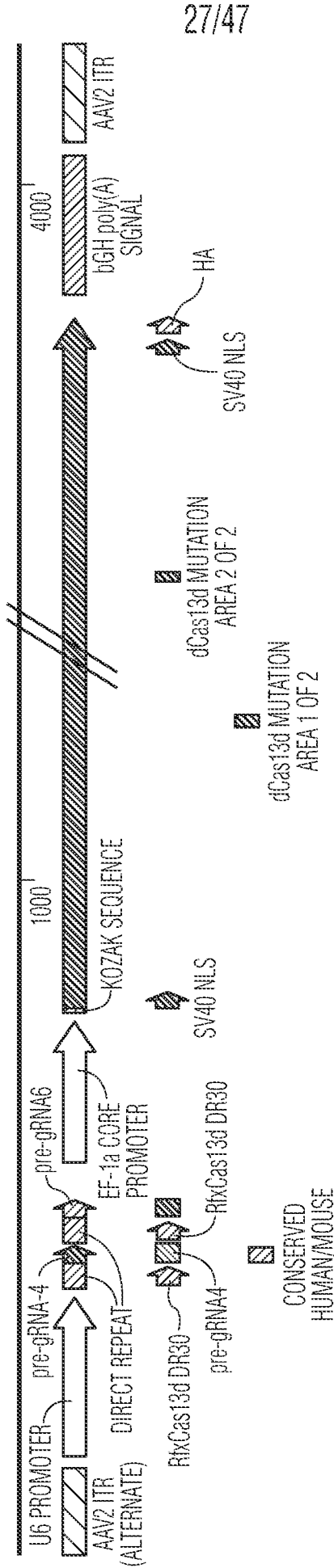


FIG. 10A

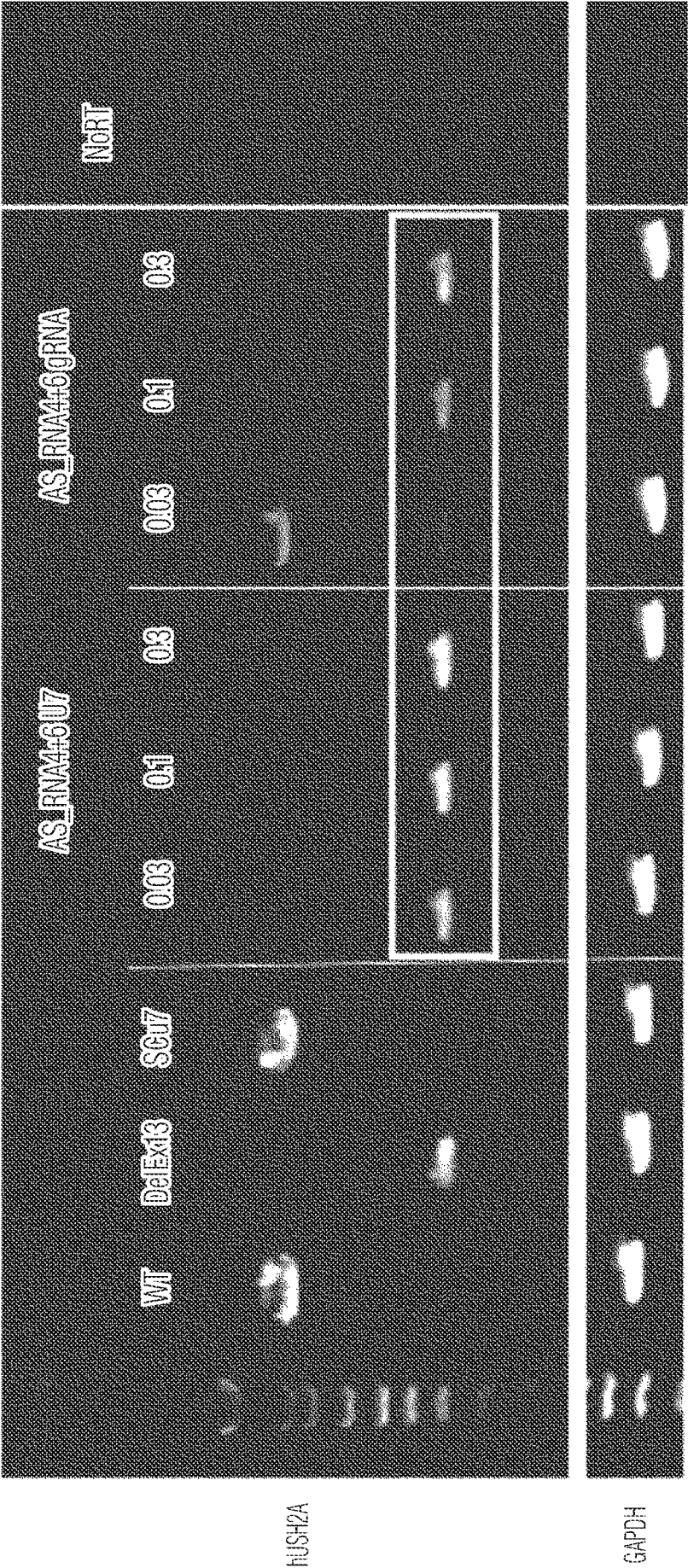


FIG. 10B

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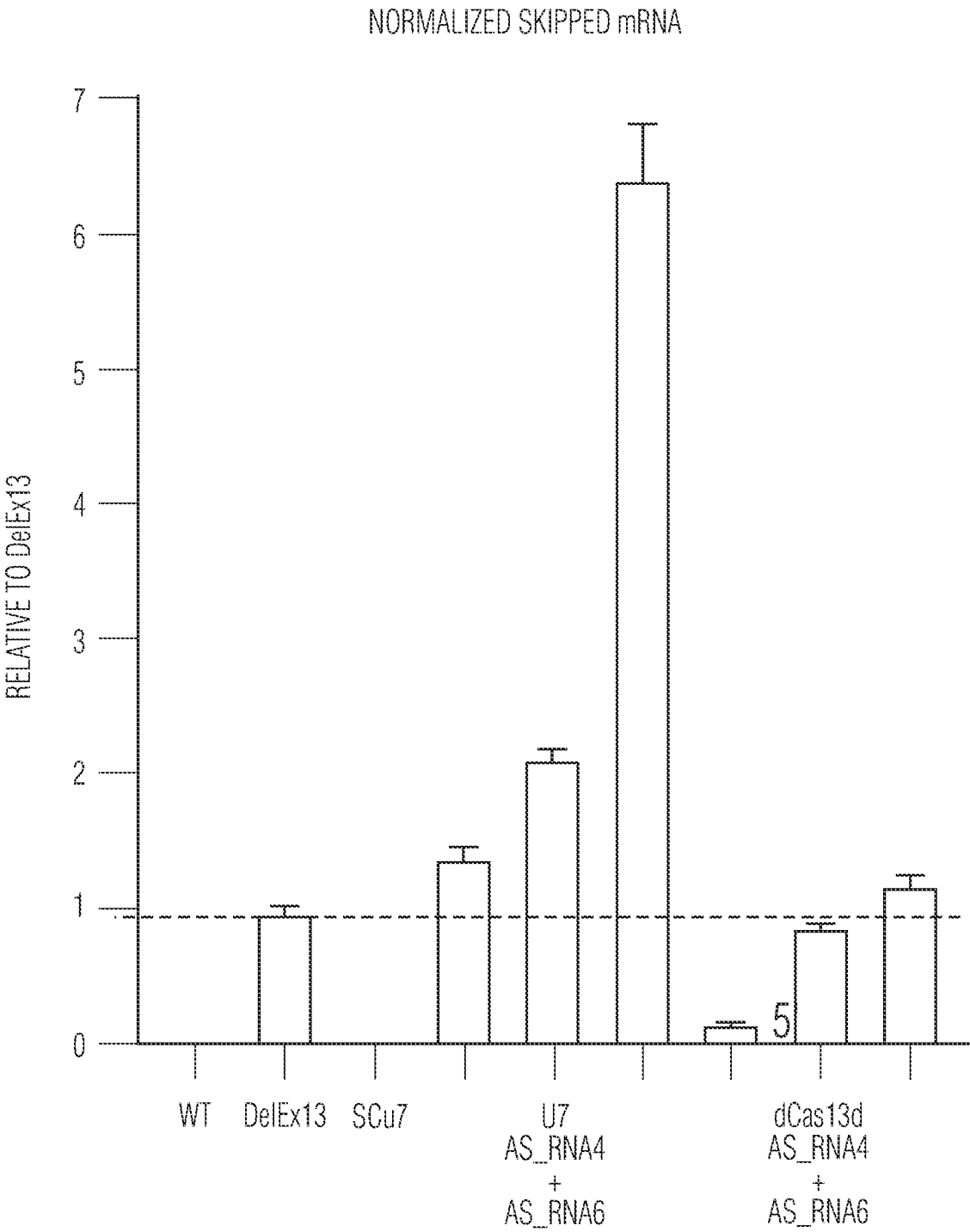


FIG. 10C

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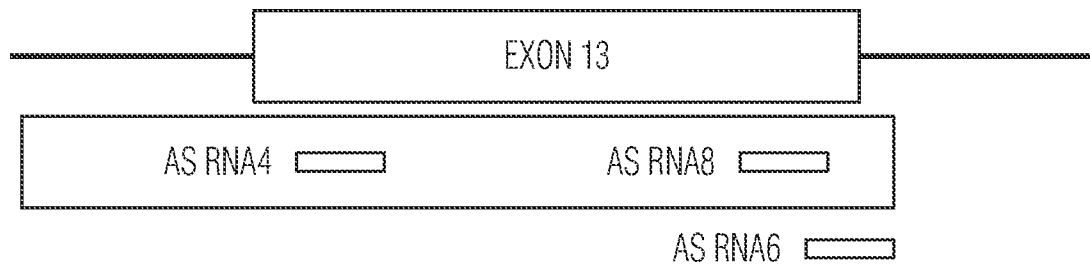


FIG. 11A

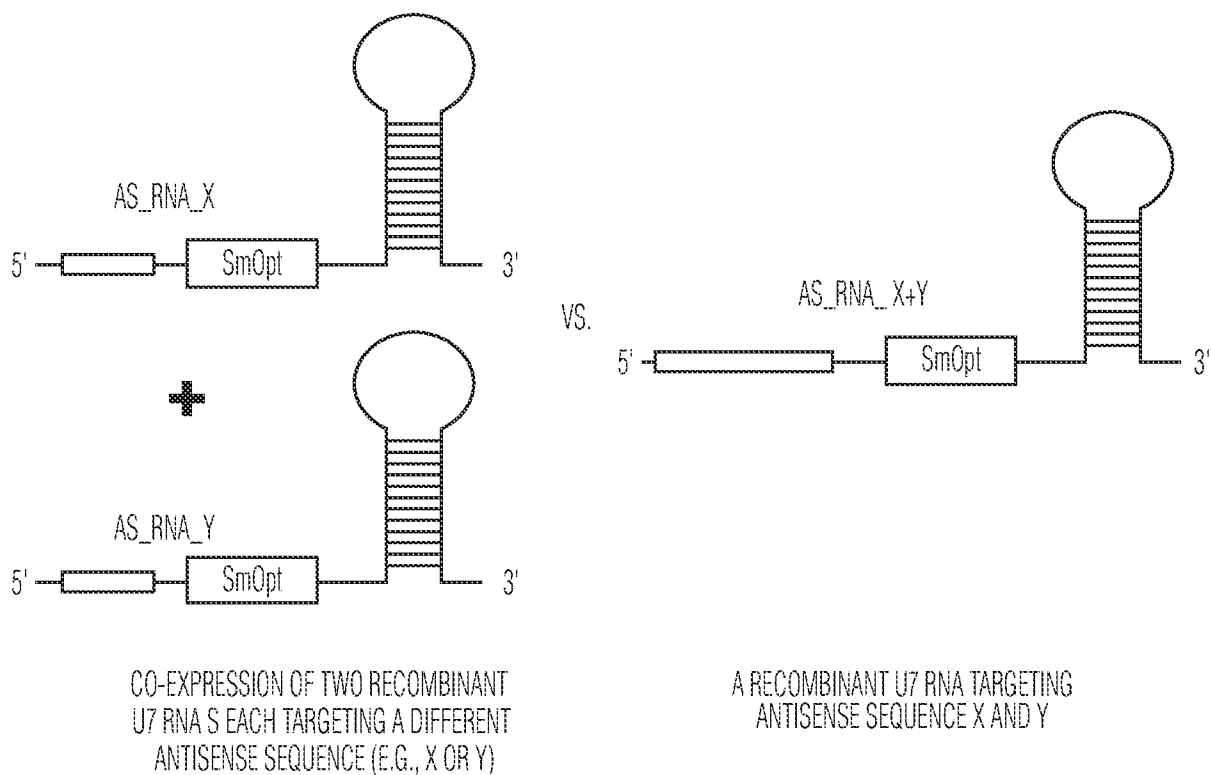


FIG. 11B

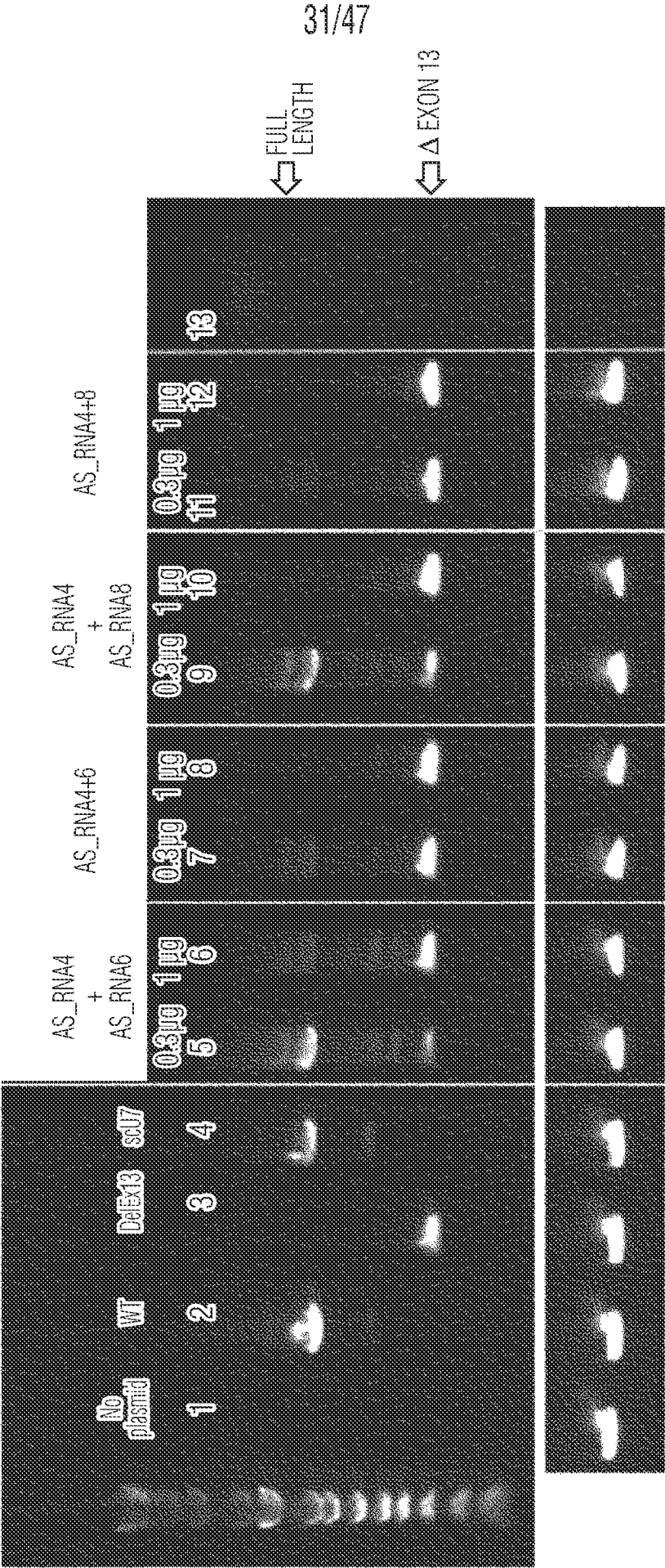


FIG. 11C

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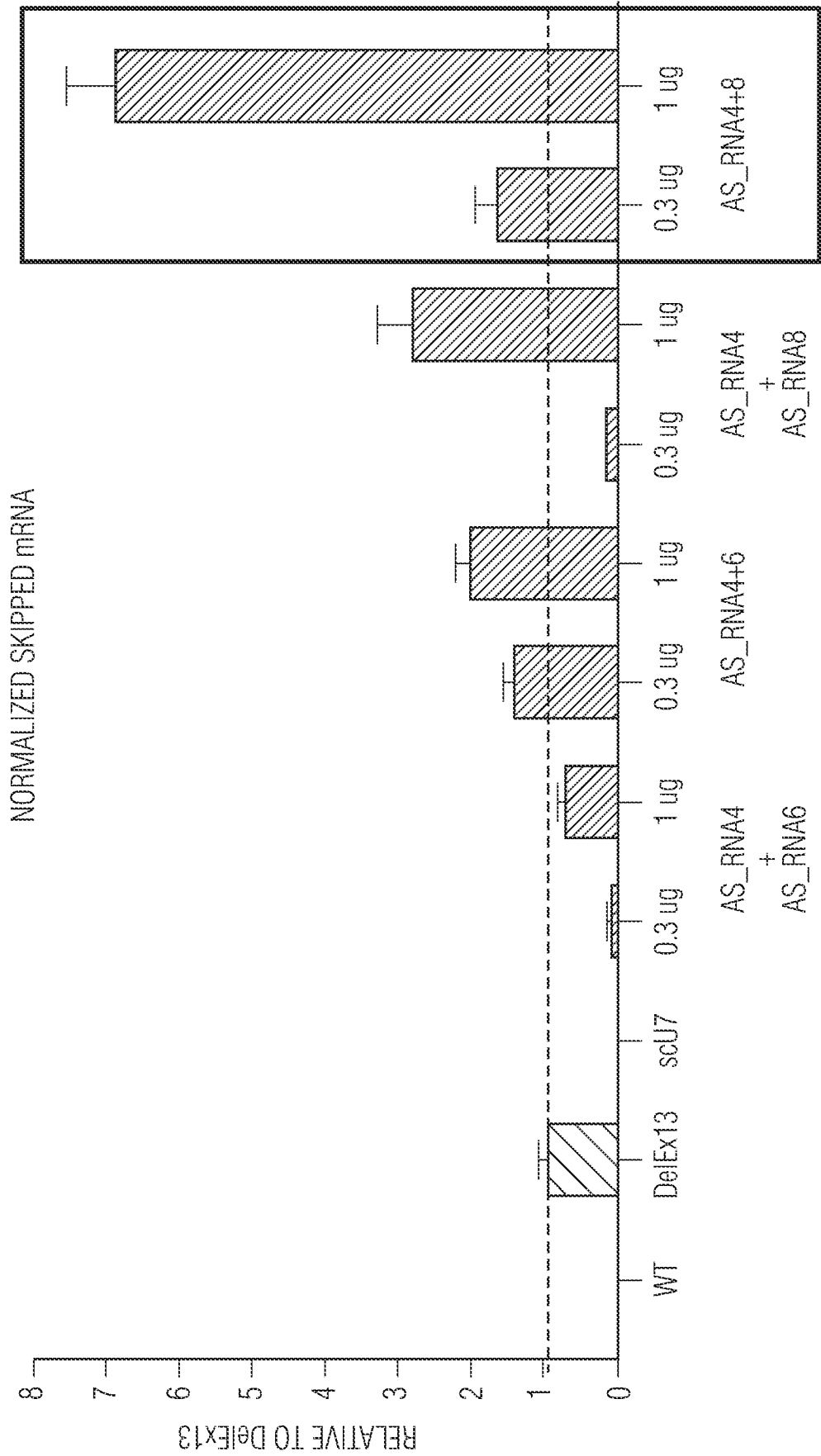


FIG. 11D

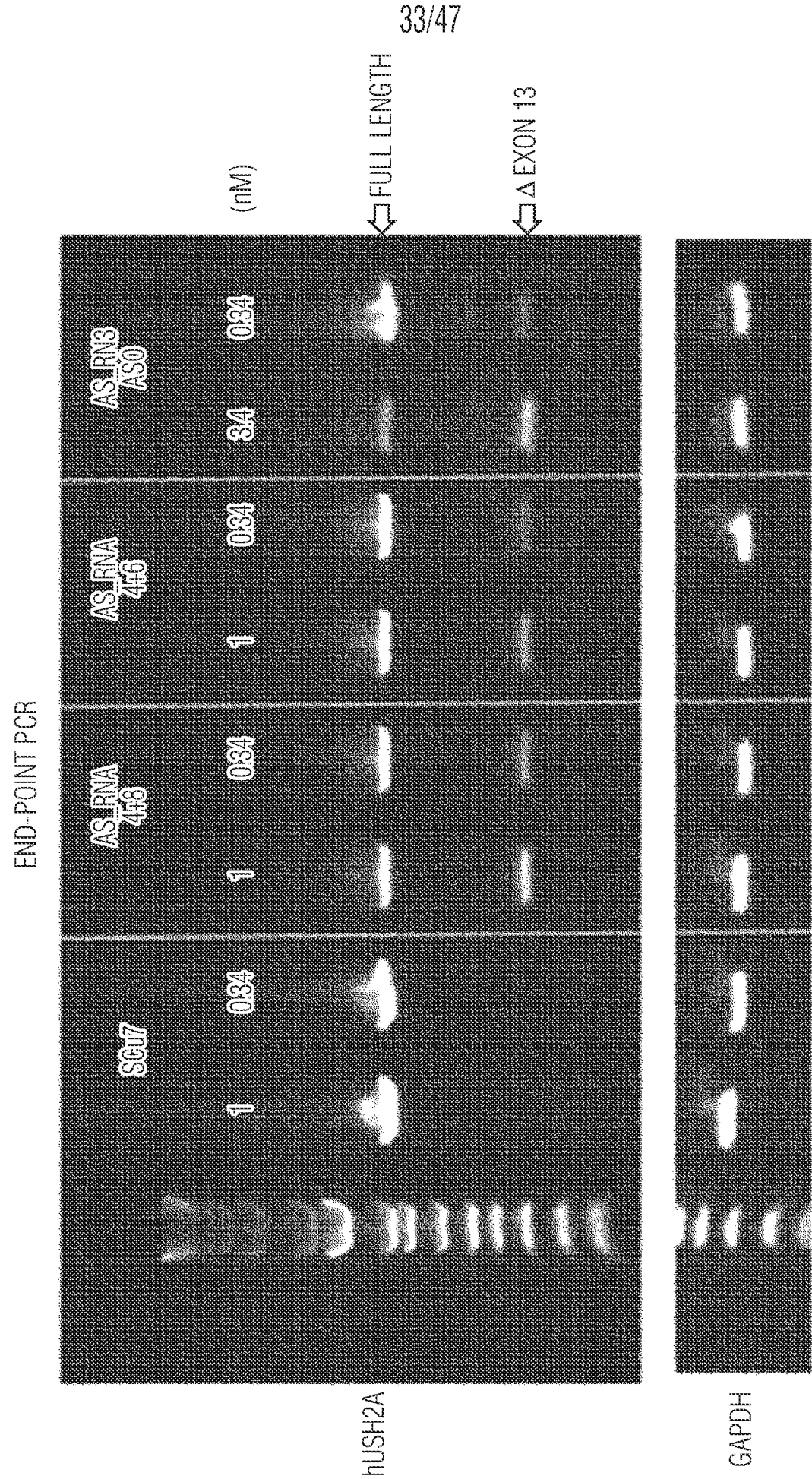


FIG. 12A

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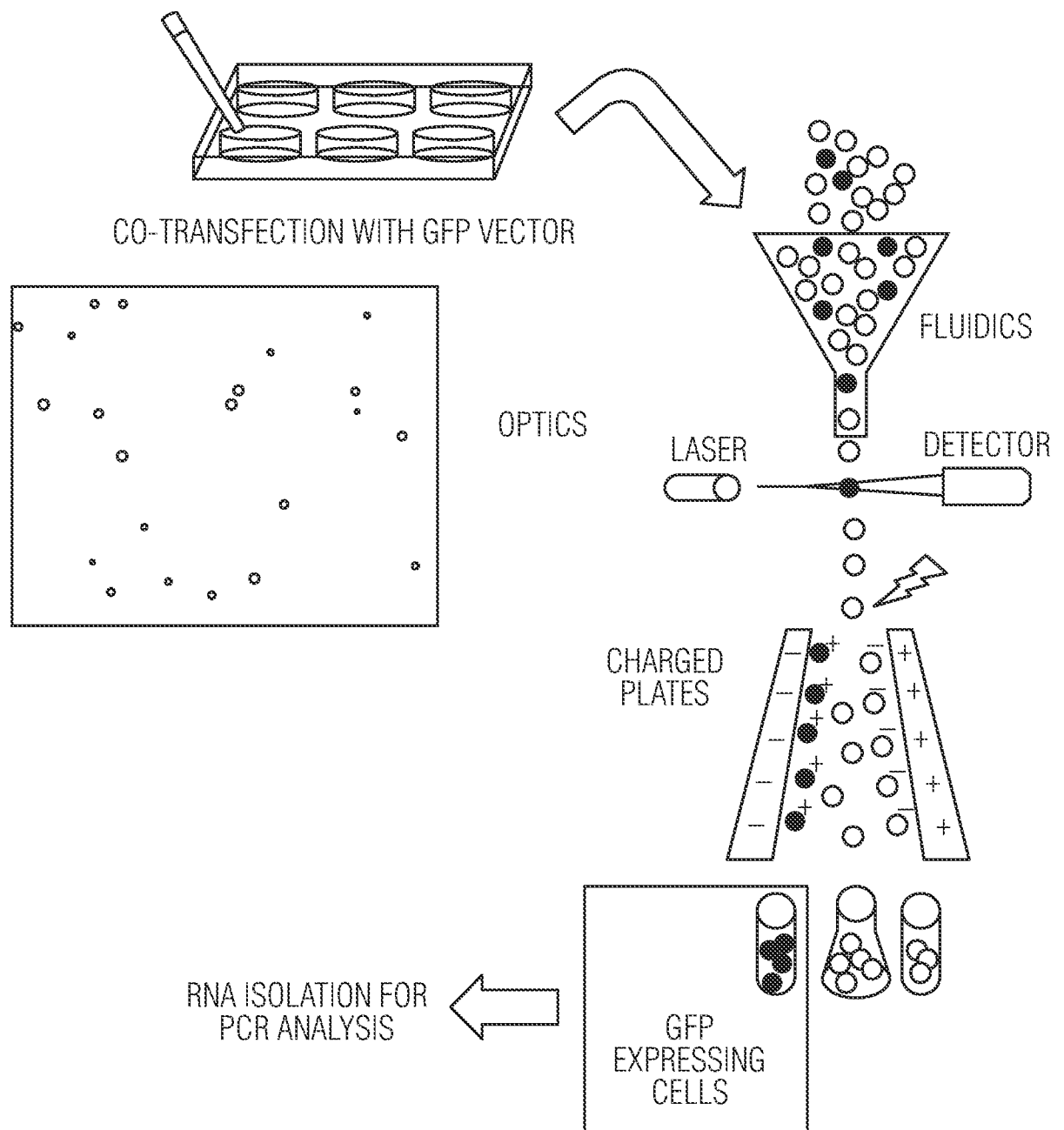


FIG. 12B

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END-POINT PCR

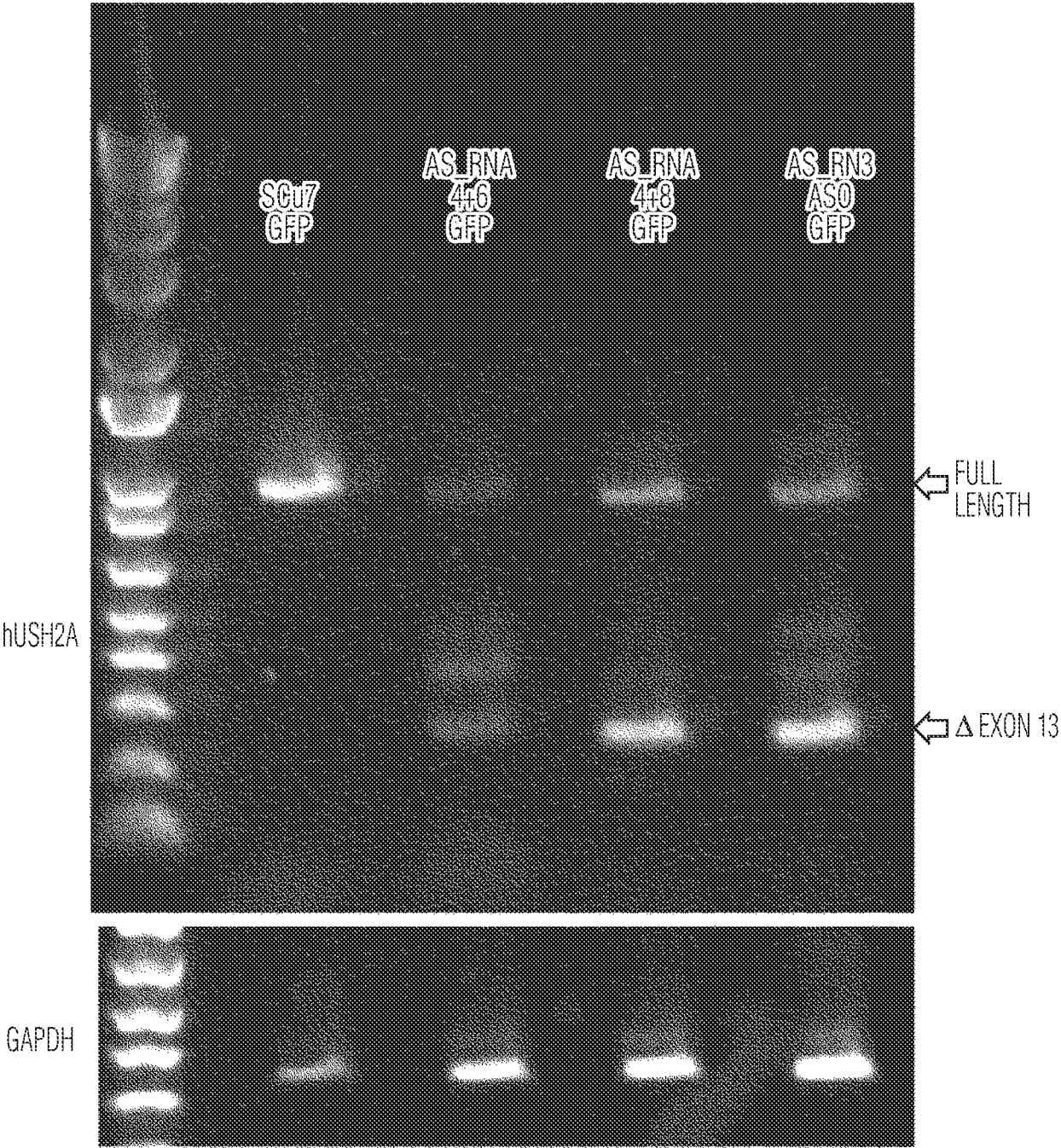


FIG. 12C

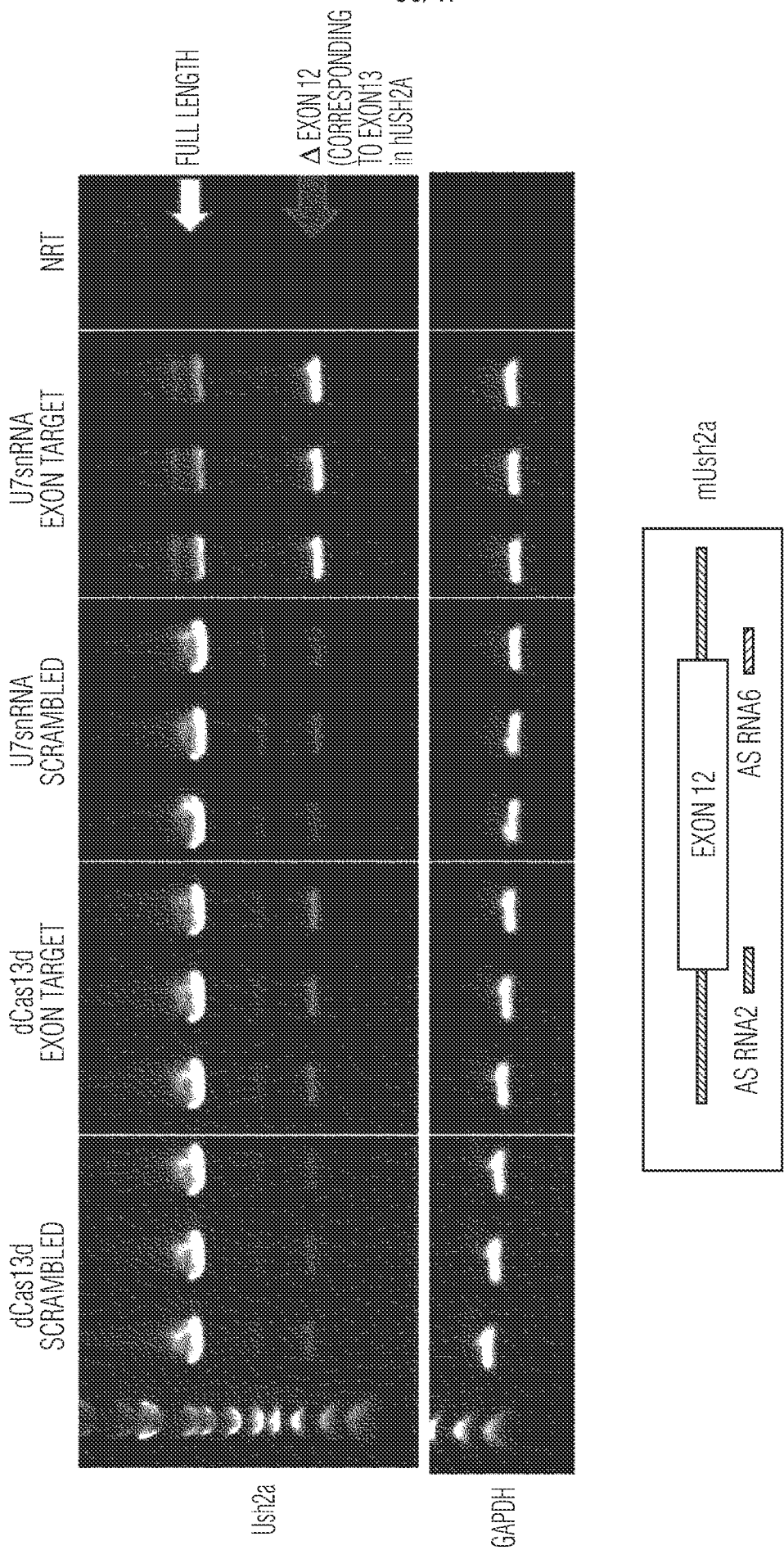


FIG. 13

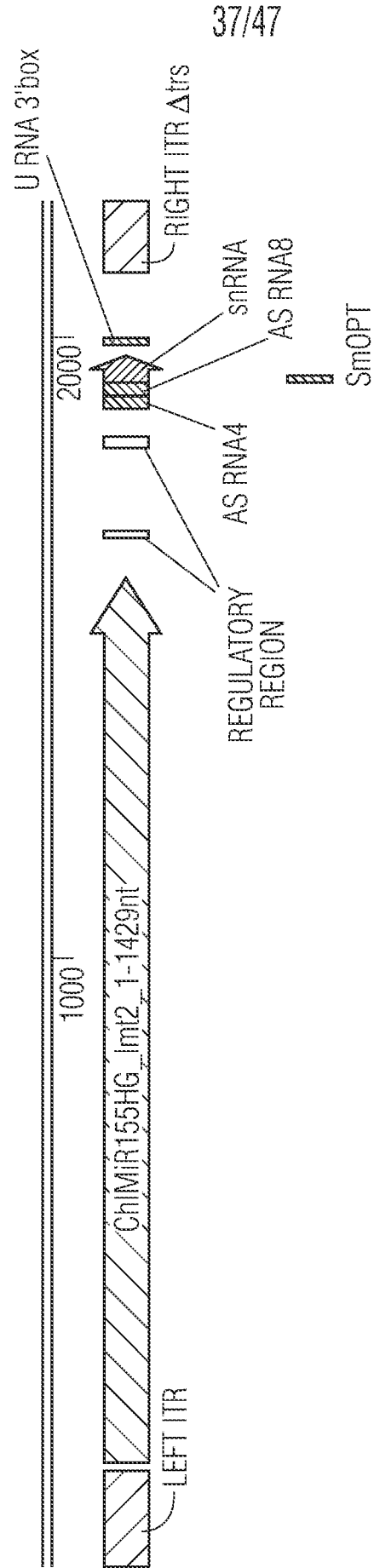


FIG. 14A

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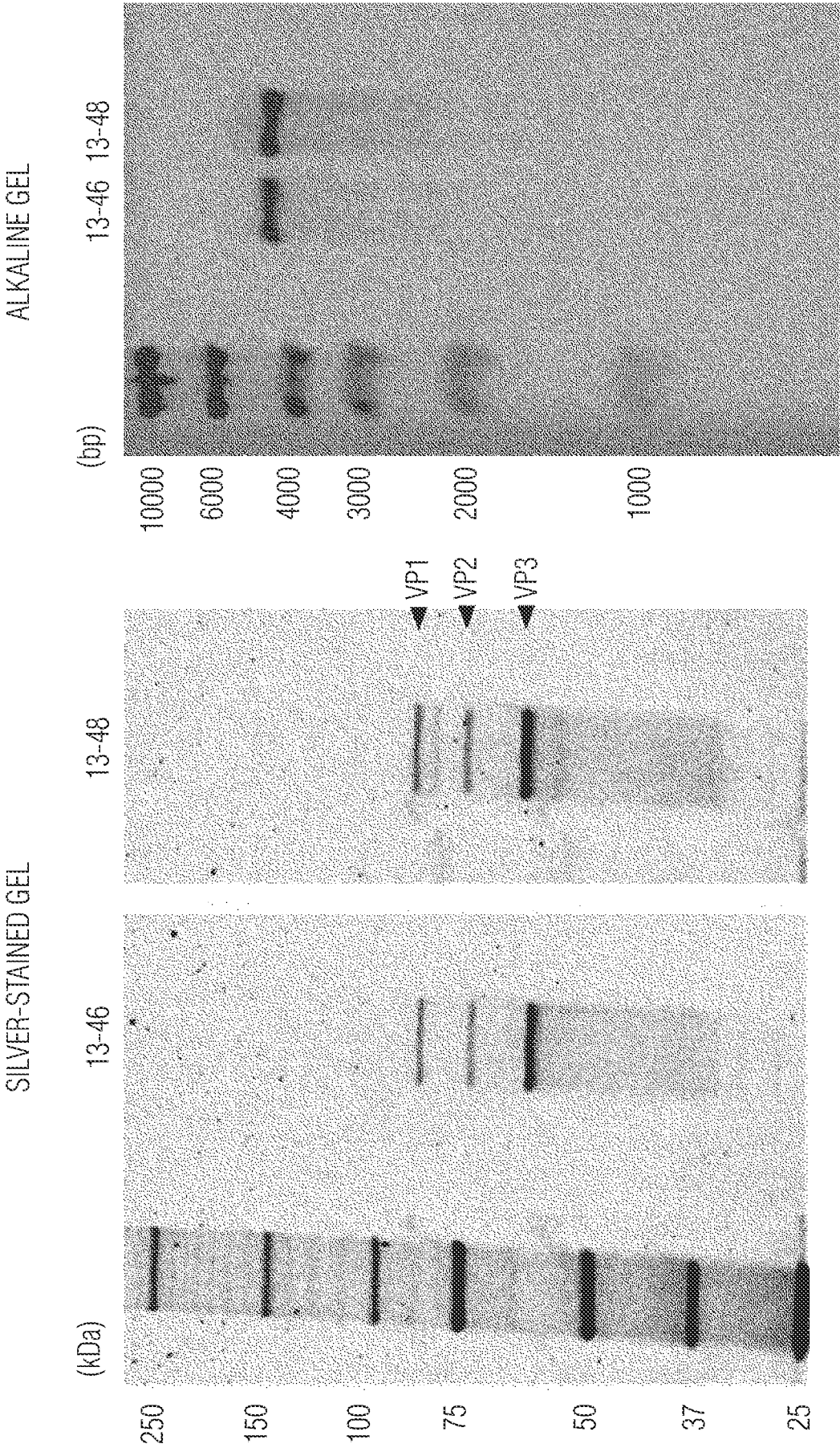


FIG. 14B



FIG. 14C

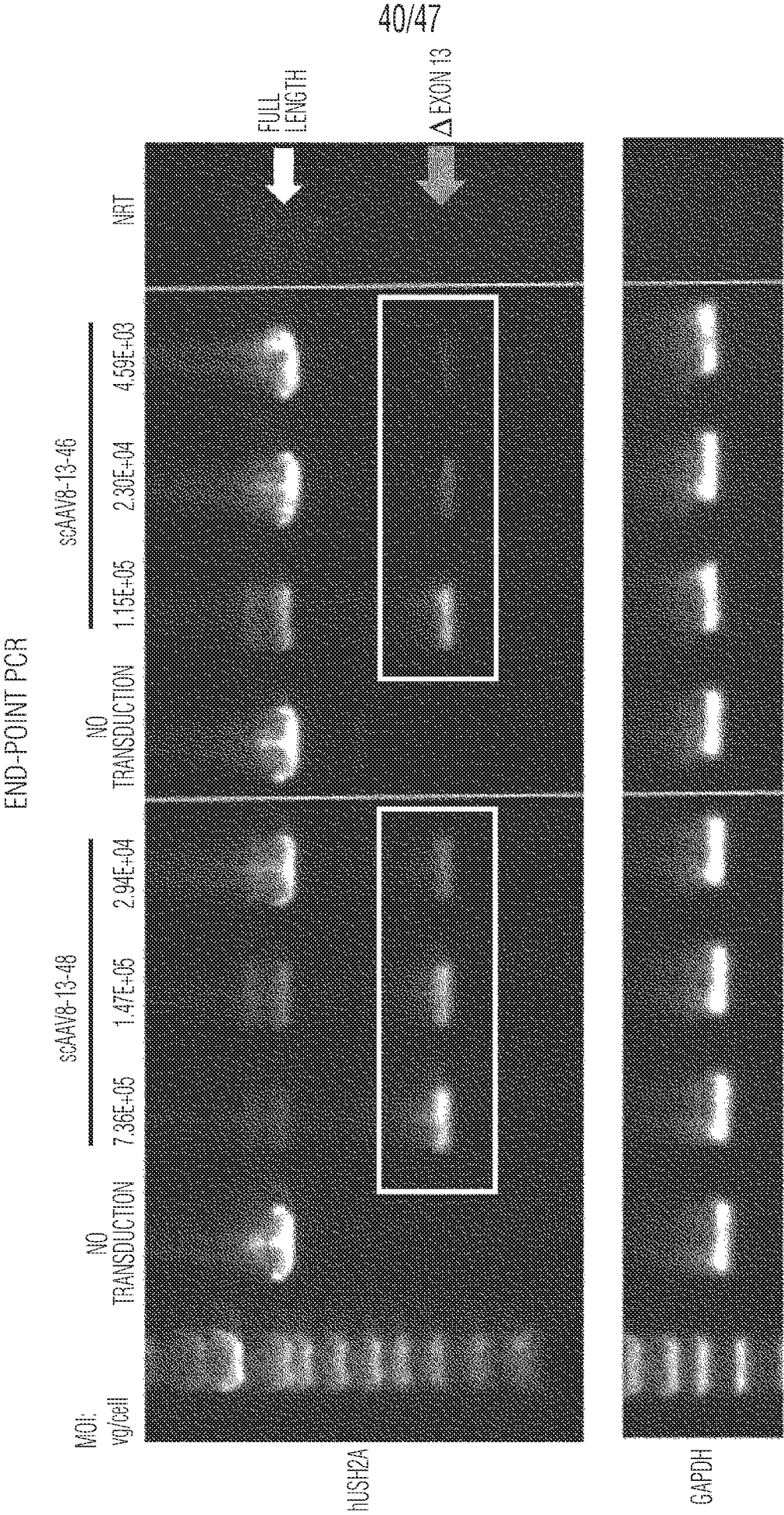
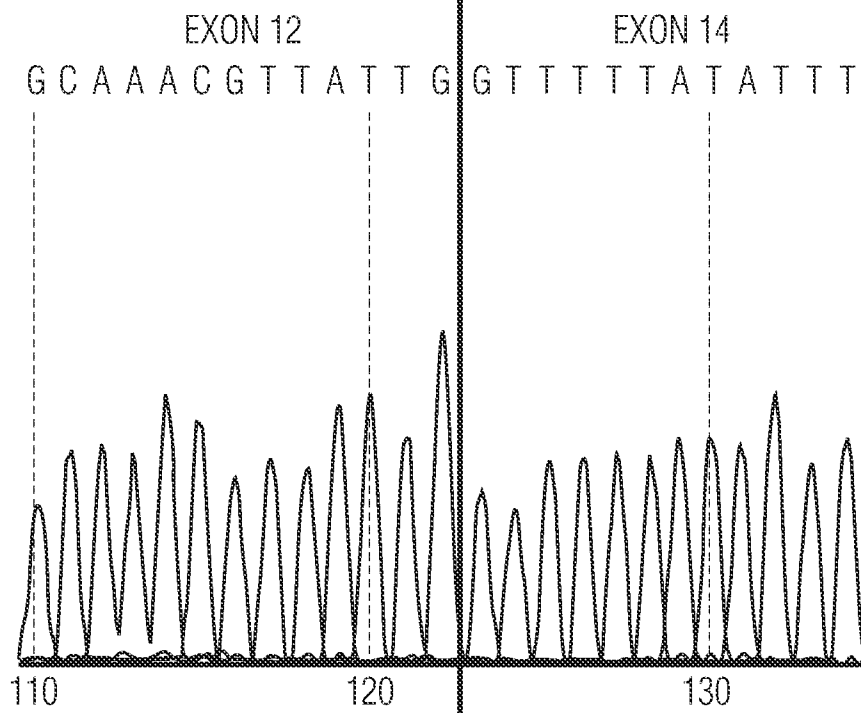


FIG. 15A

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SEQ ID NO: 71

13-48



SEQ ID NO: 71

13-46

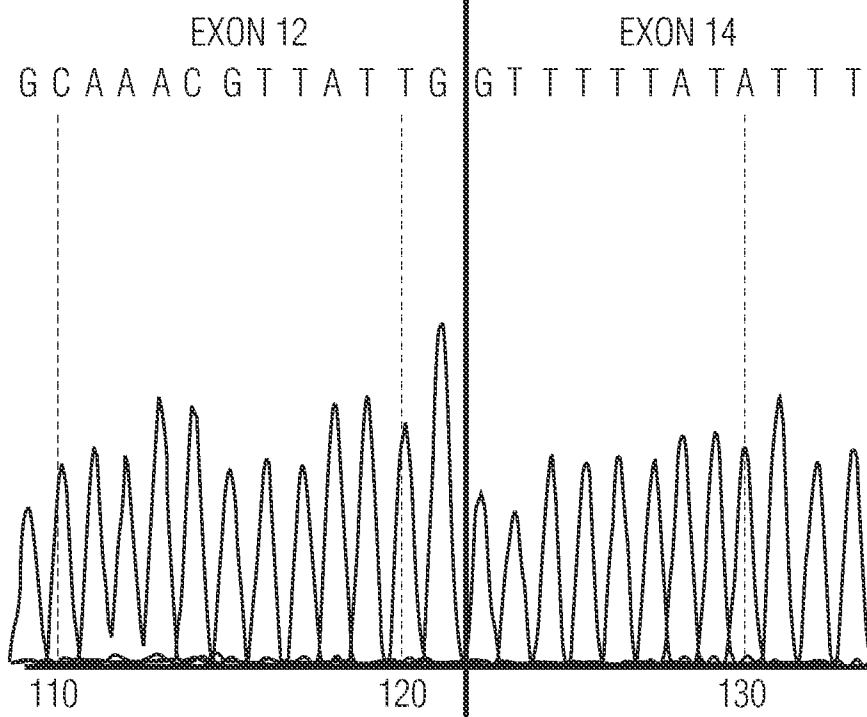


FIG. 15B

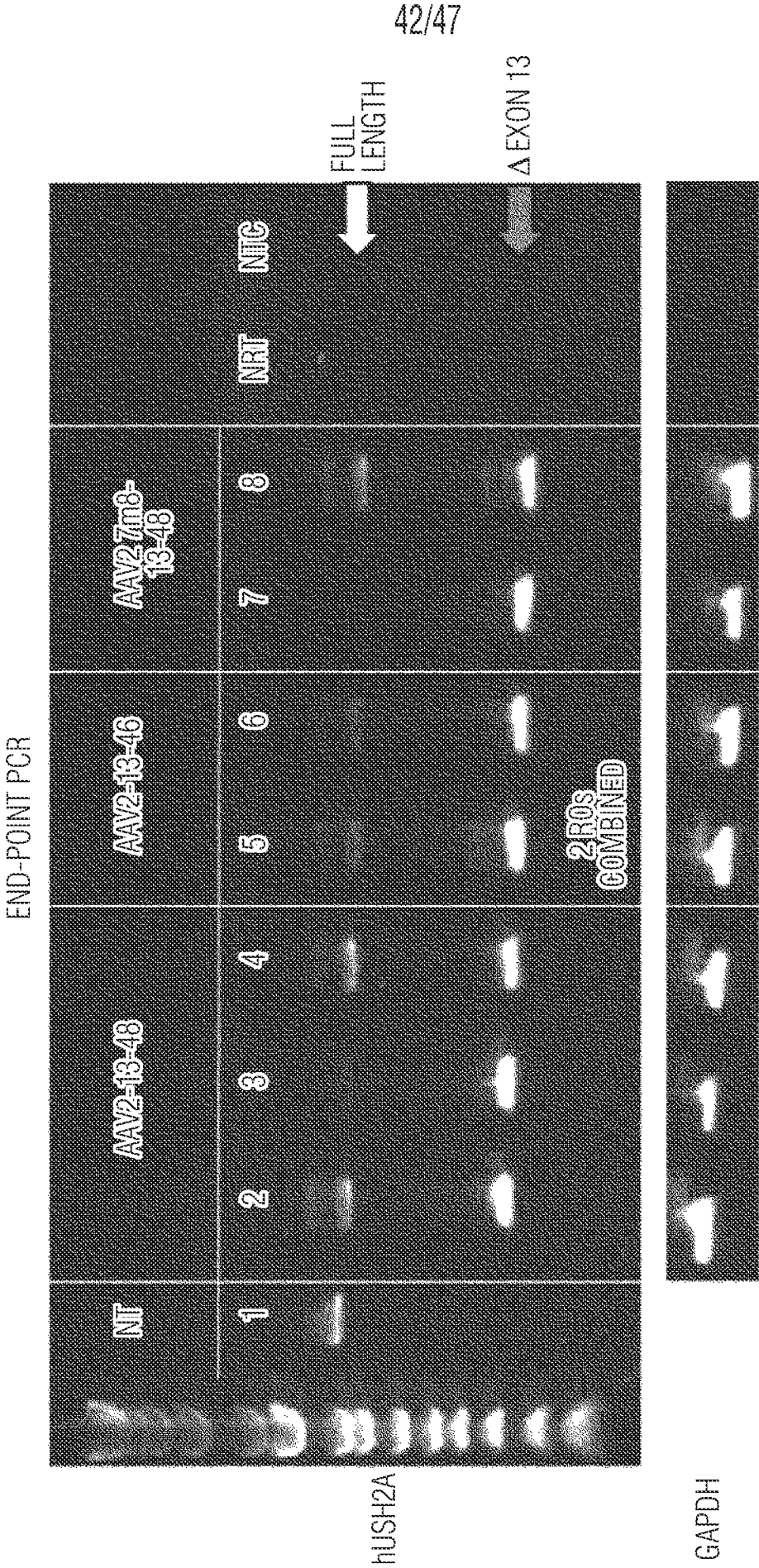
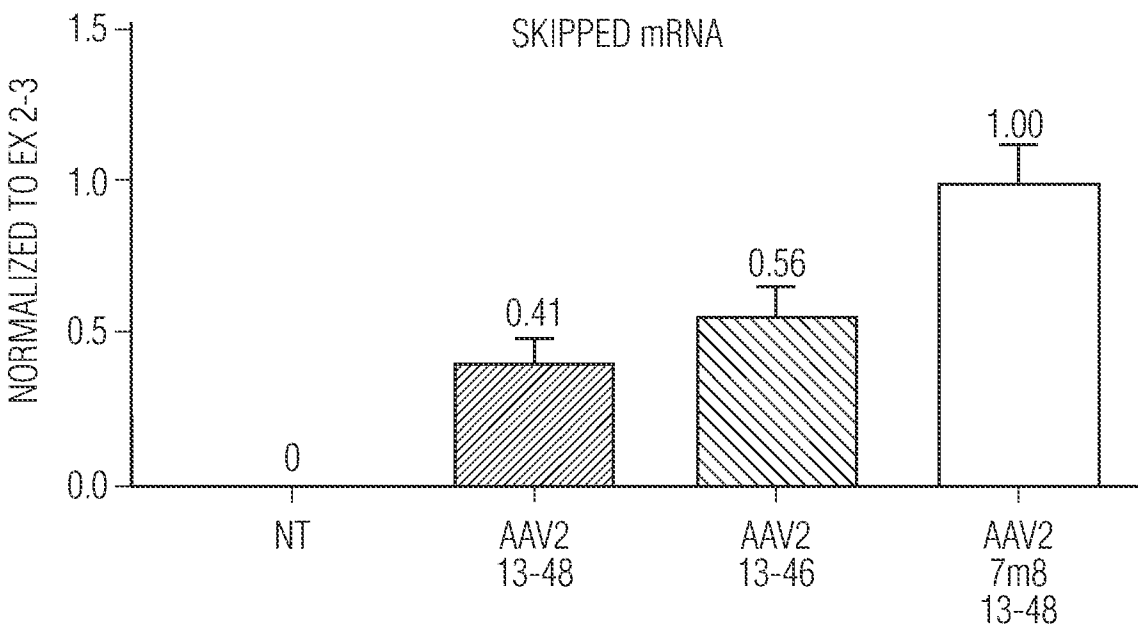


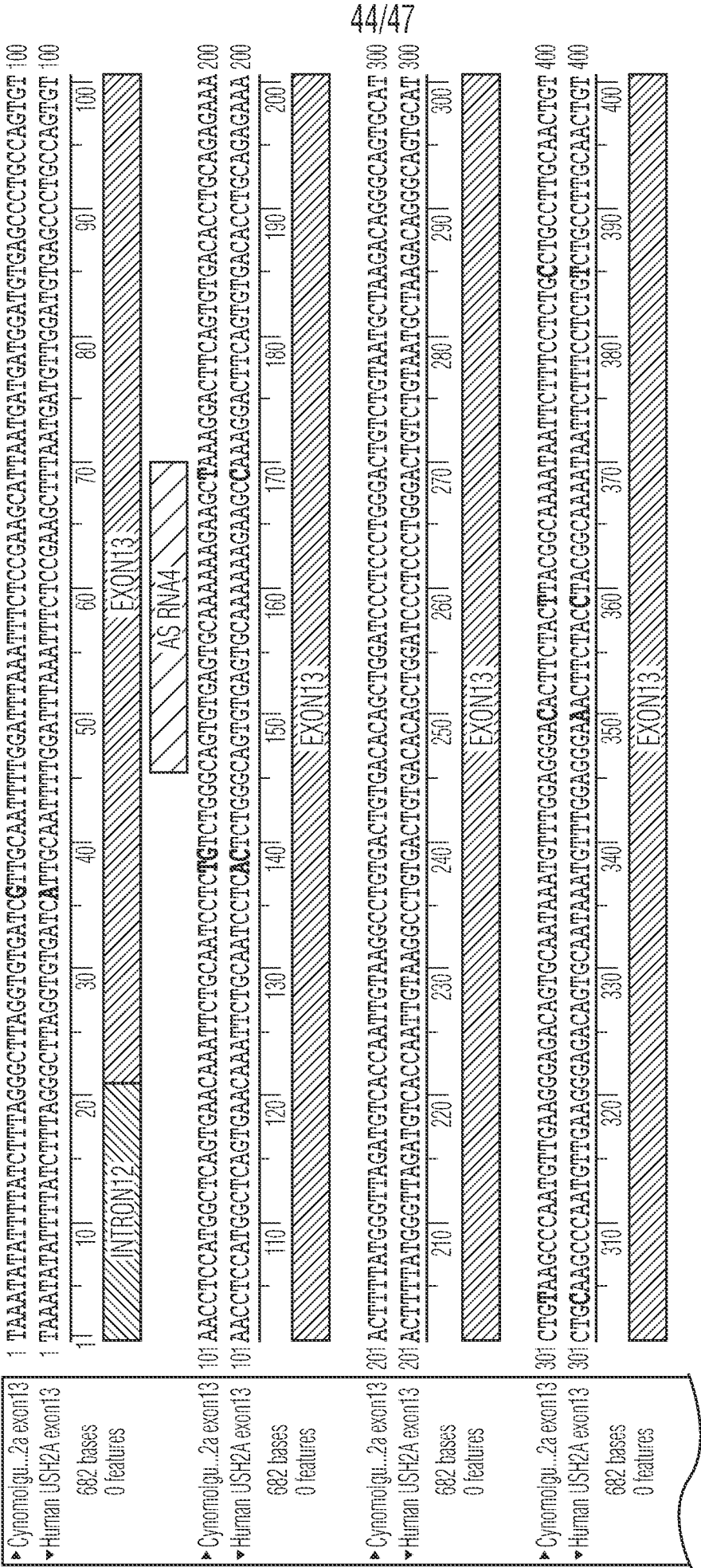
FIG. 16A

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VECTOR TREATMENT:	AAV2 13-48	AAV2 13-46	AAV2 7m8 13-48
RELATIVE	0.41	0.56	1.00

FIG. 16B



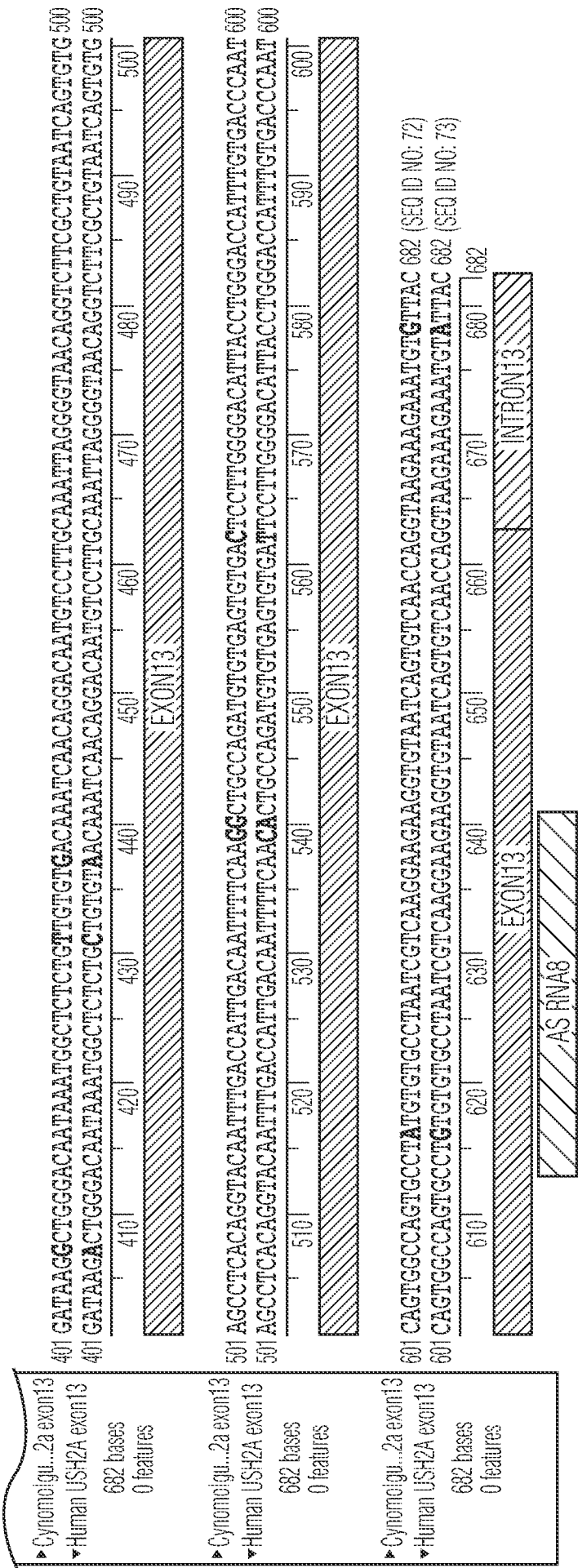


FIG. 17Aii

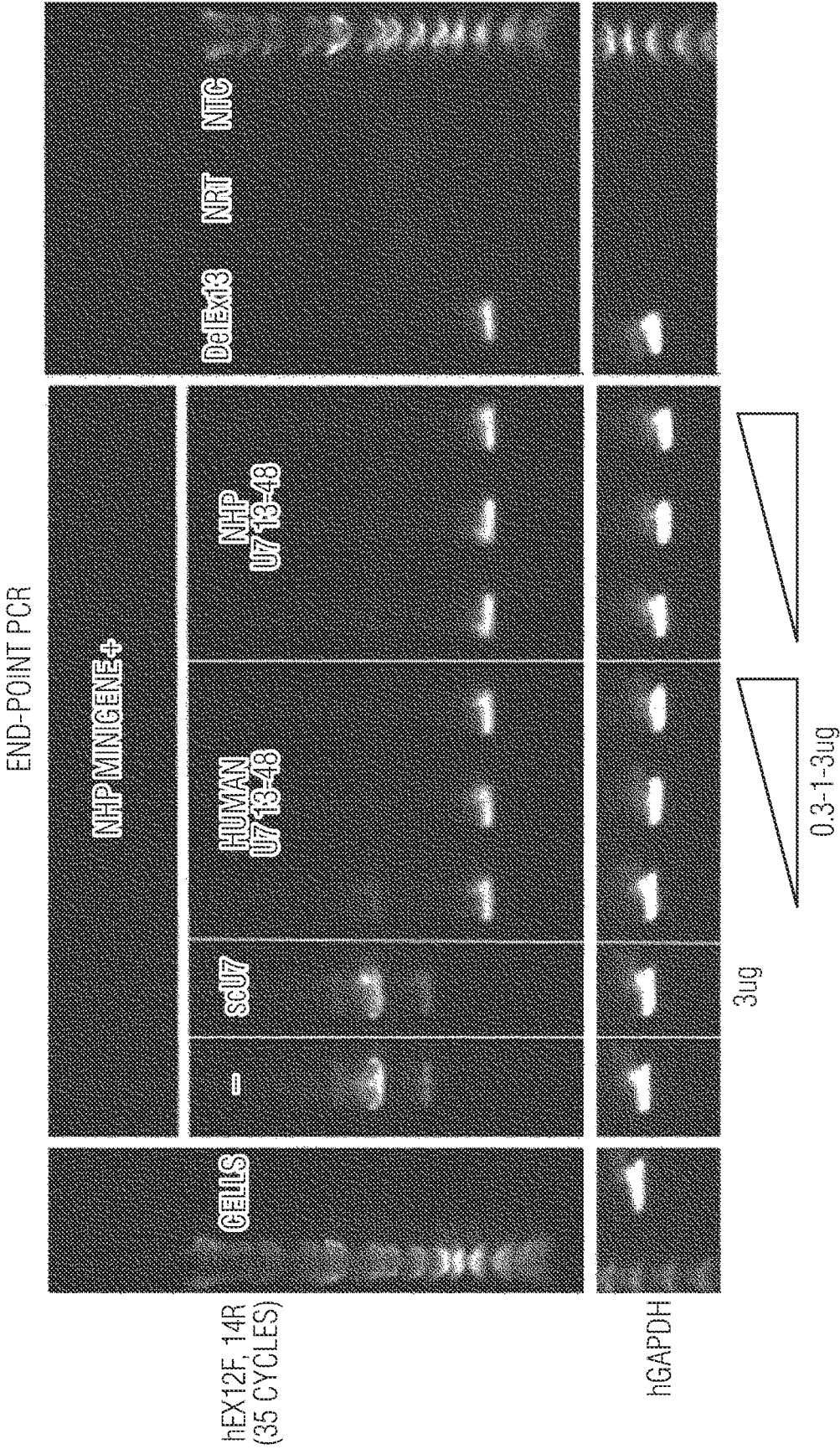
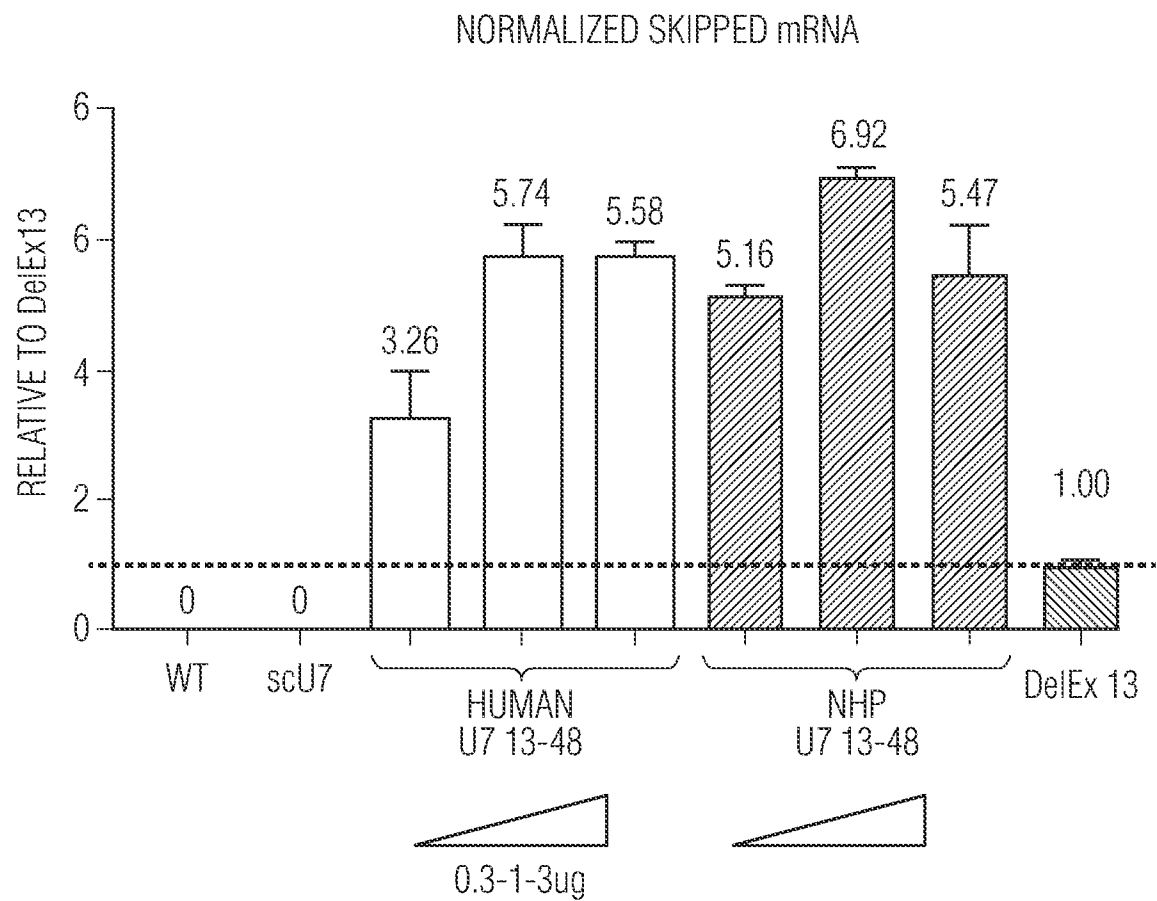


FIG. 17B

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PLASMID NAME:	0.3ug NHP MINIGENE +						DelEx13
	HUMAN U7 13-48			NHP U7 13-48			
PLASMID CONCENTRATION, ug:	0.3	1	3	0.3	1	3	0.3
RELATIVE TO DelEX13	3.26	5.74	5.58	5.16	6.92	5.47	1.00

FIG. 17C