

METHODS AND COMPOSITIONS FOR ADMINISTERING OTOFERLIN DUAL VECTOR SYSTEMS**Sequence Listing**

The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML copy, created on February 16, 2024, is named 51471-014WO5_Sequence_Listing_2_16_24.xml and is 188,879 bytes in size.

Field of the Invention

Described herein are compositions and methods for the treatment of sensorineural hearing loss and auditory neuropathy, particularly forms of the disease that are associated with biallelic mutations in otoferlin (OTOF), by way of OTOF gene therapy. The disclosure provides dual vector systems that include a first nucleic acid vector that contains a polynucleotide encoding an N-terminal portion of an OTOF protein (e.g., an OTOF isoform 5 protein) and a second nucleic acid vector that contains a polynucleotide encoding a C-terminal portion of an OTOF protein (e.g., an OTOF isoform 5 protein). These vectors can be used to increase the expression of or provide wild-type OTOF to a subject, such as a human subject suffering from sensorineural hearing loss.

Background

Sensorineural hearing loss is a type of hearing loss caused by defects in the cells of the inner ear or the neural pathways that project from the inner ear to the brain. Although sensorineural hearing loss is often acquired, and can be caused by noise, infections, head trauma, ototoxic drugs, or aging, there are also congenital forms of sensorineural hearing loss associated with autosomal recessive mutations. One such form of autosomal recessive sensorineural hearing loss is associated with mutation of the otoferlin (OTOF) gene, which is implicated in prelingual nonsyndromic hearing loss. In recent years, efforts to treat hearing loss have increasingly focused on gene therapy as a possible solution; however, OTOF is too large to allow for treatment using standard gene therapy approaches. There is a need for new therapeutics to treat OTOF-related sensorineural hearing loss.

Summary of the Invention

The invention provides methods for treating a subject having biallelic otoferlin (OTOF) mutations by administering to an inner ear of the subject an effective amount of an OTOF dual vector system. The OTOF dual vector system may be administered in an amount of 1×10^{13} vector genomes (vg)/mL to 1×10^{14} vg/mL in a volume of 200-250 μ L. The subject may be, e.g., a pediatric subject with congenital auditory neuropathy and may be determined to have profound sensorineural hearing loss prior to treatment (e.g., based on ABR measurements). The methods described herein may be used to instate hearing and may result in an improvement in ABR measurements and/or behavioral audiometry in the treated subjects.

In a first aspect, the invention provides a method of treating a human subject having biallelic OTOF mutations (e.g., a biallelic likely pathogenic or pathogenic mutation in OTOF), the method including the step of administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of

an OTOF dual vector system (e.g., the combined amount of both vectors is 1×10^{13} vg/mL to 1×10^{14} vg/mL) in a volume of 200-250 μ L by intracochlear injection, in which the OTOF dual vector system includes: a first adeno-associated virus (AAV) vector including a first inverted terminal repeat (ITR) sequence; a promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and a second AAV vector including a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence; in which the first coding polynucleotide and the second coding polynucleotide that encode the OTOF protein do not overlap, and in which neither the first nor second AAV vector encodes the full-length OTOF protein. In some embodiments, the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to about 1:3 (e.g., 3:1 to 1:3, 2:1 to 1:2, or 1:1).

In another aspect, the invention provides a method of improving hearing in a human subject, the method including the steps of selecting a subject having biallelic OTOF mutations (e.g., a biallelic likely pathogenic or pathogenic mutation in OTOF) and administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of an OTOF dual vector system (e.g., the combined amount of both vectors is 1×10^{13} vg/mL to 1×10^{14} vg/mL) in a volume of 200-250 μ L by intracochlear injection, in which the OTOF dual vector system includes: a first AAV vector including a first ITR sequence; a promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and a second AAV vector including a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence; in which the first coding polynucleotide and the second coding polynucleotide that encode the OTOF protein do not overlap, and in which neither the first nor second AAV vector encodes the full-length OTOF protein. In some embodiments, the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to 1:3 (e.g., 3:1 to 1:3, 2:1 to 1:2, or 1:1). In some embodiments, the subject has or is identified as having profound sensorineural hearing loss (e.g., a subject with absence of an ABR neural signal in response to a click stimulus at or below 85 dB normalized hearing level in the ear(s) to be treated or a subject with ≥ 90 dB hearing level in the ear(s) to be treated) (e.g., the method includes a step of selecting a subject having biallelic OTOF mutations and profound sensorineural hearing loss). In some embodiments, the subject has or is identified as having present outer hair cell function (e.g., the method includes a step of selecting a subject having biallelic OTOF mutations, profound sensorineural hearing loss, and present outer hair cell function). In some embodiments, the subject is determined to have present outer hair cell function based on detectable otoacoustic emissions (e.g., presence of otoacoustic emissions (≥ 6 dB signal-to-noise ratio) at ≥ 3 frequencies from 1 to 8 kHz in the ear(s) to be treated). In some embodiments, the subject is determined

to have present outer hair cell function based on a present cochlear microphonic (e.g., in the ear(s) to be treated).

In another aspect, the invention provides an aqueous suspension containing an OTOF dual vector system, 10 mM sodium phosphate or disodium phosphate, 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 at a pH of 7.4, in which the OTOF dual vector system includes: a first AAV vector including a first ITR sequence; a promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and a second AAV vector including a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence; in which the first coding polynucleotide and the second coding polynucleotide that encode the OTOF protein do not overlap, and in which neither the first nor second AAV vector encodes the full-length OTOF protein. In some embodiments, the suspension contains 10 mM sodium phosphate. In some embodiments, the suspension contains 10 mM disodium phosphate. In some embodiments, the ratio of the first vector to the second vector is about 3:1 to about 1:3. In some embodiments, the first vector and second vector are matched in titer and mixed at an approximately equal ratio. In some embodiments, the ratio of the first vector to the second vector is about 1:1. In some embodiments, the suspension has a titer of 1×10^{13} vg/mL to 1×10^{14} vg/mL. In some embodiments, the suspension has a titer of 1×10^{13} vg/mL to 5×10^{13} vg/mL. In some embodiments, the suspension has a titer of 3×10^{13} vg/mL. In some embodiments, the suspension has a titer of 5×10^{13} vg/mL to 1×10^{14} vg/mL. In some embodiments, the suspension has a titer of 7.3×10^{13} vg/mL. In some embodiments, the suspension is formulated for intracochlear injection.

In some embodiments, the OTOF protein is an OTOF isoform 5 protein. In some embodiments, the protein is an OTOF isoform 1 protein.

In some embodiments, the first AAV vector and the second AAV vector include an AAV1 capsid. In some embodiments, the first AAV vector and the second AAV vector include an Anc80 capsid. In some embodiments, the first AAV vector and the second AAV vector include an AAV9 capsid. In some embodiments, the first AAV vector and the second AAV vector include an AAV8 capsid. In some embodiments, the first AAV vector and the second AAV vector include an AAV2 capsid. In some embodiments, the first AAV vector and the second AAV vector include an AAV2quad(Y-F) capsid. In some embodiments, the first AAV vector and the second AAV vector include an AAV6 capsid. In some embodiments, the first AAV vector and the second AAV vector include an An80L65 capsid. In some embodiments, the first AAV vector and the second AAV vector include a DJ/9 capsid. In some embodiments, the first AAV vector and the second AAV vector include a 7m8 capsid. In some embodiments, the first AAV vector and the second AAV vector include a PHP.B capsid.

In some embodiments, the promoter is a ubiquitous promoter. In some embodiments, the ubiquitous promoter is a CAG promoter, a cytomegalovirus (CMV) promoter (e.g., the CMV immediate-early enhancer and promoter, a CMVmini promoter, a minCMV promoter, a CMV-TATA+INR promoter, or a min CMV-T6 promoter), the chicken β -actin promoter, a truncated CMV-chicken β -actin promoter

(smCBA), the CB7 promoter, the hybrid CMV enhancer/human β -actin promoter, the CASI promoter, the dihydrofolate reductase (DHFR) promoter, the human β -actin promoter, a β -globin promoter (e.g., a minimal β -globin promoter), an HSV promoter (e.g., a minimal HSV ICP0 promoter or a truncated HSV ICP0 promoter), an SV40 promoter (e.g., an SV40 minimal promoter), the EF1 α promoter, and the PGK promoter. In some embodiments, the ubiquitous promoter is a CAG promoter. In some embodiments, the ubiquitous promoter is a CMV promoter. In some embodiments, the ubiquitous promoter is the hybrid CMV enhancer/human β -actin promoter. In some embodiments, the ubiquitous promoter is the smCBA promoter. In some embodiments, the smCBA promoter comprises or consists of the sequence of SEQ ID NO: 44.

10 In some embodiments, the promoter is a Myosin 15 (Myo15) promoter. In some embodiments, the Myo15 promoter comprises a first region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 7 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 9 and/or SEQ ID NO: 10 joined (e.g., operably linked) to a second region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 8 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 14 and/or SEQ ID NO: 15, optionally containing a linker comprising one to one hundred nucleotides (e.g., 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-60, 1-70, 1-80, 1-90, 10-20, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 20-30, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, or 20-100 nucleotides) between the first region and the second region. In some embodiments, the first region is directly fused to the second region. In some embodiments, the first region comprises or consists of the sequence of SEQ ID NO: 7. In some embodiments, the second region comprises or consists of the sequence of SEQ ID NO: 8. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 19. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 21. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 22. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 36. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 37. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 42. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 43.

30 In some embodiments, the Myo15 promoter comprises a first region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 8 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 14 and/or SEQ ID NO: 15, joined (e.g., operably linked) to a second region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 7 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 9 and/or SEQ ID NO: 10, optionally containing a linker including one to one hundred nucleotides (e.g., 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-60, 1-70, 1-80, 1-90, 10-20, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 20-30, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, or 20-100 nucleotides) between the first region and the second region. In some embodiments, the first region is directly fused to the second region. In some embodiments, the first region comprises or consists of the sequence of SEQ ID NO: 8. In some embodiments, the second region comprises or consists of the

sequence of SEQ ID NO: 7. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 20. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 41.

5 In some embodiments, the Myo15 promoter comprises a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 7 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 9 and/or SEQ ID NO: 10. In some embodiments, the region comprises or consists of the sequence of SEQ ID NO: 7.

10 In some embodiments, the Myo15 promoter comprises a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 8 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 14 and/or SEQ ID NO: 15. In some embodiments, the region comprises or consists of the sequence of SEQ ID NO: 8.

15 In some embodiments, the functional portion of SEQ ID NO: 7 contains the sequence of SEQ ID NO: 9. In some embodiments, the functional portion of SEQ ID NO: 7 contains the sequence of SEQ ID NO: 10. In some embodiments, the functional portion of SEQ ID NO: 7 contains the sequence of SEQ ID NO: 11. In some embodiments, the functional portion of SEQ ID NO: 7 contains the sequence of SEQ ID NO: 12. In some embodiments, the functional portion of SEQ ID NO: 20 7 contains the sequence of SEQ ID NO: 13. In some embodiments, the functional portion of SEQ ID NO: 7 contains the sequence of SEQ ID NO: 33.

In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 14. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 15. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 34. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 35. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 14 and the sequence of SEQ ID NO: 15. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 16. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 17. In some embodiments, the functional portion of SEQ ID NO: 30 8 contains the sequence of SEQ ID NO: 18. In some embodiments, the functional portion of SEQ ID NO: 8 contains the sequence of SEQ ID NO: 38.

In some embodiments, the Myo15 promoter has at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the nucleic acid sequence of any one of SEQ ID NOs: 33-41. In some embodiments, the Myo15 promoter comprises or 35 consists of the sequence of SEQ ID NO: 33. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 34. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 35. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 36. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 37. In some embodiments, the Myo15 promoter comprises or 40 consists of the sequence of SEQ ID NO: 38. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 39. In some embodiments, the Myo15 promoter comprises or

consists of the sequence of SEQ ID NO: 40. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 41.

In some embodiments, the Myo15 promoter comprises a first region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 23 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 25 joined (e.g., operably linked) to a second region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 24 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 26 and/or SEQ ID NO: 27, optionally containing a linker comprising one to four hundred nucleotides (e.g., 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-60, 1-70, 1-80, 1-90, 1-100, 1-125, 1-150, 1-175, 1-200, 1-225, 1-250, 1-275, 1-300, 1-325, 1-350, 1-375, 1-400, 10-20, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 20-30, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, 20-100, 30-100, 40-100, 50-100, 50-150, 50-200, 50-250, 50-300, 50-350, 50-400, 100-150, 100-200, 100-250, 100-300, 100-350, 100-400, 150-200, 150-250, 150-300, 150-350, 150-400, 200-250, 200-300, 200-350, 200-400, 250-300, 250-350, 250-400, 300-400, or 350-400 nucleotides) between the first region and the second region. In some embodiments, the first region is directly fused to the second region. In some embodiments, the first region comprises or consists of the sequence of SEQ ID NO: 23. In some embodiments, the second region comprises or consists of the sequence of SEQ ID NO: 24. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 31. In some embodiments, the Myo15 promoter comprises or consists of the sequence of SEQ ID NO: 32.

In some embodiments, the Myo15 promoter comprises a first region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 24 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 26 and/or SEQ ID NO: 27, joined (e.g., operably linked) to a second region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 23 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 25, optionally containing a linker including one to four hundred nucleotides (e.g., 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-60, 1-70, 1-80, 1-90, 1-100, 1-125, 1-150, 1-175, 1-200, 1-225, 1-250, 1-275, 1-300, 1-325, 1-350, 1-375, 1-400, 10-20, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 20-30, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, 20-100, 30-100, 40-100, 50-100, 50-150, 50-200, 50-250, 50-300, 50-350, 50-400, 100-150, 100-200, 100-250, 100-300, 100-350, 100-400, 150-200, 150-250, 150-300, 150-350, 150-400, 200-250, 200-300, 200-350, 200-400, 250-300, 250-350, 250-400, 300-400, or 350-400 nucleotides) between the first region and the second region. In some embodiments, the first region is directly fused to the second region. In some embodiments, the first region comprises or consists of the sequence of SEQ ID NO: 24. In some embodiments, the second region comprises or consists of the sequence of SEQ ID NO: 23.

In some embodiments, the Myo15 promoter comprises a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 23 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 25. In some embodiments, the region comprises or consists of the sequence of SEQ ID NO: 23.

In some embodiments, the Myo15 promoter comprises a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 24 or a functional portion or derivative thereof including the sequence of SEQ ID NO: 26 and/or SEQ ID NO: 27. In some embodiments, the region comprises or consists of the sequence of SEQ ID NO: 24.

In some embodiments, the functional portion of SEQ ID NO: 23 contains the sequence of SEQ ID NO: 25.

In some embodiments, the functional portion of SEQ ID NO: 24 contains the sequence of SEQ ID NO: 26. In some embodiments, the functional portion of SEQ ID NO: 24 contains the sequence of SEQ ID NO: 27. In some embodiments, the functional portion of SEQ ID NO: 24 contains the sequence of SEQ ID NO: 26 and the sequence of SEQ ID NO: 27. In some embodiments, the functional portion of SEQ ID NO: 24 contains the sequence of SEQ ID NO: 28. In some embodiments, the functional portion of SEQ ID NO: 24 contains the sequence of SEQ ID NO: 29. In some embodiments, the functional portion of SEQ ID NO: 24 contains the sequence of SEQ ID NO: 30.

In some embodiments, the first and second recombinogenic regions are the same.

In some embodiments, the first recombinogenic region and/or the second recombinogenic region is an AK recombinogenic region. In some embodiments, the AK recombinogenic region comprises or consists of the sequence of SEQ ID NO: 47.

In some embodiments, the first recombinogenic region and/or the second recombinogenic region is an AP gene fragment. In some embodiments, the AP gene fragment comprises or consists of the sequence of any one of SEQ ID NOs: 48-53. In some embodiments, the AP gene fragment comprises or consists of the sequence of SEQ ID NO: 51.

In some embodiments, each of the first and second coding polynucleotides encode about half of the OTOF isoform 5 protein sequence.

In some embodiments, the first coding polynucleotide encodes amino acids 1-802 of SEQ ID NO: 1. In some embodiments of any of the foregoing aspects, the second coding polynucleotide encodes amino acids 803-1997 of SEQ ID NO: 1.

In some embodiments, the first and second coding polynucleotides are divided at an OTOF exon boundary. In some embodiments, the first and second coding polynucleotides are divided at the boundary between exons 20 and 21 of OTOF. In some embodiments, the first and second coding polynucleotides are divided at the boundary between exons 21 and 22 of OTOF.

In some embodiments, the first coding polynucleotide consists of exons 1-20 of a polynucleotide encoding the OTOF isoform 5 protein and the second coding polynucleotide consists of exons 21-45 and 47 of a polynucleotide encoding the OTOF isoform 5 protein (e.g., a polynucleotide encoding a human OTOF isoform 5 protein).

In some embodiments, the first coding polynucleotide consists of exons 1-21 of a polynucleotide encoding the OTOF isoform 5 protein and the second coding polynucleotide consists of exons 22-45 and 47 of a polynucleotide encoding the OTOF isoform 5 protein (e.g., a polynucleotide encoding a human OTOF isoform 5 protein).

In some embodiments, the first and second coding polynucleotides that encode the OTOF isoform 5 protein do not comprise introns.

In some embodiments, the OTOF isoform 5 protein is a human OTOF isoform 5 protein (e.g., the protein having the sequence of SEQ ID NO: 1).

In some embodiments, the OTOF isoform 5 protein comprises the sequence of SEQ ID NO: 1 or a variant thereof having one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) conservative amino acid substitutions. In some embodiments, no more than 10% (10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or fewer) of the amino acids in the OTOF isoform 5 protein variant are conservative amino acid substitutions. In some embodiments, the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 1. In some embodiments, the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 2. In some embodiments, the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 3.

In some embodiments, the N-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 58 or a variant thereof having one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) conservative amino acid substitutions. In some embodiments, no more than 10% (10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or fewer) of the amino acids in the N-terminal portion of the OTOF isoform 5 protein variant are conservative amino acid substitutions. In some embodiments, the N-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 58. In some embodiments, the N-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 56.

In some embodiments, the C-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 59 or a variant thereof having one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) conservative amino acid substitutions. In some embodiments, no more than 10% (10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or fewer) of the amino acids in the C-terminal portion of the OTOF isoform 5 protein variant are conservative amino acid substitutions. In some embodiments, the C-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 59. In some embodiments, the C-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 57.

In some embodiments, the ITRs in the first vector and second vector are AAV2 ITRs. In some embodiments, the ITRs in the first vector and second vector have at least 80% sequence identity (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) to AAV2 ITRs.

In some embodiments, the poly(A) sequence is a bovine growth hormone (bGH) poly(A) signal sequence.

In some embodiments, the splice donor sequence in the first vector comprises or consists of the sequence of SEQ ID NO: 54.

In some embodiments, the splice acceptor sequence in the second vector comprises or consists of the sequence of SEQ ID NO: 55.

In some embodiments, the first AAV vector comprises a Kozak sequence 3' of the promoter and 5' of the first coding polynucleotide that encodes the N-terminal portion of the OTOF isoform 5 protein.

In some embodiments of, the first AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 2272 to 6041 of SEQ ID NO: 60. In some embodiments, the first AAV vector

contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 2049 to 6264 of SEQ ID NO: 60.

5 In some embodiments, the first AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 182 to 3949 of SEQ ID NO: 62. In some embodiments, the first AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 19 to 4115 of SEQ ID NO: 62.

10 In some embodiments, the first AAV vector contains a polynucleotide sequence comprising the sequence of positions 2267 to 6014 of SEQ ID NO: 64. In some embodiments, the first AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of positions 2049 to 6237 of SEQ ID NO: 64.

In some embodiments, the first AAV vector contains a polynucleotide sequence comprising the sequence of positions 177 to 3924 of SEQ ID NO: 65. In some embodiments, the first AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of positions 19 to 4090 of SEQ ID NO: 65.

15 In some embodiments, the second AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 2267 to 6476 of SEQ ID NO: 61. In some embodiments, the second AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 2049 to 6693 of SEQ ID NO: 61.

20 In some embodiments, the second AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 187 to 4396 of SEQ ID NO: 63. In some embodiments, the second AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 19 to 4589 of SEQ ID NO: 63.

25 In some embodiments, the first AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 235 to 4004 of SEQ ID NO: 66. In some embodiments, the first AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 12 to 4227 of SEQ ID NO: 66.

30 In some embodiments, the first AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 230 to 3977 of SEQ ID NO: 68. In some embodiments, the first AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 12 to 4200 of SEQ ID NO: 68.

In some embodiments, the second AAV vector contains a polynucleotide sequence comprising the sequence of nucleotides 229 to 4438 of SEQ ID NO: 67. In some embodiments, the second AAV vector contains a polynucleotide sequence comprising or consisting of the sequence of nucleotides 12 to 4655 of SEQ ID NO: 67.

35 In some embodiments, the subject is less than 18 years of age. In some embodiments, the subject is less than 18 years of age and at least 7 years of age. In some embodiments, the subject is older than 24 months of age and younger than 18 years of age. In some embodiments, the subject is older than 24 months of age and younger than 7 years of age (e.g., younger than 6 years of age, younger than 5 years of age, younger than 4 years of age, or younger than 3 years of age). In some embodiments, 40 the subject is 24 months of age or younger (e.g., younger than 24 months of age, younger than 18

months of age, younger than 12 months of age, younger than 6 months of age, or younger than 3 months of age).

In some embodiments, the subject has or is identified as having profound sensorineural hearing loss (e.g., based on ABR measurements, such as ≥ 90 dB hearing level (HL) or absence of an ABR neural signal in response to a click stimulus at or below 85 decibels normalized Hearing Level (dB nHL), e.g., profound deafness secondary to biallelic OTOF mutations).

In some embodiments, the subject has or is identified as having behavioral open-set word detection scores consistent with the speech criteria listed on a cochlear implant label (e.g., behavioral open-set word detection scores of $< 30\%$ in the ear(s) to be treated).

In some embodiments, the subject has or is identified as having congenital auditory neuropathy.

In some embodiments, the subject has or is identified as having present outer hair cell function.

In some embodiments, the subject has or is identified as having detectable otoacoustic emissions (OAEs, e.g., ≥ 6 dB signal-to-noise ratio at ≥ 3 frequencies in a distortion product (DP) Gram measured from 1 to 8 kHz).

In some embodiments, a cochlear microphonic is present in the ear(s) to be treated.

In some embodiments, the administering is unilateral. In some embodiments, the administering is bilateral.

In some embodiments, the administering to an inner ear includes intracochlear injection via a catheter placed through the round window membrane of the cochlea (e.g., into the inner ear perilymph).

In some embodiments, the administering further includes creating a fenestration in the lateral semicircular canal. In some embodiments, the injection is performed using a syringe and syringe pump (e.g., a syringe is attached to the catheter and the syringe and catheter combination are loaded onto the syringe pump). In some embodiments, the administering is at a rate of 0.8-1 mL/hr (e.g., 0.9 mL/hr).

In some embodiments, the administering occurs once per ear.

In some embodiments, the first vector and the second vector are administered at about a 1:1 ratio (e.g., at a 1:1 ratio).

In some embodiments, the dual vector system is formulated as an aqueous suspension. In some embodiments, the first vector and second vector are matched in titer and mixed at an approximately equal ratio (i.e., the titer of 1×10^{13} vg/mL to 1×10^{14} vg/mL is produced by combining the first vector and second vector in an approximately 1:1 ratio). In some embodiments, the suspension includes (in addition to the AAV vectors) 10 mM sodium phosphate or disodium phosphate (also called sodium phosphate dibasic and having the formula Na_2HPO_4), 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 at a pH of about 7.4. In some embodiments, the suspension includes 10 mM sodium phosphate. In some embodiments, the suspension includes 10 mM disodium phosphate.

In some embodiments, the method further includes administering a corticosteroid (e.g., prednisone) to the subject for the first four weeks after the administering of the OTOF dual vector system. In some embodiments, the method includes administering 1 mg/kg of the corticosteroid daily during the first two weeks after the administering of the OTOF dual vector system, administering 0.5 mg/kg daily during the third week after the administering of the OTOF dual vector system, and administering 0.25 mg/kg daily during the fourth week after the administering of the OTOF dual vector system.

In some embodiments, the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 2.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 4.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.

In some embodiments, the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 9.0×10^{13} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 8.0×10^{13} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 7.0×10^{13} vg/mL in a volume of 200-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 6.0×10^{13} vg/mL in a volume of 200-250 μ L.

In some embodiments, the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 210-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 220-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 230-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 240-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 2.0×10^{13} vg/mL to 9.0×10^{13} vg/mL in a volume of 240-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL to 8.0×10^{13} vg/mL in a volume of 240-250 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL to 8.0×10^{13} vg/mL in a volume of 240 μ L.

In some embodiments, the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL in a volume of 240 μ L. In some embodiments, the OTOF dual vector system is administered in an amount of 7.3×10^{13} vg/mL in a volume of 240 μ L.

In some embodiments of any of the foregoing aspects, the method further includes identifying the subject as having a mutation in OTOF (e.g., a biallelic likely pathogenic or pathogenic mutation) prior to administering the composition.

In some embodiments of any of the foregoing aspects, the method further includes identifying the subject as having profound sensorineural hearing loss prior to administering the composition.

In some embodiments of any of the foregoing aspects, the subject has or has been identified as having Deafness, Autosomal Recessive 9 (DFNB9).

In some embodiments of any of the foregoing aspects, the method further includes the step of evaluating the hearing of the subject prior to administering the dual vector system.

In some embodiments of any of the foregoing aspects, the method further includes identifying the subject as having present outer hair cell function prior to administering the dual vector system (e.g., based on having detectable otoacoustic emissions (OAEs, e.g., ≥ 6 dB signal-to-noise ratio at ≥ 3

frequencies in a distortion product (DP) Gram measured from 1 to 8 kHz and/or a present cochlear microphonic in the ear(s) to be treated).

In some embodiments of any of the foregoing aspects, the method increases OTOF expression in a cochlear hair cell. In some embodiments, the cochlear hair cell is an inner hair cell.

5 In some embodiments of any of the foregoing aspects, the method further includes evaluating the hearing of the subject after administering the dual vector system.

In some embodiments of any of the foregoing aspects, the method increases OTOF expression in a cell (e.g., a cochlear hair cell), improves hearing (e.g., as assessed by standard tests, such as audiometry, auditory brainstem response (ABR), electrocochleography (ECOG), and otoacoustic
10 emissions), prevents or reduces hearing loss, delays the development of hearing loss, slows the progression of hearing loss, improves speech discrimination, or improves hair cell function.

In some embodiments of any of the foregoing aspects, the method improves one or more parameters selected from the subject's auditory brainstem response (ABR), behavioral audiometry, and score in one or more hearing questionnaires or behavioral tasks. In some embodiments, the hearing
15 questionnaires and behavioral tasks include one or more of the Auditory Skills Checklist, Open & Closed set task, Early Speech Perception test, Pediatric Speech Intelligibility test, Lexical Neighborhood Test Multisyllabic Lexical Neighborhood Test, Consonant-Nucleus-Consonant test, Bamford-Kowal-Bench sentence test, LittlEARS® Auditory Questionnaire, MacArthur-Bates Communicative Development Inventories Words and Gestures, Quality of Life–Cochlear Implant, Pediatric Quality of Life Inventory,
20 Hearing Environments and Reflection on Quality of Life (HEAR-QL)-26, HEAR-QL-28, The Health Utilities Index 3, Vanderbilt Fatigue Scales, and AzBio test. In some embodiments, the method improves the subject's score in the LittlEARS® Auditory Questionnaire. In some embodiments, the method improves hearing thresholds by at least 55 decibels hearing level with air conduction as assessed by behavioral pure tone audiometry. In some embodiments, the method results in a positive ABR wave V amplitude
25 response.

In another aspect, the invention provides a kit including a suspension described herein. In some embodiments, the kit further includes one or more of a syringe, syringe pump, and catheter.

In another aspect, the invention provides a syringe containing a suspension described herein.

In another aspect, the invention provides a method of administering a nucleic acid vector to the
30 inner ear of a human subject by creating an opening in the round window membrane of the cochlea and an opening in a second location in the inner ear and inserting a catheter through the opening in the round window membrane to infuse the nucleic acid vector into the perilymph. In some embodiments, the opening made in the second location in the inner ear is made in a semicircular canal. In some
35 embodiments, the semicircular canal is a lateral semicircular canal. In some embodiments, the semicircular canal is a posterior semicircular canal. In some embodiments, the semicircular canal is a superior semicircular canal. In some embodiments, a surgical procedure is performed to access the middle ear before creating the opening in the round window membrane and lateral semicircular canal. In some embodiments, the surgical procedure involves a mastoidectomy and opening a facial recess. In some embodiments, the surgical procedure is a standard surgical procedure for cochlear implant surgery.
40 In some embodiments, a syringe and/or a syringe pump are used in combination with the catheter for said administering (e.g., a syringe is attached to the catheter and the syringe and catheter combination are

loaded onto the syringe pump). In some embodiments, the nucleic acid vector (e.g., a solution containing the nucleic acid vector) is administered at a rate of 0.5-1 mL/hr (e.g., 0.6-1 mL/hr, 0.7-1 mL/hr, 0.8-1 mL/hr, or 0.9-1 mL/hour, such as 0.9 mL/hr). In some embodiments, the nucleic acid vector is a viral vector. In some embodiments, the viral vector is a lentivirus vector, an adenovirus vector, or an adeno-associated virus (AAV) vector. In some embodiments, the viral vector is an AAV vector. In some

5 embodiments, the human subject has or is at risk of developing sensorineural hearing loss. In some embodiments, the hearing loss is genetic hearing loss. In some embodiments, the genetic hearing loss is autosomal dominant hearing loss, autosomal recessive hearing loss, or X-linked hearing loss. In some

10 embodiments, the nucleic acid vector contains a polynucleotide encoding a protein that is absent or dysfunctional in the subject (e.g., a protein that is not produced or that does not function properly due to a genetic mutation associated with the hearing loss, e.g., the polynucleotide encodes a functional protein that is deficient in the subject due to a genetic mutation) or a polynucleotide encoding an expression product designed to correct a genetic mutation in the subject (e.g., correct a genetic mutation associated with the hearing loss via gene editing). In some embodiments, the hearing loss is acquired hearing loss.

15 In some embodiments, the acquired hearing loss is noise-induced hearing loss, age-related hearing loss, disease or infection-related hearing loss, head trauma-related hearing loss, or ototoxic drug-induced hearing loss. In some embodiments, the nucleic acid vector includes a polynucleotide encoding an expression product that induces or increases cochlear hair cell regeneration, increases cochlear supporting cell numbers, prevents or reduces cochlear hair cell damage, prevents or reduces cochlear

20 hair cell death, increases cochlear hair cell maturation, improves cochlear hair cell function, or promotes or increases cochlear hair cell survival. In some embodiments, the human subject has or is at risk of developing vestibular dysfunction. In some embodiments, the vestibular dysfunction is vertigo, dizziness, imbalance (e.g., loss of balance or a balance disorder), oscillopsia, or bilateral vestibulopathy. In some

25 embodiments, the vestibular dysfunction is associated with a genetic mutation. In some embodiments, the nucleic acid vector contains a polynucleotide encoding a protein that is absent or dysfunctional in the subject (e.g., a protein that is not produced or that does not function properly in the inner ear of the subject due to the genetic mutation associated with the vestibular dysfunction, e.g., the polynucleotide encodes a functional protein that is deficient in the subject due to the genetic mutation) or a polynucleotide encoding an expression product designed to correct a genetic mutation in the subject

30 (e.g., correct a genetic mutation associated with the vestibular dysfunction via gene editing). In some embodiments, the vestibular dysfunction is associated with damage to or loss of vestibular cells (e.g., vestibular hair cells or supporting cells, such as damage to or loss of vestibular cells, related to disease or infection, head trauma, ototoxic drugs (e.g., vestibulotoxic drugs), or aging). In some embodiments, the nucleic acid vector includes a polynucleotide encoding an expression product that induces or increases

35 vestibular hair cell regeneration, increases vestibular supporting cell numbers, prevents or reduces vestibular hair cell damage, prevents or reduces vestibular hair cell death, increases vestibular hair cell maturation, improves vestibular hair cell function, or promotes or increases vestibular hair cell survival.

Definitions

As used herein, the term “about” refers to a value that is within 10% above or below the value being described.

As used herein, “administration” refers to providing or giving a subject a therapeutic agent (e.g., a composition containing a first nucleic acid vector containing a polynucleotide that encodes an N-terminal portion of an otoferlin protein and a second nucleic acid vector containing a polynucleotide that encodes a C-terminal portion of an otoferlin protein), by any effective route. Exemplary routes of administration are described herein below.

As used herein, the term “cell type” refers to a group of cells sharing a phenotype that is statistically separable based on gene expression data. For instance, cells of a common cell type may share similar structural and/or functional characteristics, such as similar gene activation patterns and antigen presentation profiles. Cells of a common cell type may include those that are isolated from a common tissue (e.g., epithelial tissue, neural tissue, connective tissue, or muscle tissue) and/or those that are isolated from a common organ, tissue system, blood vessel, or other structure and/or region in an organism.

As used herein, the term “cochlear hair cell” refers to group of specialized cells in the inner ear that are involved in sensing sound. There are two types of cochlear hair cells: inner hair cells and outer hair cells. Damage to cochlear hair cells and genetic mutations that disrupt cochlear hair cell function are implicated in hearing loss and deafness.

As used herein, the terms “conservative mutation,” “conservative substitution,” and “conservative amino acid substitution” refer to a substitution of one or more amino acids for one or more different amino acids that exhibit similar physicochemical properties, such as polarity, electrostatic charge, and steric volume. These properties are summarized for each of the twenty naturally occurring amino acids in table 1 below.

Table 1. Representative physicochemical properties of naturally occurring amino acids

Amino Acid	3 Letter Code	1 Letter Code	Side-chain Polarity	Electrostatic character at physiological pH (7.4)	Steric Volume [†]
Alanine	Ala	A	nonpolar	neutral	small
Arginine	Arg	R	polar	cationic	large
Asparagine	Asn	N	polar	neutral	intermediate
Aspartic acid	Asp	D	polar	anionic	intermediate
Cysteine	Cys	C	nonpolar	neutral	intermediate
Glutamic acid	Glu	E	polar	anionic	intermediate
Glutamine	Gln	Q	polar	neutral	intermediate
Glycine	Gly	G	nonpolar	neutral	small

Amino Acid	3 Letter Code	1 Letter Code	Side-chain Polarity	Electrostatic character at physiological pH (7.4)	Steric Volume [†]
Histidine	His	H	polar	Both neutral and cationic forms in equilibrium at pH 7.4	large
Isoleucine	Ile	I	nonpolar	neutral	large
Leucine	Leu	L	nonpolar	neutral	large
Lysine	Lys	K	polar	cationic	large
Methionine	Met	M	nonpolar	neutral	large
Phenylalanine	Phe	F	nonpolar	neutral	large
Proline	Pro	P	non-polar	neutral	intermediate
Serine	Ser	S	polar	neutral	small
Threonine	Thr	T	polar	neutral	intermediate
Tryptophan	Trp	W	nonpolar	neutral	bulky
Tyrosine	Tyr	Y	polar	neutral	large
Valine	Val	V	nonpolar	neutral	intermediate
[†] based on volume in Å ³ : 50-100 is small, 100-150 is intermediate, 150-200 is large, and >200 is bulky					

From this table it is appreciated that the conservative amino acid families include (i) G, A, V, L, and I; (ii) D and E; (iii) C, S and T; (iv) H, K and R; (v) N and Q; and (vi) F, Y and W. A conservative mutation or substitution is therefore one that substitutes one amino acid for a member of the same amino acid family (e.g., a substitution of Ser for Thr or Lys for Arg).

As used herein, the terms “effective amount,” “therapeutically effective amount,” and a “sufficient amount” of a composition, vector construct, or viral vector described herein refer to a quantity sufficient to, when administered to the subject in need thereof, including a mammal, for example a human, effect beneficial or desired results, including clinical results, and, as such, an “effective amount” or synonym thereto depends upon the context in which it is being applied. For example, in the context of treating sensorineural hearing loss, it is an amount of the composition, vector construct, or viral vector sufficient to achieve a treatment response as compared to the response obtained without administration of the composition, vector construct, or viral vector. The amount of a given composition described herein that will correspond to such an amount will vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, weight) or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. Also, as used herein, a “therapeutically effective amount” of a composition, vector construct, or viral vector of the present disclosure is an amount which results in a beneficial or desired result in a subject as compared to a control. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not include amounts of

each ingredient that would have been effective if administered individually. As defined herein, a therapeutically effective amount of a composition, vector construct, viral vector or cell of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art. Dosage regime may be adjusted to provide the optimum therapeutic response.

5 As used herein, the term “endogenous” describes a molecule (e.g., a polypeptide, nucleic acid, or cofactor) that is found naturally in a particular organism (e.g., a human) or in a particular location within an organism (e.g., an organ, a tissue, or a cell, such as a human cell, e.g., a human cochlear hair cell).

As used herein, the term “express” refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g.,
10 by splicing, editing, 5’ cap formation, and/or 3’ end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

As used herein, the term “exogenous” describes a molecule (e.g., a polypeptide, nucleic acid, or cofactor) that is not found naturally in a particular organism (e.g., a human) or in a particular location within an organism (e.g., an organ, a tissue, or a cell, such as a human cell, e.g., a human cochlear hair
15 cell). Exogenous materials include those that are provided from an external source to an organism or to cultured matter extracted therefrom.

As used herein, the term “hair cell-specific expression” refers to production of an RNA transcript or polypeptide primarily within hair cells (e.g., cochlear hair cells) as compared to other cell types of the inner ear (e.g., spiral ganglion neurons, glia, or other inner ear cell types). Hair cell-specific expression of
20 a transgene can be confirmed by comparing transgene expression (e.g., RNA or protein expression) between various cell types of the inner ear (e.g., hair cells vs. non-hair cells) using any standard technique (e.g., quantitative RT PCR, immunohistochemistry, Western Blot analysis, or measurement of the fluorescence of a reporter (e.g., GFP) operably linked to a promoter). A hair cell-specific promoter induces expression (e.g., RNA or protein expression) of a transgene to which it is operably linked that is
25 at least 50% greater (e.g., 50%, 75%, 100%, 125%, 150%, 175%, 200% greater or more) in hair cells (e.g., cochlear hair cells) compared to at least 3 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, or more) of the following inner ear cell types: Border cells, inner phalangeal cells, inner pillar cells, outer pillar cells, first row Deiter cells, second row Deiter cells, third row Deiter cells, Hensen’s cells, Claudius cells, inner sulcus cells, outer sulcus cells, spiral prominence cells, root cells, interdental cells, basal cells of the stria vascularis,
30 intermediate cells of the stria vascularis, marginal cells of the stria vascularis, spiral ganglion neurons, Schwann cells.

As used herein, the terms “increasing” and “decreasing” refer to modulating resulting in, respectively, greater or lesser amounts, of function, expression, or activity of a metric relative to a reference. For example, subsequent to administration of a composition in a method described herein, the
35 amount of a marker of a metric (e.g., ABR threshold) as described herein may be increased or decreased in a subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% or more relative to the amount of the marker prior to administration. Generally, the metric is measured subsequent to administration at a time that the administration has had the recited effect, e.g., at least one week, one month, 3 months, or 6 months, after a treatment regimen
40 has begun.

As used herein, the term “intron” refers to a region within the coding region of a gene, the nucleotide sequence of which is not translated into the amino acid sequence of the corresponding protein. The term intron also refers to the corresponding region of the RNA transcribed from a gene. Introns are transcribed into pre-mRNA, but are removed during processing, and are not included in the mature mRNA.

As used herein, “locally” or “local administration” means administration at a particular site of the body intended for a local effect and not a systemic effect. Examples of local administration are epicutaneous, inhalational, intra-articular, intrathecal, intravaginal, intravitreal, intrauterine, intra-lesional administration, lymph node administration, intratumoral administration, administration to the inner ear, and administration to a mucous membrane of the subject, wherein the administration is intended to have a local and not a systemic effect.

As used herein, the term “operably linked” refers to a first molecule that can be joined to a second molecule, wherein the molecules are so arranged that the first molecule affects the function of the second molecule. The term “operably linked” includes the juxtaposition of two or more components (e.g., a promoter and another sequence element) such that both components function normally and allow for the possibility that at least one of the components can mediate a function that is exerted upon at least one of the other components. The two molecules may or may not be part of a single contiguous molecule and may or may not be adjacent. For example, a promoter is operably linked to a transcribable polynucleotide molecule if the promoter modulates transcription of the transcribable polynucleotide molecule of interest in a cell. In additional embodiments, two portions of a transcription regulatory element are operably linked to one another if they are joined such that the transcription-activating functionality of one portion is not adversely affected by the presence of the other portion. Two transcription regulatory elements may be operably linked to one another by way of a linker nucleic acid (e.g., an intervening non-coding nucleic acid) or may be operably linked to one another with no intervening nucleotides present.

As used herein, the terms “otoferlin isoform 5” and “OTOF isoform 5” refer to an isoform of the gene associated with nonsyndromic recessive deafness DFNB9. The human isoform of the gene is associated with reference sequence NM_001287489, and the transcript includes exons 1-45 and 47 of human otoferlin but lacks exon 46 of the OTOF gene. The human OTOF isoform 5 protein is also known as Otoferlin isoform e. The terms “otoferlin isoform 5” and “OTOF isoform 5” also refer to variants of the wild-type OTOF isoform 5 protein and polynucleotides encoding the same, such as variant proteins having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the amino acid sequence of a wild-type OTOF isoform 5 protein (e.g., SEQ ID NO: 1) or polynucleotides having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the polynucleotide sequence of a wild-type OTOF isoform 5 gene, provided that the OTOF isoform 5 analog encoded retains the therapeutic function of wild-type OTOF isoform 5. OTOF isoform 5 protein variants can have one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) conservative amino acid substitutions relative to a wild-type OTOF isoform 5 (e.g., SEQ ID NO: 1), provided that the that the OTOF isoform 5 variant retains the therapeutic function of wild-type OTOF isoform 5 and has no more than 10% amino acid substitutions in an N-terminal portion of the amino acid sequence and no more than 10% amino acid substitutions in a C-terminal portion of the amino

acid sequence. As used herein, OTOF isoform 5 may refer to the protein localized to inner hair cells or to the gene encoding this protein, depending upon the context, as will be appreciated by one of skill in the art. OTOF isoform 5 may refer to human OTOF isoform 5 or to a homolog from another mammalian species. Murine otoferlin contains one additional exon relative to human otoferlin (48 exons in murine
5 otoferlin), and the exons of murine otoferlin that correspond to those that encode human OTOF isoform 5 are 1-5, 7-46, and 48. The exon numbering convention used herein is based on the exons currently understood to be present in the consensus transcripts of human OTOF.

As used herein, the term "plasmid" refers to an extrachromosomal circular double stranded DNA molecule into which additional DNA segments may be ligated. A plasmid is a type of vector, a nucleic acid
10 molecule capable of transporting another nucleic acid to which it has been linked. Certain plasmids are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial plasmids having a bacterial origin of replication and episomal mammalian plasmids). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Certain plasmids are capable of
15 directing the expression of genes to which they are operably linked.

As used herein, the terms "nucleic acid" and "polynucleotide," used interchangeably herein, refer to a polymeric form of nucleosides in any length. Typically, a polynucleotide is composed of nucleosides that are naturally found in DNA or RNA (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine) joined by phosphodiester bonds.
20 However, the term encompasses molecules containing nucleosides or nucleoside analogs containing chemically or biologically modified bases, modified backbones, etc., whether or not found in naturally occurring nucleic acids, and such molecules may be preferred for certain applications. Where this application refers to a polynucleotide it is understood that both DNA, RNA, and in each case both single- and double-stranded forms (and complements of each single-stranded molecule) are provided.
25 "Polynucleotide sequence" as used herein can refer to the polynucleotide material itself and/or to the sequence information (i.e., the succession of letters used as abbreviations for bases) that biochemically characterizes a specific nucleic acid. A polynucleotide sequence presented herein is presented in a 5' to 3' direction unless otherwise indicated.

As used herein, the terms "complementarity" or "complementary" of nucleic acids means that a
30 nucleotide sequence in one strand of nucleic acid, due to orientation of its nucleobase groups, forms hydrogen bonds with another sequence on an opposing nucleic acid strand. The complementary bases in DNA are typically A with T and C with G. In RNA, they are typically C with G and U with A. Complementarity can be perfect or substantial/sufficient. Perfect complementarity between two nucleic acids means that the two nucleic acids can form a duplex in which every base in the duplex is bonded to
35 a complementary base by Watson-Crick pairing. "Substantial" or "sufficient" complementary means that a sequence in one strand is not completely and/or perfectly complementary to a sequence in an opposing strand, but that sufficient bonding occurs between bases on the two strands to form a stable hybrid complex in set of hybridization conditions (e.g., salt concentration and temperature). Such conditions can be predicted by using the sequences and standard mathematical calculations to predict the T_m (melting
40 temperature) of hybridized strands, or by empirical determination of T_m by using routine methods. T_m includes the temperature at which a population of hybridization complexes formed between two nucleic

acid strands are 50% denatured (i.e., a population of double-stranded nucleic acid molecules becomes half dissociated into single strands). At a temperature below the T_m , formation of a hybridization complex is favored, whereas at a temperature above the T_m , melting or separation of the strands in the hybridization complex is favored. T_m may be estimated for a nucleic acid having a known G+C content in an aqueous 1 M NaCl solution by using, e.g., $T_m = 81.5 + 0.41(\% \text{ G+C})$, although other known T_m computations take into account nucleic acid structural characteristics.

As used herein, the term "promoter" refers to a recognition site on DNA that is bound by an RNA polymerase. The polymerase drives transcription of the transgene. Exemplary promoters suitable for use with the compositions and methods described herein include ubiquitous promoters (e.g., the CAG promoter, cytomegalovirus (CMV) promoter, and smCBA promoter) and cochlear hair cell-specific promoters (e.g., the Myosin 15 (Myo15) promoter).

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

$$100 \text{ multiplied by (the fraction } X/Y \text{)}$$

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

The term "derivative" as used herein refers to a nucleic acid, peptide, or protein or a variant or analog thereof comprising one or more mutations and/or chemical modifications as compared to a corresponding full-length wild-type nucleic acid, peptide, or protein. Non-limiting examples of chemical modifications involving nucleic acids include, for example, modifications to the base moiety, sugar moiety, phosphate moiety, phosphate-sugar backbone, or a combination thereof.

As used herein, the term "pharmaceutical composition" refers to a mixture containing a therapeutic agent, optionally in combination with one or more pharmaceutically acceptable excipients, diluents, and/or carriers, to be administered to a subject, such as a mammal, e.g., a human, in order to prevent, treat or control a particular disease or condition affecting or that may affect the subject.

As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions and/or dosage forms, which are suitable for contact with the tissues of a subject, such as a mammal (e.g., a human) without excessive toxicity, irritation, allergic response and other problem complications commensurate with a reasonable benefit/risk ratio. Preferably, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

As used herein, the term "recombinogenic region" refers to a region of homology that mediates recombination between two different sequences.

As used herein, the term "regulatory sequence" includes promoters, enhancers, and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the polynucleotides that encode OTOF. Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185 (Academic Press, San Diego, CA, 1990); incorporated herein by reference.

As used herein, the term "sample" refers to a specimen (e.g., blood, blood component (e.g., serum or plasma), urine, saliva, amniotic fluid, cerebrospinal fluid, tissue (e.g., placental or dermal), pancreatic fluid, chorionic villus sample, and cells) isolated from a subject.

As used herein, the term "transfection" refers to any of a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, lipofection, calcium-phosphate precipitation, DEAE-dextran transfection, Nucleofection, squeeze-poration, sonoporation, optical transfection, Magnetofection, impalefection and the like.

As used herein, the terms "subject" and "patient" refer to an animal (e.g., a mammal, such as a human), veterinary animals (e.g., cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models of diseases (e.g., mice, rats). A subject to be treated according to the methods described herein may be one who has been diagnosed with hearing loss (e.g., hearing loss associated with a mutation in OTOF), or one at risk of developing these conditions. Diagnosis may be performed by any method or technique known in the art. One skilled in the art will understand that a subject to be treated according to the present disclosure may have been subjected to standard tests or may have been identified, without examination, as one at risk due to the presence of one or more risk factors associated with the disease or condition.

As used herein, the terms "transduction" and "transduce" refer to a method of introducing a vector construct or a part thereof into a cell. Wherein the vector construct is contained in a viral vector such as for example an AAV vector, transduction refers to viral infection of the cell and subsequent transfer and integration of the vector construct or part thereof into the cell genome.

As used herein, "treatment" and "treating" of a state, disorder or condition can include: (1) preventing, delaying, or reducing the incidence and/or likelihood of the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition, but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or sub-clinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state,

disorder or condition or at least one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

As used herein, the term “vector” includes a nucleic acid vector, e.g., a DNA vector, such as a plasmid, an RNA vector, virus, or other suitable replicon (e.g., viral vector). A variety of vectors have been developed for the delivery of polynucleotides encoding exogenous proteins into a prokaryotic or eukaryotic cell. Examples of such expression vectors are disclosed in, e.g., WO94/11026; incorporated herein by reference as it pertains to vectors suitable for the expression of a gene of interest. Expression vectors suitable for use with the compositions and methods described herein contain a polynucleotide sequence as well as, e.g., additional sequence elements used for the expression of proteins and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Certain vectors that can be used for the expression of OTOF as described herein include vectors that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of OTOF contain polynucleotide sequences that enhance the rate of translation of these genes or improve the stability or nuclear export of the mRNA that results from gene transcription. These sequence elements include, e.g., 5' and 3' untranslated regions and a polyadenylation signal site in order to direct efficient transcription of the gene carried on the expression vector. The expression vectors suitable for use with the compositions and methods described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker include genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, or nourseothricin.

As used herein, the term “wild-type” refers to a genotype with the highest frequency for a particular gene in a given organism.

Brief Description of the Drawings

FIGS. 1A-1D are a series of graphs of ABR responses measured at Week 6 after a single, unilateral administration of a composition containing an OTOF dual hybrid vector system to the right ear of a subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. ABR responses were measured in response to a click stimulus (FIGS. 1A-1B), 2000 Hz (FIG. 1C), and 500 Hz (FIG. 1D). Neural ABR (Wave V) responses were present at 90 dB nHL in the treated ear and were absent at Baseline.

FIG. 2 is a series of graphs of audiograms (behavioral pure tones) measured at Baseline and at Week 4 and Week 6 after a single, unilateral administration of a composition containing an OTOF dual hybrid vector system to the right ear of the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. Responses from the right (treated) ear are shown using circles and triangles and responses from the left ear (without the cochlear implant on) are shown using Xs. The treated ear showed profound hearing loss to severe/profound hearing loss from Baseline to Week 4, and the audiogram was essentially stable from Week 4 to Week 6, which may be due to testing difficulties. The untreated ear

without the cochlear implant on showed profound hearing loss that did not change from Baseline to Week 6.

FIG. 3 is a series of graphs of ABR responses measured at Baseline in the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. ABR responses were measured in response to a click stimulus (top) and 2000 Hz (bottom).

FIG. 4 is a graph of a behavioral pure tone audiogram showing responses measured at Baseline, Week 4, and Week 12 in the treated ear of the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. The treated ear exhibited profound hearing impairment at Baseline and showed responses to multiple pitches at Week 4. At Week 12, the treated ear exhibited responses representative of moderate hearing loss. Arrows indicate no response at the maximum level tested.

FIG. 5 is a drawing depicting the surgical approach and route of administration used for administration of the OTOF dual hybrid vector system. The surgical approach used to access the middle ear was similar to that used for cochlear implant surgery. The OTOF dual hybrid vector system was administered via insertion of a catheter through the round window membrane into the inner ear perilymph for infusion. A fenestration was also made in the lateral semicircular canal. AAV, adeno-associated virus; LSCC, lateral semicircular canal; RWM, round window membrane.

FIG. 6 is a series of graphs of behavioral pure tone audiometry responses measured in the treated ear and the untreated (cochlear implant) ear of the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. Hearing thresholds for the treated ear improved from profound sensorineural hearing loss to moderate sensorineural hearing loss at Week 12. No improvement was seen in the untreated ear with the cochlear implant turned off. Arrow = no response; HL, hearing level; AC, air conduction.

FIG. 7 is a series of graphs of ABR thresholds measured in the treated ear and the untreated ear of the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. A positive ABR wave response in hearing intensity from Baseline through Week 12 at thresholds of 40–80 dB was reported in the treated ear; no response was elicited at 100 dB at Baseline. No improvement in hearing intensity was seen in the untreated ear in comparison with the treated ear. Arrow = no response; Week 6: Could not test at 1000 and 4000 Hz due to subject waking up; Week 12: Subject woke up after starting 4000 Hz trial at 60 dB in the treated ear, therefore could not proceed to find threshold. nHL, normalized hearing level.

FIG. 8 is a graph showing the LittleEARS auditory questionnaire score from Baseline to Week 12 from the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. An improvement in the subject's global auditory skill development was observed at Week 12 from Baseline, according to parental reports and as indicated by the LittleEARS auditory questionnaire. Parents and investigator reported that the subject could hear sounds when the opposite ear cochlear implant was turned off, indicating improved hearing acuity in the treated ear. Parents also reported more natural vocalizations when the opposite ear cochlear implant was turned off.

FIG. 9 is a graph showing measurements of cervical vestibular evoked myogenic potentials (cVEMP) from Baseline to Week 12 from the subject with biallelic mutations in the OTOF gene who was dosed at 10 months old. Vestibular responses were present at Baseline and continued to be present through Week 12 (comparable to Baseline). CI, cochlear implant.

Detailed Description

Described herein are compositions and methods for the treatment of sensorineural hearing loss or auditory neuropathy in a subject having biallelic OTOF mutations (such as a mammalian subject, for instance, a human) by administering a first nucleic acid vector (e.g., an AAV vector) containing a promoter and a polynucleotide encoding an N-terminal portion of an otoferlin (OTOF) protein (e.g., a polynucleotide encoding a long isoform of an OTOF protein, such as a wild-type (WT) human OTOF isoform 5 protein or a wild-type human OTOF isoform 1 protein) and a second nucleic acid vector (e.g., an AAV vector) containing a polynucleotide encoding a C-terminal portion of an OTOF protein and a polyadenylation (poly(A)) sequence. When introduced into a mammalian cell, such as a cochlear hair cell, the polynucleotides encoded by the two nucleic acid vectors can combine to form a nucleic acid molecule that encodes the full-length OTOF protein. The compositions and methods described herein can, therefore, be used to induce or increase expression of WT OTOF in cochlear hair cells of a subject who has an OTOF deficiency (e.g., low OTOF expression or an OTOF mutation that impairs OTOF expression or function).

15

Otoferlin

OTOF is a 230 kDa membrane protein that contains at least six C2 domains implicated in calcium, phospholipid, and protein binding. Human OTOF is encoded by a gene that contains 47 exons, and the full-length protein is made up of 1,997 amino acids. OTOF is located at ribbon synapses in inner hair cells, where it is believed to function as a calcium sensor in synaptic vesicle fusion, triggering the fusion of neurotransmitter-containing vesicles with the plasma membrane. It has also been implicated in vesicle replenishment and clathrin-mediated endocytosis, and has been shown to interact with Myosin VI, Rab8b, SNARE proteins, calcium channel Cav1.3, Ergic2, and AP-2. The mechanism by which OTOF mediates exocytosis and the physiological significance of its interactions with its binding partners remain to be determined.

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There are multiple long and short isoforms of the Otoferlin gene. Studies of human genetic deafness have suggested that long isoforms are important for inner ear function. However, the role of these individual long isoforms and other protein variants in inner ear function is not understood. To develop effective gene transfer therapies for patients who experience deafness secondary to genetically driven Otoferlin deficiency, a cDNA sequence that encodes functional OTOF isoforms in the ear must be identified.

30

Otoferlin-associated hearing loss

OTOF was first identified by a study investigating the genetics of a non-syndromic form of deafness, autosomal recessive deafness-9 (DFNB9). Mutations in OTOF have since been found to cause sensorineural hearing loss in patients throughout the world, with many patients carrying OTOF mutations having auditory neuropathy, a disorder in which the inner ear detects sound, but is unable to properly transmit sound from the ear to the brain. These patients have an abnormal auditory brainstem response (ABR) and impaired speech discrimination with initially normal otoacoustic emissions (OAEs). Patients carrying homozygous or compound heterozygous mutations often develop hearing loss in early

40

childhood, and the severity of hearing impairment has been found to vary with the location and type of mutation in OTOF.

The compositions and methods described herein can be used to treat sensorineural hearing loss or auditory neuropathy by administering a first nucleic acid vector containing a polynucleotide encoding an N-terminal portion of an OTOF protein and a second nucleic acid vector containing a polynucleotide encoding a C-terminal portion of an OTOF protein. The full-length OTOF coding sequence is too large to include in the type of vector that is commonly used for gene therapy (e.g., an adeno-associated virus (AAV) vector, which is thought to have a packaging limit of 5 kb). The compositions and methods described herein overcome this problem by dividing the OTOF coding sequence between two different nucleic acid vectors (e.g., AAV vectors) that can combine in a cell to reconstitute the full-length OTOF sequence. In some embodiments, the OTOF protein encoded by the vectors is an OTOF isoform 5 protein (e.g., a wild-type human OTOF isoform 5 protein). In some embodiments, the OTOF protein encoded by the vectors is an OTOF isoform 1 protein (e.g., a wild-type human OTOF isoform 1 protein). These compositions and methods can be used to treat subjects having one or more mutations in the OTOF gene, e.g., an OTOF mutation that reduces OTOF expression, reduces OTOF function, or is associated with hearing loss (e.g., a frameshift mutation, a nonsense mutation, a deletion, or a missense substitution). When the first and second nucleic acid vectors are administered in a composition, the polynucleotides encoding the N-terminal and C-terminal portions of OTOF can combine within a cell (e.g., a human cell, e.g., a cochlear hair cell) to form a single nucleic acid molecule that contains the full-length OTOF coding sequence (e.g., through homologous recombination and/or splicing).

The nucleic acid vectors (e.g., AAV vectors) used in the compositions and methods described herein include polynucleotide sequences that encode wild-type OTOF isoform 5, or a variant thereof, such as a polynucleotide sequences that, when combined, encode a protein having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the amino acid sequence of wild-type mammalian (e.g., human or mouse) OTOF isoform 5. The polynucleotides used in the nucleic acid vectors described herein can encode an N-terminal portion and a C-terminal portion of an OTOF isoform 5 amino acid sequence in Table 2 below (e.g., two portions that, when combined, encode a full-length OTOF isoform 5 amino acid sequence listed in Table 2, e.g., SEQ ID NO: 1).

According to the methods described herein, a subject can be administered a composition containing a first nucleic acid vector and a second nucleic acid vector that contain an N-terminal and C-terminal portion, respectively, of a polynucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1, or a polynucleotide sequence encoding an amino acid sequence having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the amino acid sequence of SEQ ID NO: 1, or a polynucleotide sequence encoding an amino acid sequence that contains one or more conservative amino acid substitutions relative to SEQ ID NO: 1 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more conservative amino acid substitutions), provided that the OTOF analog encoded retains the therapeutic function of wild-type OTOF isoform 5 (e.g., the ability to regulate exocytosis at ribbon synapses or rescue or improve ABR response in an animal model of hearing loss related to Otoferlin gene deficiency (e.g., OTOF mutation)). No more than 10% of the amino acids in the N-terminal portion of the human OTOF isoform 5 protein and no more

than 10% of the amino acids in the C-terminal portion of the human OTOF isoform 5 protein may be replaced with conservative amino acid substitutions. The OTOF isoform 5 protein may be encoded by a polynucleotide having the sequence of SEQ ID NO: 2 or SEQ ID NO: 3. The OTOF isoform 5 protein may also be encoded by a polynucleotide having single nucleotide variants (SNVs) that have been found to be non-pathogenic in human subjects. The OTOF isoform 5 protein may be a human OTOF isoform 5 protein or may be a homolog of the human isoform 5 protein from another mammalian species (e.g., mouse, rat, cow, horse, goat, sheep, donkey, cat, dog, rabbit, guinea pig, or other mammal).

Table 2: OTOF Sequences

SEQ ID NO.	Sequence Name	Sequence
1	Human OTOF isoform 5 protein (NP_001274418.1), also called human otoferlin isoform e, 1997 aa	MALLIHLKTVSELRGRGDRIAKVTFRGQSFYSRVLENCEDVADFDE TFRWPVASSIDRNEMLEIQVFNYSKVFSNKLIGTFRMVLQKVVEES HVEVTDTLIDDNNAIIKTSLCVEVRYQATDGTVGSWDDGDFLGDES LQEEEKDSQETDGLLPGSRPSSRPPGEKSFRFRAGRSVFSAMKLGK NRSHKEEPQRPDEPAVLEMEDLDHLAIRLGDGLDPDSVSLASVTAL TTNVSNNKRSKPDIKMEPSAGRPMQDYQVSITVIEARQLVGLNMDPVV CVEVGDDKKYTSMEKSTNCPYYNEYFVDFHVSFVDFDKIIKISVI HKNLLRSGTLVGSFKMDVGTVYSQPEHQFHHKWAILSDPDDISS GLKGYVKCDVAVVGKGDNIKTPHNETDEDDIEGNLLPEGVPPE RQWARFYVKIYRAEGLPRMNTSLMANVKKAFIGENKDLVDPYVQV FFAGQKKGKTSVQKSSYEPLWNEQVVFTDLFPPLCKRMKVQIRDSD KVNDVAIGTHFIDLKISNDGDKGFLPTLGPVWVNMVYSTRNYTLL DEHQDLNEGLGEGVSFRARLLLGLAVEIVDTSNPELTSSTEVQVEQ ATPISESCAGKMEEFFLFGAFLEASMDRRNGDKPITFEVTIGNYGN EVDGLSRPQRPQRPKEPGDEEEVDLIQNASDDEAGDAGDLASVSS TPPMRPQVTDNRNYFHLPLYERKPCYIKSWWPDQRRRLYNANIMD HIADKLEEGLNDIQEMIKTEKSYPERRLRGVLEELSCGCCRFSLAD KDQGHSSRTRLDRELRKSCMRELENMGQQARMLRAQVKRHTVRD KLRLCQNFLQKLRFLADEPQHSIPDIFIWMMSNNKRVAYARVPSKD LLFSIVEEETGKDCAKVKTLFLKLPKRGFGSAGWTVQAKVELYLW LGLSKQRKEFLCGLPCGFQEVKAAQGLGLHAFPPVSLVYTKKQAF QLRAHMYQARSLFAADSSGLSDPFARVFFINQSQCTEVLNETLCPT WDQMLVFDNLELYGEAHELRDDPPIIVIEIYDQDSMGKADFMGRTF AKPLVKMADEAYCPRFPFPQLEYYQIYRGNATAGDLLAFAELLQIG PAGKADLPPINGPVDVDRGPIMPVPMGIRPVLSKYRVEVLFWGLRD LKRNVLAQVDRPRVDIECAGKGVQSSLIHNYKKNPNFNTLVKWFV DLPENELLHPLNIRVVDCRAFGRYTLVGSHAVSSLRFFIYRPPDRS APSWNTTVRLRLRCRVLCNGGSSSHSTGEVVVTMEPEVPIKKLET MVKLDATSEAVVKVDVAEEEEKEKKKKKGTAEEPEEEEPDESMLD WWSKYFASIDTMKEQLRQQEPGIDLEEKEEVDNTEGLKGSMDK

SEQ ID NO.	Sequence Name	Sequence
		EKARAAKEEKKKKTQSSGSGQGSEAPEKKKPKIDELKVYPKELESE FDNFEDWLHTFNLLRGKTGDDDEDGSTEEERIVGRFKGSLCVYKVPL PEDVSREAGYDSTYGMFQGIPSNPINVLVRVYVVRATDLHPADIN GKADPYIAIRLGKTDIRDKENYISKQLNPVFGKSFIEASFPMESMLT VAVYDWDLVGTDDLIGETKIDLENRFYSKHRATCGIAQTYSTHGYN WRDPMKPSQILTRLCKDGKVDGPHFGPPGRVKVANRVFTGPSEIE DENGQRKPTDEHVALLALRHWEDIPRAGCRLVPEHVETRPLNPD KPGIEQGRLELWVDMFPMMPAPGTPLDISPRKPKKYELRVIIWNT DEVVLEDDDDFTGEKSSDIFVRGWLKGQEDKQDQTDVHYHSLTGE GNFNWRYLFPFDYLAEEKIVISKKESMFSWDETEYKIPARLTQIW DADHFSADDFLGAIELDLNRFPRGAKTAKQCTMEMATGEVDVPLV SIFKQKRVKGWWPLLARNENDEFELTGKVEAELHLLTAEAEKNPV GLARNEPDPLEKPNRPDTAFVWFLNPLKSIKYLICTRYKWLIIKIVLAL LGLMLGLFLYSLPGYMKKLLGA
2	DNA sequence encoding the human otoferlin isoform 5 protein (SEQ ID NO: 1), 5994 bp, corresponds to the coding sequence documented in NM_001287489	ATGGCCTTGCTCATCCACCTCAAGACAGTCTCGGAGCTGCGGG GCAGGGGCGACCGGATCGCCAAAGTGACTTTCCGAGGGCAATC CTTCTACTCTCGGGTCCTGGAGAACTGTGAGGATGTGGCTGACT TTGATGAGACATTTCCGGTGGCCGGTGGCCAGCAGCATCGACAG AAATGAGATGCTGGAGATTCAGGTTTTCAACTACAGCAAAGTCTT CAGCAACAAGCTCATCGGGACCTTCCGCATGGTGCTGCAGAAG GTGGTAGAGGAGAGCCATGTGGAGGTGACTGACACGCTGATTG ATGACAACAATGCTATCATCAAGACCAGCCTGTGCGTGAGGTC CGGTATCAGGCCACTGACGGCACAGTGGGCTCCTGGGACGATG GGGACTTCCTGGGAGATGAGTCTCTTCAAGAGGAAGAGAAGGA CAGCCAAGAGACGGATGGACTGCTCCCAGGCTCCCGGCCAGC TCCCGGCCCCCAGGAGAGAAGAGCTTCCGGAGAGCCGGGAGG AGCGTGTTCTCCGCCATGAAGCTCGGCAAAAACCGGTCTCACAA GGAGGAGCCCCAAAGACCAGATGAACCGGCGGTGCTGGAGAT GGAAGACCTTGACCATCTGGCCATTGGCTAGGAGATGGACTG GATCCCGACTCGGTGTCTCTAGCCTCAGTCACAGCTCTCACCAC TAATGTCTCCAACAAGCGATCTAAGCCAGACATTAAGATGGAGC CAAGTGCTGGGCGGCCCATGGATTACCAGGTCAGCATCACGGT GATCGAGGCCCGGCAGCTGGTGGGCTTGAACATGGACCCTGTG GTGTGCGTGAGGTGGGTGACGACAAGAAGTACACATCCATGA AGGAGTCCACTAACTGCCCCTATTACAACGAGTACTTCGTCTTC GACTTCCATGTCTCTCCGGATGTCATGTTTGACAAGATCATCAAG ATTCGGTGATTCACTCCAAGAACCTGCTGCGCAGTGGCACCCCT GGTGGGCTCCTTCAAAATGGACGTGGGAACCGTGTACTCGCAG CCAGAGCACCAGTTCATCACAAGTGGGCCATCCTGTCTGACCC

SEQ ID NO.	Sequence Name	Sequence
		CGATGACATCTCCTCGGGGCTGAAGGGCTACGTGAAGTGTGAC GTTGCCGTGGTGGGCAAAGGGGACAACATCAAGACGCCCCACA AGGCCAATGAGACCGACGAAGATGACATTGAGGGGAACCTTGCT GCTCCCCGAGGGGGTGCCCCCGAACGCCAGTGGGCCCCGGTT CTATGTGAAAATTTACCGAGCAGAGGGGCTGCCCCGTATGAACA CAAGCCTCATGGCCAATGTAAAGAAGGCTTTCATCGGTGAAAAC AAGGACCTCGTGGACCCCTACGTGCAAGTCTTCTTTGCTGGCCA GAAGGGCAAGACTTCAGTGCAGAAGAGCAGCTATGAGCCCCTG TGAATGAGCAGGTCGTCTTTACAGACCTCTTCCCCCACTCTG CAAACGCATGAAGGTGCAGATCCGAGACTCGGACAAGGTCAAC GACGTGGCCATCGGCACCCACTTCATTGACCTGCGCAAGATTTCT TAATGACGGAGACAAAGGCTTCTGCCCACACTGGGCCCAGCC TGGGTGAACATGTACGGCTCCACACGTAACACGCTGCTGGA TGAGCATCAGGACCTGAACGAGGGCCTGGGGGAGGGTGTGTC CTTCCGGGCCCGGCTCCTGCTGGGCCTGGCTGTGGAGATCGTA GACACCTCCAACCCTGAGCTCACCAGCTCCACAGAGGTGCAGG TGGAGCAGGCCACGCCCATCTCGGAGAGCTGTGCAGGTAAAAT GGAAGAATTCTTTCTTTTGAGCCTTCTGGAGGCCTCAATGA TCGACCGGAGAAACGGAGACAAGCCCATCACCTTTGAGGTCAC CATAGGCAACTATGGGAACGAAGTTGATGGCCTGTCCCGGCC CAGCGGCCTCGGCCCCGGAAGGAGCCGGGGGATGAGGAAGAA GTAGACCTGATTGAGAACGCAAGTGATGACGAGGCCGGTGATG CCGGGGACCTGGCCTCAGTCTCCTCCACTCCACCAATGCGGCC CCAGGTCACCGACAGGAACCTACTTCCATCTGCCCTACCTGGAGC GAAAGCCCTGCATCTACATCAAGAGCTGGTGGCCGGACCAGCG CCGCCGCCTCTACAATGCCAACATCATGGACCACATTGCCGACA AGCTGGAAGAAGGCCTGAACGACATACAGGAGATGATCAAAAC GGAGAAGTCCTACCCTGAGCGTCGCCTGCGGGGCGTCCTGGA GGAGCTGAGCTGTGGCTGCTGCCGCTTCTCTCCCTCGCTGAC AAGGACCAGGGCCACTCATCCCGCACCAGGCTTGACCGGGAGC GCCTCAAGTCCTGCATGAGGGAGCTGGAAAACATGGGGCAGCA GGCCAGGATGCTGCGGGCCCAGGTGAAGCGGCACACGGTGCG GGACAAGCTGAGGCTGTGCCAGAACTTCTGCAGAAGCTGCGC TTCCTGGCGGACGAGCCCCAGCACAGCATTCCCACATCTTCAT CTGGATGATGAGCAACAACAAGCGTGTGCCTATGCCCGTGTG CCCTCCAAGGACCTGCTCTTCTCCATCGTGGAGGAGGAGACTG GCAAGGACTGCGCCAAGGTCAAGACGCTCTTCTTAAGCTGCC AGGGAAGCGGGGCTTCGGCTCGGCAGGCTGGACAGTGCAGGC CAAGGTGGAGCTGTACCTGTGGCTGGGCCTCAGCAAACAGCGC

SEQ ID NO.	Sequence Name	Sequence
		AAGGAGTTCCTGTGCGGCCTGCCCTGTGGCTTCCAGGAGGTCA AGGCAGCCCAGGGCCTGGGCCTGCATGCCTTCCCACCCGTGAG CCTGGTCTACACCAAGAAGCAGGCGTTCCAGCTCCGAGCGCAC ATGTACCAGGCCCGCAGCCTCTTTGCCGCCGACAGCAGCGGAC TCTCAGACCCCTTTGCCCGCGTCTTCTTCATCAATCAGAGTCAG TGCACAGAGGTGCTGAATGAGACCCTGTGTCCCACCTGGGACC AGATGCTGGTGTTCGACAACCTGGAGCTCTATGGTGAAGCTCAT GAGCTGAGGGACGATCCGCCCATCATTGTCATTGAAATCTATGA CCAGGATTCCATGGGCAAAGCTGACTTCATGGGCCGGACCTTC GCCAAACCCCTGGTGAAGATGGCAGACGAGGCGTACTGCCAC CCCGCTTCCCACCTCAGCTCGAGTACTACCAGATCTACCGTGGC AACGCCACAGCTGGAGACCTGCTGGCGGCCTTCGAGCTGCTGC AGATTGGACCAGCAGGGAAGGCTGACCTGCCCCCATCAATGG CCCGGTGGACGTGGACCGAGGTCCCATCATGCCCGTGCCCATG GGCATCCGGCCCGTGCTCAGCAAGTACCGAGTGGAGGTGCTGT TCTGGGGCCTACGGGACCTAAAGCGGGTGAACCTGGCCCAGGT GGACCGGCCACGGGTGGACATCGAGTGTGCAGGGAAGGGGGT GCAGTCGTCCCTGATCCACAATTATAAGAAGAACCCCAACTTCA ACACCCTCGTCAAGTGGTTTGAAGTGGACCTCCCAGAGAACGA GCTGCTGCACCCGCCCTTGAACATCCGTGTGGTGGACTGCCGG GCCTTCGGTTCGCTACACACTGGTGGGCTCCCATGCCGTGAGCT CCCTGCGACGCTTCATCTACCGGCCCCCAGACCGCTCGGCCCC CAGCTGGAACACCACGGTCAGGCTTCTCCGGCGCTGCCGTGTG CTGTGCAATGGGGGCTCCTCCTCTCACTCCACAGGGGAGGTTG TGGTGACTATGGAGCCAGAGGTACCCATCAAGAACTGGAGAC CATGGTGAAGCTGGACGCGACTTCTGAAGCTGTTGTCAAGGTG GATGTGGCTGAGGAGGAGAAGGAGAAGAAGAAGAAGAAGG GCACTGCCGAGGAGCCAGAGGAGGAGGAGCCAGACGAGAGCA TGCTGGAAGTGGTGGTCCAAGTACTTTGCCTCCATTGACACCATG AAGGAGCAACTTCGACAACAAGAGCCCTCTGGAATTGACTTGGA GGAGAAGGAGGAAGTGGACAATACCGAGGGCCTGAAGGGGTC AATGAAGGGCAAGGAGAAGGCAAGGGCTGCCAAAGAGGAGAAG AAGAAGAAAACTCAGAGCTCTGGCTCTGGCCAGGGGTCCGAGG CCCCCGAGAAGAAGAAACCAAGATTGATGAGCTTAAGGTATAC CCCAAAGAGCTGGAGTCCGAGTTTGATACTTTGAGGACTGGCT GCACACTTTCAACTTGCTTCGGGGCAAGACCGGGGATGATGAG GATGGCTCCACCGAGGAGGAGCGCATTGTGGGACGCTTCAAGG GCTCCCTCTGCGTGTACAAAGTGCCACTCCCAGAGGACGTGTC CCGGGAAGCCGGCTACGACTCCACCTACGGCATGTTCCAGGGC

SEQ ID NO.	Sequence Name	Sequence
		ATCCCGAGCAATGACCCCATCAATGTGCTGGTCCGAGTCTATGT GGTCCGGGGCCACGGACCTGCACCCTGCTGACATCAACGGCAAA GCTGACCCCTACATCGCCATCCGGCTAGGCAAGACTGACATCC GCGACAAGGAGAACTACATCTCCAAGCAGCTCAACCCTGTCTTT GGGAAGTCCTTTGACATCGAGGCCTCCTTCCCCATGGAATCCAT GCTGACGGTGGCTGTGTATGACTGGGACCTGGTGGGCACTGAT GACCTCATTGGGGAACCAAGATCGACCTGGAGAACCGCTTCTA CAGCAAGCACCGCGCCACCTGCGGCATCGCCAGACCTACTCC ACACATGGCTACAATATCTGGCGGGACCCCATGAAGCCCAGCC AGATCCTGACCCGCCTCTGCAAAGACGGCAAAGTGGACGGCCC CCACTTTGGGCCCCCTGGGAGAGTGAAGGTGGCCAACCGCGTC TTCAGTGGGCCCTCTGAGATTGAGGACGAGAACGGTCAGAGGA AGCCCACAGACGAGCATGTGGCGCTGTTGGCCCTGAGGCACTG GGAGGACATCCCCCGCGCAGGCTGCCGCTGGTGCCAGAGCA TGTGGAGACGAGGCGCTGCTCAACCCCGACAAGCCGGGCATC GAGCAGGGCCGCCTGGAGCTGTGGGTGGACATGTTCCCCATGG ACATGCCAGCCCCTGGGACGCCTCTGGACATCTCACCTCGGAA GCCAAGAAGTACGAGCTGCGGGTCATCATCTGGAACACAGAT GAGGTGGTCTTGGAGGACGACGACTTCTTACAGGGGAGAAGT CCAGTGACATCTTCGTGAGGGGGTGGCTGAAGGGCCAGCAGGA GGACAAGCAGGACACAGACGTCCACTACCACTCCCTCACTGGC GAGGGCAACTTCAACTGGCGCTACCTGTTCCCCTTCGACTACCT GGCGGCGGAGGAGAAGATCGTCATCTCCAAGAAGGAGTCCATG TTCTCCTGGGACGAGACCGAGTACAAGATCCCCGCGCGGCTCA CCCTGCAGATCTGGGATGCGGACCACTTCTCCGCTGACGACTT CCTGGGGGCCATCGAGCTGGACCTGAACCGGTTCCCGCGGGG CGCAAAGACAGCCAAGCAGTGCACCATGGAGATGGCCACCGGG GAGGTGGACGTGCCCCCTCGTGTCCATCTTCAAGCAAAAGCGCG TCAAAGGCTGGTGGCCCCTCCTGGCCCGCAATGAGAACGATGA GTTTGAGCTCACGGGCAAGGTGGAGGCTGAGCTGCATTTACTG ACAGCAGAGGAGGCAGAGAAGAACCCAGTGGGCCTGGCCCGC AATGAACCTGACCCCTAGAGAAACCAACCGGCCCGACACGG CCTTCGTCTGGTTCCTCAACCCTCTCAAGTCCATCAAGTACCTCA TCTGCACCCGGTACAAGTGGCTCATCATCAAGATCGTGCTGGCG CTGTTGGGGCTGCTCATGTTGGGGCTCTTCTCTACAGCCTCCC TGGCTACATGGTCAAAAAGCTCCTTGGGGCATGA
3	Codon-optimized (CO) DNA sequence encoding the human	ATGGCACTGCTGATCCACCTGAAAACCGTCTCCGAAGTGAAGG CAGAGGGGACAGAATCGCTAAAGTCACCTTCCGGGGACAGAGC TTTACAGCAGGGTGCTGGAGAAGTGCAGGACGTGGCCGACT

SEQ ID NO.	Sequence Name	Sequence
	otoferlin isoform 5 protein (SEQ ID NO: 1), 5994 bp	TTGACGAGACATTCAGGTGGCCCGTGGCCAGCTCCATCGATCG CAATGAGATGCTGGAGATCCAGGTGTTTAACTATAGCAAGGTGT TCTCCAATAAGCTGATCGGCACCTTCCGGATGGTGTGCAGAAG GTGGTGGAGGAGTCCCACGTGGAGGTGACCGACACACTGATCG ACGATAACAATGCCATCATCAAGACATCCCTGTGCGTGGAGGTG CGCTACCAGGCCACCGATGGCACAGTGGGCTCTTGGGACGATG GCGACTTCCTGGGCGATGAGTCCCTGCAGGAGGAGGAGAAGGA CTCTCAGGAGACAGATGGCCTGCTGCCTGGCTCCCGGCCATCT AGCCGCCCCCTGGCGAGAAGTCTTTTAGGAGAGCCGGCAGGT CCGTGTTCTCTGCCATGAAGCTGGGCAAGAACAGGAGCCACAA GGAGGAGCCTCAGAGGCCCGACGAGCCAGCCGTGCTGGAGAT GGAGGACCTGGATCACCTGGCCATCAGACTGGGCGATGGCCTG GACCCTGATAGCGTGTCCCTGGCCTCCGTGACCGCCCTGACCA CAAACGTGTCTAATAAGCGGAGCAAGCCAGACATCAAGATGGAG CCATCTGCCGGCAGGCCCATGGATTACCAGGTGAGCATCACAG TGATCGAGGCCAGACAGCTGGTGGGCCTGAACATGGACCCCGT GGTGTGCGTGGAAGTGGGCGACGATAAGAAGTACACCTCCATG AAGGAGTCTACAACTGTCCATACTACAACGAGTACTTCGTGTTT GATTTCCACGTGAGCCCCGACGTGATGTTGATAAGATCATCAA GATCAGCGTGATCCACTCCAAGAATCTGCTGCGGTCTGGCACC CTGGTGGGAAGCTTTAAGATGGACGTGGGCACAGTGTACTCTCA GCCTGAGCACCAGTTCCACCACAAGTGGGCCATCCTGAGCGAT CCAGACGATATCTCCTCTGGCCTGAAGGGCTATGTGAAGTGCGA CGTGGCAGTGGTGGGCAAGGGCGATAACATCAAGACCCACAC AAGGCCAATGAGACAGACGAGGACGATATCGAGGGAAACCTGC TGCTGCCAGAGGGAGTGCCACCCGAGAGGCAGTGGGCCAGGT TCTACGTGAAGATCTATAGGGCAGAGGGCCTGCCTAGGATGAA CACCAGCCTGATGGCCAATGTGAAGAAGGCCTTCATCGGCGAG AACAAGGACCTGGTGGATCCCTACGTGCAGGTGTTCTTTGCCG GCCAGAAGGGCAAGACCTCCGTGCAGAAGAGCTCCTATGAGCC TCTGTGGAATGAGCAGGTGGTGTGTTACAGACCTGTTCCCTCCAC TGTGCAAGAGGATGAAGGTGCAGATCAGAGACTCTGATAAGGT GAACGACGTGGCCATCGGCACCCACTTTATCGATCTGAGGAAG ATCAGCAATGACGGCGATAAGGGCTTCCTGCCACCCTGGGCC CCGCCTGGGTGAACATGTACGGCAGCACCAGAAATTATACACTG CTGGACGAGCACCAGGATCTGAACGAGGGCCTGGGCGAGGGC GTGAGCTTTAGAGCCAGGCTGCTGCTGGGCCTGGCCGTGGAGA TCGTGGACACCTCCAATCCCGAGCTGACCTCTAGCACAGAGGT GCAGGTGGAGCAGGCCACACCTATCTCTGAGAGCTGTGCCGGC

SEQ ID NO.	Sequence Name	Sequence
		AAGATGGAGGAGTTCTTTCTGTTTGGCGCCTTCCTGGAGGCCTC CATGATCGACCGGCGCAACGGCGATAAGCCTATCACCTTCGAG GTGACAATCGGCAACTACGGCAATGAGGTGGACGGCCTGTCTC GGCCCCAGCGCCCAAGGCCAGAAAGGAGCCTGGCGACGAGG AGGAGGTGGATCTGATCCAGAACGCCAGCGACGATGAGGCAGG CGACGCAGGCGATCTGGCCTCCGTGTCCTCTACCCCCCTATG CGGCCACAGGTGACAGACCGCAATTACTTTACCTGCCTTATCT GGAGCGCAAGCCATGCATCTACATCAAGTCTTGGTGGCCCGAT CAGAGGAGACGGCTGTATAACGCCAATATCATGGACCACATCGC CGATAAGCTGGAGGAGGGCCTGAATGACATCCAGGAGATGATC AAGACCGAGAAGTCCTATCCAGAGCGCAGGCTGAGGGCGTGCG TGGAGGAGCTGAGCTGTGGCTGCTGTAGATTCCTGTCCCTGGC CGACAAGGATCAGGGGCACTCATCACGGACACGGCTGGACCGG GAGCGGCTGAAATCATGTATGCGGGAGCTGGAAAATATGGGAC AGCAGGCAAGGATGCTGCGCGCCCAGGTGAAGAGGCACACCG TGAGAGACAAGCTGCGGCTGTGCCAGAACTTCCTGCAGAACT GCGCTTTCTGGCCGATGAGCCACAGCACAGCATCCCCGACATC TTCATCTGGATGATGTCCAACAATAAGAGAGTGGCCTACGCCCG GGTGCCCTCTAAGGATCTGCTGTTTAGCATCGTGGAGGAGGAG ACAGGCAAGGACTGTGCCAAGGTGAAGACCCTGTTCTCTGAAGC TGCCTGGCAAGAGAGGCTTTGGCAGCGCCGGATGGACCGTGCA GGCAAAGGTGGAGCTGTATCTGTGGCTGGGCCTGTCTAAGCAG CGGAAGGAGTTCCTGTGCGGCCTGCCCTGTGGCTTTCAGGAGG TGAAGGCAGCACAGGACTGGGACTGCACGCCTTCCCCCCCCGT GAGCCTGGTGACACCAAGAAGCAGGCCTTTCAGCTGAGGGCC CATATGTACCAGGCCAGGTCTCTGTTCCGCCCGATAGCTCCG GACTGAGCGACCCTTTTGCCAGGGTGTTCTTTATCAATCAGAGC CAGTGACAGAGGTGCTGAACGAGACCCTGTGCCAACATGGG ATCAGATGCTGGTGTTCGACAACCTGGAGCTGTACGGAGAGGC ACACGAGCTGAGGGACGATCCACCCATCATCGTGATCGAGATCT ATGATCAGGACTCCATGGGCAAGGCCGATTTTCATGGGCAGGAC CTTTGCCAAGCCCCTGGTGAAGATGGCCGACGAGGCCTACTGC CCTCCAAGATTCCCCCTCAGCTCGAGTACTATCAGATCTATAG GGGAAATGCAACCGCCGAGACCTGCTGGCCGCCTTTGAGCTG CTGCAGATCGGCCCCGCCGAAAGGCAGACCTGCCACCCATCA ACGGCCCAGTGGATGTGGACAGAGGCCCATCATGCCTGTGCC AATGGGCATCAGACCAGTGCTGTCCAAGTACAGGGTGGAGGTG CTGTTCTGGGGACTGCGCGACCTGAAGAGGGTGAATCTGGCCC AGGTGGATAGGCCAGAGTGGACATCGAGTGCGCCGAAAGG

SEQ ID NO.	Sequence Name	Sequence
		GCGTGCAGTCTAGCCTGATCCACAACCTATAAGAAGAACCCAAAT TTCAACACCCTGGTGAAGTGGTTTGAGGTGGATCTGCCCGAGAA TGAGCTGCTGCACCCTCCACTGAACATCCGGGTGGTGGACTGT AGAGCCTTCGGCAGGTACACCCTGGTGGGCAGCCACGCCGTGA GCAGCCTGAGGAGGTTTATCTACAGGCCCCCTGACAGGTCCGC CCCTTCTTGGAATACCACAGTGAGACTGCTGCGGCGCTGCAGG GTGCTGTGCAACGGAGGCAGCTCCTCTCACTCTACCGGCGAGG TGGTGGTGACAATGGAGCCTGAGGTACCCATCAAGAAGCTGGA GACCATGGTGAAGCTGGATGCCACAAGCGAGGCAGTGGTGAAG GTGGACGTGGCAGAGGAGGAGAAGGAGAAGAAGAAGAAG AAGGGAACCGCCGAGGAGCCTGAGGAAGAGGAGCCAGATGAG AGCATGCTGGACTGGTGGTCCAAGTACTTCGCCTCTATCGACAC AATGAAGGAGCAGCTGAGACAGCAGGAGCCTAGCGGCATCGAT CTGGAGGAGAAGGAGGAGGTGGACAATACCGAGGGCCTGAAG GGCTCCATGAAGGGCAAGGAGAAGGCAAGGGCAGCAAAGGAA GAGAAGAAGAAGAAGACCCAGAGCAGCGGCTCTGGACAGGGCA GCGAGGCACCAGAGAAGAAGAAGCCTAAGATCGATGAGCTGAA GGTGTACCCAAAGGAGCTGGAGTCCGAGTTCGATAATTTTGAGG ACTGGCTGCACACCTTCAACCTGCTGCGCGCAAGACAGGCGA CGATGAGGACGGCAGCACCGAGGAGGAGAGAATCGTGGGCCG GTTTAAGGGCTCCCTGTGCGTGTACAAGGTGCCACTGCCTGAG GACGTGAGCAGGGAGGCCGGATACGACTCTACCTATGGCATGT TCCAGGGCATCCCCTCTAATGATCCTATCAACGTGCTGGTGCGC GTGTATGTGGTGAGGGCCACAGATCTGCACCCCGCCGACATCA ACGGCAAGGCCGACCCTTACATCGCCATCCGCTGGGCAAGAC CGATATCAGGGACAAGGAGAATTATATCTCCAAGCAGCTGAACC CCGTGTTTCGGCAAGTCTTTTGACATCGAGGCCAGCTTCCCTATG GAGTCCATGCTGACCGTGGCCGTGTACGATTGGGACCTGGTGG GCACCGACGATCTGATCGGCGAGACAAAGATCGATCTGGAGAA TCGCTTTTATTCTAAGCACAGGGCAACCTGCGGAATCGCACAGA CCTACAGCACACACGGCTATAACATCTGGCGCGACCCCATGAA GCCTAGCCAGATCCTGACAAGGCTGTGCAAGGATGGCAAGGTG GACGGACCACACTTCGGACCACCCGGCAGAGTGAAGGTGGCCA ATCGGGTGTTTACAGGCCCTTCCGAGATCGAGGATGAGAACGG CCAGCGCAAGCCAACCGACGAGCACGTGGCCCTGCTGGCCCT GAGGCACTGGGAGGATATCCCAAGGGCCGGATGTAGGCTGGTG CCTGAGCACGTGGAGACCAGACCACTGCTGAATCCAGACAAGC CAGGAATCGAGCAGGGCAGGCTGGAGCTGTGGGTGGATATGTT CCCAATGGACATGCCAGCCCCAGGAACACCCCTGGATATCTCC

SEQ ID NO.	Sequence Name	Sequence
		CCTAGAAAGCCAAAGAAGTACGAGCTGAGAGTGATCATCTGGAA CACAGACGAGGTGGTGTCTGGAGGACGATGACTTCTTTACCGGC GAGAAGTCTAGCGATATCTTTGTGCGCGGATGGCTGAAGGGAC AGCAGGAGGACAAGCAGGATACAGACGTGCACTACCACTCCCT GACCGGCGAGGGCAATTTCAACTGGAGATACCTGTTCCCTTTTG ATTATCTGGCCGCCGAGGAGAAGATCGTGATCTCTAAGAAGGAG AGCATGTTTTCTGGGACGAGACAGAGTATAAGATCCCAGCCAG ACTGACCCTGCAGATCTGGGATGCCGACCACTTCAGCGCCGAT GACTTTCTGGGCGCCATCGAGCTGGACCTGAACCGGTTCCCAA GAGGCGCCAAGACCGCCAAGCAGTGCACAATGGAGATGGCAAC CGGAGAGGTGGACGTGCCTCTGGTGTCTATCTTCAAGCAGAAG CGGGTGAAGGGATGGTGGCCACTGCTGGCCAGGAACGAGAAT GATGAGTTTGAGCTGACAGGCAAGGTGGAGGCAGAGCTGCACC TGCTGACCGCCGAGGAGGCAGAGAAGAACCCAGTGGGCCTGG CCAGGAATGAGCCCGACCTCTGGAGAAGCCAAACAGGCCCGA TACAGCCTTCGTGTGGTTTCTGAATCCTCTGAAGAGCATCAAGT ACCTGATCTGTACCAGGTATAAGTGGCTGATCATCAAGATCGTG CTGGCCCTGCTGGGACTGCTGATGCTGGGCCTGTTTCTGTACTC CCTGCCCGGCTATATGGTGAAGAAGCTGCTGGGCGCCTGA
4	Human OTOF isoform 1 protein (NP_919224.1), also called human otoferlin isoform a, 1997 aa	MALLIHLKTVSELRGRGDRIAKVTRFGQSFYSRVLENCEDVADFDE TFRWPVASSIDRNEMLEIQVFNYSKVSFKNLIGTFRMVLQKVVEES HVEVTDTLIDDNNAIKTSLCVEVRYQATDGTVGSWDDGDFLGDES LQEEEKDSQETDGLLPGSRPSSRPPGEKSFRRAGRSVFSAMKLGK NRSHKEEPQRPDEPAVLEMEDLDHLAIRLGDGLDPDSVSLASVTAL TTNVSNNKRSKPDIKMEPSAGRPMDYQVSITVIEARQLVGLNMDPVV CVEVGDDKKYTSMKESTNCPYYNEYFVDFHVSFVDFHVSFVDFHVS HKNLLRSGTLVGSFKMDVGTVYSQPEHQFHKKWAILSDPDDISS GLKGYVKCDVAVVGKGDNIKTPHNETDEDDIEGNLLLPEGVPPE RQWARFYVKIYRAEGLPRMNTSLMANVKKAFIGENKDLVDPYVQV FFAGQKGKTSVQKSSYEPLWNEQVVFTDLFPPLCKRMKVQIRDSD KVNDVAIGTHFIDLRKISNDGDKGFLPTLGPWVNNMYGSTRNYTLL DEHQDLNEGLGEGVSFRARLLLGLAVEIVDTSNPELTSSTEVEQVEQ ATPISESCAGKMEEFFLFGAFLEASMIDRRNGDKPITFEVTIGNYGN EVDGLSRPQRPRPRKEPGDEEEVDLIQNASDDEAGDAGDLASVSS TPMMPQVTDRNYFHLPLYLERKPCYIKSWWPDQRRRLYNANIMD HIADKLEEGLNDIQEMIKTEKSYPERRLRGVLEELSCGCCRFSLAD KDQGHSSRTRLDRERLKS CMRELENMGQQARMLRAQVKRHTVRD KLRLCQNFLQKLRFLADEPQHSIPDIFIWMMSSNNKRVAYARVPSKD LLFSIVEEETGKDCAKVKTLFLKLPGKRGFGSAGWTVQAKVELYLW

SEQ ID NO.	Sequence Name	Sequence
		LGLSKQRKEFLCGLPCGFQEVKAAQGLGLHAFPPVSLVYTKKQAF QLRAHMYQARSLFAADSSGLSDPFARVFFINQSQCTEVLNETLCPT WDQMLVFDNLELYGEAHEL RDDPPIIVIEIYDQDSMGKADFMGRTF AKPLVKMADEAYCPRFPQLEYYQIYRGNATAGDLLAAFELLQIG PAGKADLPPINGPVDVDRGPIMPVPMGIRPVLSKYRVEVLFWGLRD LKR VNLAQVDRPRVDIECAGKGVQSSLIHNYKKNPNFNTLVKWFEV DLPENELLHPLNIRVVDCRAFGRYTLVGSHAVSSLRRIYRPPDRS APSWNTTVRLLRRRCRVLCNGGSSSHSTGEVVVTMEPEVPIKKLET MVKLDATSEAVVKVDVAEEEEKEKKKKKGTAEPEEEEEPDESMLD WWSKYFASIDTMKEQLRQQEPGIDLEEKEEVDNTEGLKGS MKGK EKARAAKEEKKKKTQSSSGSQGSEAPEKKKPKIDELKVYPKELESE FDNFEDWLHTFNLLRGKTGDDDEDGSTEERIVGRFKGSLCVYKVPL PEDVSREAGYDSTYGMFQGIPSNDPINVLVRVYVVRATDLHPADIN GKADPYIAIRLGKTDIRDKENYISKQLNPVFGKSF DIEASFPMESMLT VAVYDWDLVGTDDLIGETKIDLENRFYSKHRATCGIAQTYSTHGYN I WRDPMKPSQILTRLCKDGKVDGPHFGPPGRVKVANRVFTGPSEIE DENGQRKPTDEHVALLALRH WEDIPRAGCRLVPEHVETRPLLPD KPGIEQGRLELWVDMFPM DMPAPGTPLDISPRKPKKYELRVIIWNT DEVVLEDDDDFFTGEKSSDIFVRGWLKGQQEDKQD TDVHYHSLTGE GNFNWRYLFPFDYLA AEEKIVISKKESMFSWDETEYKIPARLTQIW DADHFSADDFLGAIELDLNRFPRGAKTAKQCTMEMATGEVDVPLV SIFKQKRVKGWWPLLARNENDEFELTGKVEAELHLLTAEAEKNPV GLARNEPDPLEKPNRPDTSFIWFLNPLKSARYFLWHTYRWLLLKLL LLLLLLLLLALFLYSVPGYLVKKILGA
5	DNA sequence encoding the human otoferlin isoform 1 protein (SEQ ID NO: 4), 5979 bp, corresponds to the coding sequence documented in NM_001100395	ATGGCCCTGATTGTTCACTCAAGACTGTCTCAGAGCTCCGAGG CAAAGGTGACCGGATTGCCAAAGTCACTTTCCGAGGGCAGTCTT TCTACTCCCGGGTCCTGGAGAACTGCGAGGGTGTGGCTGACTT TGATGAGACGTTCCGGTGGCCAGTGGCCAGCAGCATCGACCGG AATGAAGTGTTGGAGATTCAGATTTTCAACTACAGCAAAGTCTTC AGCAACAAGCTGATAGGGACCTTCTGCATGGTGCTGCAGAAAGT GGTGGAGGAGAATCGGGTAGAGGTGACCGACACGCTGATGGAT GACAGCAATGCTATCATCAAGACCAGCCTGAGCATGGAGGTCC GGTATCAGGCCACAGATGGCACTGTGGGCCCCTGGGATGATGG AGACTTCCTGGGAGATGAATCCCTCCAGGAGGAGAAGGACAGC CAGGAGACAGATGGGCTGCTACCTGGTTCCCGACCCAGCACCC GGATATCTGGCGAGAAGAGCTTTCGCAGCAAAGGCAGAGAGAA GACCAAGGGAGGCAGAGATGGCGAGCACAAAGCGGGAAGGAG TGTGTTCTCGGCCATGAAACTCGGCAAACTCGGTCCCACAAAG AGGAGCCCCAAAGACAAGATGAGCCAGCAGTGCTGGAGATGGA

SEQ ID NO.	Sequence Name	Sequence
		GGACCTGGACCACCTAGCCATTCAGCTGGGGGATGGGCTGGAT CCTGACTCCGTGTCTCTAGCCTCGGTCACCGCTCTCACCAGCAA TGTCTCCAACAAACGGTCTAAGCCAGATATTAAGATGGAGCCCA GTGCTGGAAGGCCCATGGATTACCAGGTCAGCATCACAGTGATT GAGGCTCGGCAGCTGGTGGGCTTGAACATGGACCCTGTGGTGT GTGTGGAGGTGGGTGATGACAAGAAATACACGTCAATGAAGGA GTCCACAACTGCCCTTACTACAACGAGTACTTTGTCTTCGACTT CCATGTCTCTCCTGATGTCATGTTTGACAAGATCATCAAGATCTC GGTTATCCATTCTAAGAACCTGCTTCGGAGCGGCACCCTGGTGG GTTCTTCAAAATGGATGTGGGGACTGTGTATTCCCAGCCTGAA CACCAGTTCCATCACAAATGGGCCATCCTGTCAGACCCCGATGA CATCTCTGCTGGGTTGAAGGGTTATGTAAAGTGTGATGTCGCTG TGGTGGGCAAGGGGAGACAACATCAAGACACCCCAAGGCCAA CGAGACGGATGAGGACGACATTGAAGGGAACCTTGCTGCTCCCC GAGGGCGTGCCCCCGAACGGCAGTGGGCACGGTTCTATGTGA AAATTTACCGAGCAGAGGGACTGCCCCGGATGAACACAAGCCT CATGGCCAACGTGAAGAAGGCGTTCATCGGTGAGAACAAGGAC CTCGTCGACCCCTATGTGCAAGTCTTCTTTGCTGGACAAAAGGG CAAAACATCAGTGCAGAAGAGCAGCTATGAGCCGCTATGGAATG AGCAGGTCGTCTTCACAGACTTGTTCCCCCACTCTGCAAACGC ATGAAGGTGCAGATCCGGGACTCTGACAAGGTCAATGATGTGG CCATCGGCACCCACTTCATCGACCTGCGCAAGATTTCCAACGAT GGAGACAAAGGCTTCCTGCCTACCCTCGGTCCAGCCTGGGTGA ACATGTACGGCTCCACGCGCAACTACACACTGCTGGACGAGCA CCAGGACTTGAATGAAGGCCTGGGGGAGGGTGTGTCTTCCGG GCCCCGCTCATGTTGGGACTAGCTGTGGAGATCCTGGACACCT CCAACCCAGAGCTCACCAGCTCCACGGAGGTGCAGGTGGAGCA GGCCACGCCTGTCTCGGAGAGCTGCACAGGGAGAATGGAAGAA TTTTTTCTATTTGGAGCCTTCTTGAAGCCTCAATGATTGACCGG AAAAATGGGGACAAGCCAATTACCTTTGAGGTGACCATAGGAAA CTACGGCAATGAAGTCGATGGTATGTCCCGGCCCTGAGGCCT CGGCCCCGGAAGAGCCTGGGGATGAAGAAGAGGTAGACCTGA TTCAGAACTCCAGTGACGATGAAGGTGACGAAGCCGGGGACCT GGCCTCGGTGTCCTCCACCCACCTATGCGGCCCCAGATCACG GACAGGAACTATTTCCACCTGCCCTACCTGGAGCGCAAGCCCT GCATCTATATCAAGAGCTGGTGGCCTGACCAGAGGCGGCGCCT CTACAATGCCAACATCATGGATCACATTGCTGACAAGCTGGAAG AAGGCCTGAATGATGTACAGGAGATGATCAAAACGGAGAAGTCC TACCCGGAGCGCCGCTGCGGGGTGTGCTAGAGGAACTCAGCT

SEQ ID NO.	Sequence Name	Sequence
		GTGGCTGCCACCGCTTCCTCTCCCTCTCGGACAAGGACCAGGG CCGCTCGTCCCGCACCAGGCTGGATCGAGAGCGTCTTAAGTCC TGTATGAGGGAGTTGGAGAGCATGGGACAGCAGGCCAAGAGCC TGAGGGCTCAGGTGAAGCGGCACACTGTTCTGGGACAAGCTGAG GTCATGCCAGAATTTCTGCAGAAGCTACGCTTCCTGGCGGATG AGCCCCAGCACAGCATTCTGATGTGTTCAATTTGGATGATGAGC AACAACAAACGTATCGCCTATGCCCCGCTGCCTTCCAAAGACCT GCTCTTCTCCATCGTGGAGGAGGAAGTGGGCAAGGACTGCGCC AAAGTCAAGACCTCTTCTGAAGCTGCCAGGGAAGAGGGGCT TCGGCTCGGCAGGCTGGACAGTACAGGCCAAGCTGGAGCTCTA CCTGTGGCTGGGCCTCAGCAAGCAGCGAAAGGACTTCCTGTGT GGTCTGCCCTGTGGCTTCGAGGAGGTCAAGGCAGCCCCAAGGCC TGGGCCTGCATTCTTCCGCCCATCAGCCTAGTCTACACCAAG AAGCAAGCCTTCAGCTCCGAGCACACATGTATCAGGCCCGAA GCCTCTTTGCTGCTGACAGCAGTGGGCTCTCTGATCCCTTTGCC CGTGTCTTCTTCATCAACCAGAGCCAATGCACTGAGGTTCTAAA CGAGACACTGTGTCCCACCTGGGACCAGATGCTGGTATTTGACA ACCTGGAGCTGTACGGTGAAGCTCACGAGTTACGAGATGATCC CCCCATCATTGTCATTGAAATCTACGACCAGGACAGCATGGGCA AAGCCGACTTCATGGGCCGGACCTTCGCCAAGCCCCCTGGTGAA GATGGCAGATGAAGCATACTGCCACCTCGCTTCCCGCCGCAG CTTGAGTACTACCAGATCTACCGAGGCAGTGCCACTGCCGGAG ACCTACTGGCTGCCTTCGAGCTGCTGCAGATTGGGCCATCAGG GAAGGCTGACCTGCCACCCATCAATGGCCCAGTGGACATGGAC AGAGGGCCCATCATGCCTGTGCCCGTGGGAATCCGGCCAGTGC TCAGCAAGTACCGAGTGGAGGTGCTGTTCTGGGGCCTGAGGGA CCTAAAGAGGGTGAACCTGGCCCAGGTGGACCGACCACGGGTG GACATCGAGTGTGCAGGAAAGGGGGTACAATCCTCCCTGATTCA CAATTATAAGAAGAACCCCAACTTCAACACGCTGGTCAAGTGGT TTGAAGTGGACCTCCCGGAGAATGAGCTCCTGCACCCACCTT GAACATCCGAGTGGTAGATTGCCGGGCCCTTTGGACGATACACC CTGGTGGGTTCCACGCAGTCAGCTCACTGAGGCGCTTCATCTA CCGACCTCCAGACCGCTCAGCCCCCAACTGGAACACCACAGGG GAGGTTGTAGTAAGCATGGAGCCTGAGGAGCCAGTTAAGAAGC TGGAGACCATGGTGAAGTGGATGCGACTTCTGATGCTGTGGTC AAGGTGGATGTGGCTGAAGATGAGAAGGAAAGGAAGAAGAAGA AAAAGAAAGGCCCGTCAGAGGAGCCAGAGGAGGAAGAGCCCG ATGAGAGCATGCTGGATTGGTGGTCCAAGTACTTCGCCTCCATC GACACAATGAAGGAGCAACTTCGACAACATGAGACCTCTGGAAC

SEQ ID NO.	Sequence Name	Sequence
		TGACTTGGAAGAGAAGGAAGAGATGGAAAGCGCTGAGGGCCTG AAGGGACCAATGAAGAGCAAGGAGAAGTCCAGAGCTGCAAAGG AGGAGAAAAAGAAGAAAAACCAGAGCCCTGGCCCTGGCCAGGG ATCGGAGGCTCCTGAGAAGAAGAAAGCCAAGATCGATGAGCTTA AGGTGTACCCCAAGGAGCTGGAATCGGAGTTTGACAGCTTTGA GGACTGGCTGCACACCTTCAACCTGTTGAGGGGCAAGACGGGA GATGATGAGGATGGCTCCACAGAGGAGGAGCGCATAGTAGGCC GATTCAAGGGCTCCCTCTGTGTGTACAAAGTGCCACTCCCAGAA GATGTATCTCGAGAAGCTGGCTATGATCCCACCTATGGAATGTT CCAGGGCATCCCAAGCAATGACCCCATCAATGTGCTGGTCCGA ATCTATGTGGTCCGGGCCACAGACCTGCACCCGGCCGACATCA ATGGCAAAGCTGACCCCTATATTGCCATCAAGTTAGGCAAGACC GACATCCGAGACAAGGAGAACTACATCTCCAAGCAGCTCAACCC TGTGTTTGGGAAGTCCTTTGACATTGAGGCCTCCTTCCCCATGG AGTCCATGTTGACAGTGGCCGTGTACGACTGGGATCTGGTGGG CACTGATGACCTCATCGGAGAAACCAAGATTGACCTGGAAAACC GCTTCTACAGCAAGCATCGCGCCACCTGCGGCATCGCACAGAC CTATTCCATACATGGCTACAATATCTGGAGGGACCCCATGAAGC CCAGCCAGATCCTGACACGCCTCTGTAAAGAGGGCAAAGTGGA CGGCCCCCACTTTGGTCCCCATGGGAGAGTGAGGGTTGCCAAC CGTGTCTTCACGGGGCCTTCAGAAATAGAGGATGAGAATGGTCA GAGGAAGCCACAGATGAGCACGTGGCACTGTCTGCTCTGAGA CACTGGGAGGACATCCCCGGGTGGGCTGCCGCCTTGTGCCG GAACACGTGGAGACCAGGCCGCTGCTCAACCCTGACAAGCCAG GCATTGAGCAGGGCCGCCTGGAGCTGTGGGTGGACATGTTCCC CATGGACATGCCAGCCCCTGGGACACCTCTGGATATATCCCCCA GGAAACCCAAGAAGTACGAGCTGCGGGTCATCGTGTGGAACAC AGACGAGGTGGTCCTGGAAGACGATGATTTCTTCACGGGAGAG AAGTCCAGTGACATTTTTGTGAGGGGGTGGCTGAAGGGCCAGC AGGAGGACAAACAGGACACAGATGTCCACTATCACTCCCTCACG GGGAGGGGCAACTTCAACTGGAGATACCTCTTCCCCTTCGACTA CCTAGCGGCCGAAGAGAAGATCGTTATGTCCAAAAAGGAGTCTA TGTTCTCCTGGGATGAGACGGAGTACAAGATCCCTGCGCGGCT CACCTGCAGATCTGGGACGCTGACCACTTCTCGGCTGACGAC TTCCTGGGGGCTATCGAGCTGGACCTGAACCGGTTCCCGAGGG GCGCTAAGACAGCCAAGCAGTGCACCATGGAGATGGCCACCGG GGAGGTGGACGTACCCCTGGTTTCCATCTTTAAACAGAAACGTG TCAAAGGCTGGTGGCCCCCTCTGGCCCGCAATGAGAATGATGA GTTTGAGCTCACAGGCAAAGTGGAGGCGGAGCTACACCTACTC

SEQ ID NO.	Sequence Name	Sequence
		ACGGCAGAGGAGGCAGAGAAGAACCCTGTGGGCCTGGCTCGC AATGAACCTGATCCCCTAGAAAAACCCAACCGGCCTGACACGGC ATTCGTCTGGTTCTGAACCCACTCAAATCTATCAAGTACCTCAT CTGCACCCGGTACAAGTGGCTGATCATCAAGATCGTGCTGGCG CTGCTGGGGCTGCTCATGCTGGCCCTCTTCCTTTACAGCCTCCC AGGCTACATGGTCAAGAAGCTCCTAGGGGCCTGA
6	Codon-optimized (CO) DNA sequence encoding the human otoferlin isoform 1 protein (SEQ ID NO: 4), 5994 bp	ATGGCACTGCTGATCCACCTGAAAACCGTCTCCGAAGTGAAGG CAGAGGGGACAGAATCGCTAAAGTCACCTTCCGGGGACAGAGC TTTTACAGCAGGGTGCTGGAGAACTGCGAGGACGTGGCCGACT TTGACGAGACATTCAGGTGGCCCGTGCCAGCTCCATCGATCG CAATGAGATGCTGGAGATCCAGGTGTTTAACTATAGCAAGGTGT TCTCCAATAAGCTGATCGGCACCTTCCGGATGGTGTGCAGAAG GTGGTGGAGGAGTCCCACGTGGAGGTGACCGACACACTGATCG ACGATAACAATGCCATCATCAAGACATCCCTGTGCGTGGAGGTG CGCTACCAGGCCACCGATGGCACAGTGGGCTCTTGGGACGATG GCGACTTCCTGGGCGATGAGTCCCTGCAGGAGGAGGAGAAGGA CTCTCAGGAGACAGATGGCCTGCTGCCTGGCTCCCGGCCATCT AGCCGCCCCCTGGCGAGAAGTCTTTTAGGAGAGCCGGCAGGT CCGTGTTCTCTGCCATGAAGCTGGGCAAGAACAGGAGCCACAA GGAGGAGCCTCAGAGGCCCGACGAGCCAGCCGTGCTGGAGAT GGAGGACCTGGATCACCTGGCCATCAGACTGGGCGATGGCCTG GACCTGATAGCGTGTCCCTGGCCTCCGTGACCGCCCTGACCA CAAACGTGTCTAATAAGCGGAGCAAGCCAGACATCAAGATGGAG CCATCTGCCGGCAGGCCCATGGATTACCAGGTGAGCATCACAG TGATCGAGGCCAGACAGCTGGTGGGCCTGAACATGGACCCCGT GGTGTGCGTGGAAGTGGGCGACGATAAGAAGTACACCTCCATG AAGGAGTCTACAACTGTCCATACTACAACGAGTACTTCGTGTTT GATTTCCACGTGAGCCCCGACGTGATGTTTCGATAAGATCATCAA GATCAGCGTGATCCACTCCAAGAATCTGCTGCGGTCTGGCACC CTGGTGGGAAGCTTTAAGATGGACGTGGGCACAGTGTACTCTCA GCCTGAGCACCAGTTCCACCACAAGTGGGCCATCCTGAGCGAT CCAGACGATATCTCCTCTGGCCTGAAGGGCTATGTGAAGTGCGA CGTGGCAGTGGTGGGCAAGGGCGATAACATCAAGACCCACAC AAGGCCAATGAGACAGACGAGGACGATATCGAGGGAAACCTGC TGCTGCCAGAGGGAGTGCCACCCGAGAGGCAGTGGGCCAGGT TCTACGTGAAGATCTATAGGGCAGAGGGCCTGCCTAGGATGAA CACCAGCCTGATGGCCAATGTGAAGAAGGCCTTCATCGGCGAG AACAAAGACCTGGTGGATCCCTACGTGCAGGTGTTCTTTGCCG GCCAGAAGGGCAAGACCTCCGTGCAGAAGAGCTCCTATGAGCC

SEQ ID NO.	Sequence Name	Sequence
		TCTGTGGAATGAGCAGGTGGTGTTCACAGACCTGTTCCCTCCAC TGTGCAAGAGGATGAAGGTGCAGATCAGAGACTCTGATAAGGT GAACGACGTGGCCATCGGCACCCACTTTATCGATCTGAGGAAG ATCAGCAATGACGGCGATAAGGGCTTCCTGCCCACCCTGGGCC CCGCCTGGGTGAACATGTACGGCAGCACCAGAAATTATACACTG CTGGACGAGCACCAGGATCTGAACGAGGGCCTGGGCGAGGGC GTGAGCTTTAGAGCCAGGCTGCTGCTGGGCCTGGCCGTGGAGA TCGTGGACACCTCCAATCCCGAGCTGACCTCTAGCACAGAGGT GCAGGTGGAGCAGGCCACACCTATCTCTGAGAGCTGTGCCGGC AAGATGGAGGAGTTCTTTCTGTTTGGCGCCTTCCTGGAGGCCTC CATGATCGACCGGCGCAACGGCGATAAGCCTATCACCTTCGAG GTGACAATCGGCAACTACGGCAATGAGGTGGACGGCCTGTCTC GGCCCCAGCGCCCAAGGCCAGAAAGGAGCCTGGCGACGAGG AGGAGGTGGATCTGATCCAGAACGCCAGCGACGATGAGGCAGG CGACGCAGGCGATCTGGCCTCCGTGTCCTCTACCCCCCTATG CGGCCACAGGTGACAGACCGCAATTACTTTACCTGCCTTATCT GGAGCGCAAGCCATGCATCTACATCAAGTCTTGGTGGCCCGAT CAGAGGAGACGGCTGTATAACGCCAATATCATGGACCACATCGC CGATAAGCTGGAGGAGGGCCTGAATGACATCCAGGAGATGATC AAGACCGAGAAGTCTATCCAGAGCGCAGGCTGAGGGGCGTG TGGAGGAGCTGAGCTGTGGCTGCTGTAGATTCTGTCCCTGGC CGACAAGGATCAGGGGCACTCATCACGGACACGGCTGGACCGG GAGCGGCTGAAATCATGTATGCGGGAGCTGGAAAATATGGGAC AGCAGGCAAGGATGCTGCGCGCCCAGGTGAAGAGGCACACCG TGAGAGACAAGCTGCGGCTGTGCCAGAACTTCCTGCAGAAGCT GCGCTTTCTGGCCGATGAGCCACAGCACAGCATCCCCGACATC TTCATCTGGATGATGTCCAACAATAAGAGAGTGGCCTACGCCCG GGTGCCCTCTAAGGATCTGCTGTTTAGCATCGTGAGGAGGAG ACAGGCAAGGACTGTGCCAAGGTGAAGACCCTGTTCTGAAGC TGCCTGGCAAGAGAGGCTTTGGCAGCGCCGATGGACCGTGCA GGCAAAGGTGGAGCTGTATCTGTGGCTGGGCCTGTCTAAGCAG CGGAAGGAGTTCCTGTGCGGCCTGCCCTGTGGCTTTCAGGAGG TGAAGGCAGCACAGGGACTGGGACTGCACGCCTTCCCCCCGT GAGCCTGGTGTACACCAAGAAGCAGGCCTTTCAGCTGAGGGCC CATATGTACCAGGCCAGGTCTCTGTTCCGCCCGATAGCTCCG GACTGAGCGACCTTTTGCCAGGGTGTCTTTATCAATCAGAGC CAGTGCACAGAGGTGCTGAACGAGACCCTGTGCCAACATGGG ATCAGATGCTGGTGTTCGACAACCTGGAGCTGTACGGAGAGGC ACACGAGCTGAGGGACGATCCACCCATCATCGTGATCGAGATCT

SEQ ID NO.	Sequence Name	Sequence
		ATGATCAGGACTCCATGGGCAAGGCCGATTTTCATGGGCAGGAC CTTTGCCAAGCCCCTGGTGAAGATGGCCGACGAGGCCTACTGC CCTCCAAGATTCCCCCTCAGCTCGAGTACTATCAGATCTATAG GGGAAATGCAACCGCCGGAGACCTGCTGGCCGCCTTTGAGCTG CTGCAGATCGGCCCCGCGGAAAGGCAGACCTGCCACCCATCA ACGGCCCAGTGGATGTGGACAGAGGCCCATCATGCCTGTGCC AATGGGCATCAGACCAGTGTGTCCAAGTACAGGGTGGAGGTG CTGTTCTGGGGACTGCGCGACCTGAAGAGGGTGAATCTGGCCC AGGTGGATAGGCCCAGAGTGGACATCGAGTGCGCCGAAAGG GCGTGCAGTCTAGCCTGATCCACAATAAGAAGAACCCAAAT TTCAACACCCTGGTGAAGTGGTTTGAGGTGGATCTGCCCGAGAA TGAGCTGCTGCACCCTCCACTGAACATCCGGGTGGTGGACTGT AGAGCCTTCGGCAGGTACACCCTGGTGGGCAGCCACGCCGTGA GCAGCCTGAGGAGGTTTCATCTACAGGCCCTGACAGGTCCGC CCCTTCTTGAATACCACAGTGAGACTGCTGCGGCGCTGCAGG GTGCTGTGCAACGGAGGCAGCTCCTCTCACTCTACCGGCGAGG TGGTGGTGACAATGGAGCCTGAGGTACCATCAAGAAGCTGGA GACCATGGTGAAGCTGGATGCCACAAGCGAGGCAGTGGTGAAG GTGGACGTGGCAGAGGAGGAGAAGGAGAAGAAGAAGAAG AAGGGAACCGCCGAGGAGCCTGAGGAAGAGGAGCCAGATGAG AGCATGCTGGACTGGTGGTCCAAGTACTTCGCCTCTATCGACAC AATGAAGGAGCAGCTGAGACAGCAGGAGCCTAGCGGCATCGAT CTGGAGGAGAAGGAGGAGGTGGACAATACCGAGGGCCTGAAG GGCTCCATGAAGGGCAAGGAGAAGGCAAGGGCAGCAAAGGAA GAGAAGAAGAAGAAGACCCAGAGCAGCGGCTCTGGACAGGGCA GCGAGGCACCAGAGAAGAAGAAGCCTAAGATCGATGAGCTGAA GGTGTACCCAAAGGAGCTGGAGTCCGAGTTCGATAATTTTGAGG ACTGGCTGCACACCTTCAACCTGCTGCGCGGCAAGACAGGCGA CGATGAGGACGGCAGCACCGAGGAGGAGAGAATCGTGGGCCG GTTTAAGGGCTCCCTGTGCGTGTACAAGGTGCCACTGCCTGAG GACGTGAGCAGGGAGGCCGATACGACTCTACCTATGGCATGT TCCAGGGCATCCCCTCTAATGATCCTATCAACGTGCTGGTGC GTGTATGTGGTGAGGGCCACAGATCTGCACCCCGCCGACATCA ACGGCAAGGCCGACCTTACATCGCCATCCGCCTGGGCAAGAC CGATATCAGGGACAAGGAGAATTATATCTCCAAGCAGCTGAACC CCGTGTTTCGGCAAGTCTTTTGACATCGAGGCCAGCTTCCCTATG GAGTCCATGCTGACCGTGCCGTGTACGATTGGGACCTGGTGG GCACCGACGATCTGATCGGCGAGACAAAGATCGATCTGGAGAA TCGCTTTTATTCTAAGCACAGGGCAACCTGCGGAATCGCACAGA

SEQ ID NO.	Sequence Name	Sequence
		CCTACAGCACACACGGCTATAACATCTGGCGCGACCCCATGAA GCCTAGCCAGATCCTGACAAGGCTGTGCAAGGATGGCAAGGTG GACGGACCACACTTCGGACCACCCGGCAGAGTGAAGGTGGCCA ATCGGGTGTTTACAGGCCCTTCCGAGATCGAGGATGAGAACGG CCAGCGCAAGCCAACCGACGAGCACGTGGCCCTGCTGGCCCT GAGGCACTGGGAGGATATCCCAAGGGCCGGATGTAGGCTGGTG CCTGAGCACGTGGAGACCAGACCACTGCTGAATCCAGACAAGC CAGGAATCGAGCAGGGCAGGCTGGAGCTGTGGGTGGATATGTT CCCAATGGACATGCCAGCCCCAGGAACACCCCTGGATATCTCC CCTAGAAAGCCAAAGAAGTACGAGCTGAGAGTGATCATCTGGAA CACAGACGAGGTGGTGCTGGAGGACGATGACTTCTTTACCGGC GAGAAGTCTAGCGATATCTTTGTGCGCGGATGGCTGAAGGGAC AGCAGGAGGACAAGCAGGATACAGACGTGCACTACCACTCCCT GACCGGCGAGGGCAATTTCAACTGGAGATACCTGTTCCCTTTTG ATTATCTGGCCGCCGAGGAGAAGATCGTGATCTCTAAGAAGGAG AGCATGTTTTCTGGGACGAGACAGAGTATAAGATCCCAGCCAG ACTGACCCTGCAGATCTGGGATGCCGACCACTTCAGCGCCGAT GACTTTCTGGGCGCCATCGAGCTGGACCTGAACCGTTCCCAA GAGGCGCCAAGACCGCCAAGCAGTGACAATGGAGATGGCAAC CGGAGAGGTGGACGTGCCTCTGGTGTCTATCTTCAAGCAGAAG AGGGTGAAGGGCTGGTGGCCACTGCTGGCCAGAAACGAGAATG ATGAGTTTGAGCTGACAGGCAAGGTGGAGGCAGAGCTGCACCT GCTGACCGCCGAGGAGGCAGAGAAGAACCCAGTGGGCCTGGC CAGGAATGAGCCCGACCTCTGGAGAAGCCAAACAGGCCCGAC ACCAGCTTCATCTGGTTTCTGAATCCTCTGAAGTCCGCCCGGTA CTTCCTGTGGCACACCTATCGCTGGCTGCTGCTGAAGCTGTTAT TACTGTTATTACTGCTGCTGCTGCTGGCCCTGTTTCTGTACAGC GTGCCCGGCTATCTGGTGAAGAAGATCCTGGGCGCCTGA

Expression of OTOF in mammalian cells

Mutations in OTOF have been linked to sensorineural hearing loss and auditory neuropathy. The compositions and methods described herein increase the expression of WT OTOF isoform 5 protein by administering a first nucleic acid vector that contains a polynucleotide encoding an N-terminal portion of an OTOF isoform 5 protein and a second nucleic acid vector that contains a polynucleotide encoding a C-terminal portion of an OTOF isoform 5 protein. In order to utilize nucleic acid vectors for therapeutic application in the treatment of sensorineural hearing loss and auditory neuropathy, they can be directed to the interior of the cell, and, in particular, to specific cell types. A wide array of methods has been established for the delivery of proteins to mammalian cells and for the stable expression of genes encoding proteins in mammalian cells.

Polynucleotides encoding OTOF

One platform that can be used to achieve therapeutically effective intracellular concentrations of OTOF isoform 5 in mammalian cells is via the stable expression of the gene encoding OTOF isoform 5 (e.g., by integration into the nuclear or mitochondrial genome of a mammalian cell, or by episomal concatemer formation in the nucleus of a mammalian cell). The gene is a polynucleotide that encodes the primary amino acid sequence of the corresponding protein. In order to introduce exogenous genes into a mammalian cell, genes can be incorporated into a vector. Vectors can be introduced into a cell by a variety of methods, including transformation, transfection, transduction, direct uptake, projectile bombardment, and by encapsulation of the vector in a liposome. Examples of suitable methods of transfecting or transforming cells include calcium phosphate precipitation, electroporation, microinjection, infection, lipofection and direct uptake. Such methods are described in more detail, for example, in Green, et al., *Molecular Cloning: A Laboratory Manual*, Fourth Edition (Cold Spring Harbor University Press, New York 2014); and Ausubel, et al., *Current Protocols in Molecular Biology* (John Wiley & Sons, New York 2015), the disclosures of each of which are incorporated herein by reference.

OTOF isoform 5 can also be introduced into a mammalian cell by targeting vectors containing portions of a gene encoding an OTOF isoform 5 protein to cell membrane phospholipids. For example, vectors can be targeted to the phospholipids on the extracellular surface of the cell membrane by linking the vector molecule to a VSV-G protein, a viral protein with affinity for all cell membrane phospholipids. Such a construct can be produced using methods well known to those of skill in the field.

Recognition and binding of the polynucleotide encoding an OTOF isoform 5 protein by mammalian RNA polymerase is important for gene expression. As such, one may include sequence elements within the polynucleotide that exhibit a high affinity for transcription factors that recruit RNA polymerase and promote the assembly of the transcription complex at the transcription initiation site. Such sequence elements include, e.g., a mammalian promoter, the sequence of which can be recognized and bound by specific transcription initiation factors and ultimately RNA polymerase.

Polynucleotides suitable for use in the compositions and methods described herein also include those that encode an OTOF protein downstream of a mammalian promoter (e.g., a polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein downstream of a mammalian promoter). Promoters that are useful for the expression of an OTOF protein in mammalian cells include ubiquitous promoters and cochlear hair cell-specific promoters. Ubiquitous promoters include the CAG promoter, a cytomegalovirus (CMV) promoter (e.g., the CMV immediate-early enhancer and promoter, a CMVmini promoter, a minCMV promoter, a CMV-TATA+INR promoter, or a min CMV-T6 promoter), the chicken β -actin promoter, the smCBA promoter, the CB7 promoter, the hybrid CMV enhancer/human β -actin promoter, the CASI promoter, the dihydrofolate reductase (DHFR) promoter, the human β -actin promoter, a β -globin promoter (e.g., a minimal β -globin promoter), an HSV promoter (e.g., a minimal HSV ICP0 promoter or a truncated HSV ICP0 promoter), an SV40 promoter (e.g., an SV40 minimal promoter), the EF1 α promoter, and the PGK promoter. Cochlear hair cell-specific promoters include the Myosin 15 (Myo15) promoter. Myo15 promoter sequences for use in the methods and compositions described herein are described below and in Table 3. Alternatively, promoters derived from viral genomes can also be used for the stable expression of these agents in mammalian cells. Examples of functional viral

promoters that can be used to promote mammalian expression of these agents include adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, *tk* promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of Moloney virus, Epstein barr virus (EBV) promoter, and the Rous sarcoma virus (RSV) promoter.

5

Murine Myosin 15 promoters

In some embodiments, the Myo15 promoter for use in the compositions and methods described herein includes polynucleotide sequences from regions of the murine Myo15 locus that are capable of expressing a transgene specifically in hair cells, or variants thereof, such as a polynucleotide sequences that have at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to regions of the murine Myo15 locus that are capable of expressing a transgene specifically in hair cells. These regions include polynucleotide sequences immediately preceding the murine Myo15 translation start site and an upstream regulatory element that is located over 5 kb from the murine Myo15 translation start site. The murine Myo15 promoter for use in the compositions and methods described herein can optionally include a linker operably linking the regions of the murine Myo15 locus that are capable of expressing a transgene specifically in hair cells, or the regions of the murine Myo15 locus can be joined directly without an intervening linker.

In some embodiments, the murine Myo15 promoter for use in the compositions and methods described herein contains a first region (an upstream regulatory element) having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to a region containing the first non-coding exon of the murine Myo15 gene (nucleic acids from -6755 to -7209 with respect to the murine Myo15 translation start site, the sequence of which is set forth in SEQ ID NO: 7) or a functional portion or derivative thereof joined (e.g., operably linked) to a second region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the polynucleotide sequence immediately preceding the murine Myo15 translation start site (nucleic acids from -1 to -1157 with respect to the murine Myo15 translation start site, the sequence of which is set forth in SEQ ID NO: 8) or a functional portion or derivative thereof. The functional portion of SEQ ID NO: 7 may have the sequence of nucleic acids from -7166 to -7091 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 9) and/or the sequence of nucleic acids from -7077 to -6983 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 10). The first region may contain the polynucleotide sequence of SEQ ID NO: 9 fused to the polynucleotide sequence of SEQ ID NO: 10 with no intervening nucleic acids, as set forth in SEQ ID NO: 11, or the first region may contain the polynucleotide sequence of SEQ ID NO: 10 fused to the polynucleotide sequence of SEQ ID NO: 9 with no intervening nucleic acids, as set forth in SEQ ID NO: 12. Alternatively, the first region may contain the sequences of SEQ ID NO: 9 and SEQ ID NO: 10 joined by the endogenous intervening polynucleotide sequence (e.g., the first region may have or include the sequence of nucleic acids from -7166 to -6983 with respect to the murine Myo15 translation start site, as set forth in SEQ ID NO: 13 and SEQ ID NO: 33) or a nucleic acid linker. In a murine Myo15 promoter in which the first region contains both SEQ ID NO: 9 and SEQ ID NO: 10, the two sequences can be included in any order (e.g., SEQ ID NO: 9 may be joined to (e.g., precede) SEQ ID NO: 10, or SEQ ID NO: 10 may be joined to (e.g., precede) SEQ ID NO: 9). The functional portion of SEQ ID NO: 8 may

have the sequence of nucleic acids from -590 to -509 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 14) and/or the sequence of nucleic acids from -266 to -161 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 15). In some embodiments, the sequence containing SEQ ID NO: 14 has the sequence of SEQ ID NO: 34. In some embodiments, the sequence containing SEQ ID NO: 15 has the sequence of SEQ ID NO: 35. The second region may contain the polynucleotide sequence of SEQ ID NO: 14 fused to the polynucleotide sequence of SEQ ID NO: 15 with no intervening nucleic acids, as set forth in SEQ ID NO: 16, or the second region may contain the polynucleotide sequence of SEQ ID NO: 15 fused to the polynucleotide sequence of SEQ ID NO: 14 with no intervening nucleic acids, as set forth in SEQ ID NO: 17. The second region may contain the nucleic acid sequence of SEQ ID NO: 34 fused to the nucleic acid sequence of SEQ ID NO: 35 with no intervening nucleic acids, as set forth in SEQ ID NO: 38, or the second region may contain the nucleic acid sequence of SEQ ID NO: 35 fused to the nucleic acid sequence of SEQ ID NO: 34 with no intervening nucleic acids. Alternatively, the second region may contain the sequences of SEQ ID NO: 14 and SEQ ID NO: 15 joined by the endogenous intervening polynucleotide sequence (e.g., the second region may have the sequence of nucleic acids from -590 to -161 with respect to the murine Myo15 translation start site, as set forth in SEQ ID NO: 18) or a nucleic acid linker. In a murine Myo15 promoter in which the second region contains both SEQ ID NO: 14 and SEQ ID NO: 15, the two sequences can be included in any order (e.g., SEQ ID NO: 14 may be joined to (e.g., precede) SEQ ID NO: 15, or SEQ ID NO: 15 may be joined to (e.g., precede) SEQ ID NO: 14).

The first region and the second region of the murine Myo15 promoter can be joined directly or can be joined by a nucleic acid linker. For example, the murine Myo15 promoter can contain the sequence of SEQ ID NO: 7 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 9-13 and 33, e.g., SEQ ID NOs 9 and 10) fused to the sequence of SEQ ID NO: 8 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 14-18, 34, 35, and 38, e.g., SEQ ID NOs 14 and 15) with no intervening nucleic acids. For example, the polynucleotide sequence of the murine Myo15 promoter that results from direct fusion of SEQ ID NO: 7 to SEQ ID NO: 8 is set forth in SEQ ID NO: 19. Alternatively, a linker can be used to join the sequence of SEQ ID NO: 7 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 9-13 and 33, e.g., SEQ ID NOs 9 and 10) to the sequence of SEQ ID NO: 8 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 14-18, 34, 35, and 38, e.g., SEQ ID NOs 14 and 15). Exemplary Myo15 promoters containing functional portions of both SEQ ID NO: 7 and SEQ ID NO: 8 are provided in SEQ ID NOs: 21, 22, 36, 37, 42, and 43.

The length of a nucleic acid linker for use in a murine Myo15 promoter described herein can be about 5 kb or less (e.g., about 5 kb, 4.5 kb, 4 kb, 3.5 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 450 bp, 400 bp, 350 bp, 300 bp, 250 bp, 200 bp, 150 bp, 100 bp, 90 bp, 80 bp, 70 bp, 60 bp, 50 bp, 40 bp, 30 bp, 25 bp, 20 bp, 15 bp, 10 bp, 5 bp, 4 bp, 3 bp, 2 bp, or less). Nucleic acid linkers that can be used in the murine Myo15 promoter described herein do not disrupt the ability of the murine Myo15 promoter of the invention to induce transgene expression in hair cells.

In some embodiments, the sequence of SEQ ID NO: 7 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 9-13 and 33, e.g., SEQ ID NOs 9 and 10) is joined (e.g., operably linked) to the sequence of SEQ ID NO: 8 or a functional portion or derivative thereof (e.g., any one or

more of SEQ ID NOs: 14-18, 34, 35, and 38, e.g., SEQ ID NOs 14 and 15), and, in some embodiments, the order of the regions is reversed (e.g., the sequence of SEQ ID NO: 8 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 14-18, 34, 35, and 38, e.g., SEQ ID NOs 14 and 15) is joined (e.g., operably linked) to the sequence of SEQ ID NO: 7 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 9-13 and 33, e.g., SEQ ID NOs 9 and 10)). For example, the polynucleotide sequence of a murine Myo15 promoter that results from direct fusion of SEQ ID NO: 8 to SEQ ID NO: 7 is set forth in SEQ ID NO: 20. An example of a murine Myo15 promoter in which a functional portion or derivative of SEQ ID NO: 8 precedes a functional portion or derivative of SEQ ID NO: 7 is provided in SEQ ID NO: 41. Regardless of order, the sequence of SEQ ID NO: 7 or a functional portion or derivative thereof and the sequence of SEQ ID NO: 8 or a functional portion or derivative thereof can be joined by direct fusion or a nucleic acid linker, as described above.

In some embodiments, the murine Myo15 promoter for use in the compositions and methods described herein contains a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to a region containing the first non-coding exon of the murine Myo15 gene (nucleic acids from -6755 to -7209 with respect to the murine Myo15 translation start site, the sequence of which is set forth in SEQ ID NO: 7) or a functional portion or derivative thereof. The functional portion of SEQ ID NO: 7 may have the sequence of nucleic acids from -7166 to -7091 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 9) and/or the sequence of nucleic acids from -7077 to -6983 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 10). The murine Myo15 promoter may contain the polynucleotide sequence of SEQ ID NO: 9 fused to the polynucleotide sequence of SEQ ID NO: 10 with no intervening nucleic acids, as set forth in SEQ ID NO: 11, or the murine Myo15 promoter may contain the polynucleotide sequence of SEQ ID NO: 10 fused to the polynucleotide sequence of SEQ ID NO: 9 with no intervening nucleic acids, as set forth in SEQ ID NO: 12. Alternatively, the murine Myo15 promoter may contain the sequences of SEQ ID NO: 9 and SEQ ID NO: 10 joined by the endogenous intervening polynucleotide sequence (e.g., the first region may have or include the sequence of nucleic acids from -7166 to -6983 with respect to the murine Myo15 translation start site, as set forth in SEQ ID NO: 13 and SEQ ID NO: 33) or a polynucleotide linker. In a murine Myo15 promoter that contains both SEQ ID NO: 9 and SEQ ID NO: 10, the two sequences can be included in any order (e.g., SEQ ID NO: 9 may be joined to (e.g., precede) SEQ ID NO: 10, or SEQ ID NO: 10 may be joined to (e.g., precede) SEQ ID NO: 9).

In some embodiments, the murine Myo15 promoter for use in the compositions and methods described herein contains a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the polynucleotide sequence immediately upstream of the murine Myo15 translation start site (nucleic acids from -1 to -1157 with respect to the murine Myo15 translation start site, the sequence of which is set forth in SEQ ID NO: 8) or a functional portion or derivative thereof. The functional portion of SEQ ID NO: 8 may have the sequence of nucleic acids from -590 to -509 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 14) and/or the sequence of nucleic acids from -266 to -161 with respect to the murine Myo15 translation start site (set forth in SEQ ID NO: 15). In some embodiments, the sequence containing SEQ ID NO: 14 has the sequence of SEQ ID NO: 34. In some embodiments, the sequence containing SEQ ID NO: 15 has the sequence of SEQ ID NO: 35. The murine Myo15 promoter may contain the polynucleotide

sequence of SEQ ID NO: 14 fused to the polynucleotide sequence of SEQ ID NO: 15 with no intervening nucleic acids, as set forth in SEQ ID NO: 16, or the murine Myo15 promoter may contain the polynucleotide sequence of SEQ ID NO: 15 fused to the polynucleotide sequence of SEQ ID NO: 14 with no intervening nucleic acids, as set forth in SEQ ID NO: 17. The murine Myo15 promoter may contain the

5 nucleic acid sequence of SEQ ID NO: 34 fused to the nucleic acid sequence of SEQ ID NO: 35 with no intervening nucleic acids, as set forth in SEQ ID NO: 38, or the murine Myo15 promoter may contain the nucleic acid sequence of SEQ ID NO: 35 fused to the nucleic acid sequence of SEQ ID NO: 41 with no intervening nucleic acids. Alternatively, the murine Myo15 promoter may contain the sequences of SEQ ID NO: 14 and SEQ ID NO: 15 joined by the endogenous intervening polynucleotide sequence (e.g., the

10 second region may have the sequence of nucleic acids from -590 to -161 with respect to the murine Myo15 translation start site, as set forth in SEQ ID NO: 18) or a nucleic acid linker. In a murine Myo15 promoter that contains both SEQ ID NO: 14 and SEQ ID NO: 15, the two sequences can be included in any order (e.g., SEQ ID NO: 14 may be joined to (e.g., precede) SEQ ID NO: 15, or SEQ ID NO: 15 may be joined to (e.g., precede) SEQ ID NO: 14).

15 In some embodiments, the murine Myo15 promoter for use in the compositions and methods described herein contains a functional portion or derivative of a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to a region containing the first non-coding exon of the Myo15 gene (nucleic acids from -6755 to -7209 with respect to the murine Myo15 translation start site, the sequence of which is set forth in SEQ ID NO:

20 7) flanked on either side by a functional portion or derivative of a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the nucleic acid sequence immediately upstream of the murine Myo15 translation start site (nucleic acids from -1 to -1157 with respect to the murine Myo15 translation start site, the sequence of which is set forth in SEQ ID NO: 8). For example, a functional portion or derivative of SEQ ID NO: 8, such as SEQ

25 ID NO: 14 or 34 may be directly fused or joined by a nucleic acid linker to a portion of SEQ ID NO: 7, such as any one of SEQ ID NOs: 9-13 and 33, which is directly fused or joined by a nucleic acid linker to a different functional portion of SEQ ID NO: 8, such as SEQ ID NO: 15 or 35. In other embodiments, a functional portion or derivative of SEQ ID NO: 8, such as SEQ ID NO: 15 or 35 may be directly fused or joined by a nucleic acid linker to a portion of SEQ ID NO: 7, such as any one of SEQ ID NOs: 9-13 and

30 33, which is directly fused or joined by a nucleic acid linker to a different functional portion of SEQ ID NO: 8, such as SEQ ID NO: 14 or 34. For example, polynucleotides having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the nucleic acid sequence of SEQ ID NOs: 34, 33, and 35 can be fused to produce a polynucleotide having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

35 more, sequence identity) to the nucleic acid sequence of SEQ ID NO: 39. In some embodiments, polynucleotides having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the nucleic acid sequence of SEQ ID NOs: 35, 33, and 34 can be fused to produce a polynucleotide having at least 85% sequence identity (e.g., 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the nucleic acid sequence of SEQ ID NO: 40.

Human Myosin 15 promoters

5 The polynucleotides of the compositions and methods described herein may also include nucleic acid sequences from regions of the human Myo15 locus that are capable of expressing a transgene specifically in hair cells, or variants thereof, such as a nucleic acid sequences that have at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to regions of the human Myo15 locus that are capable of expressing a transgene specifically in hair cells. The polynucleotides of the compositions and methods described herein can optionally include a linker operably linking the regions of the human Myo15 locus that are capable of expressing a transgene specifically in hair cells, or the regions of the human Myo15 locus can be joined directly without an intervening linker.

15 In some embodiments, the human Myo15 promoter for use in the compositions and methods described herein contains a first region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the sequence set forth in SEQ ID NO: 23 or a functional portion or derivative thereof joined (e.g., operably linked) to a second region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the sequence set forth in SEQ ID NO: 24 or a functional portion or derivative thereof. The functional portion of SEQ ID NO: 23 may have the sequence set forth in SEQ ID NO: 25. The functional portion of SEQ ID NO: 24 may have the sequence set forth in SEQ ID NO: 26 and/or the sequence set forth in SEQ ID NO: 27. The second region may contain the nucleic acid sequence of SEQ ID NO: 26 fused to the nucleic acid sequence of SEQ ID NO: 27 with no intervening nucleic acids, as set forth in SEQ ID NO: 28, or the second region may contain the nucleic acid sequence of SEQ ID NO: 27 fused to the nucleic acid sequence of SEQ ID NO: 26 with no intervening nucleic acids, as set forth in SEQ ID NO: 29. Alternatively, the second region may contain the sequences of SEQ ID NO: 26 and SEQ ID NO: 27 joined by the endogenous intervening nucleic acid sequence (as set forth in SEQ ID NO: 30) or a nucleic acid linker. In a human Myo15 promoter in which the second region contains both SEQ ID NO: 26 and SEQ ID NO: 27, the two sequences can be included in any order (e.g., SEQ ID NO: 26 may be joined to (e.g., precede) SEQ ID NO: 27, or SEQ ID NO: 27 may be joined to (e.g., precede) SEQ ID NO: 26).

35 The first region and the second region of the human Myo15 promoter can be joined directly or can be joined by a nucleic acid linker. For example, the human Myo15 promoter can contain the sequence of SEQ ID NO: 23 or a functional portion or derivative thereof (e.g., SEQ ID NO: 25) fused to the sequence of SEQ ID NO: 24 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 26-30, e.g., SEQ ID NOs: 26 and/or 27) with no intervening nucleic acids. Alternatively, a linker can be used to join the sequence of SEQ ID NO: 23 or a functional portion or derivative thereof (e.g., SEQ ID NO: 25) to the sequence of SEQ ID NO: 24 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 26-30, e.g., SEQ ID NOs: 26 and/or 27). Exemplary human

Myo15 promoters containing functional portions of both SEQ ID NO: 23 and SEQ ID NO: 24 are provided in SEQ ID NOs: 31 and 32.

In some embodiments, the sequence of SEQ ID NO: 23 or a functional portion or derivative thereof (e.g., SEQ ID NO: 25) is joined (e.g., operably linked) to the sequence of SEQ ID NO: 24 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 26-30, e.g., SEQ ID NOs: 26 and 27), and, in some embodiments, the order of the regions is reversed (e.g., the sequence of SEQ ID NO: 24 or a functional portion or derivative thereof (e.g., any one or more of SEQ ID NOs: 26-30, e.g., SEQ ID NOs: 26 and/or 27) is joined (e.g., operably linked) to the sequence of SEQ ID NO: 23 or a functional portion or derivative thereof (e.g., SEQ ID NO: 25)). Regardless of order, the sequence of SEQ ID NO: 23 or a functional portion or derivative thereof and the sequence of SEQ ID NO: 24 or a functional portion or derivative thereof can be joined by direct fusion or a nucleic acid linker, as described above.

In some embodiments, the human Myo15 promoter for use in the compositions and methods described herein contain a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to a region containing the sequence set forth in SEQ ID NO: 23 or a functional portion or derivative thereof. The functional portion of SEQ ID NO: 23 may have the sequence of nucleic acids set forth in SEQ ID NO: 25.

In some embodiments, the human Myo15 promoter for use in the compositions and methods described herein contains a region having at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the sequence set forth in SEQ ID NO: 18 or a functional portion or derivative thereof. The functional portion of SEQ ID NO: 24 may have the sequence set forth in SEQ ID NO: 26 and/or the sequence set forth in SEQ ID NO: 27. The human Myo15 promoter may contain the nucleic acid sequence of SEQ ID NO: 26 fused to the nucleic acid sequence of SEQ ID NO: 27 with no intervening nucleic acids, as set forth in SEQ ID NO: 28, or the human Myo15 promoter may contain the nucleic acid sequence of SEQ ID NO: 27 fused to the nucleic acid sequence of SEQ ID NO: 26 with no intervening nucleic acids, as set forth in SEQ ID NO: 29. Alternatively, the human Myo15 promoter may contain the sequences of SEQ ID NO: 26 and SEQ ID NO: 27 joined by the endogenous intervening nucleic acid sequence (e.g., as set forth in SEQ ID NO: 30) or a nucleic acid linker. In a human Myo15 promoter that contains both SEQ ID NO: 26 and SEQ ID NO: 27, the two sequences can be included in any order (e.g., SEQ ID NO: 26 may be joined to (e.g., precede) SEQ ID NO: 27, or SEQ ID NO: 27 may be joined to (e.g., precede) SEQ ID NO: 26).

The length of a nucleic acid linker for use in a human Myo15 promoter described herein can be about 5 kb or less (e.g., about 5 kb, 4.5, kb, 4, kb, 3.5 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 450 bp, 400 bp, 350 bp, 300 bp, 250 bp, 200 bp, 150 bp, 100 bp, 90 bp, 80 bp, 70 bp, 60 bp, 50 bp, 40 bp, 30 bp, 25 bp, 20 bp, 15, bp, 10 bp, 5 bp, 4 bp, 3 bp, 2 bp, or less). Nucleic

acid linkers that can be used in the human Myo15 promoters described herein do not disrupt the ability of the Myo15 promoter of the invention to induce transgene expression in hair cells.

The foregoing Myo15 promoter sequences are summarized in Table 3, below.

5 **Table 3: Exemplary nucleotide sequences for use in the Myo15 promoter described herein**

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
7	Region containing non-coding exon 1 of murine Myo15 (-6755 to -7209)	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCTAGT TCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTAAGCTTCTGC CACTGGCTCCGGCATTGCAGAGAGAAGAGAAGGGGCGGCA GAGCTGAACCTTAGCCTTGCCCTTCTGGGTACCTTCTGAGC CTCACTGTCTTCTGTGAGATGGGCAAAGTGCGGGTGTGACTC CTTGGCAACGGTGTTACACCAGGGCAGGTAAAGTTGTAGTTA TTTGTGGGGTACACCAGGACTGTAAAGGTGTAAGTAT
8	Region immediately preceding the translation start site of murine Myo15 (-1 to -1157)	GGTCTCACCCAGCATTTTCACTTCTAATAAGTTCAAATGTGAT ACGGCACCTTTCTAAAAATTAGTTTTCAGGGAAATAGGGTTCA AAACTGGTAGTGGTAGGGTCCATTCTCACGACCCCCAGGCCT GCTAACCCCTGACCAAGCTACCTATTACTTACCCTCCTCTTTCT CCTCCTCCTCTTTCTCCTTCTCCTGCTTCCCCTCTTCCTTCTC CCTCCCTTCTCTCCCTCCTCCCCCTCCTTGGCTGTGATCAG ATCCAGAGCCTGAATGAGCCTCCTGACCCACACCCCCACTA GCATGGGCCTGCAAGTGCCCAGAAGTCCCTCCTGCCTCCTA AACTGCCCAGCCGATCCATTAGCTCTTCTTCTTCCAGTGA AAGAAGCAGGCACAGCCTGTCCCTCCCGTTCTACAGAAAGG AAGCTACAGCACAGGGAGGGCCAAAGGCCTTCTGGGACTA GACAGTTGATCAACAGCAGGACTGGAGAGCTGGGCTCCATTT TTGTTCTTGGTGCCCTGCCCCTCCCATGACCTGCAGAGAC ATTCAGCCTGCCAGGCTTTATGAGGTGGGAGCTGGGCTCTC CCTGATGTATTATTAGCTCCCTGGAGTTGGCCAGCTCCTGT TACACTGGCCACAGCCCTGGGCATCCGCTTCTCACTTCTAGT TTCCCCTCCAAGGTAATGTGGTGGGTCATGATCATTCTATCCT GGCTTCAGGGACCTGACTCCACTTTGGGGCCATTCGAGGGG TCTAGGGTAGATGATGTCCCCCTGTGGGGATTAATGTCCTGC TCTGTAAACTGAGCTAGCTGAGATCCAGGAGGGCTTGGCCA GAGACAGCAAGTTGTTGCCATGGTGACTTTAAAGCCAGGTTG CTGCCCCAGCACAGGCCTCCAGTCTACCCCTACTAGAAAAC

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		AACACCCAGGCACTTTCCACCACCTCTCAAAGGTGAAACCCA AGGCTGGTCTAGAGAATGAATTATGGATCCTCGCTGTCCGTG CCACCCAGCTAGTCCCAGCGGCTCAGACACTGAGGAGAGAC TGAGGTTTCAGCTACAAGCAAAAAGACCTAGCTGGTCTCCAA GCAGTGTCTCCAAGTCCCTGAACCTGTGACACCTGCCCCAG GCATCATCAGGCACAGAGGGCCACC
9	Portion of SEQ ID NO: 7 (-7166 to -7091)	CCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAAT AATAGATGTCATTAAATATACATTGGGCCCCAGG
10	Portion of SEQ ID NO: 7 (-7077 to -6983)	AGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGACATAGGA CCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCCACAGGA CCCAGGTAAGGG
11	Portion of SEQ ID NO: 7 (SEQ ID NO: 9 fused to SEQ ID NO: 10)	CCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAAT AATAGATGTCATTAAATATACATTGGGCCCCAGGAGCCTGAG CCTCCTTTCCATCTCTGTGGAGGCAGACATAGGACCCCCAAC AAACAGCATGCAGGTTGGGAGCCAGCCACAGGACCCAGGTA AGGG
12	Portion of SEQ ID NO: 7 (SEQ ID NO: 10 fused to SEQ ID NO: 9)	AGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGACATAGGA CCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCCACAGGA CCCAGGTAAGGGCCCATGTCAGCTGCTTGTGCTTTCCAGAGA CAAAACAGGAATAATAGATGTCATTAAATATACATTGGGCCCC AGG
13	Portion of SEQ ID NO: 7 (-7166 to -6983)	CCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAAT AATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGG
14	Portion of SEQ ID NO: 8 (-590 to -509)	TGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTCC CTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCCCTG
15	Portion of SEQ ID NO: 8 (-266 to -161)	CACAGGCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAG GCACTTTCCACCACCTCTCAAAGGTGAAACCAAGGCTGGTC TAGAGAATGAATTATGGATCCT
16	Portion of SEQ ID NO: 8 (SEQ ID NO: 14 fused to SEQ ID NO: 15)	TGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTCC CTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCCCTG CACAGGCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAG GCACTTTCCACCACCTCTCAAAGGTGAAACCAAGGCTGGTC TAGAGAATGAATTATGGATCCT

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
17	Portion of SEQ ID NO: 8 (SEQ ID NO: 15 fused to SEQ ID NO: 14)	CACAGGCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAG GCACTTTCCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTC TAGAGAATGAATTATGGATCCTTGAGGTGGGAGCTGGGCTCT CCCTGATGTATTATTCAGCTCCCTGGAGTTGGCCAGCTCCTG TTACACTGGCCACAGCCCTG
18	Portion of SEQ ID NO: 8 (-590 to -161)	TGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTCC CTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCCCTGG GCATCCGCTTCTCACTTCTAGTTTCCCCTCCAAGGTAATGTG GTGGGTCATGATCATTCTATCCTGGCTTCAGGGACCTGACTC CACTTTGGGGCCATTGAGGGGTCTAGGGTAGATGATGTCC CCCTGTGGGGATTAATGTCCTGCTCTGTAAACTGAGCTAGC TGAGATCCAGGAGGGCTTGGCCAGAGACAGCAAGTTGTTGC CATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACAGGCCT CCCAGTCTACCCTCACTAGAAAACAACACCCAGGCACCTTCC ACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGAGAATG AATTATGGATCCT
19	SEQ ID NO: 7 fused to SEQ ID NO: 8	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCTAGT TCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTAAGCTTCTGC CACTGGCTCCGGCATTGCAGAGAGAAGAGAAGGGGCGGCA GAGCTGAACCTTAGCCTTGCCCTTCTGGGTACCCTTCTGAGC CTCACTGTCTTCTGTGAGATGGGCAAAGTGCGGGTGTGACTC CTTGGCAACGGTGTTACACCAGGGCAGGTAAAGTTGTAGTTA TTTGTGGGGTACACCAGGACTGTTAAAGGTGTAAGTATGGTC TCACCCAGCATTTTCACTTCTAATAAGTTCAAATGTGATACGG CACCTTTCTAAAAATTAGTTTTCAGGGAAATAGGGTTCAAAAC TGGTAGTGGTAGGGTCCATTCTACGACCCCCAGGCCTGCT AACCTGACCAAGCTACCTATTACTTACCCTCCTCTTCTCCT CCTCCTCTTCTCCTTCTCCTGCTTCCCCTCTTCTTCTCCT CCCTCCTCTCCCTCCTCCCCCTCCTTGGCTGTGATCAGATC CAGAGCCTGAATGAGCCTCCTGACCCACACCCCCACTAGC ATGGGCCTGCAAGTGCCCAGAAGTCCCTCCTGCCTCCTAAAC TGCCCAGCCGATCCATTAGCTCTTCTTCTTCCCAGTGAAAG AAGCAGGCACAGCCTGTCCCTCCCGTTCTACAGAAAGGAAG CTACAGCACAGGGAGGGCCAAAGGCCTTCTGGGACTAGAC

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		AGTTGATCAACAGCAGGACTGGAGAGCTGGGCTCCATTTTG TTCCTTGGTGCCCTGCCCTCCCCATGACCTGCAGAGACATT CAGCCTGCCAGGCTTTATGAGGTGGGAGCTGGGCTCTCCCT GATGTATTATTACAGCTCCCTGGAGTTGGCCAGCTCCTGTTAC ACTGGCCACAGCCCTGGGCATCCGCTTCTCACTTCTAGTTTC CCCTCCAAGGTAATGTGGTGGGTCATGATCATTCTATCCTGG CTTCAGGGACCTGACTCCACTTTGGGGCCATTCGAGGGGTC TAGGGTAGATGATGTCCCCCTGTGGGGATTAATGTCCTGCTC TGTAAACTGAGCTAGCTAGATCCAGGAGGGCTTGGCCAG AGACAGCAAGTTGTTGCCATGGTGACTTTAAAGCCAGGTTGC TGCCCCAGCACAGGCCTCCCAGTCTACCCTCACTAGAAAACA ACACCCAGGCACTTTCCACCACCTCTCAAAGGTGAAACCCAA GGCTGGTCTAGAGAATGAATTATGGATCCTCGCTGTCCGTGC CACCCAGCTAGTCCCAGCGGCTCAGACACTGAGGAGAGACT GTAGGTTACAGTACAAGCAAAAAGACCTAGCTGGTCTCCAAG CAGTGTCTCCAAGTCCCTGAACCTGTGACACCTGCCCCAGG CATCATCAGGCACAGAGGGCCACC
20	SEQ ID NO: 8 fused to SEQ ID NO: 7	GGTCTACCCAGCATTTTCACTTCTAATAAGTTCAAATGTGAT ACGGCACCTTTCTAAAAATTAGTTTTAGGGAAATAGGGTTCA AACTGGTAGTGGTAGGGTCCATTCTCACGACCCCCAGGCCT GCTAACCTGACCAAGCTACCTATTACTTACCCTCCTCTTTCT CCTCCTCCTCTTTCTCCTTCTCCTGCTTCCCCCTCTTCCTTCTC CCTCCCTTCTCCTCCTCCTCCCCCTCCTTGGCTGTGATCAG ATCCAGAGCCTGAATGAGCCTCCTGACCCCACACCCCCACTA GCATGGGCCTGCAAGTGCCAGAAAGTCCCTCCTGCCTCCTA AACTGCCCAGCCGATCCATTAGCTCTTCCTTCTTCCCAGTGA AAGAAGCAGGCACAGCCTGTCCCTCCCGTTCTACAGAAAGG AAGCTACAGCACAGGGAGGGCCAAAGGCCTTCTGGGACTA GACAGTTGATCAACAGCAGGACTGGAGAGCTGGGCTCCATTT TTGTTCTTGGTGCCCTGCCCTCCCCATGACCTGCAGAGAC ATTCAGCCTGCCAGGCTTTATGAGGTGGGAGCTGGGCTCTC CCTGATGTATTATTACAGCTCCCTGGAGTTGGCCAGCTCCTGT TACACTGGCCACAGCCCTGGGCATCCGCTTCTCACTTCTAGT TTCCCCTCCAAGGTAATGTGGTGGGTCATGATCATTCTATCCT GGCTTCAGGGACCTGACTCCACTTTGGGGCCATTCGAGGGG TCTAGGGTAGATGATGTCCCCCTGTGGGGATTAATGTCCTGC TCTGTAAACTGAGCTAGCTAGATCCAGGAGGGCTTGGCCA GAGACAGCAAGTTGTTGCCATGGTGACTTTAAAGCCAGGTTG

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		CTGCCCCAGCACAGGCCTCCCAGTCTACCCTCACTAGAAAAC AACACCCAGGCACCTTCCACCACCTCTCAAAGGTGAAACCCA AGGCTGGTCTAGAGAATGAATTATGGATCCTCGCTGTCCGTG CCACCCAGCTAGTCCCAGCGGCTCAGACACTGAGGAGAGAC TGTAGGTTCAAGTACAAGCAAAAAGACCTAGCTGGTCTCCAA GCAGTGTCTCCAAGTCCCTGAACCTGTGACACCTGCCCCAG GCATCATCAGGCACAGAGGGCCACCCTGCAGCTCAGCCTAC TACTTGCTTTCCAGGCTGTTCCCTAGTTCCCATGTGCTGCTT GTGCTTTCCAGAGACAAAACAGGAATAATAGATGTCATTAAAT ATACATTGGGCCCCAGGCGGTCAATGTGGCAGCCTGAGCCT CCTTTCCATCTCTGTGGAGGCAGACATAGGACCCCCAACAAA CAGCATGCAGGTTGGGAGCCAGCCACAGGACCCAGGTAAGG GGCCCTGGGTCTTAAGCTTCTGCCACTGGCTCCGGCATTG CAGAGAGAAGAGAAGGGGCGGCAGAGCTGAACCTTAGCCTT GCCTTCTGGGTACCCTTCTGAGCCTCACTGTCTTCTGTGAG ATGGGCAAAGTGCGGGTGTGACTCCTTGGCAACGGTGTTAC ACCAGGGCAGGTAAAGTTGTAGTTATTTGTGGGGTACACCAG GACTGTAAAGGTGTAACCTAT
21	Portion of SEQ ID NO: 7 that contains SEQ ID NO: 9 and SEQ ID NO: 10 fused to portion of SEQ ID NO: 8 that contains SEQ ID NO: 14 and SEQ ID NO: 15	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCCCTAGT TCCCATGTGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTAAGCTTCTGC CACTGGCTCCGGCATTGCAGAGAGAAGAGAAGGGGCGGCA GACTGGAGAGCTGGGCTCCATTTTGTTCCTTGGTGCCCTGC CCCTCCCCATGACCTGCAGAGACATTCAGCCTGCCAGGCTTT ATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTC CCTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCCCTG GGCATCCGCTTCTCACTTCTAGTTTCCCTCCAAGGTAATGT GGTGGGTCATGATCATTCTATCCTGGCTTCAGGGACCTGACT CCACTTTGGGGCCATTGAGGGGTCTAGGGTAGATGATGTC CCCCTGTGGGGATTAATGTCCTGCTCTGTAAACTGAGCTAG CTGAGATCCAGGAGGGCTTGGCCAGAGACAGCAAGTTGTTG CCATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACAGGC CTCCCAGTCTACCCTCACTAGAAAACAACCCAGGCACCTTT CCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGAGAA TGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGTCCC

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		AGCGGCTCAGACACTGAGGAGAGACTGTAGGTTTCAGCTACA AGCAAAAAGACCTAGCTGGTCTCCAAGCAGTGTCTCCAAGTC CCTGAACCTGTGACACCTGCCCCAGGCATCATCAGGCACAG AGGGCCACC
22	Portion of SEQ ID NO: 7 that contains SEQ ID NO: 9 and SEQ ID NO: 10 fused to portion of SEQ ID NO: 8 that contains SEQ ID NO: 14 and SEQ ID NO: 15	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCTAGT TCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTTTTATGAGGT GGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTCCCTGGAG TTGGCCAGCTCCTGTTACACTGGCCACAGCCCTGGGCATCC GCTGCCATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACA GGCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAGGCAC TTTCCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGA GAATGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGT CCCAGCGGCTCAGACACTG
23	Region 1 of the human Myo15 promoter	GTATGCCTTTTGAGATGGATGCAGCAGGTTCTGTGAGGCTGC CAGGAGGGGTAGAGTTCCCGGGGGCCTCGGGCCCCGCTGG AGTGTGGAGCAGGCCCATGCTCAGCTCTCCAGGCTGTTTCGT GGCTCCCCTGTCAGCTGCTCACTCCTTTCCAGAGACAAAACA GGAATAATAGACATCATTAAATATACATAGGGCCCCAGGCGG TCGGCGTGGTGGGCTGGGCCTCCCTTCC
24	Region 2 of human Myo15 promoter	TGCCCTGCCTTCTGAGCCGGCAGCCTGGCTCCCCACCCCAT GTATTATTCAGCTCCTGAGAGCCAGCCAGCTCCTGTTACACT GACCGCAGCCCAGCACCTGCTCTGCCCATTCCTCCTCCC TTGCCTAGGACCTAGAGGGTTCAAAGTTCTCCTCCAAGATGA CTTGGTGGGCTTTGGCCATCCCACCCTAGGCCCCACTTCTG GCCCAGTGCAGGTGTGCTGGTGATTTAGGGCAGGTGGCATT CCATCTCTGTGGCTCAATGTCTTCCTCTGTGAAGCCGAAGTG ACCCAAGGGCTCCCTTCATGGGGTTGAGCCAGCTGTGGCCC AGGGAGGGCCTAACCAGGATGAGCACTGATGTTGCCATGAC GACTCCGAGGCCAGAATGTCTCCCCAGCACAGGCCTCATA GGCAGGCTTCCCCATCCTGGTAAACAACACCCACACACTTTC TACTACTGCTCTAGGGTGAACCCAAGGCGCTCTAGAGGAGA TGAATTATGGATCCGCCCTCCCGGAATCCTGGCTCGGCCCTC CCCACGCCACCCAGGGCCAGTCGGGTCTGCTCACAGCCCGA GGAGGCCGCGTGTCCAGCCGCGGCAAGAGACAGAGCAGG

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		TCCCTGTGTCTCCAAGTCCCTGAGCCCGTGACACCGGCCCC AGGCCCTGTAGAGAGCAGGCAGCCACC
25	Portion of SEQ ID NO: 23	CCCCTGTCAGCTGCTCACTCCTTTCCAGAGACAAAACAGGAA TAATAGACATCATTAATATACATAGGGCCCCAGG
26	Portion of SEQ ID NO: 24	TGAGCCGGCAGCCTGGCTCCCCACCCCATGTATTATTCAGCT CCTGAGAGCCAGCCAGCTCCTGTTACACTGACCGCAGCCC
27	Portion of SEQ ID NO: 24	CACAGGCCTCATAGGCAGGCTTCCCCATCCTGGTAAACAACA CCCACACACTTTCTACTACTGCTCTAGGGTGAAACCCAAGGC GCTCTAGAGGAGATGAATTATGGATCC
28	Portion of SEQ ID NO: 24 (SEQ ID NO: 26 fused to SEQ ID NO: 27)	TGAGCCGGCAGCCTGGCTCCCCACCCCATGTATTATTCAGCT CCTGAGAGCCAGCCAGCTCCTGTTACACTGACCGCAGCCCC ACAGGCCTCATAGGCAGGCTTCCCCATCCTGGTAAACAACAC CCACACACTTTCTACTACTGCTCTAGGGTGAAACCCAAGGCG CTCTAGAGGAGATGAATTATGGATCC
29	Portion of SEQ ID NO: 24 (SEQ ID NO: 27 fused to SEQ ID NO: 26)	CACAGGCCTCATAGGCAGGCTTCCCCATCCTGGTAAACAACA CCCACACACTTTCTACTACTGCTCTAGGGTGAAACCCAAGGC GCTCTAGAGGAGATGAATTATGGATCCTGAGCCGGCAGCCT GGCTCCCCACCCCATGTATTATTCAGCTCCTGAGAGCCAGCC AGCTCCTGTTACACTGACCGCAGCCC
30	Portion of SEQ ID NO: 24 (contiguous sequence including SEQ ID NO: 26 and SEQ ID NO: 27)	TGAGCCGGCAGCCTGGCTCCCCACCCCATGTATTATTCAGCT CCTGAGAGCCAGCCAGCTCCTGTTACACTGACCGCAGCCCA GCACCTGCTCTGCCCATCCCCCTCCTCCCTTGCTAGGACCT AGAGGGTTCAAAGTTCTCCTCCAAGATGACTTGGTGGGCTTT GGCCATCCCACCCTAGGCCCCACTTCTGGCCCAGTGCAGGT GTGCTGGTGATTTAGGGCAGGTGGCATTCCATCTCTGTGGCT CAATGTCTTCCTCTGTGAAGCCGAAGTGACCCAAGGGCTCCC TTCATGGGGTTGAGCCAGCTGTGGCCCAGGGAGGGCCTAAC CAGGATGAGCACTGATGTTGCCATGACGACTCCGAGGCCAG AATGTCTCCCCCAGCACAGGCCTCATAGGCAGGCTTCCCCAT CCTGGTAAACAACACCCACACACTTTCTACTACTGCTCTAGG GTGAAACCCAAGGCGCTCTAGAGGAGATGAATTATGGATCC
31	Polynucleotide containing SEQ ID NO: 23 and SEQ ID NO: 24	GTATGCCTTTTGAGATGGATGCAGCAGGTTCTGTGAGGCTGC CAGGAGGGGTAGAGTTCCCGGGGGCCTCGGGCCCCGCTGG AGTGTGGAGCAGGCCCATGCTCAGCTCTCCAGGCTGTTTCGT GGCTCCCCTGTCAGCTGCTCACTCCTTTCCAGAGACAAAACA GGAATAATAGACATCATTAATATACATAGGGCCCCAGGCGG TCGGCGTGGTGGGCTGGGCCTCCCTTCCCCATAACACTGAG

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		CTGCTCTGCTGGGCCAACCGTGCTCCTGGGCCAGCCAGAGG ACCCCCATGAGGCGGCATGCAGGCGGGAGCAGGCCACAG AACGCAGGTAAGGAGACCTTAGCCTAGAGTCCTTGGGGTCT GTCAGTGGCCACCCTCGCATCCCAGGCTGCAGGAACTGAG GCCCAGAGAGGACAAGGACTTTCCTGGACCCACACAGCCAG TCAGTGACAGAGCCTAGGGTCTGAGCCAGGCCTGACCCAAC CTCCATTTCTGCCTCTCTACCCCTGCCCCGCCCCAACACAC ACACACACACAAGTGGAGTTCCTGAAACGCCCTCCTTGC CCTGCCTTCTGAGCCGGCAGCCTGGCTCCCCACCCCATGTA TTATTCAGCTCCTGAGAGCCAGCCAGCTCCTGTTACACTGAC CGCAGCCCAGCACCTGCTCTGCCATTCCCCTCCTCCCTTGC CTAGGACCTAGAGGGTTCAAAGTTCTCCTCCAAGATGACTTG GTGGGCTTTGGCCATCCCACCCTAGGCCCACTTCTGGCCC AGTGCAGGTGTGCTGGTGATTTAGGGCAGGTGGCATTCCAT CTCTGTGGCTCAATGTCTTCCTCTGTGAAGCCGAAGTGACCC AAGGGCTCCCTTCATGGGGTTGAGCCAGCTGTGGCCCAGGG AGGGCCTAACCAGGATGAGCACTGATGTTGCCATGACGACT CCGAGGCCAGAATGTCTCCCCAGCACAGGCCTCATAGGCA GGCTTCCCCATCCTGGTAAACAACACCCACACACTTTCTACT ACTGCTCTAGGGTGAACCCAAGGCGCTCTAGAGGAGATGA ATTATGGATCCGCCCTCCCGGAATCCTGGCTCGGCCCTCCC CACGCCACCCAGGGCCAGTCGGGTCTGCTCACAGCCCGAG GAGGCCGCGTGTCCAGCCGCGGGCAAGAGACAGAGCAGGT CCCTGTGTCTCCAAGTCCCTGAGCCCGTGACACCGGCCCA GGCCCTGTAGAGAGCAGGCAGCCACC
32	Polynucleotide containing SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27	GCAGGCCCATGCTCAGCTCTCCAGGCTGTTCTGGCTCCCC TGTCAGCTGCTCACTCCTTTCCAGAGACAAAACAGGAATAAT AGACATCATTAAATATACATAGGGCCCCAGGCGGTGGCGTG GTGGGCTGGGCCTCCCTTCCCCATAAACTGAGCTGCTCTG CTGGGCCAACCGTGCTCCTGGGCCAGCCAGAGGACCCCAT GAGGCGGCATGCAGGCGGGGAGCAGGCCACAGAACGCAGG TAAGGAGACCTTGCCTTCTGAGCCGGCAGCCTGGCTCCCCA CCCCATGTATTATTAGCTCCTGAGAGCCAGCCAGCTCCTGT TAACTGACCGCAGCCAGCACCTGCTCTGCCATTCCCCTC CTCCCTTGCCTAGGACCTAGAGGGTTCAAAGTTCTCCTCAA GATGACTTGGTGGGCTTTGGCCATCGGGCCTAACCAGGATG AGCACTGATGTTGCCATGACGACTCCGAGGCCAGAATGTCTC CCCCAGCACAGGCCTCATAGGCAGGCTTCCCCATCCTGGTA

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		AACAACACCCACACACTTTCTACTACTGCTCTAGGGTGAAAC CCAAGGCGCTCTAGAGGAGATGAATTATGGATCCGCCCTCC CGGAATCCTGGCTCGGCCCTCCCCACGC
33	Portion of SEQ ID NO: 7 that contains SEQ ID NO: 9 and SEQ ID NO: 10	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCTAGT TCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTCT
34	Portion of SEQ ID NO: 8 that contains SEQ ID NO: 14	TTTATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAG CTCCCTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCC CTGGGCATCCGC
35	Portion of SEQ ID NO: 8 that contains SEQ ID NO: 15	TGCCATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACAG GCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAGGCACT TTCCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGAG AATGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGTC CCAGCGGCTCAGACACTG
36	SEQ ID NO: 33 fused to SEQ ID NO: 34	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCTAGT TCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTCTTTTATGAGGT GGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTCCCTGGAG TTGGCCAGCTCCTGTTACACTGGCCACAGCCCTGGGCATCC GC
37	SEQ ID NO: 33 fused to SEQ ID NO: 35	CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCTAGT TCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAA TAATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTTGCCATGGTG ACTTTAAAGCCAGGTTGCTGCCCCAGCACAGGCCTCCAGTC TACCCTCACTAGAAAACAACACCCAGGCACTTTCCACCACCT CTCAAAGGTGAAACCCAAGGCTGGTCTAGAGAATGAATTATG GATCCTCGCTGTCCGTGCCACCCAGCTAGTCCCAGCGGCTC AGACACTG

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
38	SEQ ID NO: 34 fused to SEQ ID NO: 35	TTTATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAG CTCCCTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCC CTGGGCATCCGCTGCCATGGTGACTTTAAAGCCAGGTTGCTG CCCCAGCACAGGCCTCCCAGTCTACCCTCACTAGAAAAACAAC ACCCAGGCACTTTCCACCACCTCTCAAAGGTGAAACCCAAGG CTGGTCTAGAGAATGAATTATGGATCCTCGCTGTCCGTGCCA CCCAGCTAGTCCCAGCGGCTCAGACACTG
39	SEQ ID NO: 34 fused to SEQ ID NO: 33, which is fused to SEQ ID NO: 35	TTTATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAG CTCCCTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCC CTGGGCATCCGCTGCAGCTCAGCCTACTACTTGCTTTCCAG GCTGTTCCCTAGTTCCCATGTCAGCTGCTTGTGCTTTCCAGAG ACAAAACAGGAATAATAGATGTCATTAAATATACATTGGGCCC CAGGCGGTCAATGTGGCAGCCTGAGCCTCCTTTCCATCTCTG TGGAGGCAGACATAGGACCCCCAACAAACAGCATGCAGGTT GGGAGCCAGCCACAGGACCCAGGTAAGGGGCCCTGGGTCC TTTGCCATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACA GGCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAGGCAC TTTCCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGA GAATGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGT CCCAGCGGCTCAGACACTG
40	SEQ ID NO: 35 fused to SEQ ID NO: 33, which is fused to SEQ ID NO: 34	TGCCATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACAG GCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAGGCACT TTCCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGAG AATGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGTC CCAGCGGCTCAGACACTGCTGCAGCTCAGCCTACTACTTGCT TTCCAGGCTGTTCCCTAGTTCCCATGTCAGCTGCTTGTGCTTTC CAGAGACAAAACAGGAATAATAGATGTCATTAAATATACATTG GGCCCCAGGCGGTCAATGTGGCAGCCTGAGCCTCCTTTCCA TCTCTGTGGAGGCAGACATAGGACCCCCAACAAACAGCATG CAGGTTGGGAGCCAGCCACAGGACCCAGGTAAGGGGCCCT GGGTCTTTTTATGAGGTGGGAGCTGGGCTCTCCCTGATGTA TTATTCAGCTCCCTGGAGTTGGCCAGCTCCTGTTACACTGGC CACAGCCCTGGGCATCCGC
41	SEQ ID NO: 34 fused to SEQ ID NO: 35, which is fused to SEQ ID NO: 33	TTTATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAG CTCCCTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCC CTGGGCATCCGCTGCCATGGTGACTTTAAAGCCAGGTTGCTG CCCCAGCACAGGCCTCCCAGTCTACCCTCACTAGAAAAACAAC ACCCAGGCACTTTCCACCACCTCTCAAAGGTGAAACCCAAGG

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
		CTGGTCTAGAGAATGAATTATGGATCCTCGCTGTCCGTGCCA CCCAGCTAGTCCCAGCGGCTCAGACACTGCTGCAGCTCAGC CTACTACTTGCTTTCCAGGCTGTTCCCTAGTTCCCATGTCAGCT GCTTGTGCTTTCCAGAGACAAAACAGGAATAATAGATGTCATT AAATATACATTGGGCCCCAGGCGGTCAATGTGGCAGCCTGA GCCTCCTTTCCATCTCTGTGGAGGCAGACATAGGACCCCCAA CAAACAGCATGCAGGTTGGGAGCCAGCCACAGGACCCAGGT AAGGGGCCCTGGGTCTT
42	Portion of SEQ ID NO: 7 that contains SEQ ID NO: 9 and SEQ ID NO: 10 fused to portion of SEQ ID NO: 8 that contains SEQ ID NO: 14 and SEQ ID NO: 15	TGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCCCTAGTT CCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAAT AATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTAAGCTTCTGC CACTGGCTCCGGCATTGCAGAGAGAAGAGAAGGGGCGGCA GACTGGAGAGCTGGGCTCCATTTTTGTTCTTGGTGCCCTGC CCCTCCCCATGACCTGCAGAGACATTGAGCCTGCCAGGCTTT ATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTCAGCTC CCTGGAGTTGGCCAGCTCCTGTTACACTGGCCACAGCCCTG GGCATCCGCTTCTCACTTCTAGTTTCCCCTCCAAGGTAATGT GGTGGGTCATGATCATTCTATCCTGGCTTCAGGGACCTGACT CCACTTTGGGGCCATTGAGGGGTCTAGGGTAGATGATGTC CCCCTGTGGGGATTAATGTCTGCTCTGTAAACTGAGCTAG CTGAGATCCAGGAGGGCTTGGCCAGAGACAGCAAGTTGTTG CCATGGTGACTTTAAAGCCAGGTTGCTGCCCCAGCACAGGC CTCCCAGTCTACCCTCACTAGAAAACAACACCCAGGCACTTT CCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGAGAA TGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGTCCC AGCGGCTCAGACACTGAGGAGAGACTGTAGGTTGAGCTACA AGCAAAAAGACCTAGCTGGTCTCCAAGCAGTGTCTCCAAGTC CCTGAACCTGTGACACCTGCCCCAGGCATCATCAGGCACAG AGGGCCACC
43	Portion of SEQ ID NO: 7 that contains SEQ ID NO: 9 and SEQ ID NO: 10 fused to portion of SEQ ID NO: 8 that	TGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCCCTAGTT CCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAAACAGGAAT AATAGATGTCATTAAATATACATTGGGCCCCAGGCGGTCAAT GTGGCAGCCTGAGCCTCCTTTCCATCTCTGTGGAGGCAGAC ATAGGACCCCCAACAACAGCATGCAGGTTGGGAGCCAGCC ACAGGACCCAGGTAAGGGGCCCTGGGTCTTTTTATGAGGT

SEQ ID NO.	Description of polynucleotide sequence	Polynucleotide Sequence
	contains SEQ ID NO: 14 and SEQ ID NO: 15	GGGAGCTGGGCTCTCCCTGATGTATTATTTCAGCTCCCTGGAG TTGGCCAGCTCCTGTTACACTGGCCACAGCCCTGGGCATCC GCTGCCATGGTGA CTTTAAAGCCAGGTTGCTGCCCCAGCACA GGCCTCCCAGTCTACCCTCACTAGAAAACAACACCCAGGCAC TTTCCACCACCTCTCAAAGGTGAAACCCAAGGCTGGTCTAGA GAATGAATTATGGATCCTCGCTGTCCGTGCCACCCAGCTAGT CCCAGCGGCTCAGACACTG

Additional Myo15 promoters useful in conjunction with the compositions and methods described herein include nucleic acid molecules that have at least 85% sequence identity (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to the polynucleotide sequences set forth in Table 3, as well as functional portions or derivatives of the polynucleotide sequences set forth in Table 3. The Myo15 promoters listed in Table 3 are characterized in International Application Publication Nos. WO2019210181A1 and WO2020163761A1, which are incorporated herein by reference.

In embodiments in which an smCBA promoter is included in a dual vector system described herein (e.g., in the first vector in a dual vector system), the smCBA promoter may have the sequence of the smCBA promoter described in U.S. Patent No. 8,298,818, which is incorporated herein by reference. In some embodiments, the smCBA promoter has the sequence of:

GGTACCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATG
GAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGA
CCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGAC
TTTCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACAT
CTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCAC
TCTCCCCATCTCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATTAT
TTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCG
GGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCA
ATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTATGGCGAGGCGGCGGCGGCGGCG
GCCCTATAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGCGCTGCCTTCG
CCCCGTGCCCCGCTCCGCCGCCGCTCGCGCGCCCCGCCCGGCTCTGACTGAC
CGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAAT
TAGCGCTTGGTTTAATGACGGCTTGTTCCTTTCTGTGGCTGCGTGAAAGCCTTGAG
GGGCTCCGGGAGCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTCTTTTCTAC
AGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCA (SEQ ID NO:
44).

Once a polynucleotide encoding OTOF has been incorporated into the nuclear DNA of a mammalian cell or stabilized in an episomal monomer or concatemer, the transcription of this polynucleotide can be induced by methods known in the art. For example, expression can be induced by exposing the mammalian cell to an external chemical reagent, such as an agent that modulates the binding of a transcription factor and/or RNA polymerase to the mammalian promoter and thus regulates gene expression. The chemical reagent can serve to facilitate the binding of RNA polymerase and/or transcription factors to the mammalian promoter, e.g., by removing a repressor protein that has bound the promoter. Alternatively, the chemical reagent can serve to enhance the affinity of the mammalian promoter for RNA polymerase and/or transcription factors such that the rate of transcription of the gene located downstream of the promoter is increased in the presence of the chemical reagent. Examples of chemical reagents that potentiate polynucleotide transcription by the above mechanisms include tetracycline and doxycycline. These reagents are commercially available (Life Technologies, Carlsbad, CA) and can be administered to a mammalian cell in order to promote gene expression according to established protocols.

Other DNA sequence elements that may be included in the nucleic acid vectors for use in the compositions and methods described herein include enhancer sequences. Enhancers represent another class of regulatory elements that induce a conformational change in the polynucleotide containing the gene of interest such that the DNA adopts a three-dimensional orientation that is favorable for binding of transcription factors and RNA polymerase at the transcription initiation site. Thus, polynucleotides for use in the compositions and methods described herein include those that encode an OTOF protein and additionally include a mammalian enhancer sequence. Many enhancer sequences are now known from mammalian genes, and examples include enhancers from the genes that encode mammalian globin, elastase, albumin, α -fetoprotein, and insulin. Enhancers for use in the compositions and methods described herein also include those that are derived from the genetic material of a virus capable of infecting a eukaryotic cell. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. Additional enhancer sequences that induce activation of eukaryotic gene transcription are disclosed in Yaniv, et al., *Nature* 297:17 (1982). An enhancer may be spliced into a vector containing a polynucleotide encoding an OTOF protein, for example, at a position 5' or 3' to this gene. In a preferred orientation, the enhancer is positioned at the 5' side of the promoter, which in turn is located 5' relative to the polynucleotide encoding an OTOF protein.

The nucleic acid vectors described herein may include a Woodchuck Posttranscriptional Regulatory Element (WPRE). The WPRE acts at the mRNA level, by promoting nuclear export of transcripts and/or by increasing the efficiency of polyadenylation of the nascent transcript, thus increasing the total amount of mRNA in the cell. The addition of the WPRE to a vector can result in a substantial improvement in the level of transgene expression from several different promoters, both in vitro and in vivo. The WPRE can be located in the second nucleic acid vector between the polynucleotide encoding a C-terminal portion of an OTOF protein and the poly(A) sequence. In some embodiments of the compositions and methods described herein, the WPRE has the sequence:

GATCCAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTA
 TGTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATT
 GCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGATAAATCCTGGTTGCTGTCTCTTT
 ATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCT
 5 GACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGAC
 TTTGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCC
 GCTGCTGGACAGGGGCTCGGCTGTTGGGCACCTGACAATCCGTGGTGTGTCGGG
 GAAATCATCGTCCTTTCTTGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCG
 GGACGTCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGC
 10 GGCCTGCTGCCGGCTCTGCGGCCTCTTCGCGTCTTCGA (SEQ ID NO: 45).

In other embodiments, the WPRE has the sequence:

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTG
 15 CTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTC
 CCGTATGGCTTTCATTTTCTCCTCCTTGATAAATCCTGGTTAGTTCTTGCCACGGCG
 GAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCA
 CTGACAATCCGTGGTGTGTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAC
 CATCTAGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGC
 20 TGCAATAACAAGTTAACAACAACAATTGCATTATTTATGTTTCAGGTTCAGGGGG
 AGATGTGGGAGGTTTTTTAA (SEQ ID NO: 46)

Dual hybrid vectors for expressing OTOF

An OTOF isoform 5 protein (e.g., an OTOF isoform 5 protein having the sequence of SEQ ID NO:
 25 1) can be expressed in mammalian cells using a dual hybrid vector system. This approach uses two
 nucleic acid vectors (e.g., two adeno-associated virus vectors) to express a single, large protein. Each of
 the two nucleic acid vectors (e.g., two adeno-associated virus vectors) contains a portion of a
 polynucleotide that encodes the protein (e.g., one vector contains a polynucleotide encoding an N-
 terminal portion of the protein and the other vector contains a polynucleotide encoding a C-terminal
 30 portion of the protein, and the polynucleotide encoding the N-terminal portion of the protein and the
 polynucleotide encoding the C-terminal portion of the protein do not overlap). The dual hybrid vectors also
 feature an overlapping region at which homologous recombination can occur (e.g., a recombinogenic
 region that is contained within each vector) and splice donor and splice acceptor sequences (e.g., the first
 vector contains a splice donor sequence and the second vector contains a splice acceptor sequence).
 35 The recombinogenic region is 3' of the splice donor sequence in the first nucleic acid vector and 5' of the
 splice acceptor sequence in the second nucleic acid vector. The first and second polynucleotide
 sequences can then join to form a single sequence based on one of two mechanisms: 1) recombination
 at the overlapping region, or 2) concatemerization of the ITRs. The remaining recombinogenic region(s)

and/or the concatemerized ITRs can be removed by splicing, leading to the formation of a contiguous polynucleotide sequence that encodes the full-length protein of interest.

Recombinogenic regions that can be used in the compositions and methods described herein include the F1 phage AK gene having a sequence of: GGGATTTTGCCGATTTCGGCCTATTGGTTAA

- 5 AAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAAT (SEQ ID NO: 47) and alkaline phosphatase (AP) gene fragments as described in US Patent No. 8,236,557, which are incorporated herein by reference. In some embodiments, the AP gene fragment has the sequence of:

10 CCCCCGGGTGCGCGGCGTCGGTGGTGCCGGCGGGGGGCGCCAGGTGCGAGGCGG
TGTAGGGCTCCAGGCAGGCGGCGAAGGCCATGACGTGCGCTATGAAGGTCTGCTC
CTGCACGCCGTGAACCAGGTGCGCCTGCGGGCCGCGCGCGAACACCGCCACGTC
CTCGCCTGCGTGGGTCTCTTCGTCCAGGGGCACTGCTGACTGCTGCCGATACTCG
GGGCTCCCGCTCTCGCTCTCGGTAACATCCGGCCGGGCGCCGTCCTTGAGCACAT
15 AGCCTGGACCGTTTCCGTATAGGAGGACCGTG TAGGCCTTCCTGTCCCGGGCCTTG
CCAGCGGCCAGCCCGATGAAGGAGCTCCCTCGCAGGGGGTAGCCTCCGAAGGAG
AAGACGTGGGAGTGGTCGGCAGTGACGAGGCTCAGCGTGTCTCCTCGCTGGTGA
GCTGGCCCCGCCCTCTCAATGGCGTCGTGGAACATGATCGTCTCAGTCAGTGCCCCGG
TAAGCCCTGCTTTCATGATGACCATGGTCGATGCGACCACCCTCCACGAAGAGGAA
GAAGCCGCGGGGGTGTCTGCTCAGCAGGCGCAGGGCAGCCTCTGTCTATCTCCATC
20 AGGGAGGGGTCCAGTGTGGAGTCTCGGTGGATCTCGTATTTTCATGTCTCCAGGCTC
AAAGAGACCCATGAGATGGGTACAGACGGGTCCAGGGAAGCCTGCATGAGCTCA
GTGCGGTTCCACACGTACCGGGCACCCCTGGCGTTCGCCGAGCCATTCTGCACCA
GATTCTTCCCGTCCAGCCTGGTCCCACCTTGGCTGTAGTCATCTGGGTACTCAGGG
TCTGGGGTTCCCATGCGAAACATGTACTTTCGGCCTCCA (SEQ ID NO: 48).

25

In some embodiments, the AP gene fragment has the sequence of:

30 CCCCCGGGTGCGCGGCGTCGGTGGTGCCGGCGGGGGGCGCCAGGTGCGAGGCGG
TGTAGGGCTCCAGGCAGGCGGCGAAGGCCATGACGTGCGCTATGAAGGTCTGCTC
CTGCACGCCGTGAACCAGGTGCGCCTGCGGGCCGCGCGCGAACACCGCCACGTC
CTCGCCTGCGTGGGTCTCTTCGTCCAGGGGCACTGCTGACTGCTGCCGATACTCG
GGGCTCCCGCTCTCGCTCTCGGTAACATCCGGCCGGGCGCCGTCCTTGAGCACAT
AGCCTGGACCGTTTCCGTATAGGAGGACCGTG TAGGCCTTCCTGTCCCGGGCCTTG
CCAGCGGCCAGCCCGATGAAGGAGCTCCCTCGCAGGGGGTAGCCTCCGAAGGAG
35 AAGACGTGGGAGTGGTCGGCAGTGACGAGGCTCAGCGTGTCTCCTCG CTGGTGA
(SEQ ID NO: 49).

In some embodiments, the AP gene fragment has the sequence of:

40 GCTGGCCCCGCCCTCTCAATGGCGTCGTGGAACATGATCGTCTCAGTCAGTGCCCCGG
TAAGCCCTGCTTTCATGATGACCATGGTCGATGCGACCACCCTCCACGAAGAGGAA

GAAGCCGCGGGGTGTCTGCTCAGCAGGCGCAGGGCAGCCTCTGTCATCTCCATC
 AGGGAGGGGTCCAGTGTGGAGTCTCGGTGGATCTCGTATTTTCATGTCTCCAGGCTC
 AAAGAGACCCATGAGATGGGTACAGACGGGTCCAGGGAAGCCTGCATGAGCTCA
 GTGCGGTTCCACACGTACCGGGCACCCCTGGCGTTCGCCGAGCCATTCTGCACCA
 5 GATTCTTCCCGTCCAGCCTGGTCCCACCTTGGCTGTAGTCATCTGGGTA CT CAGGG
 TCTGGGGTTCCCATGCGAAACATGTACTTTTCGGCCTCCA (SEQ ID NO: 50).

In some embodiments, the AP gene fragment has the sequence of:

CCCCGGGTGCGCGGCGTCCGGTGGTGCCGGCGGGGGGCGCCAGGTGCGAGGCGG
 10 TGTAGGGCTCCAGGCAGGCGGCGAAGGCCATGACGTGCGCTATGAAGGTCTGCTC
 CTGCACGCCGTGAACCAGGTGCGCCTGCGGGCCGCGCGCGAACACCGCCACGTC
 CTCGCCTGCGTGGGTCTCTTCGTCCAGGGGCACTGCTGACTGCTGCCGATACTCG
 GGGCTCCCGCTCTCGCTCTCGGTAACATCCGGCCGGGCGCCGTCCTTGAGCACAT
 AGCCTGGACCGTTTC (SEQ ID NO: 51)

15

In some embodiments, the AP gene fragment has the sequence of:

CGTATAGGAGGACCGTGTAGGCCTTCCTGTCCCGGGCCTTGCCAGCGGCCAGCCC
 GATGAAGGAGCTCCCTCGCAGGGGGTAGCCTCCGAAGGAGAAGACGTGGGAGTGG
 20 TCGGCAGTGACGAGGCTCAGCGTGTCTCCTCGCTGGTGAGCTGGCCCGCCCTCT
 CAATGGCGTGTGGAACATGATCGTCTCAGTCAGTGCCCGGTAAGCCCTGCTTTCA
 TGATGACCATGGTCGATGCGACCACCCTCCACGAAGAGGAAGAAGCCGCGGGGGT
 GTCTGCTCAGCAGG (SEQ ID NO: 52).

25 In some embodiments, the AP gene fragment has the sequence of:

CGCAGGGCAGCCTCTGTCATCTCCATCAGGGAGGGGTCCAGTGTGGAGTCTCGGT
 GGATCTCGTATTTTCATGTCTCCAGGCTCAAAGAGACCCATGAGATGGGTACAGAC
 GGGTCCAGGGAAGCCTGCATGAGCTCAGTGCGGTTCCACACGTACCGGGCACCCCT
 30 GGCGTTCGCCGAGCCATTCTGCACCAGATTCTTCCCGTCCAGCCTGGTCCCACCT
 TGGCTGTAGTCATCTGGGTA CT CAGGGTCTGGGGTTCCCATGCGAAACATGTACTTT
 CGGCCTCCA (SEQ ID NO: 53).

35 An exemplary splice donor sequence for use in the methods and compositions described herein
 can include the sequence:

GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTGAGACAGAGAA
 GACTCTTGCGTTTCTGA (SEQ ID NO: 54).

40 An exemplary splice acceptor sequence for use in the methods and compositions described
 herein can include the sequence:

TAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAG (SEQ ID NO: 55).

Additional examples of splice donor and splice acceptor sequences are known in the art.

Dual hybrid vectors for use in the methods and compositions described herein are designed such that approximately half of the OTOF gene is contained within each vector (e.g., each vector contains a polynucleotide that encodes approximately half of the OTOF isoform 5 protein). The determination of how to split the polynucleotide sequence between the two nucleic acid vectors can be made based on the size of the promoter and the locations of the portions of the polynucleotide that encode the OTOF C2 domains. When a short promoter is used in the dual hybrid vector system (e.g., a promoter that is 1 kb or shorter, e.g., approximately 1 kb, 950 bp, 900 bp, 850 bp, 800 bp, 750 bp, 700 bp, 650 bp, 600 bp, 550 bp, 500 bp, 450 bp, 400 bp, 350 bp, 300 bp or shorter), such as CAG, CMV, smCBA, or a Myo15 promoter having a sequence that is 1 kb or shorter (e.g., a Myo15 promoter described hereinabove, e.g., a Myo15 promoter having the sequence of SEQ ID NO: 21 or SEQ ID NO: 42), the OTOF polynucleotide sequence can be divided between the two nucleic acid vectors at an exon boundary that occurs after the portion of the polynucleotide that encodes the C2D domain and before the portion of the polynucleotide that encodes C2E domain, for example, the exon 26/27 boundary. The nucleic acid vectors containing promoters of this size can optionally contain OTOF UTRs (e.g., full-length 5' and 3' UTRs). When a long promoter is used in the dual hybrid vector system (e.g., a promoter that is longer than 1 kb, e.g., 1.1 kb, 1.25 kb, 1.5 kb, 1.75 kb, 2 kb, 2.5 kb, 3 kb or longer), such as a Myo15 promoter that is longer than 1 kb (e.g., SEQ ID NO: 19), the OTOF polynucleotide sequence can be divided between the two nucleic acid vectors at an exon boundary that occurs after the portion of the polynucleotide that encodes the C2C domain, and either before the portion of the polynucleotide that encodes the C2D domain, such as the exon 19/20 boundary, the exon 20/21 boundary, the exon 21/22 boundary, or within the portion of the polynucleotide that encodes the C2D domain, such as the exon 25/26 boundary. A short promoter (e.g., a CMV promoter, CAG promoter, smCBA promoter, or a Myo15 promoter having a sequence that is 1 kb or shorter, e.g., a Myo15 promoter having the sequence of SEQ ID NO: 21 or SEQ ID NO: 42) can also be used in the dual vector systems designed for large promoters, in which case additional elements (e.g., OTOF UTR sequences) may be included in the first vector (e.g., the vector containing the portion of the polynucleotide the encodes the C2C domain).

One exemplary dual hybrid vector system that uses a short promoter includes a first nucleic acid vector containing a CAG promoter operably linked to exons 1-26 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), a splice donor sequence 3' of the polynucleotide sequence, and a recombinogenic region 3' of the splice donor sequence; and a second nucleic acid vector containing a recombinogenic region, a splice acceptor sequence 3' of the recombinogenic region, a polynucleotide 3' of the splice acceptor sequence that contains exons 27-45 and 47 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), and a poly(A) sequence (e.g., a bGH poly(A) signal sequence). The first and second nucleic acid vectors can also contain full-length 5' and 3' OTOF UTRs, respectively (e.g., the 127 bp human OTOF 5' UTR can be included in the first nucleic acid vector, and the 1035 bp human OTOF 3' UTR can be included in the second nucleic acid vector). Another exemplary dual hybrid vector system that uses a short promoter includes a first nucleic acid vector containing a smCBA promoter or a Myo15 promoter

that is 1 kb or shorter (e.g., a Myo15 promoter having the sequence of SEQ ID NO: 21 or SEQ ID NO: 42) operably linked to exons 1-20 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), a splice donor sequence 3' of the polynucleotide sequence, and a recombinogenic region 3' of the splice donor sequence; and a second nucleic acid vector containing a recombinogenic region, a splice acceptor sequence 3' of the recombinogenic region, a polynucleotide 3' of the splice acceptor sequence that contains exons 21-45 and 47 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF, e.g., SEQ ID NO: 1), and a poly(A) sequence (e.g., a bGH poly(A) signal sequence). The first nucleic acid vector can also contain the full-length 5' OTOF UTRs (e.g., the 127 bp human OTOF 5' UTR can be included in the first nucleic acid vector). The CMV promoter can be used in place of the CAG, smCBA, or Myo15 promoter in either of the foregoing dual vector systems.

An exemplary dual hybrid vector system that uses a long promoter includes a first nucleic acid vector containing a Myo15 promoter that is longer than 1 kb (e.g., SEQ ID NO: 19) operably linked to exons 1-19 or exons 1-20 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), a splice donor sequence 3' of the polynucleotide sequence, and a recombinogenic region 3' of the splice donor sequence; and a second nucleic acid vector containing a recombinogenic region, a splice acceptor sequence 3' of the recombinogenic region, a polynucleotide 3' of the splice acceptor sequence that contains exons 20-45 and 47 (when the first nucleic acid vector contains exons 1-19 of the polynucleotide) or exons 21-45 and 47 (when the first nucleic acid vector contains exons 1-20 of the polynucleotide) of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), and a poly(A) sequence (e.g., a bGH poly(A) signal sequence). Neither the first nor the second nucleic acid vector in the foregoing Myo15 promoter dual hybrid vector system contains an OTOF UTR. A short promoter (e.g., a CMV promoter, CAG promoter, smCBA promoter, or a Myo15 promoter having a sequence that is 1 kb or shorter, e.g., a Myo15 promoter having the sequence of SEQ ID NO: 21 or SEQ ID NO: 42) can also be used in the foregoing dual vector systems designed for large promoters. If these dual vector systems contain a short promoter, they may also include a 5' OTOF UTR in the first vector.

To accommodate an OTOF UTR, the OTOF coding sequence can be divided in a different position. For example, in a dual hybrid vector system in which the first nucleic acid vector contains a Myo15 promoter that is longer than 1 kb (e.g., SEQ ID NO: 19) operably linked to exons 1-25 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), a splice donor sequence 3' of the polynucleotide sequence, and a recombinogenic region 3' of the splice donor sequence; and in which the second nucleic acid vector contains a recombinogenic region, a splice acceptor sequence 3' of the recombinogenic region, a polynucleotide 3' of the splice acceptor sequence that contains exons 26-45 and 47 of a polynucleotide encoding an OTOF protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1), and a poly(A) sequence (e.g., a bGH poly(A) signal sequence), the second nucleic acid can also contain a full-length OTOF 3' UTR (e.g., the 1035 bp human OTOF UTR). A short promoter (e.g., a CMV promoter, CAG promoter, smCBA promoter, or a Myo15 promoter having a sequence that is 1 kb or shorter, e.g., a Myo15 promoter having the sequence of SEQ ID NO: 21 or SEQ ID NO: 42) can also be used in the foregoing dual vector system designed for large promoters. If these dual vector systems contain a short promoter, they may also include a 5' OTOF UTR in the first vector.

The polynucleotide sequence encoding an OTOF isoform 5 protein can be a cDNA sequence (e.g., a sequence that does not include introns). In some embodiments, the first and/or the second nucleic acid vector in the dual vector system can include intronic sequence. The intronic sequence may be included between one or more exons in the OTOF coding sequence, or the intronic sequence can be included between an exon of the coding sequence and another component of the nucleic acid vector (e.g., between an exon of the OTOF coding sequence and the splice donor sequence in the first nucleic acid vector or between an exon of the OTOF coding sequence and the splice acceptor sequence in the second nucleic acid vector).

In some embodiments, the polynucleotide encoding OTOF isoform 5 is divided between the first and second nucleic acid vectors (e.g., AAV vectors) in the dual vector system at the exon 20/21 boundary. In some embodiments, the polynucleotide encoding OTOF isoform 5 is divided between the first and second nucleic acid vectors (e.g., AAV vectors) in the dual vector system at the exon 21/22 boundary.

When the polynucleotide encoding OTOF isoform 5 is divided between the first and second nucleic acid vectors (e.g., AAV vectors) at the exon 20/21 boundary, the polynucleotide sequence encoding the N-terminal portion of OTOF has the sequence of:

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ATGGCCTTGCTCATCCACCTCAAGACAGTCTCGGAGCTGCGGGGCAGGGGCGACC
GGATCGCCAAAGTGACTTTCCGAGGGCAATCCTTCTACTCTCGGGTCCTGGAGAAC
TGTGAGGATGTGGCTGACTTTGATGAGACATTTCCGGTGGCCGGTGGCCAGCAGCAT
CGACAGAAATGAGATGCTGGAGATTCAAGTTTTCAACTACAGCAAAGTCTTCAGCAA
CAAGCTCATCGGGACCTTCCGCATGGTGTGAGAAAGGTGGTAGAGGAGAGCCAT
GTGGAGGTGACTGACACGCTGATTGATGACAACAATGCTATCATCAAGACCAGCCT
GTGCGTGGAGGTCCGGTATCAGGCCACTGACGGCACAGTGGGCTCCTGGGACGAT
GGGGACTTCCTGGGAGATGAGTCTCTTCAAGAGGAAGAGAAGGACAGCCAAGAGA
CGGATGGACTGCTCCCAGGCTCCCGGCCAGCTCCCGGCCCCAGGAGAGAAGA
GCTTCCGGAGAGCCGGGAGGAGCGTGTCTCCGCCATGAAGCTCGGCAAAAACCG
GTCTACAAGGAGGAGCCCCAAAGACCAGATGAACCGGCGGTGCTGGAGATGGAA
GACCTTGACCATCTGGCCATTCGGCTAGGAGATGGACTGGATCCCGACTCGGTGTC
TCTAGCCTCAGTCACAGCTCTCACCATAATGTCTCCAACAAGCGATCTAAGCCAGA
CATTAAAGATGGAGCCAAGTGCTGGGCGGCCCATGGATTACCAGGTCAGCATCACGG
TGATCGAGGCCCGGCAGCTGGTGGGCTTGAACATGGACCCTGTGGTGTGCGTGGA
GGTGGGTGACGACAAGAAGTACACATCCATGAAGGAGTCCACTAACTGCCCCTATT
ACAACGAGTACTTCGTCTTCGACTTCCATGTCTCTCCGGATGTCATGTTTGACAAGA
TCATCAAGATTTCCGGTGATTCACTCCAAGAACCTGCTGCGCAGTGGCACCCCTGGTG
GGCTCCTTCAAAATGGACGTGGGAACCGTGTACTCGCAGCCAGAGCACCAGTTCCA
TCACAAGTGGGCCATCCTGTCTGACCCGATGACATCTCCTCGGGGCTGAAGGGCT
ACGTGAAGTGTGACGTTGCCGTGGTGGGCAAAGGGGACAACATCAAGACGCCCCA
CAAGGCCAATGAGACCGACGAAGATGACATTGAGGGGAACCTTGCTGCTCCCCGAG
GGGGTGCCCCCGAACGCCAGTGGGCCCGTTCTATGTGAAAATTTACCGAGCAG
AGGGGCTGCCCCGTATGAACACAAGCCTCATGGCCAATGTAAAGAAGGCTTTCATC
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GGTGAAAACAAGGACCTCGTGGACCCCTACGTGCAAGTCTTCTTTGCTGGCCAGAA
GGGCAAGACTTCAGTGCAGAAGAGCAGCTATGAGCCCCTGTGGAATGAGCAGGTC
GTCTTTACAGACCTCTTCCCCCACTCTGCAAACGCATGAAGGTGCAGATCCGAGA
CTCGGACAAGGTCAACGACGTGGCCATCGGCACCCACTTCATTGACCTGCGCAAGA
5 TTTCTAATGACGGAGACAAAGGCTTCCTGCCCACACTGGGCCAGCCTGGGTGAAC
ATGTACGGCTCCACACGTAACCTACACGCTGCTGGATGAGCATCAGGACCTGAACGA
GGGCCTGGGGGAGGGTGTGTCCTTCGGGGCCGGCTCCTGCTGGGCCTGGCTGT
GGAGATCGTAGACACCTCCAACCCTGAGCTCACCAGCTCCACAGAGGTGCAGGTG
GAGCAGGCCACGCCATCTCGGAGAGCTGTGCAGGTAAAATGGAAGAATTCTTTCT
10 CTTTGGAGCCTTCCTGGAGGCCTCAATGATCGACCGGAGAAACGGAGACAAGCCCA
TCACCTTTGAGGTACCATAGGCAACTATGGGAACGAAGTTGATGGCCTGTCCCGG
CCCCAGCGGCCTCGGCCCCGGAAGGAGCCGGGGGATGAGGAAGAAGTAGACCTG
ATTCAGAACGCAAGTGATGACGAGGCCGGTGATGCCGGGGACCTGGCCTCAGTCT
CCTCCACTCCACCAATGCGGCCCCAGGTACCGACAGGAACTACTTCCATCTGCCC
15 TACCTGGAGCGAAAGCCCCTGCATCTACATCAAGAGCTGGTGGCCGGACCAGCGCC
GCCGCCTCTACAATGCCAACATCATGGACCACATTGCCGACAAGCTGGAAGAAGGC
CTGAACGACATACAGGAGATGATCAAAACGGAGAAGTCCTACCCTGAGCGTCGCCT
GCGGGGCGTCCTGGAGGAGCTGAGCTGTGGCTGCTGCCGCTTCCTCTCCCTCGCT
GACAAGGACCAGGGCCACTCATCCCGCACCAGGCTTGACCGGGAGCGCCTCAAGT
20 CCTGCATGAGGGAGCTG (SEQ ID NO: 56).

When the polynucleotide encoding OTOF isoform 5 is divided between the first and second nucleic acid vectors (e.g., AAV vectors) at the exon 20/21 boundary, the polynucleotide sequence encoding the C-terminal portion of OTOF has the sequence of:

25 GAAAACATGGGGCAGCAGGCCAGGATGCTGCGGGCCCAGGTGAAGCGGCACACG
GTGCGGGACAAGCTGAGGCTGTGCCAGAACTTCCTGCAGAAGCTGCGCTTCCTGG
CGGACGAGCCCCAGCACAGCATTCCCGACATCTTCATCTGGATGATGAGCAACAAC
AAGCGTGTGCGCTATGCCCCTGTGCCCTCCAAGGACCTGCTCTTCTCCATCGTGGA
30 GGAGGAGACTGGCAAGGACTGCGCCAAGGTCAAGACGCTCTTCCTTAAGCTGCCA
GGGAAGCGGGGCTTCGGCTCGGCAGGCTGGACAGTGCAGGCCAAGGTGGAGCTG
TACCTGTGGCTGGGCCTCAGCAAACAGCGCAAGGAGTTCCTGTGCGGCCTGCCCT
GTGGCTTCCAGGAGGTCAAGGCAGCCCAGGGCCTGGGCCTGCATGCCTTCCCACC
CGTCAGCCTGGTCTACACCAAGAAGCAGGCGTTCCAGCTCCGAGCGCACATGTACC
35 AGGCCCGCAGCCTCTTTGCCGCCGACAGCAGCGGACTCTCAGACCCCTTTGCCCG
CGTCTTCTTCATCAATCAGAGTCAGTGCACAGAGGTGCTGAATGAGACCCTGTGTCC
CACCTGGGACCAGATGCTGGTGTTCGACAACCTGGAGCTCTATGGTGAAGCTCATG
AGCTGAGGGACGATCCGCCCATCATTGTGATTGAAATCTATGACCAGGATTCCATGG
GCAAAGCTGACTTCATGGGCCGGACCTTCGCCAAACCCCTGGTGAAGATGGCAGAC
40 GAGGCGTACTGCCACCCCGCTTCCCACTCAGCTCGAGTACTACCAGATCTACCG
TGGCAACGCCACAGCTGGAGACCTGCTGGCGGCCTTCGAGCTGCTGCAGATTGGA

CCAGCAGGGAAGGCTGACCTGCCCCCATCAATGGCCCGGTGGACGTGGACCGAG
GTCCCATCATGCCCCGTGCCCATGGGCATCCGGCCCCGTGCTCAGCAAGTACCGAGT
GGAGGTGCTGTTCTGGGGCCTACGGGACCTAAAGCGGGTGAACCTGGCCCAGGTG
GACCGGCCACGGGTGGACATCGAGTGTGCAGGGAAGGGGGTGCAGTCTGCCCTG
5 ATCCACAATTATAAGAAGAACCCCAACTTCAACACCCTCGTCAAGTGGTTTGAAGTG
GACCTCCCAGAGAACGAGCTGCTGCACCCGCCCTTGAACATCCGTGTGGTGGACT
GCCGGGCCTTCGGTCGCTACACACTGGTGGGCTCCCATGCCGTCAGTCCCTGCG
ACGCTTCATCTACCGGCCCCCAGACCGCTCGGCCCCCAGCTGGAACACCACGGTC
AGGCTTCTCCGGCGCTGCCGTGTGCTGTGCAATGGGGGCTCCTCCTCTCACTCCAC
10 AGGGGAGGTTGTGGTGAATATGGAGCCAGAGGTACCCATCAAGAACTGGAGACC
ATGGTGAAGCTGGACGCGACTTCTGAAGCTGTTGTCAAGGTGGATGTGGCTGAGGA
GGAGAAGGAGAAGAAGAAGAAGAAGAGGCACTGCGGAGGAGCCAGAGGAGGA
GGAGCCAGACGAGAGCATGCTGGACTGGTGGTCCAAGTACTTTGCCTCCATTGACA
CCATGAAGGAGCAACTTCGACAACAAGAGCCCTCTGGAATTGACTTGGAGGAGAAG
15 GAGGAAGTGGACAATACCGAGGGCCTGAAGGGGTCAATGAAGGGCAAGGAGAAGG
CAAGGGCTGCCAAAGAGGAGAAGAAGAAGAAAACCTCAGAGCTCTGGCTCTGGCCA
GGGGTCCGAGGGCCCCGAGAAGAAGAAACCCAAGATTGATGAGCTTAAGGTATACC
CCAAAGAGCTGGAGTCCGAGTTTGATAACTTTGAGGACTGGCTGCACACTTTCACT
TGCTTCGGGGCAAGACCGGGGATGATGAGGATGGCTCCACCGAGGAGGAGCGCAT
20 TGTGGGACGCTTCAAGGGCTCCCTCTGCGTGTACAAAGTGCCACTCCCAGAGGACG
TGTCCCGGGAAGCCGGCTACGACTCCACCTACGGCATGTTCCAGGGCATCCCGAG
CAATGACCCCATCAATGTGCTGGTCCGAGTCTATGTGGTCCGGGCCACGGACCTGC
ACCCTGCTGACATCAACGGCAAAGCTGACCCCTACATCGCCATCCGGCTAGGCAAG
ACTGACATCCGCGACAAGGAGAACTACATCTCCAAGCAGCTCAACCCTGTCTTTGG
25 GAAGTCCTTTGACATCGAGGCCTCCTTCCCCATGGAATCCATGCTGACGGTGGCTG
TGTATGACTGGGACCTGGTGGGCACTGATGACCTCATTGGGGAAACCAAGATCGAC
CTGGAGAACCGCTTCTACAGCAAGCACCGCGCCACCTGCGGCATCGCCAGACCT
ACTCCACACATGGCTACAATATCTGGCGGGACCCCATGAAGCCAGCCAGATCCTG
ACCCGCCTCTGCAAAGACGGCAAAGTGACGGCCCCCACTTTGGGCCCCCTGGGA
30 GAGTGAAGGTGGCCAACCGCGTCTTCACTGGGCCCTCTGAGATTGAGGACGAGAA
CGGTCAGAGGAAGCCACAGACGAGCATGTGGCGCTGTTGGCCCTGAGGCACTGG
GAGGACATCCCCCGCGCAGGCTGCCGCCTGGTGCCAGAGCATGTGGAGACGAGG
CCGCTGCTCAACCCCGACAAGCCGGGCATCGAGCAGGGCCGCCTGGAGCTGTGG
GTGGACATGTTCCCATGGACATGCCAGCCCCTGGGACGCCTCTGGACATCTCACC
35 TCGGAAGCCCAAGAAGTACGAGCTGCGGGTCATCATCTGGAACACAGATGAGGTG
GTCTTGAGGACGACGACTTCTTACAGGGGAGAAGTCCAGTGACATCTTCGTGAG
GGGGTGGCTGAAGGGCCAGCAGGAGGACAAGCAGGACACAGACGTCCACTACCAC
TCCCTCACTGGCGAGGGCAACTTCAACTGGCGCTACCTGTTCCCTTCGACTACCT
GGCGGCGGAGGAGAAGATCGTCATCTCCAAGAAGGAGTCCATGTTCTCCTGGGAC
40 GAGACCGAGTACAAGATCCCCGCGCGGCTCACCTGCAGATCTGGGATGCGGACC
ACTTCTCCGCTGACGACTTCTGCGGGGCCATCGAGCTGGACCTGAACCGGTTCCCG

CGGGGCGCAAAGACAGCCAAGCAGTGCACCATGGAGATGGCCACCGGGGAGGTG
GACGTGCCCCCTCGTGTCCATCTTCAAGCAAAAGCGCGTCAAAGGCTGGTGGCCCT
CCTGGCCCCGAATGAGAACGATGAGTTTGAGCTCACGGGCAAGGTGGAGGCTGAG
CTGCATTTACTGACAGCAGAGGAGGCAGAGAAGAACCCAGTGGGCCTGGCCCCGCA
5 ATGAACCTGACCCCCTAGAGAAACCAACCGGCCCGACACGGCCTTCGTCTGGTTC
CTCAACCCTCTCAAGTCCATCAAGTACCTCATCTGCACCCGGTACAAGTGGCTCATC
ATCAAGATCGTGGCTGGCGCTGTTGGGGCTGCTCATGTTGGGGCTCTTCCTCTACAG
CCTCCCTGGCTACATGGTCAAAAAGCTCCTTGGGGCATGA (SEQ ID NO: 57).

10 In embodiments in which the polynucleotide encoding OTOF isoform 5 is divided between the first and second nucleic acid vectors (e.g., AAV vectors) at the exon 20/21 boundary, the N-terminal portion of the OTOF polypeptide has the sequence of:

MALLIHLKTVSELRGRGDRIAKVTFRGQSFYSRVLENCEDVADFDETRWPVASSIDRN
15 EMLEIQVFNYSKVFSNKLIGTRFMVLQKVVEESHVEVTDTLIDDNNAIKTSLCDEVRYQA
TDGTVGSWDDGDFLGDESLQEEEKDSQETDGLLPGSRPSSRPPGEKSFRRAGRSVFS
AMKLGKNRSHKEEPQRPDEPAVLEMEDLDHLAIRLGDGLDPDSVSLASVTALTNNVSNK
RSKPDIKMEPSAGRPMQVYSITVIEARQLVGLNMDPVVCVEVGDDKKYTSMKESTNC
PYYNEYFVDFHVSFVDFDKIIKISVIHSKNLLRSGTLVGSFKMDVGTVYSQPEHQFHH
20 KWAILSDPDDISSGLKGYVKCDVAVVGKGDNIKTPHNETDEDDIEGNLLLPEGVPPER
QWARFYVKIYRAEGLPRMNTSLMANVKKAFIGENKDLVDPYVQVFFAGQKGTSVQKS
SYEPLWNEQVVFTDLFPPLCKRMKVQIRSDKVNDVAIGTHFIDLRKISNDGDKGLPTL
GPAWVNMYGSTRNYTLLDEHQDLNEGLGEGVSFRARLLLGLAVEIVDTSNPELTSSTEV
QVEQATPISESCAGKMEEFFLFGAFLEASMDRRNGDKPITFEVTIGNYGNEVDGLSRPQ
25 RPRPRKEPGDEEEVDLIQNASDDEAGDAGDLASVSSTPPMRPQVTDNRNYFHLPLYLERK
PCIIYKSWWPDQRRRLYNANIMDHIADKLEGLNDIQEMIKTEKSYPERRLRGVLEELSC
GCCRFSLADKQGHSSRTRLDRERLKSCEMREL (SEQ ID NO: 58).

30 In embodiments in which the polynucleotide encoding OTOF isoform 5 is divided between the first and second nucleic acid vectors (e.g., AAV vectors) at the exon 20/21 boundary, the C-terminal portion of the OTOF polypeptide has the sequence of:

ENMGQQARMLRAQVKRHTVRDKLRLCQNFLLQKLRFLADEPQHSIPDIFIWMMSNNKRV
AYARVPSKDLLFSIVEEETGKDCAKVKTLFLKLPKRGFGSAGWTVQAKVELYLWLGLS
35 KQRKEFLCGLPCGFQEVKAAQGLGLHAFPPVSLVYTKKQAFQLRAHMYQARSIFAADS
SGLSDPFARVFFINQSQCTEVLNETLCPTWDQMLVFDNLELYGEAHEL RDDPPIVIEIYD
QDSMGKADFMGRFTAKPLVKMADEAYCPRFPQLEYYQIYRGNATAGDLLAAFELLQI
GPAGKADLPPINGPVDVDRGPIMPVPMGIRPVLSKYRVEVLFWGLRDLKRVNLAQVDRP
RVDIECAGKGVQSSLIHNYKKNPNFNTLVKWEVDLPENELLHPPLNIRVVDCRAFGRYT
40 LVGSHAVSSLRRIYRPPDRSAPSWNTTVRLRLRCRVLCNGGSSSHSTGEVVVTMEPE
VPIKKLETMVKLDATSEAVVKVDVAEEEEKEKKKKKKGTAEPEEEEEPEDESMLDWWISKY

FASIDTMKEQLRQQEPSGIDLEEKEEVDNTEGLKGSMTGKEKARAAKEEKKKKTQSSG
SGQGSEAPEKKKPKIDELKVYPKELESEFDNFEDWLHTFNLLRGKTGDDDEDGSTEERI
VGRFKGSLCVYKVPLPEDVSREAGYDSTYGMFQGIPSNDPINVLVRVYVVRATDLHPAD
INGKADPYIAIRLGKTDIRDKENYISKQLNPVFGKSFDFEASFPMESMLTVAVYDWDLVGT
5 DDLIGETKIDLENRFYSKHRATCGIAQTYSTHGYNIWRDPMKPSQILTRLCKDGKVDGPH
FGPPGRVKVANRVFTGPSEIEDENGQRKPTDEHVALLALRHWDIPRAGCRLVPEHVE
TRPLLNPDKPGIEQGRLELWVDMFPMDMAPGTPLDISPRKPKKYELRVIWNTDEVVLE
DDFFTGEKSSDIFVRGWLGKQKQEDKQDQTDVHYHSLTGEGNFNWRYLFPFDYLAEEK
IVISKESMFSWDETEYKIPARLTLQIWDADHFSADDFLGAIELDLNRFRGAKTAKQCT
10 MEMATGEVDVPLVSIFKQKRVKGGWWPLLARNENDEFELTGKVEAELHLLTAEAAEKNP
VGLARNEPDLEKPNRPDTAFVWFLNPLKSIKYLICTRYKWLIIKIVLALLGLLMLGLFLYS
LPGYMKVKKLLGA (SEQ ID NO: 59).

Transfer plasmids that may be used to produce the nucleic acid vectors for use in the
15 compositions and methods described herein are provided in Table 4. A transfer plasmid (e.g., a
plasmid containing a DNA sequence to be delivered by a nucleic acid vector, e.g., to be delivered
by an AAV) may be co-delivered into producer cells with a helper plasmid (e.g., a plasmid
providing proteins necessary for AAV manufacture) and a rep/cap plasmid (e.g., a plasmid that
provides AAV capsid proteins and proteins that insert the transfer plasmid DNA sequence into the
20 capsid shell) to produce a nucleic acid vector (e.g., an AAV vector) for administration. Nucleic
acid vectors (e.g., a nucleic acid vector (e.g., an AAV vector) containing a polynucleotide
encoding an N-terminal portion of OTOF isoform 5 and a nucleic acid vector (e.g., an AAV vector)
containing a polynucleotide encoding a C-terminal portion of OTOF isoform 5) can be combined
(e.g., in a single formulation) prior to administration. The following transfer plasmids are designed
25 to produce nucleic acid vectors (e.g., AAV vectors) for co-formulation or co-administration (e.g.,
administration simultaneously or sequentially) in a dual hybrid vector system: SEQ ID NO: 60 and
SEQ ID NO: 61; SEQ ID NO: 62 and SEQ ID NO: 63; SEQ ID NO: 64 and SEQ ID NO: 61; SEQ
ID NO: 65 and SEQ ID NO: 63; SEQ ID NO: 66 and SEQ ID NO: 67; and SEQ ID NO: 68 and
SEQ ID NO: 67.

30

Table 4: Transfer plasmids for the production of dual hybrid vector systems

SEQ ID NO.	Description	Plasmid Sequence
60	5' transgene plasmid containing the following features: Lambda (Biologically inert and inactivated DNA derived from bacteriophage lambda to reduce off-target DNA	TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACA CATGCAGCTCCCGGATAGAGGTCATCCTCCTGACCA TTCCATCATTCCAGTCGAACTCACACACAACACCAAA TGCATTTAAGTCGCTTGAAATTGCTATAAGCAGAGCAT GTTGCGCCAGCATGATTAATACAGCATTTAATACAGAG CCGTGTTTATTGAGTCGGTATTGAGAGTCTGACCAGAA ATTATTAATCTGGTGAAGTTATTCCTCTGTGATTACGTC

SEQ ID NO.	Description	Plasmid Sequence
	encapsidation) at positions 53-2027	ATGGTCGATTTCAATTTCTATTGATGCTTTCCAGTCGTA ATCAATGATGATTTTTTTGATGTTTGACCTCTGTTTCATA TCCTCACAGATAAAAAATCGCCCTCACACTGGAGGGC
	ITR at positions 2049-2178	AAAGAAGATTTCCAATAATCAGAACAAGTCGGCTCCTG TTTAGTTACGAGCGACATTGCTCCGTGTATTCACTCGT TGGAATGAATACACAGTGCAGTGTATTATTCTGTTATTTA
	Myo15 promoter at positions 2272-3236	TGCCAAAAATTAAGGCCACTATCAGGCAGCTTTGTTGT TCTGTTTACCAAGTTCTCTGGCAATCATTGCCGTCGTT CGTATTGCCCATTTATCGACATATTTCCCATCTTCCTAT
	Kozak sequence (Site to initiate protein translation) at positions 3253-3262	ACAGGAAACATTTCTTCAGGCTTAACCATGCATTCCGA TTGCAGCTTGCATCCATTGCATCGCTTGAATTGTCCAC ACCATTGATTTTTATCAATAGTCGTAGTTTAACGGATAG
	N-terminal portion of human OTOF isoform 5 at positions 3259-5664	TCCTGGTATTGTTCCATCACATCCTGAGGATGCCCTTC GAACTCTTCAAATTCTTCTCCTAATATCACCTTAAATA GTGGATTGCGGTAGTAAAGATTGTGCCTGTCTTTTAAC CACATCAGGCTCGGTGGTTCTCGTGTACCCCTACAGC
	Splice donor (APSD) sequence at positions 5665-5748	GAGAAATCGGATAAACTATTACAACCCCTACAGTTTGT AGAGTATAGAAAATGATCCACTCGTTATTCTCGGACGA GTGTTTCAGTAATGAACCTCTGGAGAGAACCATCTATAT
	AP head sequence (recombinogenic region) at positions 5755-6041	GATCGTTATCTGGGTTTGACTTCTGCTTTTAAGCCCAG ATAACTTGCCCTGAATATGTTAATGAGAGAATCGGTATT CCTCATGTGTGGCATGTTTTCGTCTTTGCTCTTGCATTT
	ITR at positions 6135-6264	TCACTAGCAATTAATGTGCATCGATTATCAGCTATTGC CAGCGCCAGATATAAGCGATTAAAGCTAAGAAAACGCA TTAAGGTGCAAAACGATAAAGTGCGATCAGTAATTCAA
	Lambda at positions 6275-8287	AACCTTACAGGAGAGCAATCTATGGTTTTGTGCTCAGC CCTTAATGAAGGCAGGTAGTATGTGGTTACATCAAAAC AATCCCACATATTAGTGAGTTGATTGAGCTTGGTGTG
	Ori (origin of replication) at positions 8344-8932	TTGAACAAAACTTTTCCCGATGGAATGGAAAGCATAT ATTATTCCCTATTGAGGATATTTACTGGACTGAATTAGT TGCCAGCTATGATCCATATAATATTGAGATAAAGCCAA
	KanR (antibiotic resistance gene) at positions 9110-9919	GGCCAATATCTAAGTAACTAGATAAGAGGAATCGATTT TCCCTTAATTTTCTGGCGTCCACTGCATGTTATGCCGC GTTCCGCCAGGCTTGCTGTACCATGTGCGCTGATTCTT
	Transgene to be transferred into vector in dual vector system at positions 2049-6264	GCGCTCAATACGTTGCAGGTTGCTTTCAATCTGTTTGT GGTATTCAGCCAGCACTGTAAGGTCTATCGGATTTAGT GCGCTTTCTACTCGTGATTTGCGTTTGCGATTACGCGA GAGAATAGGGCGGTAACTGGTTTTGCGCTTACCCCA

SEQ ID NO.	Description	Plasmid Sequence
		ACCAACAGGGGATTGCTGCTTTCCATTGAGCCTGTTA CTCTGCGCGACGTTGCGGGCGGCGTGTTGTGCATCC ATCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTGTG GCAGTTGTAGTCCTGAACGAAAACCCCCGCGATTGG CACGTTGGCAGCTAATCCGGAATCGCACTTACGGCCA ATGCTTCGTTTCGTATCACACACCCCAAAGCCTTCTGC TTTGAATGCTGCCCTTCTTCAGGGCTTAATTTTAAGA GCGTCACCTTCATGGTGGTCAGTGCGTCCTGCTGATG TGCTCAGGCACGATTTAATTAAGGCCTTAATTAGGCTG CGCGCTCGCTCGCTCACTGAGGCCGCCGGGCAAAG CCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCA GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAAC TCCATCACTAGGGGTTCTTGTAGTTAATGATTAACCC GCCATGCTACTTATCTACGTAGCCATGCTCTAGGAAGA TCGGAATTCGCCCTTAAGCTAGCGGCGCGCCCAATTC TGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTTCT AGTTCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAAA ACAGGAATAATAGATGTCATTAAATATACATTGGGCCC CAGGCGGTCAATGTGGCAGCCTGAGCCTCCTTTCCAT CTCTGTGGAGGCAGACATAGGACCCCCAACAAACAGC ATGCAGGTTGGGAGCCAGCCACAGGACCCAGGTAAG GGGCCCTGGGTCCTTAAGCTTCTGCCACTGGCTCCGG CATTGCAGAGAGAAGAGAAGGGGCGGCAGACTGGAG AGCTGGGCTCCATTTTGTTCCTTGGTGCCCTGCCCT CCCCATGACCTGCAGAGACATTCAGCCTGCCAGGCTT TATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTATTC AGCTCCCTGGAGTTGGCCAGCTCCTGTTACACTGGCC ACAGCCCTGGGCATCCGCTTCTCACTTCTAGTTTCCCC TCCAAGGTAATGTGGTGGGTCATGATCATTCTATCCTG GCTTCAGGGACCTGACTCCACTTTGGGGCCATTCGAG GGGTCTAGGGTAGATGATGTCCCCCTGTGGGGATTAA TGTCTGCTCTGTAAACTGAGCTAGCTGAGATCCAG GAGGGCTTGGCCAGAGACAGCAAGTTGTTGCCATGGT GACTTTAAAGCCAGGTTGCTGCCCCAGCACAGGCCTC CCAGTCTACCCTCACTAGAAAACAACACCCAGGCACTT TCCACCACCTCTCAAAGGTGAAACCAAGGCTGGTCT AGAGAATGAATTATGGATCCTCGCTGTCCGTGCCACC CAGCTAGTCCCAGCGGCTCAGACACTGAGGAGAGACT

SEQ ID NO.	Description	Plasmid Sequence
		GTAGGTTTCAGCTACAAGCAAAAAGACCTAGCTGGTCT CCAAGCAGTGTCTCCAAGTCCCTGAACCTGTGACACC TGCCCCAGGCATCATCAGGCACAGAGGGCCACCAAGA ATTCTAGCGGCCGCCACCATGGCCTTGCTCATCCACC TCAAGACAGTCTCGGAGCTGCGGGGCAGGGGCGACC GGATCGCCAAAGTGACTTTCCGAGGGCAATCCTTCTA CTCTCGGGTCCTGGAGAACTGTGAGGATGTGGCTGAC TTTGATGAGACATTTCCGTGGCCGGTGGCCAGCAGCA TCGACAGAAATGAGATGCTGGAGATTCAGGTTTTCAAC TACAGCAAAGTCTTCAGCAACAAGCTCATCGGGACCTT CCGCATGGTGCTGCAGAAGGTGGTAGAGGAGAGCCA TGTGGAGGTGACTGACACGCTGATTGATGACAACAAT GCTATCATCAAGACCAGCCTGTGCGTGGAGGTCCGGT ATCAGGCCACTGACGGCACAGTGGGCTCCTGGGACG ATGGGGACTTCTGGGAGATGAGTCTCTTCAAGAGGA AGAGAAGGACAGCCAAGAGACGGATGGACTGCTCCCA GGCTCCCGGCCCAGCTCCCGGCCCCCAGGAGAGAAG AGCTTCCGGAGAGCCGGGAGGAGCGTGTCTCCGCC ATGAAGCTCGGCAAAAACCGGTCTCACAAGGAGGAGC CCCAAAGACCAGATGAACCGGCGGTGCTGGAGATGG AAGACCTTGACCATCTGGCCATTCCGGCTAGGAGATGG ACTGGATCCCGACTCGGTGTCTCTAGCCTCAGTCACA GCTCTCACCCTAATGTCTCCAACAAGCGATCTAAGCC AGACATTAAGATGGAGCCAAGTGCTGGGCGGCCCATG GATTACCAGGTCAGCATCACGGTGATCGAGGCCCGGC AGCTGGTGGGCTTGAACATGGACCCTGTGGTGTGCGT GGAGGTGGGTGACGACAAGAAGTACACATCCATGAAG GAGTCCACTAACTGCCCCTATTACAACGAGTACTTCGT CTTCGACTTCCATGTCTCTCCGGATGTCATGTTTGACA AGATCATCAAGATTTCCGTGATTCACTCCAAGAACCTG CTGCGCAGTGGCACCCCTGGTGGGCTCCTTCAAAATGG ACGTGGGAACCGTGTACTCGCAGCCAGAGCACCAGTT CCATCACAAGTGGGCCATCCTGTCTGACCCCGATGAC ATCTCCTCGGGGCTGAAGGGCTACGTGAAGTGTGACG TTGCCGTGGTGGGCAAAGGGGACAACATCAAGACGCC CCACAAGGCCAATGAGACCGACGAAGATGACATTGAG GGGAAC TTGCTGCTCCCCGAGGGGGTGCCCCCGAA CGCCAGTGGGCCCCGGTTCTATGTGAAAATTTACCGAG

SEQ ID NO.	Description	Plasmid Sequence
		CAGAGGGGCTGCCCCGTATGAACACAAGCCTCATGGC CAATGTAAAGAAGGCTTTCATCGGTGAAAACAAGGACC TCGTGGACCCCTACGTGCAAGTCTTCTTTGCTGGCCA GAAGGGCAAGACTTCAGTGCAGAAGAGCAGCTATGAG CCCCTGTGGAATGAGCAGGTCTGCTTTACAGACCTCTT CCCCCACTCTGCAAACGCATGAAGGTGCAGATCCGA GACTCGGACAAGGTCAACGACGTGGCCATCGGCACC CACTTCATTGACCTGCGCAAGATTTCTAATGACGGAGA CAAAGGCTTCCTGCCCACACTGGGCCCAGCCTGGGTG AACATGTACGGCTCCACACGTAACACGCTGCTGG ATGAGCATCAGGACCTGAACGAGGGCCTGGGGGAGG GTGTGTCTTCCTCGGGCCCCGGCTCCTGCTGGGCCTGG CTGTGGAGATCGTAGACACCTCCAACCCTGAGCTCAC CAGCTCCACAGAGGTGCAGGTGGAGCAGGCCACGCC CATCTCGGAGAGCTGTGCAGGTAAAATGGAAGAATTC TTTCTCTTTGGAGCCTTCCTGGAGGCCTCAATGATCGA CCGGAGAAACGGAGACAAGCCCATCACCTTTGAGGTC ACCATAGGCAACTATGGGAACGAAGTTGATGGCCTGT CCCGGCCCCAGCGGCCTCGGCCCCGGAAGGAGCCG GGGGATGAGGAAGAAGTAGACCTGATTGAGAACGCAA GTGATGACGAGGCCGGTGATGCCGGGGACCTGGCCT CAGTCTCCTCCACTCCACCAATGCGGCCCCAGGTCAC CGACAGGAACACTTCCATCTGCCCTACCTGGAGCGA AAGCCCTGCATCTACATCAAGAGCTGGTGGCCGGACC AGCGCCCGCCCTCTACAATGCCAACATCATGGACCA CATTGCCGACAAGCTGGAAGAAGGCCTGAACGACATA CAGGAGATGATCAAAACGGAGAAGTCCTACCCTGAGC GTCGCCTGCGGGGCGTCCTGGAGGAGCTGAGCTGTG GCTGCTGCCGCTTCCTCTCCCTCGCTGACAAGGACCA GGGCCACTCATCCCGCACCAGGCTTGACCGGGAGCG CCTCAAGTCCTGCATGAGGGAGCTGGTAAGTATCAAG GTTACAAGACAGGTTTAAGGAGACCAATAGAACTGG GCTTGTGAGACAGAGAAGACTCTTGCGTTTCTGAGC TAGCCCCCGGGTGCGCGGCGTCGGTGGTGCCGGCG GGGGGCGCCAGGTGCGAGGCGGTGTAGGGCTCCAG GCAGGCGGCGAAGGCCATGACGTGCGCTATGAAGGT CTGCTCCTGCACGCCGTGAACCAGGTGCGCCTGCGG GCCGCGCGCAACACCGCCACGTCCTCGCCTGCGTG

SEQ ID NO.	Description	Plasmid Sequence
		GGTCTCTTCGTCCAGGGGCACTGCTGACTGCTGCCGA TACTCGGGGCTCCCGCTCTCGCTCTCGGTAACATCCG GCCGGGCGCCGTCCTTGAGCACATAGCCTGGACCGTT TCGTCGACCTCGAGTTAAGGGCGAATCCCCGATAAGG ATCTTCCTAGAGCATGGCTACGTAGATAAGTAGCATGG CGGGTTAATCATTAATACTACAAGGAACCCCTAGTGATGG AGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCAC TGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGG CTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCG CAGCCTTAATTAATCCACATCTGTATGTTTTTTATATT AATTTATTTTTGCAGGGGGGCATTGTTTGGTAGGTGA GAGTTCTGAATTGCTATGTTTAGTGAGTTGTATCTATTT ATTTTTCAATAAATAACAATTAGTTATGTGTTTGGGGGC GATCGTGAGGCAAAGAAAACCCGGCGCTGAGGCCGG GTTATCTTGTTCTCTGGTCAAATTATATAGTTGAAAAA CAAGGATGCATATATGAATGAACGATGCAGAGGCAAT GCCGATGGCGATAGTGGGTATCAGGTAGCCGCTTATG CTGGAAAGAAGCAATAACCCGCAGAAAAACAAAGCTC CAAGCTCAACAAAATAAGGGCATAGACAATAACTACC TATGTCATATACCCATACTCTCTAATCTTGCCAGTCG GCGCGTTCTGCTTCCGATTAGAAACGTCAAGGCAGCA ATCAGGATTGCAATCTTGTTCTCTGCATAGGATGACAA TGTCGCCCCAAGACCATCTCTATGAGCTGAAAAAGAAA CACAAGGAATGTAGTGGCGGAAAAGGAGATAGCAAAT GCTTACGATAACGTAAGGAATTATTACTATGTAAACAC CAGGCAAGATTCTGTTCCGTATAATTACTCCTGATAATT AATCCTTAACTTTGCCACCTGCCTTTTAAACATTCCA GTATATCACTTTTCATTCTTGCGTAGCAATATGCCCTCT CTTCAGCTATCTCAGCATTGGTGACCTTGTTTCAGAGGC GCTGAGAGATGGCCTTTTTCTGATAGATAATGTTCTGT TAAAATATCTCCGGCCTCATCTTTTGCCCGCAGGCTAA TGTCTGAAAATTGAGGTGACGGGTAAAAAATAATATCC TTGGCAACCTTTTTTATATCCCTTTTAAATTTTGGCTTA ATGACTATATCCAATGAGTCAAAAAGCTCCCCTTCAAT ATCTGTTGCCCTAAGACCTTTAATATATCGCCAAATA CAGGTAGCTTGGCTTCTACCTTCACCGTTGTTCTGCCG ATGAAATGCTAATGCATAACATCGTCTTTGGTGGTTCC CCTCATCAGTGGCTCTATCTGAACGCGCTCTCCACTG

SEQ ID NO.	Description	Plasmid Sequence
		CTTAATGACATTCCCTTTCCCGATTAAAAAATCTGTCAGA TCGGATGTGGTCGGCCCCGAAAACAGTTCTGGCAAAAC CAATGGTGTGCGCTTCAACAAACAAAAAAGATGGGAAT CCCAATGATTTCGTCATCTGCGAGGCTGTTCTTAATATC TTCAACTGTAGCTTTAGAGCGATTTATCTTCTGAACCA GACTCTTGTCATTTGTTTTGGTAAAGAGAAAAGTTTTTC CATCGATTTTATGAATATACAAATAATTGGAGCCAACCT TCAGGTGATGATTATCAGCCAGCAGAGAATTAAGGAAA ACAGACAGGTTTATTGAGCACTTATCTTTCCCTTTATTT TTGCTGCGGTAAGTCGCATAAAAAACCATTCTTCACAAT TCAATCCATTTACTATGTTATGTTCTGAGGGGAGTGAA AATCCCCTAATTTCGATGAAGATTCTTGCTAAATTGTTA TCAGCTATGCGCCGACCAGAACACCTTGCCGATCAGC CAAACGTCTAATCAGGCCACTGACTAGCGATAACTTTC CCCACAACGGAACAACCTCTCATTGCATGGGATAATTGG GTACTGTGGGTTTAGTGGTTGTAAAAACACCTGACCGC TATCCCTGATCAGTTTCTTGAAGGTAACTCATCACCC CCAAGTCTGGCTATACAGAAATCACCTGGCTCAACAG CCTGCTCAGGGTCAACGAGAATTTACATTCCGTCAGG ATAGCTTGGCTTGGAGCCTGTTGGTGCGGTCACGGAA TTACCTTCAACCTCAAGCCAGAATGCAGAATCACTGGC TTTTTTGGTTGTGCTTACCCATCTCTCCGCATCACCTTT GGTAAAGGTTCTAAGCTAAGGTGAGAACATCCCTGCC TGAACATGAGAAAAAACAGGGTACTCATACTCACTTAT TAGTGACGGCTATGAGCAAAAGGCCAGCAAAAGGCCA GGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCA TAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGA CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT AAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGT GCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC CTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTT CTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAG GTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCC CCGTTCAAGCCGACCGCTGCGCCTTATCCGGTAACTA TCGTCTTGAGTCCAACCCGTAAGACACGACTTATCG CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTG

SEQ ID NO.	Description	Plasmid Sequence
		GTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAA AGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG CTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATT ACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT CTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAAAC TCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAG GATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTT TAAATCAAGCCCAATCTGAATAATGTTACAACCAATTAA CCAATTCTGATTAGAAAACTCATCGAGCATCAAATGA AACTGCAATTTATTCATATCAGGATTATCAATACCATAT TTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTC ACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTAT CGGTCTGCGATTCCGACTCGTCCAACATCAATACAACC TATTAATTTCCCTCGTCAAAAAAAGGTTATCAAGTGA GAAATCACCATGAGTGACGACTGAATCCGGTGAGAAT GGCAAAAGTTTATGCATTTCTTCCAGACTTGTTCAACA GGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATC AACCAAACCGTTATTCATTCTGTGATTGCGCCTGAGCAA GACGAAATACGCGATCGCTGTTAAAAGGACAATTACAA ACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCA GCGCATCAACAATATTTTCACCTGAATCAGGATATTCTT CTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTG GTGAGTAACCATGCATCATCAGGAGTACGGATAAAATG CTTGATGGTGGGAAGAGGCATAAATCCGTCAGCCAG TTTAGTCTGACCATCTCATCTGTAAACATCATTGGCAAC GCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCAT CGGGCTTCCCATAACAAGCGATAGATTGTGCGACCTGA TTGCCCCGACATTATCGCGAGCCCATTATACCCATATA AATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAC GTTTCCCGTTGAATATGGCTCATAACACCCTTGTATT ACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGA TATATTTTATCTTGTGCAATGTAAACATCAGAGATTTG AGACACGGGCCAGAGCTGCA
61	3' transgene plasmid containing the following features: Lambda at positions 53-2027	TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACA CATGCAGCTCCCGGATAGAGGTCATCCTTCCTGACCA TTTCCATCATTCCAGTCGAACTCACACACAACACCAAA TGCATTTAAGTCGCTTGAAATTGCTATAAGCAGAGCAT GTTGCGCCAGCATGATTAATACAGCATTTAATACAGAG

SEQ ID NO.	Description	Plasmid Sequence
	ITR at positions 2049-2178 AP head sequence at positions 2267-2553 Splice acceptor sequence at positions 2576-2624 C-terminal portion of human OTOF isoform 5 at positions 2625-6212 bGH poly(A) sequence at positions 6255-6476 ITR at positions 6564-6693 Lambda at positions 6704-8716 Ori at positions 8773-9361 KanR at positions 9539-10,348 Transgene to be transferred into vector in dual vector system at positions 2049-6693	CCGTGTTTATTGAGTCGGTATTCAGAGTCTGACCAGAA ATTATTAATCTGGTGAAGTTATTCCTCTGTCATTACGTC ATGGTCGATTTCAATTTCTATTGATGCTTCCAGTCGTA ATCAATGATGATTTTTTGGATGTTTGACCTCTGTTCCATA TCCTCACAGATAAAAAATCGCCCTCACACTGGAGGGC AAAGAAGATTTCCAATAATCAGAACAAGTCGGCTCCTG TTTAGTTACGAGCGACATTGCTCCGTGTATTCACTCGT TGGAATGAATACACAGTGCAGTGTTTATTCTGTTATTTA TGCCAAAAATTAAGGCCACTATCAGGCAGCTTGTGT TCTGTTTACCAAGTTCTCTGGCAATCATTGCCGTCGTT CGTATTGCCCATTTATCGACATATTTCCCATCTTCCTAT ACAGGAAACATTTCTTCAGGCTTAACCATGCATTCCGA TTGCAGCTTGCATCCATTGCATCGCTTGAATTGTCCAC ACCATTGATTTTTATCAATAGTCGTAGTTTAACGGATAG TCCTGGTATTGTTCCATCACATCCTGAGGATGCCCTTC GAACTCTTCAAATTCTTCTCCTAATATCACCTTAAATA GTGGATTGCGGTAGTAAAGATTGTGCCTGTCTTTTAAC CACATCAGGCTCGGTGGTTCTCGTGTAACCCCTACAGC GAGAAATCGGATAAACTATTACAACCCCTACAGTTTGT AGAGTATAGAAAATGATCCACTCGTTATTCTCGGACGA GTGTTTCAGTAATGAACCTCTGGAGAGAACCATCTATAT GATCGTTATCTGGGTTTGACTTCTGCTTTTAAGCCCAG ATAACTTGCCCTGAATATGTTAATGAGAGAATCGGTATT CCTCATGTGTGGCATGTTTTCGTCTTTGCTCTTGCATTT TCACTAGCAATTAATGTGCATCGATTATCAGCTATTGC CAGCGCCAGATATAAGCGATTAAAGCTAAGAAAACGCA TTAAGGTGCAAAACGATAAAGTGCGATCAGTAATTCAA AACCTTACAGGAGAGCAATCTATGGTTTTGTGCTCAGC CCTTAATGAAGGCAGGTAGTATGTGGTTACATCAAAAC AATTCCCATAACATTAGTGAGTTGATTGAGCTTGGTGTG TTGAACAAAACTTTTCCCGATGGAATGGAAAGCATAT ATTATTCCCTATTGAGGATATTTACTGGACTGAATTAGT TGCCAGCTATGATCCATATAATATTGAGATAAAGCCAA GGCCAATATCTAAGTAACTAGATAAGAGGAATCGATTT TCCCTTAATTTTCTGGCGTCCACTGCATGTTATGCCGC GTTCCGCCAGGCTTGCTGTACCATGTGCGCTGATTCTT GCGCTCAATACGTTGCAGGTTGCTTTCAATCTGTTTGT GGTATTCAGCCAGCACTGTAAGGTCTATCGGATTAGT

SEQ ID NO.	Description	Plasmid Sequence
		GCGCTTTCTACTCGTGATTTGCGTTTGCGATTGAGCGA GAGAATAGGGCGGTAACTGGTTTTGCGCTTACCCCA ACCAACAGGGGATTGCTGCTTTCCATTGAGCCTGTTA CTCTGCGCGACGTTGCGGGCGGCGTGTTGTGCATCC ATCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTGTG GCAGTTGTAGTCCTGAACGAAAACCCCCGCGATTGG CACGTTGGCAGCTAATCCGGAATCGCACTTACGGCCA ATGCTTCGTTTCGTATCACACACCCCAAAGCCTTCTGC TTTGAATGCTGCCCTTCTTCAGGGCTTAATTTTAAAG GCGTCACCTTCATGGTGGTCAGTGCGTCCTGCTGATG TGCTCAGGCACGATTTAATTAAGGCCTTAATTAGGCTG CGCGCTCGCTCGCTCACTGAGGCGCGCCGGGCAAAG CCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCA GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAAC TCCATCACTAGGGGTTCTTGTAGTTAATGATTAACCC GCCATGCTACTTATCTACGTAGCCATGCTCTAGGAAGA TCGGAATTCGCCCTTAAGCTAGCGGCGCGCCCCCG GGTGCGCGGCGTCGGTGGTGCCGGCGGGGGGCGCC AGGTGCGCAGGCGGTGTAGGGCTCCAGGCAGGCGGCG AAGGCCATGACGTGCGCTATGAAGGTCTGCTCCTGCA CGCGGTGAACCAGGTGCGCCTGCGGGCCGCGCGCGA ACACCGCCACGTCCTCGCCTGCGTGGGTCTCTTCGTC CAGGGGCACTGCTGACTGCTGCCGATACTCGGGGCT CCCGCTCTCGCTCTCGGTAACATCCGGCCGGGCGCC GTCCTTGAGCACATAGCCTGGACCGTTTCCTTAAGCG ACGCATGCTCGCGATAGGCACCTATTGGTCTTACTGA CATCCACTTTGCCTTTCTCTCCACAGGAAAACATGGGG CAGCAGGCCAGGATGCTGCGGGCCCAGGTGAAGCGG CACACGGTGCGGGACAAGCTGAGGCTGTGCCAGAAC TTCCTGCAGAAGCTGCGCTTCCTGGCGGACGAGCCCC AGCACAGCATTCCCACATCTTCATCTGGATGATGAGC AACAACAAGCGTGTCGCCTATGCCCGTGTGCCCTCCA AGGACCTGCTCTTCTCCATCGTGGAGGAGGAGACTGG CAAGGACTGCGCCAAGGTCAAGACGCTCTTCCTTAAG CTGCCAGGGAAGCGGGGCTTCGGCTCGGCAGGCTGG ACAGTGAGGCCAAGGTGGAGCTGTACCTGTGGCTG GGCCTCAGCAAACAGCGCAAGGAGTTCCTGTGCGGC CTGCCCTGTGGCTTCCAGGAGGTCAAGGCAGCCCAG

SEQ ID NO.	Description	Plasmid Sequence
		GGCCTGGGCCTGCATGCCTTCCCACCCGTCAGCCTG GTCTACACCAAGAAGCAGGCGTTCCAGCTCCGAGCGC ACATGTACCAGGCCCGCAGCCTCTTTGCCGCCGACAG CAGCGGACTCTCAGACCCCTTTGCCCGCGTCTTCTTC ATCAATCAGAGTCAGTGCACAGAGGTGCTGAATGAGA CCCTGTGTCCCACCTGGGACCAGATGCTGGTGTTCGA CAACCTGGAGCTCTATGGTGAAGCTCATGAGCTGAGG GACGATCCGCCCATCATTGTCATTGAAATCTATGACCA GGATTCCATGGGCAAAGCTGACTTCATGGGCCGGACC TTCGCCAAACCCCTGGTGAAGATGGCAGACGAGGCGT ACTGCCCACCCCGCTTCCCACCTCAGCTCGAGTACTA CCAGATCTACCGTGGCAACGCCACAGCTGGAGACCTG CTGGCGGCCTTCGAGCTGCTGCAGATTGGACCAGCAG GGAAGGCTGACCTGCCCCCATCAATGGCCCGGTGG ACGTGGACCGAGGTCCCATCATGCCCGTGCCCATGG GCATCCGGCCCGTGCTCAGCAAGTACCGAGTGAGG TGCTGTTCTGGGGCCTACGGGACCTAAAGCGGGTGAA CCTGGCCCAGGTGGACCGGCCACGGGTGGACATCGA GTGTGCAGGGAAGGGGGTGCACTCGTCCCTGATCCA CAATTATAAGAAGAACCCCAACTTCAACACCCTCGTCA AGTGGTTTGAAGTGGACCTCCCAGAGAACGAGCTGCT GCACCCGCCCTTGAACATCCGTGTGGTGGACTGCCGG GCCTTCGGTTCGTACACACTGGTGGGCTCCCATGCCG TCAGCTCCCTGCGACGCTTCATCTACCGGCCCCCAAGA CCGCTCGGCCCCCAAGCTGGAACACCACGGTCAGGCT TCTCCGGCGCTGCCGTGTGCTGTGCAATGGGGGCTC CTCCTCTCACTCCACAGGGGAGGTTGTGGTGAATATG GAGCCAGAGGTACCCATCAAGAACTGGAGACCATGG TGAAGCTGGACGCGACTTCTGAAGCTGTTGTCAAGGT GGATGTGGCTGAGGAGGAGAAGGAGAAGAAGAAGAA GAAGAAGGGCACTGCGGAGGAGCCAGAGGAGGAGGA GCCAGACGAGAGCATGCTGGACTGGTGGTCCAAGTAC TTTGCTCCATTGACACCATGAAGGAGCAACTTCGACA ACAAGAGCCCTCTGGAATTGACTTGGAGGAGAAGGAG GAAGTGGACAATAACCGAGGGCCTGAAGGGGTCAATGA AGGGCAAGGAGAAGGCAAGGGCTGCCAAAGAGGAGA AGAAGAAGAAAACCTCAGAGCTCTGGCTCTGGCCAGGG GTCCGAGGCCCCCGAGAAGAAGAAACCAAGATTGAT

SEQ ID NO.	Description	Plasmid Sequence
		GAGCTTAAGGTATACCCCAAAGAGCTGGAGTCCGAGT TTGATAACTTTGAGGACTGGCTGCACACTTTCAACTTG CTTCGGGGCAAGACCGGGGATGATGAGGATGGCTCC ACCGAGGAGGAGCGCATTGTGGGACGTTCAAGGGC TCCCTCTGCGTGTAACAAGTGCCACTCCCAGAGGACG TGTCCCGGAAGCCGGCTACGACTCCACCTACGGCAT GTTCCAGGGCATCCCGAGCAATGACCCCATCAATGTG CTGGTCCGAGTCTATGTGGTCCGGGCCACGGACCTGC ACCCTGCTGACATCAACGGCAAAGCTGACCCCTACAT CGCCATCCGGCTAGGCAAGACTGACATCCGCGACAAG GAGAACTACATCTCCAAGCAGCTCAACCCTGTCTTTGG GAAGTCCTTTGACATCGAGGCCTCCTTCCCCATGGAAT CCATGCTGACGGTGGCTGTGTATGACTGGGACCTGGT GGGCACTGATGACCTCATTGGGGAAACCAAGATCGAC CTGGAGAACCGCTTCTACAGCAAGCACCGCGCCACCT GCGGCATCGCCCAGACCTACTCCACACATGGCTACAA TATCTGGCGGGACCCCATGAAGCCCAGCCAGATCCTG ACCCGCCTCTGCAAAGACGGCAAAGTGGACGGCCCC CACTTTGGGCCCCCTGGGAGAGTGAAGGTGGCCAAC CGCGTCTTCACTGGGCCCTCTGAGATTGAGGACGAGA ACGGTCAGAGGAAGCCACAGACGAGCATGTGGCGC TGTTGGCCCTGAGGCACTGGGAGGACATCCCCGCG CAGGCTGCCGCCTGGTGCCAGAGCATGTGGAGACGA GGCCGCTGCTCAACCCCGACAAGCCGGGCATCGAGC AGGGCCGCCTGGAGCTGTGGGTGGACATGTTCCCAT GGACATGCCAGCCCCTGGGACGCCTCTGGACATCTCA CCTCGGAAGCCCAAGAAGTACGAGCTGCGGGTCATCA TCTGGAACACAGATGAGGTGGTCTTGGAGGACGACGA CTTCTTCACAGGGGAGAAGTCCAGTGACATCTTCGTG AGGGGGTGGCTGAAGGGCCAGCAGGAGGACAAGCAG GACACAGACGTCCACTACCACTCCCTCACTGGCGAGG GCAACTTCAACTGGCGCTACCTGTTCCCTTCGACTAC CTGGCGGCGGAGGAGAAGATCGTCATCTCCAAGAAG GAGTCCATGTTCTCCTGGGACGAGACCGAGTACAAGA TCCCCGCGCGGCTCACCTGCAGATCTGGGATGCGG ACCACTTCTCCGCTGACGACTTCCTGGGGGCCATCGA GCTGGACCTGAACCGGTTCCCGCGGGGCGCAAAGAC AGCCAAGCAGTGACCATGGAGATGGCCACCGGGGA

SEQ ID NO.	Description	Plasmid Sequence
		GGTGGACGTGCCCCTCGTGTCCATCTTCAAGCAAAAG CGCGTCAAAGGCTGGTGGCCCCTCCTGGCCCGCAAT GAGAACGATGAGTTTGAGCTCACGGGCAAGGTGGAG GCTGAGCTGCATTTACTGACAGCAGAGGAGGCAGAGA AGAACCCAGTGGGCCTGGCCCGCAATGAACCTGACCC CCTAGAGAAACCCAACCGGCCCGACACGGCCTTCGTC TGGTTCCTCAACCCTCTCAAGTCCATCAAGTACCTCAT CTGCACCCGGTACAAGTGGCTCATCATCAAGATCGTG CTGGCGCTGTTGGGGCTGCTCATGTTGGGGCTCTTCC TCTACAGCCTCCCTGGCTACATGGTCAAAAAGCTCCTT GGGGCATGAACGGCCGCTATGCTAGCTTGGTACCAAG GGCGGATCCTGCATAGAGCTCGCTGATCAGCCTCGAC TGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCT CCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCC CACTGTCCTTTCTAATAAAATGAGGAAATTGCATCGC ATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGG GGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGA CAATAGCAGGCATCTCGAGTTAAGGGCGAATTCCCGA TAAGGATCTTCCTAGAGCATGGCTACGTAGATAAGTAG CATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGT GATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCG CTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGC CCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGA GCGCGCAGCCTTAATTAAATCCACATCTGTATGTTTTTT ATATTAATTTATTTTTTGCAGGGGGGCATTGTTTGGTAG GTGAGAGTTCTGAATTGCTATGTTTAGTGAGTTGTATC TATTTATTTTTCAATAAATACAATTAGTTATGTGTTTTGG GGGCGATCGTGAGGCAAAGAAAACCCGGCGCTGAGG CCGGGTATTCTTGTTCTCTGGTCAAATTATATAGTTG GAAAACAAGGATGCATATATGAATGAACGATGCAGAG GCAATGCCGATGGCGATAGTGGGTATCAGGTAGCCGC TTATGCTGGAAAGAAGCAATAACCCGCAGAAAAACAAA GCTCCAAGCTCAACAAAATAAGGGCATAGACAATAAC TACCTATGTCATATACCCATACTCTCTAATCTTGGCCA GTCGGCGCGTTCTGCTTCCGATTAGAAACGTCAAGGC AGCAATCAGGATTGCAATCTTGGTTCCTGCATAGGATG ACAATGTGCCCCAAGACCATCTCTATGAGCTGAAAAA GAAACACAAGGAATGTAGTGGCGGAAAAGGAGATAGC

SEQ ID NO.	Description	Plasmid Sequence
		AAATGCTTACGATAACGTAAGGAATTATTACTATGTAAA CACCAGGCAAGATTCTGTTCCGTATAATTACTCCTGAT AATTAATCCTTAACCTTTGCCACCTGCCTTTTAAAAACAT TCCAGTATATCACTTTTCATTCTTGCCTAGCAATATGCC CTCTCTTCAGCTATCTCAGCATTGGTGACCTTGTTTCAG AGGCGCTGAGAGATGGCCTTTTTCTGATAGATAATGTT CTGTTAAAATATCTCCGGCCTCATCTTTGCCCGCAGG CTAATGTCTGAAAATTGAGGTGACGGGTAAAAATAAT ATCCTTGGCAACCTTTTTATATCCCTTTTAAATTTTGG CTTAATGACTATATCCAATGAGTCAAAAAGCTCCCTT CAATATCTGTTGCCCTAAGACCTTTAATATATCGCCA AATACAGGTAGCTTGGCTTCTACCTTCACCGTTGTTCT GCCGATGAAATGCTAATGCATAACATCGTCTTTGGTG TTCCCCTCATCAGTGGCTCTATCTGAACGCGCTCTCCA CTGCTTAATGACATTCCCTTCCCGATTAAAAATCTGTC AGATCGGATGTGGTCGGCCCGAAAACAGTTCTGGCAA AACCAATGGTGTGCGCTTCAACAAACAAAAAGATGGG AATCCCAATGATTGTCATCTGCGAGGCTGTTCTTAAT ATCTTCAACTGTAGCTTTAGAGCGATTTATCTTCTGAAC CAGACTCTTGTCATTTGTTTTGGTAAAGAGAAAAGTTTT TCCATCGATTTTATGAATATACAAATAATTGGAGCCAAC CTTCAGGTGATGATTATCAGCCAGCAGAGAATTAAGGA AAACAGACAGGTTTATTGAGCACTTATCTTCCCTTTAT TTTTGCTGCGGTAAGTCGCATAAAAACCATTTCCACA ATTCAATCCATTTACTATGTTATGTTCTGAGGGGAGTG AAAATCCCCTAATTCGATGAAGATTCTTGCTAAATTGT TATCAGCTATGCGCCGACCAGAACACCTTGCCGATCA GCCAAACGTCTAATCAGGCCACTGACTAGCGATAACTT TCCCCACAACGGAACAACCTCTCATTGCATGGGATAATT GGGTAAGTGTGGGTTTAGTGGTTGTAAAAACACCTGAC CGCTATCCCTGATCAGTTTCTTGAAGGTAAACTCATCA CCCCCAAGTCTGGCTATACAGAAATCACCTGGCTCAA CAGCCTGCTCAGGGTCAACGAGAATTTACATTCCGTCA GGATAGCTTGGCTTGGAGCCTGTTGGTGCGGTCACGG AATTACCTTCAACCTCAAGCCAGAATGCAGAATCACTG GCTTTTTTGGTTGTGCTTACCCATCTCTCCGCATCACC TTTGGTAAAGGTTCTAAGCTAAGGTGAGAACATCCCTG CCTGAACATGAGAAAAACAGGGTACTCATACTCACTT

SEQ ID NO.	Description	Plasmid Sequence
		ATTAGTGACGGCTATGAGCAAAAGGCCAGCAAAAGGC CAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC CATAGGCTCCGCCCCCTGACGAGCATCACAAAATC GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACT ATAAAGATAACCAGGCGTTTCCCCCTGGAAGCTCCCTC GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGAT ACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCT TTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT AGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACC CCCCGTTGAGCCCGACCGCTGCGCCTTATCCGGTAAC TATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATC GCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGA GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGT GGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTT GGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA AAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCAC CGCTGGTAGCGGTGGTTTTTTTGTTCGAAGCAGCAG ATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTT GATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAA AACTCACGTAAAGGGATTTTGGTCATGAGATTATCAAA AAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAG TTTTAAATCAAGCCCAATCTGAATAATGTTACAACCAAT TAACCAATTCTGATTAGAAAACTCATCGAGCATCAAT GAAACTGCAATTTATTCATATCAGGATTATCAATACCAT ATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAAC TCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGT ATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAA CCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGT GAGAAATCACCATGAGTGACGACTGAATCCGGTGAGA ATGGCAAAAGTTTATGCATTTCTTCCAGACTTGTTCAA CAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGC ATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAG CAAGACGAAATACGCGATCGCTGTAAAAAGGACAATTA CAAACAGGAATCGAATGCAACCGGCGCAGGAACACTG CCAGCGCATCAACAATATTTTACCTGAATCAGGATAT TCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGC AGTGGTGAGTAACCATGCATCATCAGGAGTACGGATA AAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCA

SEQ ID NO.	Description	Plasmid Sequence
		GCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTG GCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGG CGCATCGGGCTTCCCATAACAAGCGATAGATTGTCGCA CCTGATTGCCCGACATTATCGCGAGCCCATTTATACCC ATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCC TCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTT GTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTAT GATGATATATTTTATCTTGTGCAATGTAACATCAGAGA TTTTGAGACACGGGCCAGAGCTGCA
62	5' transgene plasmid containing the following features: ITR at positions 19-161 Myo15 promoter at positions 182-1146 N-terminal portion of human OTOF isoform 5 at positions 1167-3572 Splice donor sequence at positions 3573-3656 AP head sequence at positions 3663-3949 ITR at positions 3973-4115 KanR at positions 4641-5435 pUC ori at positions 5821-6491 Transgene to be transferred into vector in dual vector system at positions 19-4115	GGGGGGGGGGGGGGGGGGTGGCCACTCCCTCTCT GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAA GGTCGCCCCGACGCCCCGGGCTTTGCCCGGGCGGCCTC AGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA CTCCATCACTAGGGGTTCTCTCAGATCTGAATTCGGTAC CTGCAGCTCAGCCTACTACTTGCTTTCCAGGCTGTCC TAGTTCCCATGTCAGCTGCTTGTGCTTTCCAGAGACAA AACAGGAATAATAGATGTCATTAAATATACATTGGGCC CCAGGCGGTCAATGTGGCAGCCTGAGCCTCCTTTCCA TCTCTGTGGAGGCAGACATAGGACCCCCAACAAACAG CATGCAGGTTGGGAGCCAGCCACAGGACCCAGGTAA GGGGCCCTGGGTCTTAAGCTTCTGCCACTGGCTCCG GCATTGCAGAGAGAAGAGAAGGGGCGGCAGACTGGA GAGCTGGGCTCCATTTTGTTCCTTGGTGCCCTGCCC CTCCCCATGACCTGCAGAGACATTACGCCTGCCAGGC TTTATGAGGTGGGAGCTGGGCTCTCCCTGATGTATTAT TCAGCTCCCTGGAGTTGGCCAGCTCCTGTTACTGAG CCACAGCCCTGGGCATCCGCTTCTCACTTCTAGTTTCC CCTCCAAGGTAATGTGGTGGGTGATGATCATTCTATCC TGGCTTCAGGGACCTGACTCCACTTTGGGGCCATTCCG AGGGGTCTAGGGTAGATGATGTCCCCCTGTGGGGATT AATGTCCTGCTCTGTAAACTGAGCTAGCTGAGATCCA GGAGGGCTTGGCCAGAGACAGCAAGTTGTTGCCATGG TGACTTTAAAGCCAGGTTGCTGCCCCAGCACAGGCCT CCCAGTCTACCCTCACTAGAAAACAACACCCAGGCAC TTTCCACCACCTCTCAAAGGTGAAACCAAGGCTGGT CTAGAGAATGAATTATGGATCCTCGCTGTCCGTGCCAC CCAGCTAGTCCCAGCGGCTCAGACACTGAGGAGAGAC TGTAGGTTGAGCTACAAGCAAAAAGACCTAGCTGGTCT

SEQ ID NO.	Description	Plasmid Sequence
		CCAAGCAGTGTCTCCAAGTCCCTGAACCTGTGACACC TGCCCCAGGCATCATCAGGCACAGAGGGCCACCGAAT TCTAGCGGCCGCCACCATGGCCTTGCTCATCCACCTC AAGACAGTCTCGGAGCTGCGGGGCAGGGGCGACCGG ATCGCCAAAGTGACTTTCCGAGGGCAATCCTTCTACTC TCGGGTCTGGAGAACTGTGAGGATGTGGCTGACTTT GATGAGACATTTCCGGTGGCCGGTGGCCAGCAGCATCG ACAGAAATGAGATGCTGGAGATTCAAGTTTTCAACTAC AGCAAAGTCTTCAGCAACAAGCTCATCGGGACCTTCC GCATGGTGCTGCAGAAGGTGGTAGAGGAGAGCCATGT GGAGGTGACTGACACGCTGATTGATGACAACAATGCT ATCATCAAGACCAGCCTGTGCGTGGAGGTCCGGTATC AGGCCACTGACGGCACAGTGGGCTCCTGGGACGATG GGGACTTCCTGGGAGATGAGTCTCTTCAAGAGGAAGA GAAGGACAGCCAAGAGACGGATGGACTGCTCCCAGG CTCCCGGCCAGCTCCCGGCCCCCAGGAGAGAAGAG CTTCCGAGAGCCGGGAGGAGCGTGTCTCCGCCAT GAAGCTCGGCAAAAACCGGTCTCACAAGGAGGAGCCC CAAAGACCAGATGAACCGGCGGTGCTGGAGATGGAA GACCTTGACCATCTGGCCATTCCGGCTAGGAGATGGAC TGGATCCCGACTCGGTGTCTCTAGCCTCAGTCACAGC TCTCACCCTAATGTCTCCAACAAGCGATCTAAGCCAG ACATTAAGATGGAGCCAAGTGCTGGGCGGCCCATGGA TTACCAGGTCAGCATCACGGTGATCGAGGCCCGGCAG CTGGTGGGCTTGAACATGGACCCTGTGGTGTGCGTGG AGGTGGGTGACGACAAGAAGTACACATCCATGAAGGA GTCCACTAACTGCCCCATTACAACGAGTACTTCGTCT TCGACTTCCATGTCTCTCCGGATGTCATGTTTGACAAG ATCATCAAGATTTCCGGTGATTCACTCCAAGAACCTGCT GCGCAGTGGCACCTGGTGGGCTCCTTCAAAATGGAC GTGGGAACCGTGTACTCGCAGCCAGAGCACCAAGTTCC ATCACAAGTGGGCCATCCTGTCTGACCCCGATGACAT CTCCTCGGGGCTGAAGGGCTACGTGAAGTGTGACGTT GCCGTGGTGGGCAAAGGGGACAACATCAAGACGCCC CACAAGGCCAATGAGACCGACGAAGATGACATTGAGG GGAAGTTGCTGCTCCCCGAGGGGGTGGCCCCCGAAC GCCAGTGGGCCCGGTTCTATGTGAAAATTTACCGAGC AGAGGGGCTGCCCCGTATGAACACAAGCCTCATGGCC

SEQ ID NO.	Description	Plasmid Sequence
		AATGTAAAGAAGGCTTTCATCGGTGAAAACAAGGACCT CGTGGACCCCTACGTGCAAGTCTTCTTGCTGGCCAG AAGGGCAAGACTTCAGTGCAGAAGAGCAGCTATGAGC CCCTGTGGAATGAGCAGGTCGTCTTTACAGACCTCTTC CCCCCACTCTGCAAACGCATGAAGGTGCAGATCCGAG ACTCGGACAAGGTCAACGACGTGGCCATCGGCACCCA CTTCATTGACCTGCGCAAGATTTCTAATGACGGAGACA AAGGCTTCCTGCCCACACTGGGCCCAGCCTGGGTGAA CATGTACGGCTCCACACGTAACTACACGCTGCTGGAT GAGCATCAGGACCTGAACGAGGGCCTGGGGGAGGGT GTGTCCTTCCGGGCCCCGGCTCCTGCTGGGCCTGGCT GTGGAGATCGTAGACACCTCCAACCCTGAGCTCACCA GCTCCACAGAGGTGCAGGTGGAGCAGGCCACGCCCA TCTCGGAGAGCTGTGCAGGTAAAATGGAAGAATTCTTT CTCTTTGGAGCCTTCCTGGAGGCCTCAATGATCGACC GGAGAAACGGAGACAAGCCCATCACCTTTGAGGTAC CATAGGCAACTATGGGAACGAAGTTGATGGCCTGTCC CGGCCCCAGCGGCCTCGGCCCCGGAAGGAGCCGGG GGATGAGGAAGAAGTAGACCTGATTGAGAACGCAAGT GATGACGAGGCCGGTGATGCCGGGGACCTGGCCTCA GTCTCCTCCACTCCACCAATGCGGCCCCAGGTACCG ACAGGAACTACTTCCATCTGCCCTACCTGGAGCGAAA GCCCTGCATCTACATCAAGAGCTGGTGGCCGGACCAG CGCCGCCGCCTCTACAATGCCAACATCATGGACCACA TTGCCGACAAGCTGGAAGAAGGCCTGAACGACATACA GGAGATGATCAAAACGGAGAAGTCCTACCCTGAGCGT CGCCTGCGGGGCGTCCTGGAGGAGCTGAGCTGTGGC TGCTGCCGCTTCCTCTCCCTCGCTGACAAGGACCAGG GCCACTCATCCCGCACCAGGCTTGACCGGGAGCGCC TCAAGTCCTGCATGAGGGAGCTGGTAAGTATCAAGGT TACAAGACAGGTTTAAGGAGACCAATAGAACTGGGC TTGTCGAGACAGAGAAGACTCTTGCGTTTCTGAGCTAG CCCCCGGGTGCGCGGCGTCGGTGGTGCCGGCGGGG GGCGCCAGGTGCGAGGCGGTGTAGGGCTCCAGGCAG GCGGCGAAGGCCATGACGTGCGCTATGAAGGTCTGCT CCTGCACGCCGTGAACCAGGTGCGCCTGCGGGCCGC GCGCGAACACCGCCACGTCCTCGCCTGCGTGGGTCT CTTCGTCCAGGGGCACTGCTGACTGCTGCCGATACTC

SEQ ID NO.	Description	Plasmid Sequence
		GGGGCTCCCGCTCTCGCTCTCGGTAACATCCGGCCG GGCGCCGTCCTTGAGCACATAGCCTGGACCGTTTCGT CGACTGGGGAGAGATCTGAGGAACCCCTAGTGATGGA GTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACT GAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGAC CTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCG CAGAGAGGGAGTGGCCAACCCCCCCCCCCCCCCCCC TGCAGCCTGGCGTAATAGCGAAGAGGCCCGCACCGAT CGCCCTTCCCAACAGTTGCGTAGCCTGAATGGCGAAT GGCGCGACGCGCCCTGTAGCGGCGCATTAAAGCGCGG CGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACT TGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTC CCTTCCTTTCTCGCCACGTTGCGCGGCTTCCCCGTCA AGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA GTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAG GGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGA CGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTT AATAGTGGACTCTTGTTCCAACTGGAACAACACTCAA CCCTATCGCGGTCTATTCTTTTGATTTATAAGGGATGTT GCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTT AACAAAAATTTTAACAAAATTCAGAAGAACTCGTCAAGA AGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCG GCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCAT CGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAA CGCTATGTCTGATAGCGGTCCGCCACACCCAGCCGG CCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCA CCATGATATTCGGCAAGCAGGCATCGCCATGGGTAC GACGAGATCCTCGCCGTCGGGCATGCTCGCCTTGAGC CTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGC TCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTC CATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCT TGGTGGTCTGAATGGGCAGGTAGCCGGATCAAGCGTAT GCAGCCGCCGATTGCATCAGCCATGATGGATACTTT CTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTG CCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCC GCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAA CGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCT CGTCTTGCAGTTCATTCAAGGGCACCGGACAGGTGCGT

SEQ ID NO.	Description	Plasmid Sequence
		CTTGACAAAAAGAACCGGGCGCCCCCTGCGCTGACAGC CGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTT GTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGC GGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATC ATGCGAAACGATCCTCATCCTGTCTCTTGATCAGATCT TGATCCCCTGCGCCATCAGATCCTTGGCGGCGAGAAA GCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACC AGAGGGCGCCCCAGCTGGCAATTCCGGTTCGCTTGCT GTCCATAAAACCGCCCAGTCTAGCTATCGCCATGTAAG CCCACTGCAAGCTACCTGCTTTCTCTTTGCGCTTGCGT TTCCCTTGTCAGATAGCCCAGTAGCTGACATTCATC CGGGGTCAGCACCGTTTCTGCGGACTGGCTTTCTACG TGAAAAGGATCTAGGTGAAGATCCTTTTGGATAATCTC ATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTG AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT GAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAA ACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTCG CGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAC GGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCT AGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCT GTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT ACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTT ACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGG CGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACAC AGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAG ATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTT CCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGC GGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTT CCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCTG GGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGA TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC AGCAACGCGGCCTTTTACGGTTCCTGGGCTTTTGCT GGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCT GATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGC TGATACCGCTCGCCGAGCCGAACGACCGAGCGCAG CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAAT ACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAT TAATGCAGGGCTGCA

SEQ ID NO.	Description	Plasmid Sequence
63	3' transgene plasmid containing the following features: ITR at positions 19-161 AP head sequence at positions 187-473 Splice acceptor sequence at positions 496-544 C-terminal portion of human OTOF isoform 5 at positions 545-4132 bGH poly(A) sequence at positions 4175-4396 ITR at positions 4447-4589 KanR at positions 5115-5909 pUC ori at positions 6295-6965 Transgene to be transferred into vector in dual vector system at positions 19-4589	GGGGGGGGGGGGGGGGGGTGGCCACTCCCTCTCT GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACAAA GGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTC AGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA CTCCATCACTAGGGGTTCCCTCAGATCTGAATTCTAGCG GCCGCCCCCGGGTGCGCGGCGTGGTGGTGGCGGC GGGGGGCGCCAGGTGCGCAGGCGGTGTAGGGCTCCA GGCAGGCGGCGAAGGCCATGACGTGCGCTATGAAGG TCTGCTCCTGCACGCCGTGAACCAGGTGCGCCTGCG GGCCGCGCGCAACACCGCCACGTCTCGCCTGCGT GGGTCTCTTCGTCCAGGGGCACTGCTGACTGCTGCCG ATACTCGGGGCTCCCGCTCTCGCTCTCGGTAACATCC GGCCGGGCGCGCTCCTTGAGCACATAGCCTGGACCG TTTCCTTAAGCGACGCATGCTCGCGATAGGCACCTATT GGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGG AAAACATGGGGCAGCAGGCCAGGATGCTGCGGGCCC AGGTGAAGCGGCACACGGTGCGGGACAAGCTGAGGC TGTGCCAGAACTTCCTGCAGAAGCTGCGCTTCCTGGC GGACGAGCCCCAGCACAGCATTCCCAGCATCTTCATC TGGATGATGAGCAACAACAAGCGTGTGCGCTATGCCC GTGTGCCCTCCAAGGACCTGCTCTTCTCCATCGTGGA GGAGGAGACTGGCAAGGACTGCGCCAAGGTCAAGAC GCTCTTCCTTAAGCTGCCAGGGAAGCGGGGCTTCGGC TCGGCAGGCTGGACAGTGCAGGCCAAGGTGGAGCTG TACCTGTGGCTGGGCCTCAGCAAACAGCGCAAGGAGT TCCTGTGCGGCCTGCCCTGTGGCTTCCAGGAGGTCAA GGCAGCCCAGGGCCTGGGCCTGCATGCCTTCCCACC CGTCAGCCTGGTCTACACCAAGAAGCAGGCGTTCCAG CTCCGAGCGCACATGTACCAGGCCCGCAGCCTCTTTG CCGCCGACAGCAGCGGACTCTCAGACCCCTTTGCCCG CGTCTTCTTCATCAATCAGAGTCAGTGCACAGAGGTGC TGAATGAGACCCTGTGTCCCACCTGGGACCAGATGCT GGTGTTGACAACCTGGAGCTCTATGGTGAAGCTCAT GAGCTGAGGGACGATCCGCCCATCATTGTCATTGAAA TCTATGACCAGGATTCCATGGGCAAAGCTGACTTCATG GGCCGGACCTTCGCCAAACCCCTGGTGAAGATGGCA GACGAGGCGTACTGCCCACCCCGCTTCCCACCTCAGC TCGAGTACTACCAGATCTACCGTGGCAACGCCACAGC

SEQ ID NO.	Description	Plasmid Sequence
		TGGAGACCTGCTGGCGGCCTTCGAGCTGCTGCAGATT GGACCAGCAGGGAAGGCTGACCTGCCCCCATCAAT GGCCCGGTGGACGTGGACCGAGGTCCCATCATGCCC GTGCCCATGGGCATCCGGCCCGTGCTCAGCAAGTACC GAGTGGAGGTGCTGTTCTGGGGCCTACGGGACCTAAA GCGGGTGAACCTGGCCCAGGTGGACCGGCCACGGGT GGACATCGAGTGTGCAGGGAAGGGGGTGCAGTCGTC CCTGATCCACAATTATAAGAAGAACCCCAACTTCAACA CCCTCGTCAAGTGGTTTGAAGTGGACCTCCCAGAGAA CGAGCTGCTGCACCCGCCCTTGAACATCCGTGTGGTG GACTGCCGGGCCTTCGGTCGCTACACACTGGTGGGCT CCCATGCCGTCAGCTCCCTGCGACGCTTCATCTACCG GCCCCAGACCGCTCGGCCCCCAGCTGGAACACCAC GGTCAGGCTTCTCCGGCGCTGCCGTGTGCTGTGCAAT GGGGGCTCCTCCTCTCACTCCACAGGGGAGGTTGTG GTGACTATGGAGCCAGAGGTACCCATCAAGAACTGG AGACCATGGTGAAGCTGGACGCGACTTCTGAAGCTGT TGTCAAGGTGGATGTGGCTGAGGAGGAGAAGGAGAA GAAGAAGAAGAAGAAGGGCACTGCGGAGGAGCCAGA GGAGGAGGAGCCAGACGAGAGCATGCTGGACTGGTG GTCCAAGTACTTTGCCTCCATTGACACCATGAAGGAGC AACTTCGACAACAAGAGCCCTCTGGAATTGACTTGGA GGAGAAGGAGGAAGTGGACAATACCGAGGGCCTGAA GGGGTCAATGAAGGGCAAGGAGAAGGCAAGGGCTGC CAAAGAGGAGAAGAAGAAGAACTCAGAGCTCTGGC TCTGGCCAGGGGTCCGAGGCCCGGAGAAGAAGAAA CCCAAGATTGATGAGCTTAAGGTATACCCCAAAGAGCT GGAGTCCGAGTTTGATAACTTTGAGGACTGGCTGCAC ACTTTCAACTTGCTTCGGGGCAAGACCGGGGATGATG AGGATGGCTCCACCGAGGAGGAGCGCATTGTGGGAC GCTTCAAGGGCTCCCTCTGCGTGTACAAAGTGCCACT CCCAGAGGACGTGTCCCGGAAGCCGGCTACGACTC CACCTACGGCATGTTCCAGGGCATCCCAGCAATGAC CCCATCAATGTGCTGGTCCGAGTCTATGTGGTCCGGG CCACGGACCTGCACCCTGCTGACATCAACGGCAAAGC TGACCCCTACATCGCCATCCGGCTAGGCAAGACTGAC ATCCGCGACAAGGAGAAGTACATCTCCAAGCAGCTCA ACCCTGTCTTTGGAAGTCCTTTGACATCGAGGCCTC

SEQ ID NO.	Description	Plasmid Sequence
		CTTCCCCATGGAATCCATGCTGACGGTGGCTGTGTAT GACTGGGACCTGGTGGGCACTGATGACCTCATTGGGG AAACCAAGATCGACCTGGAGAACCGCTTCTACAGCAA GCACCGCGCCACCTGCGGCATCGCCCAGACCTACTC CACACATGGCTACAATATCTGGCGGGACCCCATGAAG CCCAGCCAGATCCTGACCCGCCTCTGCAAAGACGGCA AAGTGGACGGCCCCCACTTTGGGCCCCCTGGGAGAG TGAAGGTGGCCAACCGCGTCTTCACTGGGCCCTCTGA GATTGAGGACGAGAACGGTCAGAGGAAGCCCACAGA CGAGCATGTGGCGCTGTTGGCCCTGAGGCACTGGGA GGACATCCCCCGCGCAGGCTGCCGCCTGGTGCCAGA GCATGTGGAGACGAGGCCGCTGCTCAACCCCGACAA GCCGGGCATCGAGCAGGGCCGCCTGGAGCTGTGGGT GGACATGTTCCCCATGGACATGCCAGCCCCTGGGACG CCTCTGGACATCTCACCTCGGAAGCCCAAGAAGTACG AGCTGCGGGTTCATCATCTGGAACACAGATGAGGTGGT CTTGAGGACGACGACTTCTTACAGGGGAGAAGTCC AGTGACATCTTCGTGAGGGGGTGGCTGAAGGGCCAG CAGGAGGACAAGCAGGACACAGACGTCCACTACCACT CCCTCACTGGCGAGGGCAACTTCAACTGGCGCTACCT GTTCCCCTTCGACTACCTGGCGGCGGAGGAGAAGATC GTCATCTCCAAGAAGGAGTCCATGTTCTCCTGGGACG AGACCGAGTACAAGATCCCCGCGCGGCTCACCCTGCA GATCTGGGATGCGGACCACTTCTCCGCTGACGACTTC CTGGGGGCCATCGAGCTGGACCTGAACCGGTTCCCG CGGGGCGCAAAGACAGCCAAGCAGTGCAACCATGGAG ATGGCCACCGGGGAGGTGGACGTGCCCCCTCGTGTCC ATCTTCAAGCAAAAGCGCGTCAAAGGCTGGTGGCCCC TCCTGGCCCGCAATGAGAACGATGAGTTTGAGCTCAC GGGCAAGGTGGAGGCTGAGCTGCATTTACTGACAGCA GAGGAGGCAGAGAAGAACCAGTGGGCCTGGCCCCG AATGAACCTGACCCCCTAGAGAAACCAACCGGCCCG ACACGGCCTTCGTCTGGTTCCTCAACCCTCTCAAGTCC ATCAAGTACCTCATCTGCACCCGGTACAAGTGGCTCAT CATCAAGATCGTGCTGGCGCTGTTGGGGCTGCTCATG TTGGGGCTCTTCTCTACAGCCTCCCTGGCTACATGG TCAAAAAGCTCCTTGGGGCATGAACGGCCGCTATGCT AGCTTGGTACCAAGGGCGGATCCTGCATAGAGCTCGC

SEQ ID NO.	Description	Plasmid Sequence
		TGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC TGTTGTTTGCCCCTCCCCGTGCCTTCCTTGACCCTG GAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGA GGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTA TTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGG GAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAG AGATCTGAGGACTAGTCCGTCGACTGGGGAGAGATCT GAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTC TGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCAA AGCCCGGGCGTCGGGGCGACCTTTGGTCGCCCGGCCT CAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCA ACCCCCCCCCCCCCCCCCCTGCAGCCTGGCGTAATAG CGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTG CGTAGCCTGAATGGCGAATGGCGCGACGCGCCCTGT AGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACG CGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCG CCCGCTCCTTTGCTTTCTTCCCTTCTTTCTCGCCAC GTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGG CTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCT CGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTA GTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTT GACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGT TCCAACTGGAACAACACTCAACCCTATCGCGGTCTAT TCTTTTGATTTATAAGGGATGTTGCCGATTTGGGCCTA TTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACA AAATTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGC GATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAG CACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCT TCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATA GCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAA TCCAGAAAAGCGGCCATTTTCCACCATGATATTGGGCA AGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCC GTCGGGCATGCTCGCCTTGAGCCTGGCGAACAGTTCG GCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCAT CCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGC TCGCTCGATGCGATGTTTCGCTTGGTGGTCAATGGG CAGGTAGCCGATCAAGCGTATGCAGCCGCCGCATTG CATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAG

SEQ ID NO.	Description	Plasmid Sequence
		GTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCC AATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGT CGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCA GCCACGATAGCCGCGCTGCCTCGTCTTGACAGTTCATT CAGGGCACCGGACAGGTGCGTCTTGACAAAAAGAACC GGGCGCCCCGCTGCGCTGACAGCCGGAACACGGCGGCA TCAGAGCAGCCGATTGTCTGTTGTGCCAGTCATAGC CGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGC GTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTC ATCCTGTCTCTTGATCAGATCTTGATCCCCTGCGCCAT CAGATCCTTGCGGCGAGAAAGCCATCCAGTTTACTTT GCAGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCT GGCAATTCCGGTTCGCTTGCTGTCCATAAACCGCCC AGTCTAGCTATCGCCATGTAAGCCCACTGCAAGCTAC CTGCTTTCTCTTGCGCTTGCGTTTTCCCTTGTCAGA TAGCCCAGTAGCTGACATTCATCCGGGGTCAGCACCG TTTCTGCGGACTGGCTTTCTACGTGAAAAGGATCTAGG TGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTT AACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGT AGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCT GCGCGTAATCTGCTGCTTGCAAACAAAAAACACCG CTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTAC CAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCG CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTT AGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACA TACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGC CAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAAGTCA AGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCT GAACGGGGGGTTTCGTGCACACAGCCAGCTTGGAGC GAACGACCTACACCGAACTGAGATACCTACAGCGTGA GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAAG GCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACA GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCC TGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTG ACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGG CGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTT TACGGTTTCTGGGCTTTTGCTGGCCTTTTGCTCACATG TTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCG

SEQ ID NO.	Description	Plasmid Sequence
		TATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAG GAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCC CCGCGCGTTGGCCGATTCATTAATGCAGGGCTGCA
64	5' transgene plasmid containing the following features: Lambda at positions 53-2027 ITR at positions 2049-2178 CMV i.e enhancer at positions 2267-2636 (part of smCBA promoter) Chicken β -actin promoter at positions 2633-2915 (part of smCBA promoter) Exon1 at positions 2916-3008 (part of smCBA promoter) Chimeric intron at positions 3008-3209 (part of smCBA promoter) Kozak sequence at positions 3226-3235 N-terminal portion of human OTOF isoform 5 at positions 3232-5637 Splice donor sequence at positions 5638-5721 AP head sequence at positions 5728-6014	TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACA CATGCAGCTCCCGGATAGAGGTCATCCTTCTGACCA TTCCATCATTCCAGTCGAACTCACACACAACACCAAA TGCATTTAAGTCGCTTGAAATTGCTATAAGCAGAGCAT GTTGCGCCAGCATGATTAATACAGCATTTAATACAGAG CCGTGTTTATTGAGTCGGTATTCAGAGTCTGACCAGAA ATTATTAATCTGGTGAAGTTATTCCTCTGTCAATACGTC ATGGTTCGATTTCAATTTCTATTGATGCTTTCCAGTCGTA ATCAATGATGTATTTTTTGATGTTTGACCTCTGTTTATA TCCTCACAGATAAAAAATCGCCCTCACACTGGAGGGC AAAGAAGATTTCCAATAATCAGAACAAGTCGGCTCCTG TTTAGTTACGAGCGACATTGCTCCGTGTATTCACTCGT TGGAATGAATACACAGTGCAGTGTTTATTCTGTTATTTA TGCCAAAAATTAAGGCCACTATCAGGCAGCTTTGTTGT TCTGTTTACCAAGTTCTCTGGCAATCATTGCCGTCGTT CGTATTGCCCATTTATCGACATATTTCCCATCTTCCTAT ACAGGAAACATTTCTTCAGGCTTAACCATGCATTCCGA TTGCAGCTTGCATCCATTGCATCGCTTGAATTGTCCAC ACCATTGATTTTTATCAATAGTCGTAGTTTAACGGATAG TCCTGGTATTGTTCCATCACATCCTGAGGATGCCCTTC GAACTCTTCAAATTCTTCTCCTAATATCACCTTAAATA GTGGATTGCGGTAGTAAAGATTGTGCCTGTCTTTAAC CACATCAGGCTCGGTGGTTCTCGTGTACCCCTACAGC GAGAAATCGGATAAACTATTACAACCCCTACAGTTTGT AGAGTATAGAAAATGATCCACTCGTTATTCTCGGACGA GTGTTTCAGTAATGAACCTCTGGAGAGAACCATCTATAT GATCGTTATCTGGGTTTGACTTCTGCTTTTAAGCCCAG ATAACTTGCCCTGAATATGTTAATGAGAGAATCGGTATT CCTCATGTGTGGCATGTTTTCGTCTTTGCTCTTGCAATT TCACTAGCAATTAATGTGCATCGATTATCAGCTATTGC CAGCGCCAGATATAAGCGATTTAAGCTAAGAAAACGCA TTAAGGTGCAAAACGATAAAGTGCGATCAGTAATTCAA AACCTTACAGGAGAGCAATCTATGGTTTTGTGCTCAGC CCTTAATGAAGGCAGGTAGTATGTGGTTACATCAAAAC

SEQ ID NO.	Description	Plasmid Sequence
	ITR at positions 6108-6237 Lambda at positions 6248-8260 Ori at positions 8317-8905 KanR at positions 9083-9892 Transgene to be transferred into vector in dual vector system at positions 2049-6237	AATCCCCATACATTAGTGAGTTGATTGAGCTTGGTGTG TTGAACAAAACCTTTTCCCGATGGAATGGAAAGCATAT ATTATCCCTATTGAGGATATTTACTGGACTGAATTAGT TGCCAGCTATGATCCATATAATATTGAGATAAAGCCAA GGCCAATATCTAAGTAACTAGATAAGAGGAATCGATTT TCCCTTAATTTTCTGGCGTCCACTGCATGTTATGCCGC GTTCCGCCAGGCTTGCTGTACCATGTGCGCTGATTCTT GCGCTCAATACGTTGCAGGTTGCTTCAATCTGTTTGT GGTATTCAGCCAGCACTGTAAGGTCTATCGGATTTAGT GCGCTTTCTACTCGTGATTTGCGTTTGCGATTGACGGA GAGAATAGGGCGGTAACTGGTTTTGCGCTTACCCCA ACCAACAGGGGATTTGCTGCTTCCATTGAGCCTGTTA CTCTGCGCGACGTTGCGGGCGGCGTGTTTGTGCATCC ATCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTGTG GCAGTTGTAGTCCTGAACGAAAACCCCCGCGATTGG CACGTTGGCAGCTAATCCGGAATCGCACTTACGGCCA ATGCTTCGTTTCGTATCACACACCCCAAAGCCTTCTGC TTTGAATGCTGCCCTTCTTCAGGGCTTAATTTTAAAGA GCGTCACCTTCATGGTGGTCAGTGCGTCCTGCTGATG TGCTCAGGCACGATTTAATTAAGGCCTTAATTAGGCTG CGCGCTCGCTCGCTCACTGAGGCGCGCCGGGCAAAG CCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCA GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGCCCAAC TCCATCACTAGGGGTTCTTGATGTTAATGATTAACCC GCCATGCTACTTATCTACGTAGCCATGCTCTAGGAAGA TCGGAATTCGCCCTTAAGCTAGCGGCGCGCCGGTACC TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGG TAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCC GCCATTGACGTCAATAATGACGTATGTTCCCATAGTA ACGCCAATAGGGACTTTCATTGACGTCAATGGGTGG AGTATTTACGGTAACTGCCCACTTGGCAGTACATCAA GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAA TGACGGTAAATGGCCCCGCTGGCATTATGCCAGTAC ATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA CGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCC CCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCC CCACCCCCAATTTTGTATTTATTTATTTTAAATATTTT

SEQ ID NO.	Description	Plasmid Sequence
		GTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGCG CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAA TCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCG AGGCGGCGGGCGGCGGGCGGCCCTATAAAAAGCGAAGC GCGCGGCGGGGCGGGAGTCGCTGCGCGCTGCCTTCGC CCCGTGCCCCGCTCCGCCGCCGCTCGCGCCGCCCG CCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGA GCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTA GCGCTTGTTTAATGACGGCTTGTTTCTTTTCTGTGGC TGCGTGAAAGCCTTGAGGGGCTCCGGGAGCTAGAGC CTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTCTACA GCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCAT CATTTTGGCAAAGAATTCTAGCGGCCGCCACCATGGC CTTGCTCATCCACCTCAAGACAGTCTCGGAGCTGCGG GGCAGGGGGCGACCGGATCGCCAAAGTGACTTTCGGA GGGCAATCCTTCTACTCTCGGGTCCTGGAGAACTGTG AGGATGTGGCTGACTTTGATGAGACATTTCCGGTGGCC GGTGGCCAGCAGCATCGACAGAAATGAGATGCTGGAG ATTCAGGTTTTCAACTACAGCAAAGTCTTCAGCAACAA GCTCATCGGGACCTTCCGCATGGTGCTGCAGAAGGTG GTAGAGGAGAGCCATGTGGAGGTGACTGACACGCTGA TTGATGACAACAATGCTATCATCAAGACCAGCCTGTGC GTGGAGGTCCGGTATCAGGCCACTGACGGCACAGTG GGCTCCTGGGACGATGGGGACTTCTGGGAGATGAG TCTCTTCAAGAGGAAGAGAAGGACAGCCAAGAGACGG ATGGACTGCTCCCAGGCTCCCGGCCAGCTCCCGGC CCCCAGGAGAGAAGAGCTTCCGGAGAGCCGGGAGGA GCGTGTTCTCCGCCATGAAGCTCGGC AAAAACCGGTC TCACAAGGAGGAGCCCCAAAGACCAGATGAACCGGC GGTGCTGGAGATGGAAGACCTTGACCATCTGGCCATT CGGCTAGGAGATGGACTGGATCCCGACTCGGTGTCTC TAGCCTCAGTCACAGCTCTCACCCTAATGTCTCCAAC AAGCGATCTAAGCCAGACATTAAGATGGAGCCAAGTG CTGGGCGGCCCATGGATTACCAGGTCAGCATCACGGT GATCGAGGCCCGGCAGCTGGTGGGCTTGAACATGGA CCCTGTGGTGTGCGTGGAGGTGGGTGACGACAAGAA GTACACATCCATGAAGGAGTCCACTAACTGCCCTATT

SEQ ID NO.	Description	Plasmid Sequence
		ACAACGAGTACTTCGTCTTCGACTTCCATGTCTCTCCG GATGTCATGTTTGACAAGATCATCAAGATTTCCGGTGAT TCACTCCAAGAACCTGCTGCGCAGTGGCACCCCTGGTG GGCTCCTTCAAAATGGACGTGGGAACCGTGTACTCGC AGCCAGAGCACCAAGTCCATCACAAGTGGGCCATCCT GTCTGACCCCGATGACATCTCCTCGGGGCTGAAGGGC TACGTGAAGTGTGACGTTGCCGTGGTGGGCAAAGGG GACAACATCAAGACGCCCCACAAGGCCAATGAGACCG ACGAAGATGACATTGAGGGGAAC TTGCTGCTCCCCGA GGGGGTGCCCCCGAACGCCAGTGGGCCCCGGTTCTA TGTGAAAATTTACCGAGCAGAGGGGCTGCCCCGTATG AACACAAGCCTCATGGCCAATGTAAAGAAGGCTTTCAT CGGTGAAAACAAGGACCTCGTGGACCCCTACGTGCAA GTCTTCTTTGCTGGCCAGAAGGGCAAGACTTCAGTGC AGAAGAGCAGCTATGAGCCCCTGTGGAATGAGCAGGT CGTCTTTACAGACCTCTTCCCCCCTCTGCAAACGCA TGAAGGTGCAGATCCGAGACTCGGACAAGGTCAACGA CGTGGCCATCGGCACCCACTTCATTGACCTGCGCAAG ATTTCTAATGACGGAGACAAAGGCTTCTGCCCCACCT GGGCCCAGCCTGGGTGAACATGTACGGCTCCACACGT AACTACACGCTGCTGGATGAGCATCAGGACCTGAACG AGGGCCTGGGGGAGGGTGTGTCCTTCCGGGCCCCGGC TCCTGCTGGGCCTGGCTGTGGAGATCGTAGACACCTC CAACCCTGAGCTCACCAGCTCCACAGAGGTGCAGGTG GAGCAGGCCACGCCATCTCGGAGAGCTGTGCAGGT AAAATGGAAGAATTCTTTCTTTGGAGCCTTCTGGA GGCCTCAATGATCGACCGGAGAAACGGAGACAAGCCC ATCACCTTTGAGGTCACCATAGGCAACTATGGGAACG AAGTTGATGGCCTGTCCCGGCCCCAGCGGCCTCGGC CCCGGAAGGAGCCGGGGGATGAGGAAGAAGTAGACC TGATTGAGAACGCAAGTGATGACGAGGCCGGTGATGC CGGGGACCTGGCCTCAGTCTCCTCCACTCCACCAATG CGGCCCCAGGTCACCGACAGGAAC TACTTCCATCTGC CCTACCTGGAGCGAAAGCCCTGCATCTACATCAAGAG CTGGTGGCCGGACCAGCGCCGCCGCTCTACAATGC CAACATCATGGACCACATTGCCGACAAGCTGGAAGAA GGCCTGAACGACATACAGGAGATGATCAAAACGGAGA AGTCCTACCCTGAGCGTCGCCTGCGGGGCGTCTCTGG

SEQ ID NO.	Description	Plasmid Sequence
		AGGAGCTGAGCTGTGGCTGCTGCCGCTTCCTCTCCCT CGCTGACAAGGACCAGGGCCACTCATCCCGCACCAG GCTTGACCGGGAGCGCCTCAAGTCCTGCATGAGGGA GCTGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGA GACCAATAGAACTGGGCTTGTCGAGACAGAGAAGAC TCTTGCGTTTTCTGAGCTAGCCCCCGGGTGCGCGGCGT CGGTGGTGCCGGCGGGGGGCGCCAGGTCGCAGGCG GTGTAGGGCTCCAGGCAGGCGGCGAAGGCCATGACG TGCGCTATGAAGGTCTGCTCCTGCACGCCGTGAACCA GGTGCGCCTGCGGGCCGCGCGCAACACCGCCACGT CCTCGCCTGCGTGGGTCTCTTCGTCCAGGGGCACTGC TGA CTGCTGCCGATACTCGGGGCTCCCGCTCTCGCTC TCGGTAACATCCGGCCGGGCGCCGTCCTTGAGCACAT AGCCTGGACCGTTTCGTGACCTCGAGTTAAGGGCGA ATTCCCATAAGGATCTTCCTAGAGCATGGCTACGTAG ATAAGTAGCATGGCGGGTTAATCATTAACTACAAGGAA CCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCG CTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTGCG CCCGACGCCCGGGCTTTGCCGGGCGGCCTCAGTGA GCGAGCGAGCGCGCAGCCTTAATTAATCCACATCTG TATGTTTTTATATTAATTTATTTTTTGAGGGGGGCAT TGTTTGGTAGGTGAGAGTTCTGAATTGCTATGTTTAGT GAGTTGTATCTATTTATTTTTCAATAAATACAATTAGTTA TGTGTTTTGGGGGCGATCGTGAGGCAAAGAAAACCCG GCGCTGAGGCCGGGTATTCTTGTTCTCTGGTCAAATT ATATAGTTGGAACAAGGATGCATATATGAATGAACG ATGCAGAGGCAATGCCGATGGCGATAGTGGGTATCAG GTAGCCGCTTATGCTGGAAGAAGCAATAACCCGCAG AAAAACAAAGCTCCAAGCTCAACAAAACTAAGGGCATA GACAATAACTACCTATGTCATATACCCATACTCTCTAAT CTTGCCAGTCGGCGCGTTCTGCTTCCGATTAGAAAC GTCAAGGCAGCAATCAGGATTGCAATCTTGGTTCCTG CATAGGATGACAATGTCGCCCCAAGACCATCTCTATGA GCTGAAAAAGAAACACAAGGAATGTAGTGGCGGAAAA GGAGATAGCAAATGCTTACGATAACGTAAGGAATTATT ACTATGTAAACACCAGGCAAGATTCTGTTCCGTATAAT TACTCCTGATAATTAATCCTTAACCTTTGCCACCTGCCT TTTAAACATTCCAGTATATCACTTTTCATTCTTGCCTA

SEQ ID NO.	Description	Plasmid Sequence
		GCAATATGCCCTCTCTTCAGCTATCTCAGCATTGGTGA CCTTGTTGAGAGGCGCTGAGAGATGGCCTTTTTCTGAT AGATAATGTTCTGTAAAAATATCTCCGGCCTCATCTTTT GCCCCGAGGCTAATGTCTGAAAATTGAGGTGACGGGT TAAAAATAATATCCTTGCCAACCTTTTTTATATCCCTTTT AAATTTTGGCTTAATGACTATATCCAATGAGTCAAAAAG CTCCCCTTCAATATCTGTTGCCCTAAGACCTTTAATAT ATCGCCAAATACAGGTAGCTTGGCTTCTACCTTCACCG TTGTTCTGCCGATGAAATGCTAATGCATAACATCGTCT TTGGTGGTTCCCCTCATCAGTGGCTCTATCTGAACGC GCTCTCCACTGCTTAATGACATTCCTTTCCCGATTAAA AAATCTGTGAGATCGGATGTGGTCGGCCCCGAAAACAG TTCTGGCAAACCAATGGTGTGCCTTCAACAAAACAAA AAAGATGGGAATCCCAATGATTCGTCTGCGAGGC TGTTCTTAATATCTTCAACTGTAGCTTTAGAGCGATTTA TCTTCTGAACCAGACTCTTGTCAATTTGTTTTGGTAAAGA GAAAAGTTTTTCCATCGATTTTATGAATATACAAATAAT TGGAGCCAACCTTCAGGTGATGATTATCAGCCAGCAG AGAATTAAGGAAAACAGACAGGTTTATTGAGCACTTAT CTTCCCTTTATTTTTGCTGCGGTAAGTCGCATAAAAAC CATTCTTACAATTCAATCCATTTACTATGTTATGTTCT GAGGGGAGTGAAAATCCCCTAATTCGATGAAGATTCT TGCTAAATTGTTATCAGCTATGCGCCGACCAGAACACC TTGCCGATCAGCCAAACGTCTAATCAGGCCACTGACTA GCGATAACTTTCCCCACAACGGAACAACTCTCATTGCA TGGGATAATTGGGTACTGTGGGTTTAGTGGTTGTAAAA ACACCTGACCGCTATCCCTGATCAGTTTCTTGAAGGTA AACTCATCACCCCAAGTCTGGCTATACAGAAATCACC TGGCTCAACAGCCTGCTCAGGGTCAACGAGAATTTAC ATTCCGTCAGGATAGCTTGGCTTGGAGCCTGTTGGTG CGGTCAACGAATTACCTTCAACCTCAAGCCAGAATGC AGAATCACTGGCTTTTTTGGTTGTGCTTACCCATCTCT CCGCATCACCTTTGGTAAAGGTTCTAAGCTAAGGTGAG AACATCCCTGCCTGAACATGAGAAAAAACAGGGTACTC ATACTCACTTATTAGTGACGGCTATGAGCAAAAGGCCA GCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTG GCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATC ACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC

SEQ ID NO.	Description	Plasmid Sequence
		GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGA AGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGC TTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAG CGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCA GTTCCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGT GCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTA TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACA CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGG ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAG AACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA CCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAA ACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCA AGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGA AGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGA TTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAA AAATGAAGTTTTAAATCAAGCCCAATCTGAATAATGTTA CAACCAATTAACCAATTCTGATTAGAAAACTCATCGA GCATCAAAATGAACTGCAATTTATTCATATCAGGATTAT CAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAG GAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAG ATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACAT CAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGT TATCAAGTGAGAAATCACCATGAGTGACGACTGAATCC GGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGAC TTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAAT CACTCGCATCAACCAAACCGTTATTCATTCTGTATTGC GCCTGAGCAAGACGAAATACGCGATCGCTGTAAAAAG GACAATTACAAACAGGAATCGAATGCAACCGGCGCAG GAACACTGCCAGCGCATCAACAATATTTTCACCTGAAT CAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCG GGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAG TACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAAT TCCGTGAGCCAGTTTAGTCTGACCATCTCATCTGTAAC ATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACA ACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGAT TGTCGCACCTGATTGCCCCGACATTATCGCGAGCCCAT

SEQ ID NO.	Description	Plasmid Sequence
		TTATACCCATATAAATCAGCATCCATGTTGGAATTTAAT CGCGGCCTCGACGTTTCCCGTTGAATATGGCTCATAA CACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTTA TTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAAC ATCAGAGATTTTGAGACACGGGCCAGAGCTGCA
65	5' transgene plasmid containing the following features: ITR at positions 19-161 CMV enhancer at positions 177-546 (part of smCBA promoter) Chicken β -actin promoter at positions 548-825 (part of smCBA promoter) Exon1 at positions 826-918 (part of smCBA promoter) Chimeric intron at positions 918-1119 (part of smCBA promoter) N-terminal portion of human OTOF isoform 5 at positions 1142-3547 Splice donor sequence at positions 3548-3631 AP head sequence at positions 3638-3924 ITR at positions 3948-4090 KanR at positions 4616-5410	GGGGGGGGGGGGGGGGGGTGGCCACTCCCTCTCT GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAA GGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTC AGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA CTCCATCACTAGGGGTTCCCTCAGATCTGAATTCGGTAC CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTC ATAGCCCATATATGGAGTTCGCGTTACATAACTTACG GTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGT AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTG GAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCA AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCA ATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTA CATGACCTTATGGGACTTTCTACTTGGCAGTACATCT ACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGC CCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTC CCCACCCCCAATTTTGTATTTATTTATTTTAAATTATTT TGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGCGG CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAA TCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCG AGGCGGCGGGCGGCGGCGGCCCTATAAAAAGCGAAGC GCGCGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGC CCCGTGCCCCGCTCCGCGCGCCCTCGCGCGGCCCG CCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGA GCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTA GCGCTTGTTTAAATGACGGCTTGTTTCTTTCTGTGGC TGCGTGAAAGCCTTGAGGGGCTCCGGGAGCTAGAGC CTCTGCTAACCATGTTTCATGCCTTCTTTTCTTCTACA GCTCCTGGGCAACGTGCTGTTATTGTGCTGTCTCAT CATTTTGGCAAAGAATTCTAGCGGCCGCCACCATGGC CTTGCTCATCCACCTCAAGACAGTCTCGGAGCTGCGG GGCAGGGGCGACCGGATCGCCAAAGTGACTTTCCGA

SEQ ID NO.	Description	Plasmid Sequence
	pUC ori at positions 5796-6466 Transgene to be transferred into vector in dual vector system at positions 19-4090	GGGCAATCCTTCTACTCTCGGGTCCTGGAGAACTGTG AGGATGTGGCTGACTTTGATGAGACATTTCCGGTGGCC GGTGGCCAGCAGCATCGACAGAAATGAGATGCTGGAG ATTCAGGTTTTCAACTACAGCAAAGTCTTCAGCAACAA GCTCATCGGGACCTTCCGCATGGTGTGCTGAGAAGGTG GTAGAGGAGAGCCATGTGGAGGTGACTGACACGCTGA TTGATGACAACAATGCTATCATCAAGACCAGCCTGTGC GTGGAGGTCCGGTATCAGGCCACTGACGGCACAGTG GGCTCCTGGGACGATGGGGACTTCCTGGGAGATGAG TCTCTTCAAGAGGAAGAGAAGGACAGCCAAGAGACGG ATGGACTGCTCCCAGGCTCCCGGCCAGCTCCCGGC CCCCAGGAGAGAAGAGCTTCCGGAGAGCCGGGAGGA GCGTGTTCTCCGCCATGAAGCTCGGC AAAACCGGTC TCACAAGGAGGAGCCCCAAAGACCAGATGAACCGGC GGTGCTGGAGATGGAAGACCTTGACCATCTGGCCATT CGGCTAGGAGATGGACTGGATCCCGACTCGGTGTCTC TAGCCTCAGTCACAGCTCTCACCCTAATGTCTCCAAC AAGCGATCTAAGCCAGACATTAAGATGGAGCCAAGTG CTGGGCGGCCCATGGATTACCAGGTCAGCATCACGGT GATCGAGGCCCCGGCAGCTGGTGGGCTTGAACATGGA CCCTGTGGTGTGCGTGGAGGTGGGTGACGACAAGAA GTACACATCCATGAAGGAGTCCACTAACTGCCCCCTATT ACAACGAGTACTTCGTCTTCGACTTCCATGTCTCTCCG GATGTCATGTTTGACAAGATCATCAAGATTTCCGTGAT TCACTCCAAGAACCTGCTGCGCAGTGGCACCCCTGGTG GGCTCCTTCAAAATGGACGTGGGAACCGTGTACTCGC AGCCAGAGCACCAAGTTCCATCACAAGTGGGCCATCCT GTCTGACCCCGATGACATCTCCTCGGGGCTGAAGGGC TACGTGAAGTGTGACGTTGCCGTGGTGGGCAAAGGG GACAACATCAAGACGCCCCACAAGGCCAATGAGACCG ACGAAGATGACATTGAGGGGAAGTCTGCTCCCCGA GGGGGTGCCCCCGAACGCCAGTGGGCCCCGTTCTA TGTGAAAATTTACCGAGCAGAGGGGCTGCCCCGTATG AACACAAGCCTCATGGCCAATGTAAAGAAGGCTTTCAT CGGTGAAAACAAGGACCTCGTGGACCCCTACGTGCAA GTCTTCTTTGCTGGCCAGAAGGGCAAGACTTCAGTGC AGAAGAGCAGCTATGAGCCCCTGTGGAATGAGCAGGT CGTCTTTACAGACCTCTTCCCCCACTCTGCAACGCA

SEQ ID NO.	Description	Plasmid Sequence
		TGAAGGTGCAGATCCGAGACTCGGACAAGGTCAACGA CGTGGCCATCGGCACCCACTTCATTGACCTGCGCAAG ATTTCTAATGACGGAGACAAAGGCTTCCTGCCCACACT GGGCCCAGCCTGGGTGAACATGTACGGCTCCACACGT AACTACACGCTGCTGGATGAGCATCAGGACCTGAACG AGGGCCTGGGGGAGGGTGTGTCTTCCGGGCCCCGGC TCCTGCTGGGCCTGGCTGTGGAGATCGTAGACACCTC CAACCCTGAGCTCACCAGCTCCACAGAGGTGCAGGTG GAGCAGGCCACGCCCATCTCGGAGAGCTGTGCAGGT AAAATGGAAGAATTCTTTCTCTTTGGAGCCTTCCTGGA GGCCTCAATGATCGACCGGAGAAACGGAGACAAGCCC ATCACCTTTGAGGTCACCATAGGCAACTATGGGAACG AAGTTGATGGCCTGTCCCGCCCCAGCGGCCTCGGC CCCGGAAGGAGCCGGGGGATGAGGAAGAAGTAGACC TGATTCAGAACGCAAGTGATGACGAGGCCGGTGATGC CGGGGACCTGGCCTCAGTCTCCTCCACTCCACCAATG CGGCCCCAGGTCACCGACAGGAACTACTTCCATCTGC CCTACCTGGAGCGAAAGCCCTGCATCTACATCAAGAG CTGGTGGCCGGACCAGCGCCGCGCCTCTACAATGC CAACATCATGGACCACATTGCCGACAAGCTGGAAGAA GGCCTGAACGACATACAGGAGATGATCAAAACGGAGA AGTCCTACCCTGAGCGTCGCCTGCGGGGCGTCCTGG AGGAGCTGAGCTGTGGCTGCTGCCGCTTCCTCTCCCT CGCTGACAAGGACCAGGGCCACTCATCCCGCACCAG GCTTGACCGGGAGCGCCTCAAGTCCTGCATGAGGGA GCTGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGA GACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGAC TCTTGCGTTTCTGAGCTAGCCCCCGGTGCGCGGCGT CGGTGGTGCCGGCGGGGGGCGCCAGTGCAGGGCG GTGTAGGGCTCCAGGCAGGCGGCGAAGGCCATGACG TGCGCTATGAAGGTCTGCTCCTGCACGCCGTGAACCA GGTGCGCCTGCGGGCCGCGCGGAACACCGCCACGT CCTCGCCTGCGTGGGTCTCTTCGTCCAGGGGCACTGC TGA CTGCTGCCGATACTCGGGGCTCCCGCTCTCGCTC TCGGTAACATCCGGCCGGGCGCCGTCCTTGAGCACAT AGCCTGGACCGTTTCGTGCGACTGGGGAGAGATCTGAG GAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGC GCGCTCGCTCGCTCACTGAGGCCGCCCCGGGCAAAGC

SEQ ID NO.	Description	Plasmid Sequence
		CCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAG TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACC CCCCCCCCCCCCCCTGCAGCCTGGCGTAATAGCG AAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCG TAGCCTGAATGGCGAATGGCGCGACGCGCCCTGTAG CGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCG CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCC GCTCCTTTTCGCTTTCTTCCCTTCTTCTCGCCACGTT CGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTC CCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGA CCCCAAAAAATTGATTAGGGTGATGGTTCACGTAGTG GGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGAC GTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCC AAACTGGAACAACACTCAACCCTATCGCGGTCTATTCT TTTGATTTATAAGGGATGTTGCCGATTTTCGGCCTATTG GTTAAAAAATGAGCTGATTTAACAAAAATTTTAACAAAA TTCAGAAGAAGTCAAGGCGATAGAAGGCGAT GCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCAC GAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCA GCAATATCACGGGTAGCCAACGCTATGTCCTGATAGC GGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCC AGAAAAGCGGCCATTTTCCACCATGATATTGGCAAGC AGGCATCGCCATGGGTACGACGAGATCCTCGCCGTC GGGCATGCTCGCCTTGAGCCTGGCGAACAGTTCGGCT GGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCT GATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCG CTCGATGCGATGTTTCGCTTGGTGGTGAATGGGCAG GTAGCCGATCAAGCGTATGCAGCCGCCGATTGCAT CAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTG AGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAAT AGCAGCCAGTCCCTTCCCGCTCAGTGACAACGTGCA GCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCC ACGATAGCCGCGCTGCCTCGTCTTGAGTTTATTAG GGCACCGGACAGGTGGTCTTGACAAAAAGAACCGG GCGCCCCCTGCGCTGACAGCCGGAACACGGCGGCATC AGAGCAGCCGATTGTCTGTTGTGCCAGTCATAGCCG AATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGT GCAATCCATCTTGTTCAATCATGCGAAACGATCCTCAT

SEQ ID NO.	Description	Plasmid Sequence
		CCTGTCTCTTGATCAGATCTTGATCCCCTGCGCCATCA GATCCTTGGCGGCGAGAAAGCCATCCAGTTTACTTTG CAGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCTG GCAATTCGGGTTGCTTGCTGTCCATAAAACCGCCCA GTCTAGCTATCGCCATGTAAGCCCACTGCAAGCTACCT GCTTTCTCTTTGCGCTTGCGTTTTCCCTTGTCAGATA GCCCAGTAGCTGACATTCATCCGGGGTCAGCACCGTT TCTGCGGACTGGCTTTCTACGTGAAAAGGATCTAGGT GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTA GAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTG CGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCT ACCAGCGGTGGTTTGTGTCGGGATCAAGAGCTACCA ACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCA GATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAG GCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC CTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCA GTGGCGATAAGTCGTGTCTTACCGGGTTGGAAGTCAAG ACGATAGTTACCGGATAAGGCGCAGCGGTGCGGGCTGA ACGGGGGGTTCGTGCACACAGCCCAGCTTGAGCGA ACGACCTACACCGAACTGAGATACCTACAGCGTGAGC TATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGC GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGG AGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG GTATCTTTATAGTCCTGTGCGGTTTCGCCACCTCTGAC TTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG GAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTA CGGTTCTGCGCTTTTGCTGGCCTTTTGCTCACATGTT CTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTA TTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAG CCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGA AGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC GCGCGTTGGCCGATTCATTAATGCAGGGCTGCA
66	5' transgene plasmid containing the following features: ITR at positions 12-141	CCTTAATTAGGCTGCGCGCTCGCTCGCTCACTGAGGC CGCCCGGGCAAAGCCCGGGCGTGGGGCGACCTTTGG TCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGAGTGGCCAACTCCATCACTAGGGGTTCTTGTA TTAATGATTAACCGCCATGCTACTTATCTACGTAGCC

SEQ ID NO.	Description	Plasmid Sequence
	Myo15 promoter at positions 235-1199	ATGCTCTAGGAAGATCGGAATTCGCCCTTAAGCTAGC GGCGCGCCCAATTCTGCAGCTCAGCCTACTACTTGCT TTCCAGGCTGTTCTAGTTCCCATGTCAGCTGCTTGTG
	Kozak sequence at positions 1216-1225	CTTTCCAGAGACAAAACAGGAATAATAGATGTCATTAA ATATACATTGGGCCCCAGGCGGTCAATGTGGCAGCCT GAGCCTCCTTTCCATCTCTGTGGAGGCAGACATAGGA
	N-terminal portion of human OTOF isoform 5 at positions 1222-3627	CCCCCAACAAACAGCATGCAGGTTGGGAGCCAGCCAC AGGACCCAGGTAAGGGGGCCCTGGGTCTTAAGCTTCT GCCACTGGCTCCGGCATTGCAGAGAGAAGAGAAGGG GCGGCAGACTGGAGAGCTGGGCTCCATTTTTGTTCT
	Splice donor sequence at positions 3628-3711	TGGTGCCCTGCCCTCCCCATGACCTGCAGAGACATT CAGCCTGCCAGGCTTTATGAGGTGGGAGCTGGGCTCT CCCTGATGTATTATTCAGCTCCCTGGAGTTGGCCAGCT
	AP head sequence at positions 3718-4004	CCTGTTACACTGGCCACAGCCCTGGGCATCCGCTTCT CACTTCTAGTTTCCCCTCCAAGGTAATGTGGTGGGTCA TGATCATTCTATCCTGGCTTCAGGGACCTGACTCCACT
	ITR at positions 4098-4227	TTGGGGCCATTGAGGGGTCTAGGGTAGATGATGTCC CCCTGTGGGGATTAATGTCCTGCTCTGTAAACTGAGC
	M13 fwd at positions 4246-4262	TAGCTGAGATCCAGGAGGGCTTGCCAGAGACAGCAA GTTGTTGCCATGGTGACTTTAAAGCCAGGTTGCTGCC
	f1 ori at positions 4404-4859	CCAGCACAGGCCTCCCAGTCTACCCTACTAGAAAAC AACACCCAGGCACTTTCCACCACCTCTCAAAGGTGAAA
	AmpR promoter at positions 4885-4989	CCCAAGGCTGGTCTAGAGAATGAATTATGGATCCTCG CTGTCCGTGCCACCCAGCTAGTCCCAGCGGCTCAGAC ACTGAGGAGAGACTGTAGGTTGAGCTACAAGCAAAAA
	KanR at positions 4990-5799	GACCTAGCTGGTCTCCAAGCAGTGTCTCCAAGTCCCT GAACCTGTGACACCTGCCCCAGGCATCATCAGGCACA GAGGGCCACCAAGAATTCTAGCGGCCGCCACCATGG
	mutBsmBI at positions 5430-5430	CCTTGCTCATCCACCTCAAGACAGTCTCGGAGCTGCG
	ori at positions 5970-6558	GGGCAGGGGCGACCGGATCGCCAAAGTGACTTTCCG AGGGCAATCCTTCTACTCTCGGGTCTGGAGAAGTGT
	CAP binding site at positions 6846-6867	GAGGATGTGGCTGACTTTGATGAGACATTTCCGTGGC CGGTGGCCAGCAGCATCGACAGAAATGAGATGCTGGA
	lac promoter at positions 6882-6912	GATTCAGGTTTTCACTACAGCAAAGTCTTCAGCAACA AGCTCATCGGGACCTTCCGCATGGTGCTGCAGAAGGT
	lac operator at positions 6920-6936	GGTAGAGGAGAGCCATGTGGAGGTGACTGACACGCT GATTGATGACAACAATGCTATCATCAAGACCAGCCTGT
	M13 rev at positions 6944-6960	GCGTGGAGGTCCGGTATCAGGCCACTGACGGCACAG

SEQ ID NO.	Description	Plasmid Sequence
	Transgene to be transferred into vector in dual vector system at positions 12-4227	TGGGCTCCTGGGACGATGGGGACTTCCTGGGAGATG AGTCTCTTCAAGAGGAAGAGAAGGACAGCCAAGAGAC GGATGGACTGCTCCCAGGCTCCCGGCCAGCTCCCG GCCCCAGGAGAGAAGAGCTTCGGAGAGCCGGGAG GAGCGTGTTCTCCGCCATGAAGCTCGGCAAAACCGG TCTCACAAGGAGGAGCCCCAAAGACCAGATGAACCGG CGGTGCTGGAGATGGAAGACCTTGACCATCTGGCCAT TCGGCTAGGAGATGGACTGGATCCCGACTCGGTGTCT CTAGCCTCAGTCACAGCTCTCACCCTAATGTCTCCAA CAAGCGATCTAAGCCAGACATTAAGATGGAGCCAAGT GCTGGGCGGCCCATGGATTACCAGGTCAGCATCACG GTGATCGAGGCCCGGCAGCTGGTGGGCTTGAACATG GACCCTGTGGTGTGCGTGGAGGTGGGTGACGACAAG AAGTACACATCCATGAAGGAGTCCACTAACTGCCCTA TTACAACGAGTACTTCGTCTTCGACTTCCATGTCTCTC CGGATGTCATGTTTGACAAGATCATCAAGATTTCCGGTG ATTCACTCCAAGAACCTGCTGCGCAGTGGCACCTGG TGGGCTCCTTCAAAATGGACGTGGGAACCGTGACTC GCAGCCAGAGCACCAGTTCCATCACAAGTGGGCCATC CTGTCTGACCCCGATGACATCTCCTCGGGGCTGAAGG GCTACGTGAAGTGTGACGTTGCCGTGGTGGGCAAAGG GGACAACATCAAGACGCCCCACAAGGCCAATGAGACC GACGAAGATGACATTGAGGGGAACCTTGCTGCTCCCG AGGGGGTGCCCCCGAACGCCAGTGGGCCCGGTTCT ATGTGAAAATTTACCGAGCAGAGGGGCTGCCCGTAT GAACACAAGCCTCATGGCCAATGTAAAGAAGGCTTTCA TCGGTGAAAACAAGGACCTCGTGGACCCCTACGTGCA AGTCTTCTTTGCTGGCCAGAAGGGCAAGACTTCAGTG CAGAAGAGCAGCTATGAGCCCCTGTGGAATGAGCAGG TCGTCTTTACAGACCTCTTCCCCCACTCTGCAAACGC ATGAAGGTGCAGATCCGAGACTCGGACAAGGTCAACG ACGTGGCCATCGGCACCCACTTCATTGACCTGCGCAA GATTTCTAATGACGGAGACAAAGGCTTCCTGCCACA CTGGGCCCAGCCTGGGTGAACATGTACGGCTCCACAC GTAATAACACGCTGCTGGATGAGCATCAGGACCTGAA CGAGGGCCTGGGGGAGGGTGTGTCCTTCGGGCCCCG GCTCCTGCTGGGCCTGGCTGTGGAGATCGTAGACACC TCCAACCCTGAGCTCACCAGCTCCACAGAGGTGCAGG

SEQ ID NO.	Description	Plasmid Sequence
		TGGAGCAGGCCACGCCCATCTCGGAGAGCTGTGCAG GTAAATGGAAGAATTCTTTCTCTTTGGAGCCTTCCTG GAGGCCTCAATGATCGACCGGAGAAACGGAGACAAGC CCATCACCTTTGAGGTCACCATAGGCAACTATGGGAA CGAAGTTGATGGCCTGTCCCGGCCCCAGCGGCCTCG GCCCCGGAAGGAGCCGGGGGATGAGGAAGAAGTAGA CCTGATTCAGAACGCAAGTGATGACGAGGCCGGTGAT GCCGGGGACCTGGCCTCAGTCTCCTCCACTCCACCAA TGCGGCCCCAGGTCACCGACAGGAACTACTTCCATCT GCCCTACCTGGAGCGAAAGCCCTGCATCTACATCAAG AGCTGGTGGCCGGACCAGCGCCGCCGCTCTACAAT GCCAACATCATGGACCACATTGCCGACAAGCTGGAAG AAGGCCTGAACGACATACAGGAGATGATCAAAACGGA GAAGTCCTACCCTGAGCGTCGCCTGCGGGGCGTCCT GGAGGAGCTGAGCTGTGGCTGCTGCCGCTTCCTCTCC CTCGCTGACAAGGACCAGGGCCACTCATCCCGCACCA GGCTTGACCGGGAGCGCCTCAAGTCCTGCATGAGGG AGCTGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGA GACCAATAGAACTGGGCTTGTCGAGACAGAGAAGAC TCTTGCGTTTCTGAGCTAGCCCCCGGGTGCGCGCGT CGGTGGTGCCGGCGGGGGGCGCCAGGTCGCAGGCG GTGTAGGGCTCCAGGCAGGCGGCGAAGGCCATGACG TGCGCTATGAAGGTCTGCTCCTGCACGCCGTGAACCA GGTGCGCCTGCGGGCCGCGCGGAACACCGCCACGT CCTCGCCTGCGTGGGTCTCTTCGTCCAGGGGCACTGC TGA CTGCTGCCGATACTCGGGGCTCCCGCTCTCGCTC TCGGTAACATCCGGCCGGGCGCCGTCCCTTGAGCACAT AGCCTGGACCGTTTCGTCGACCTCGAGTTAAGGGCGA ATTCCCGATAAGGATCTTCCTAGAGCATGGCTACGTAG ATAAGTAGCATGGCGGGTTAATCATTAACTACAAGGAA CCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCG CTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTGCG CCCGACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGA GCGAGCGAGCGCGCAGCCTTAATTAACCTAATCACT GGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCT GGCGTTACCCAACTTAATCGCCTTGACGACATCCCC CTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCAC CGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGC

SEQ ID NO.	Description	Plasmid Sequence
		GAATGGGACGCGCCCTGTAGCGGCGCATTAAAGCGCG GCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACA CTTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCT TCCCTTCCTTTCTCGCCACGTTGCGCGGCTTCCCCGT CAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGAT TTAGTGCTTTACGGCACCTCGACCCCCAAAAAAGTTGAT TAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT AGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTG TTAATAGTGGACTCTTGTTCCAACTGGAACAACACT CAACCCTATCTCGGTCTATTCTTTGATTATAAGGGAT TTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGA TTTAACAAAAATTTAACGCGAATTTTAACAAAAATTTAA CGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGC GCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAA ATATGTATCCGCTCATGAGACAATAACCCTGATAAATG CTTCAATAATATTGAAAAAGGAAGAGTATGAGCCATAT TCAACGGGAAACGTCGAGGCCGCGATTAAATTCCAAC ATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGA TAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGT ATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACA TGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAG ATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCT TCCGACCATCAAGCATTTTATCCGTACTCCTGATGATG CATGGTTACTCACCCTGCGATCCCCGAAAAACAGC ATTCCAGGTATTAGAAGAATATCCTGATTAGGTGAAA ATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTT GCATTGATTCTGTTTGTAAATTGTCTTTTAACAGCGA TCGCGTATTTTCGTCTTGCTCAGGCGCAATCACGAATGA ATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAG CGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAA TGCATAAACTTTTGCCATTCTACCGGATTGATCGTC ACTCATGGTGATTTCTCACTTGATAACCTTATTTTAC GAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGT CGGAATCGCAGACCGATACCAGGATCTTGCCATCCTA TGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAA ACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATAT GAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTT CTAACTGTCAGACCAAGTTTACTCATATATACTTTAGAT

SEQ ID NO.	Description	Plasmid Sequence
		TGATTTAAACTTCATTTTTAATTTAAAGGATCTAGGT GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTA GAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTG CGCGTAATCTGCTGCTTGCAACAAAAAACACCGCT ACCAGCGGTGGTTTGTGGCCGATCAAGAGCTACCA ACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCA GATACCAAATACTGTTCTTAGTGTAGCCGTAGTTAG GCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC CTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTGCCA GTGGCGATAAGTCGTGTCTTACCGGGTTGACTCAAG ACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGA ACGGGGGGTTCGTGCACACAGCCAGCTTGAGCGA ACGACCTACACCGAACTGAGATACCTACAGCGTGAGC TATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGC GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGG AGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTG GTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGAC TTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG GAGCCTATGAAAAACGCCAGCAACGCGGCCTTTTTTA CGGTTCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTA TTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAG CCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGA AGCGGAAGAGCGCCCAATACGAAACCGCCTCTCCCC GCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACA GGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACG CAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAG GCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGG AATTGTGAGCGGATAACAATTTACACAGGAAACAGCT ATGACCATGATTACGCCAGATTTAATTAAGG
67	3' transgene plasmid containing the following features: ITR at positions 12-141 AP head sequence at positions 229-515	CCTTAATTAGGCTGCGCGCTCGCTCGCTCACTGAGGC CGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGG TCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGAGTGGCCAACTCCATCACTAGGGGTTCTTGTTAG TTAATGATTAACCCGCCATGCTACTTATCTACGTAGCC ATGCTCTAGGAAGATCGGAATTCGCCCTTAAGCTAGC GGCGCGCCCCCGGGTGCGCGGCGTCGGTGGTGCCG

SEQ ID NO.	Description	Plasmid Sequence
	Splice acceptor sequence at positions 538-586	GCGGGGGGCGCCAGGTCGCAGGCGGTGTAGGGCTC
	C-terminal portion of human OTOF isoform 5 at positions 587-4174	CAGGCAGGCGGCGAAGGCCATGACGTGCGCTATGAA GGTCTGCTCCTGCACGCCGTGAACCAGGTGCGCCTG CGGGCCGCGCGCGAACACCGCCACGTCTCGCCTGC GTGGGTCTCTTCGTCCAGGGGCACTGCTGACTGCTGC CGATACTCGGGGCTCCCGCTCTCGCTCTCGGTAACAT CCGGCCGGGCGCCGTCTTGAGCACATAGCCTGGAC CGTTTCCTTAAGCGACGCATGCTCGCGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACA GGAAAACATGGGGCAGCAGGCCAGGATGCTGCGGGC CCAGGTGAAGCGGCACACGGTGCGGGACAAGCTGAG GCTGTGCCAGAACTTCCTGCAGAAGCTGCGCTTCCTG GCGGACGAGCCCCAGCACAGCATTCCCGACATCTTCA TCTGGATGATGAGCAACAACAAGCGTGTGCCTATGC CCGTGTGCCCTCCAAGGACCTGCTCTTCTCCATCGTG GAGGAGGAGACTGGCAAGGACTGCGCCAAGGTCAAG ACGCTCTTCCTTAAGCTGCCAGGGAAGCGGGCTTCG GCTCGGCAGGCTGGACAGTGCAGGCCAAGGTGGAGC TGTACCTGTGGCTGGGCTCAGCAAACAGCGCAAGGA GTTCTGTGCGGCCTGCCCTGTGGCTTCCAGGAGGTC AAGGCAGCCCAGGGCCTGGGCCTGCATGCCTTCCCA CCCGTCAGCCTGGTCTACACCAAGAAGCAGGCGTTCC AGCTCCGAGCGCACATGTACCAGGCCCGCAGCCTCTT TGCCGCGGACAGCAGCGGACTCTCAGACCCCTTTGCC CGCGTCTTCTTCATCAATCAGAGTCAGTGCACAGAGGT GCTGAATGAGACCCTGTGTCCACCTGGGACCAGATG CTGGTGTTGACAACCTGGAGCTCTATGGTGAAGCTC ATGAGCTGAGGGACGATCCGCCCATTGTCATTGA AATCTATGACCAGGATTCCATGGGCAAAGCTGACTTCA TGGGCCGGACCTTCGCCAAACCCCTGGTGAAGATGGC AGACGAGGCGTACTGCCACCCCGCTTCCACCTCAG CTCGAGTACTACCAGATCTACCGTGGCAACGCCACAG CTGGAGACCTGCTGGCGGCCTTCGAGCTGCTGCAGAT TGGACCAGCAGGGAAGGCTGACCTGCCCCCATCAAT GGCCCGGTGGACGTGGACCGAGGTCCCATCATGCCC GTGCCATGGGCATCCGGCCCGTGCTCAGCAAGTACC GAGTGGAGGTGCTGTTCTGGGGCCTACGGGACCTAAA GCGGGTGAACCTGGCCCAGGTGGACCGGCCACGGGT
	bGH poly(A) sequence at positions 4217-4438	
	ITR at positions 4526-4655	
	M13 fwd at positions 4674-4690	
	f1 ori at positions 4832-5287	
	AmpR promoter at positions 5313-5417	
	KanR at positions 5418-6227	
	mutBsmBI at positions 5858-5858	
	ori at positions 6398-6986	
	CAP binding site at positions 7274-7295	
	lac promoter at positions 7310-7340	
	lac operator at positions 7348-7364	
	M13 rev at positions 7372-7388	
	Transgene to be transferred into vector in dual vector system at positions 12-4655	

SEQ ID NO.	Description	Plasmid Sequence
		GGACATCGAGTGTGCAGGGAAGGGGGTGCAGTCGTC CCTGATCCACAATTATAAGAAGAACCCCAACTTCAACA CCCTCGTCAAGTGGTTTGAAGTGGACCTCCCAGAGAA CGAGCTGCTGCACCCGCCCTTGAACATCCGTGTGGTG GACTGCCGGGCCTTCGGTCGCTACACACTGGTGGGCT CCCATGCCGTCAGCTCCCTGCGACGTTTCTATCTACCG GCCCCCAGACCGCTCGGCCCCCAGCTGGAACACCAC GGTCAGGCTTCTCCGGCGCTGCCGTGTGCTGTGCAAT GGGGGCTCCTCCTCTCACTCCACAGGGGAGGTTGTG GTGACTATGGAGCCAGAGGTACCCATCAAGAACTGG AGACCATGGTGAAGCTGGACGCGACTTCTGAAGCTGT TGTCAAGGTGGATGTGGCTGAGGAGGAGAAGGAGAA GAAGAAGAAGAAGAAGGGCACTGCGGAGGAGCCAGA GGAGGAGGAGCCAGACGAGAGCATGCTGGACTGGTG GTCCAAGTACTTTGCCTCCATTGACACCATGAAGGAGC AACTTCGACAACAAGAGCCCTCTGGAATTGACTTGGA GGAGAAGGAGGAAGTGGACAATACCGAGGGCCTGAA GGGGTCAATGAAGGGCAAGGAGAAGGCAAGGGCTGC CAAAGAGGAGAAGAAGAAGAACTCAGAGCTCTGGC TCTGGCCAGGGGTCCGAGGCCCCCGAGAAGAAGAAA CCCAAGATTGATGAGCTTAAGGTATACCCCAAAGAGCT GGAGTCCGAGTTTGATAACTTTGAGGACTGGCTGCAC ACTTTCAACTTGCTTCGGGGCAAGACCGGGGATGATG AGGATGGCTCCACCGAGGAGGAGCGCATTGTGGGAC GCTTCAAGGGCTCCCTCTGCGTGTACAAAGTGCCACT CCCAGAGGACGTGTCCCGGGAAGCCGGCTACGACTC CACCTACGGCATGTTCCAGGGCATCCCAGCAATGAC CCCATCAATGTGCTGGTCCGAGTCTATGTGGTCCGGG CCACGGACCTGCACCCTGCTGACATCAACGGCAAAGC TGACCCCTACATCGCCATCCGGCTAGGCAAGACTGAC ATCCGCGACAAGGAGAACTACATCTCCAAGCAGCTCA ACCCTGTCTTTGGGAAGTCCTTTGACATCGAGGCCTC CTTCCCCATGGAATCCATGCTGACGGTGGCTGTGTAT GACTGGGACCTGGTGGGCACTGATGACCTCATTGGGG AAACCAAGATCGACCTGGAGAACCGCTTCTACAGCAA GCACCGCGCCACCTGCGGCATCGCCAGACCTACTC CACACATGGCTACAATATCTGGCGGGACCCCATGAAG CCCAGCCAGATCCTGACCCGCCTCTGCAAAGACGGCA

SEQ ID NO.	Description	Plasmid Sequence
		AAGTGGACGGCCCCCACTTTGGGCCCCCTGGGAGAG TGAAGGTGGCCAACCGCGTCTTCACTGGGCCCTCTGA GATTGAGGACGAGAACGGTCAGAGGAAGCCCACAGA CGAGCATGTGGCGCTGTTGGCCCTGAGGCACTGGGA GGACATCCCCCGCGCAGGCTGCCGCCTGGTGCCAGA GCATGTGGAGACGAGGCCGCTGCTCAACCCCGACAA GCCGGGCATCGAGCAGGGCCGCCTGGAGCTGTGGGT GGACATGTTCCCATGGACATGCCAGCCCCTGGGACG CCTCTGGACATCTCACCTCGGAAGCCCAAGAAGTACG AGCTGCGGGTCATCATCTGGAACACAGATGAGGTGGT CTTGAGGACGACGACTTCTTACAGGGGAGAAGTCC AGTGACATCTTCGTGAGGGGGTGGCTGAAGGGCCAG CAGGAGGACAAGCAGGACACAGACGTCCACTACCACT CCCTCACTGGCGAGGGCAACTTCAACTGGCGCTACCT GTTCCCTTCGACTACCTGGCGGCGGAGGAGAAGATC GTCATCTCCAAGAAGGAGTCCATGTTCTCCTGGGACG AGACCGAGTACAAGATCCCCGCGCGGCTCACCTGCA GATCTGGGATGCGGACCACTTCTCCGCTGACGACTTC CTGGGGGCCATCGAGCTGGACCTGAACCGGTTCCCG CGGGGCGCAAAGACAGCCAAGCAGTGCACCATGGAG ATGGCCACCGGGGAGGTGGACGTGCCCTCGTGTCC ATCTTCAAGCAAAAGCGCGTCAAAGGCTGGTGGCCCC TCCTGGCCCGCAATGAGAACGATGAGTTTGAGCTCAC GGGCAAGGTGGAGGCTGAGCTGCATTTACTGACAGCA GAGGAGGCAGAGAAGAACCAGTGGGCCTGGCCCGC AATGAACCTGACCCCCTAGAGAAACCAACCGGCCCG ACACGGCCTTCGTCTGGTTCCTCAACCCTCTCAAGTCC ATCAAGTACCTCATCTGCACCCGGTACAAGTGGCTCAT CATCAAGATCGTGCTGGCGCTGTTGGGGCTGCTCATG TTGGGGCTCTTCTCTACAGCCTCCCTGGCTACATGG TCAAAAAGCTCCTTGGGGCATGAACGGCCGCTATGCT AGCTTGGTACCAAGGGCGGATCCTGCATAGAGCTCGC TGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC TGTTGTTTGGCCCTCCCCCGTGCCCTTCTTGACCCTG GAAGGTGCCACTCCCCTGTCTTCTTAATAAAATGA GGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTA TTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGG GAGGATTGGGAAGACAATAGCAGGCATCTCGAGTTAA

SEQ ID NO.	Description	Plasmid Sequence
		GGGCGAATTCCCGATAAGGATCTTCCTAGAGCATGGC TACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTA CAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCT CTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCA AAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCC TCAGTGAGCGAGCGAGCGCGCAGCCTTAATTAACCTA ATTCAGTGGCCGTCGTTTTACAACGTCGTGACTGGGAA AACCCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA TCCCCCTTTGCCAGCTGGCGTAATAGCGAAGAGGCC CGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGA ATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAA GCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGC TTTCTTCCCTTCCTTTCTCGCCACGTTGCGCGGCTTTC CCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTT CCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAAC TTGATTAGGTGATGGTTCACGTAGTGGGCCATCGCC CTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCA CGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACA ACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAA GGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGA GCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAAT ATTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAA TGTGCGCGGAACCCCTATTTGTTATTTTTCTAAATACA TTCAAATATGTATCCGCTCATGAGACAATAACCCTGAT AAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGC CATATTCACGGGAAACGTCGAGGCCGCGATTAAATT CCAACATGGATGCTGATTTATATGGGTATAAATGGGCT CGCGATAATGTCTGGGCAATCAGGTGCGACAATCTATC GCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCT GAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACA GATGAGATGGTCAGACTAAACTGGCTGACGGAATTTAT GCCTCTTCCGACCATCAAGCATTTTATCCGTA CTCTG ATGATGCATGGTTACTCACCAGTGGGATCCCCGAAA AACAGCATTCCAGGTATTAGAAGAATATCCTGATTGAG GTGAAAATATTGTTGATGCGCTGGCAGTGTTCTGCG CCGGTTGCATTGATTCTGTTTGTAATTGTCCTTTTAA CAGCGATCGCGTATTTGCTGCTGCTCAGGCGCAATCA

SEQ ID NO.	Description	Plasmid Sequence
		CGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGA TGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGG AAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTG AGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTAT TTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTG GACGAGTCGGAATCGCAGACCGGATACCAGGATCTTGC CATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCAT TACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATC CTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATG AGTTTTTCTAACTGTCAGACCAAGTTTACTCATATATAC TTTAGATTGATTTAAACTTCATTTTAAATTTAAAGGAT CTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAAT CCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACC CCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCC ACCGCTACCAGCGGTGGTTTGTGGCCGGATCAAGAG CTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAG AGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGT AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTG CTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGA CTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCG GGCTGAACGGGGGGTTTCGTGCACACAGCCCAGCTTG GAGCGAACGACCTACACCGAACTGAGATACCTACAGC GTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAG AAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGG AACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAA CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACC TCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGG GGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCC TTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA CATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATA ACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCG CCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC TCTCCCCGCGGTTGGCCGATTCATTAATGCAGCTGG CACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAG CGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGC

SEQ ID NO.	Description	Plasmid Sequence
		ACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGT TGTGTGGAATTGTGAGCGGATAACAATTTACACAGGA AACAGCTATGACCATGATTACGCCAGATTTAATTAAGG
68	5' transgene plasmid containing the following features: ITR at positions 12-141 CMV i.e enhancer at positions 230-594 CMV enhancer at positions 296-599 Chicken β -actin promoter at positions 596-878 Exon 1 at positions 879-971 Chimeric intron at positions 971-1172 Kozak sequence at positions 1189-1198 N-terminal portion of human OTOF isoform 5 at positions 1195-3600 Splice donor sequence at positions 3601-3684 AP head sequence at positions 3691-3977 ITR at positions 4071-4200 M13 fwd at positions 4219-4235	CCTTAATTAGGCTGCGCGCTCGCTCGCTCACTGAGGC CGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGG TCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGAGTGGCCAACTCCATCACTAGGGGTTCTTG TAG TTAATGATTAACCCGCCATGCTACTTATCTACGTAGCC ATGCTCTAGGAAGATCGGAATTCGCCCTTAAGCTAGC GGCGCGCCGGTACCTAGTTATTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGC GTTACATAACTTACGGTAAATGGCCCCGCTGGCTGAC CGCCCAACGACCCCCGCCCATTGACGTCAATAATGAC GTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGC CCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCCAGTACATGACCTTATGGGACTTTCCTA CTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC ATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCC CCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTA TTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGG GGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGG CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAG GTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAA AGTTTCCTTTTATGGCGAGGCGGCGGGCGGCGGGC CCTATAAAAAGCGAAGCGCGCGGGCGGGCGGGAGTCG CTGCGCGCTGCCTTCGCCCCGTGCCCGCTCCGCCG CCGCCTCGCGCCGCCCCGCCCGGCTCTGACTGACCG CGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTT CTCCTCCGGGCTGTAATTAGCGCTTGTTTAATGACG GCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGG GGCTCCGGGAGCTAGAGCCTCTGCTAACCATGTTTAT GCCTTCTTCTTTTCTTACAGCTCCTGGGCAACGTGCT GGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTCTA GCGGCCGCCACCATGGCCTTGCTCATCCACCTCAAGA CAGTCTCGGAGCTGCGGGGCAGGGGCGACCGGATCG CCAAAGTGACTTTCCGAGGGCAATCCTTCTACTCTCGG

SEQ ID NO.	Description	Plasmid Sequence
	f1 ori at positions 4377-4832 AmpR promoter at positions 4858-4962 KanR at positions 4963-5772 mutBsmBI at positions 4963-5772 ori at positions 5943-6531 CAP binding site at positions 6819-6840 lac promoter at positions 6855-6885 lac operator at positions 6835-6909 M13 rev at positions 6917-6933 Transgene to be transferred into vector in dual vector system at positions 12-4200	GTCCTGGAGAACTGTGAGGATGTGGCTGACTTTGATG AGACATTTCCGGTGGCCGGTGGCCAGCAGCATCGACAG AAATGAGATGCTGGAGATTCAGGTTTTCAACTACAGCA AAGTCTTCAGCAACAAGCTCATCGGGACCTTCCGCAT GGTGCTGCAGAAAGGTGGTAGAGGAGAGCCATGTGGA GGTGACTGACACGCTGATTGATGACAACAATGCTATCA TCAAGACCAGCCTGTGCGTGGAGGTCCGGTATCAGGC CACTGACGGCACAGTGGGCTCCTGGGACGATGGGGA CTTCCTGGGAGATGAGTCTTCAAGAGGAAGAGAAG GACAGCCAAGAGACGGATGGACTGCTCCCAGGCTCC CGGCCCAGCTCCCGGCCCCCAGGAGAGAAGAGCTTC CGGAGAGCCGGGAGGAGCGTGTCTCCGCCATGAAG CTCGGCAAAAACCGGTCTCACAAGGAGGAGCCCCAAA GACCAGATGAACCGGCGGTGCTGGAGATGGAAGACC TTGACCATCTGGCCATTCCGGCTAGGAGATGGACTGGA TCCCCGACTCGGTGTCTCTAGCCTCAGTCACAGCTCTC ACCACTAATGTCTCCAACAAGCGATCTAAGCCAGACAT TAAGATGGAGCCAAGTGCTGGGCGGCCCCATGGATTAC CAGGTCAGCATCACGGTGATCGAGGCCCGGCAGCTG GTGGGCTTGAACATGGACCCTGTGGTGTGCGTGGAG GTGGGTGACGACAAGAAGTACACATCCATGAAGGAGT CCACTAACTGCCCCCTATTACAACGAGTACTTCGTCTTC GACTTCCATGTCTCTCCGGATGTCATGTTTGACAAGAT CATCAAGATTTCCGGTGATTCACTCCAAGAACCTGCTGC GCAGTGGCACCCTGGTGGGCTCCTTCAAAATGGACGT GGGAACCGTGTA CTGCGAGCCAGAGCACCAAGTTCCAT CACAAGTGGGCCATCCTGTCTGACCCCGATGACATCT CCTCGGGGCTGAAGGGCTACGTGAAGTGTGACGTTGC CGTGGTGGGCAAAGGGGACAACATCAAGACGCCCCA CAAGGCCAATGAGACCGACGAAGATGACATTGAGGGG AAGTTGCTGCTCCCCGAGGGGGTGGCCCCCGAACGC CAGTGGGCCCCGTTCTATGTGAAAATTTACCGAGCAG AGGGGCTGCCCCGTATGAACACAAGCCTCATGGCCAA TGTAAGAAGGCTTTCATCGGTGAAAACAAGGACCTC GTGGACCCCTACGTGCAAGTCTTCTTTGCTGGCCAGA AGGGCAAGACTTCAGTGCAGAAGAGCAGCTATGAGCC CCTGTGGAATGAGCAGGTCGTCTTTACAGACCTCTTCC CCCCACTCTGCAAACGCATGAAGGTGCAGATCCGAGA

SEQ ID NO.	Description	Plasmid Sequence
		CTCGGACAAGGTCAACGACGTGGCCATCGGCACCCAC TTCATTGACCTGCGCAAGATTTCTAATGACGGAGACAA AGGCTTCCTGCCCACACTGGGCCCAGCCTGGGTGAAC ATGTACGGCTCCACACGTAACCTACACGCTGCTGGATG AGCATCAGGACCTGAACGAGGGCCTGGGGGAGGGTG TGTCCTTCCGGGCCCCGGCTCCTGCTGGGCCTGGCTGT GGAGATCGTAGACACCTCCAACCCTGAGCTCACCAGC TCCACAGAGGTGCAGGTGGAGCAGGCCACGCCCATC TCGGAGAGCTGTGCAGGTAAAATGGAAGAATTCTTTCT CTTTGGAGCCTTCCTGGAGGCCTCAATGATCGACCGG AGAAACGGAGACAAGCCCATCACCTTTGAGGTCACCA TAGGCAACTATGGGAACGAAGTTGATGGCCTGTCCCG GCCCCAGCGGCCTCGGCCCCGGAAGGAGCCGGGGG ATGAGGAAGAAGTAGACCTGATTGAGAACGCAAGTGA TGACGAGGCCGGTGATGCCGGGGACCTGGCCTCAGT CTCCTCCACTCCACCAATGCGGCCCCAGGTCACCGAC AGGAACTACTTCCATCTGCCCTACCTGGAGCGAAAGC CCTGCATCTACATCAAGAGCTGGTGGCCGGACCAGCG CCGCCGCTCTACAATGCCAACATCATGGACCACATT GCCGACAAGCTGGAAGAAGGCCTGAACGACATACAGG AGATGATCAAAACGGAGAAGTCCTACCCTGAGCGTCG CCTGCGGGGCGTCCTGGAGGAGCTGAGCTGTGGCTG CTGCCGCTTCCTCTCCCTCGCTGACAAGGACCAGGGC CACTCATCCCGCACCAGGCTTGACCGGGGAGCGCCTCA AGTCCTGCATGAGGGAGCTGGTAAGTATCAAGGTTAC AAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTG TCGAGACAGAGAAGACTCTTGCGTTTCTGAGCTAGCC CCCGGGTGCGCGGCGTCGGTGGTGCCGGCGGGGGG CGCCAGGTCGCAGGCGGTGTAGGGCTCCAGGCAGGC GGCGAAGGCCATGACGTGCGCTATGAAGGTCTGCTCC TGCACGCCGTGAACCAGGTGCGCCTGCGGGCCGCGC GCGAACACCGCCACGTCCTCGCCTGCGTGGGTCTCTT CGTCCAGGGGCACTGCTGACTGCTGCCGATACTCGG GGCTCCCGCTCTCGCTCTCGGTAACATCCGGCCGGG CGCCGTCCCTTGAGCACATAGCCTGGACCGTTTCGTCG ACCTCGAGTTAAGGGCGAATTCCCGATAAGGATCTTC CTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGT TAATCATTAACACAAGGAACCCCTAGTGATGGAGTTG

SEQ ID NO.	Description	Plasmid Sequence
		GCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG CCGGGCGACCAAAGGTCGCCCACGCCCGGGCTTTG CCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCC TTAATTAACCTAATTCAGTGGCCGTCGTTTTACAACGTC GTGACTGGGAAAACCTGGCGTTACCCAACCTAATCG CCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAAT AGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGT TGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTA GCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGC GCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGC CCGCTCCTTTCGCTTTCCTCCCTTCTTCGCCCACG TTCGCCGGCTTTCGCCGTCAGCTCTAAATCGGGGGC TCCCTTTAGGGTTCGATTTAGTGCTTTACGGCACCTC GACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAG TGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTG ACGTTGGAGTCCACGTTCCTTAATAGTGGAATCTTGT CCAACTGGAACAACACTCAACCTATCTCGGTCTATT CTTTTGATTATAAGGGATTTTGCCGATTTGCGCCTATT GGTTAAAAATGAGCTGATTTAACAAAAATTAACGCG AATTTTAACAAAAATTAACGCTTACAATTTAGGTGGCA CTTTTCGGGAAATGTGCGCGGAACCCCTATTTGTTTA TTTTCTAAATACATTCAAATATGTATCCGCTCATGAGA CAATAACCCTGATAAATGCTTCAATAATTGAAAAAGG AAGAGTATGAGCCATATTCAACGGGAAACGTCGAGGC CGCGATTAAATCCAACATGGATGCTGATTTATATGGG TATAATGGGCTCGCGATAATGTGGGCAATCAGGTG CGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCC AGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCA ATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTG ACGGAATTTATGCCTCTTCGACCATCAAGCATTTTAT CCGTACTCCTGATGATGCATGGTTACTCACCCTGCGA TCCCCGAAAAACAGCATTCCAGGTATTAGAAGAATAT CCTGATTCAGGTGAAAAATATTGTTGATGCGCTGGCAGT GTTCTGCGCCGTTGCATTCGATTCCTGTTTGTAATT GTCCTTTTAACAGCGATCGCGTATTTGCTCTTGCTCAG GCGCAATCACGAATGAATAACGTTTGGTTGATGCGA GTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGA ACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCT

SEQ ID NO.	Description	Plasmid Sequence
		CACCGGATTCAGTCGTCACCTCATGGTGATTTCTCACTT GATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTG TATTGATGTTGGACGAGTCGGAATCGCAGACCGATAC CAGGATCTTGCCATCCTATGGAACGCCTCGGTGAGT TTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAAATAG GTATTGATAATCCTGATATGAATAAATTGCAGTTTCATT TGATGCTCGATGAGTTTTTCTAACTGTCAGACCAAGTT TACTCATATATACTTTAGATTGATTTAAACTTCATTTTT AATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATC TCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCAC TGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTT CTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTG CAAACAAAAAACACCGCTACCAGCGGTGGTTTGTTT GCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAA CTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTT CTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTC TGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGT TACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAG GCGCAGCGGTCTGGGCTGAACGGGGGGTTCTGTGCACA CAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGA GATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCT TCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAG CGGCAGGGTCTGGAACAGGAGAGCGCACGAGGGAGCT TCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTC GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTG ATGCTCGTCAGGGGGGCGGAGCCTATGAAAAACGC CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCT GGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCT GATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGC TGATACCGCTCGCCGACGCCGAACGACCGAGCGCAG CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAAT ACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAT TAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAG CGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCT CACTCATTAGGCACCCAGGCTTTACACTTTATGCTTC CGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACA

SEQ ID NO.	Description	Plasmid Sequence
		ATTTACACAGGAAACAGCTATGACCATGATTACGCCA GATTTAATTAAGG

AAV Vectors for nucleic acid delivery

In some embodiments, nucleic acids of the compositions and methods described herein are incorporated into recombinant AAV (rAAV) vectors and/or virions in order to facilitate their introduction into a cell. rAAV vectors useful in the compositions and methods described herein are recombinant nucleic acid constructs that include (1) a heterologous sequence to be expressed (e.g., a polynucleotide encoding an N-terminal or C-terminal portion of an OTOF protein) and (2) viral sequences that facilitate stability and expression of the heterologous genes. The viral sequences may include those sequences of AAV that are required in cis for replication and packaging (e.g., functional ITRs) of the DNA into a virion. Such rAAV vectors may also contain marker or reporter genes. Useful rAAV vectors have one or more of the AAV WT genes deleted in whole or in part but retain functional flanking ITR sequences. The AAV ITRs may be of any serotype suitable for a particular application. For use in the methods and compositions described herein, the ITRs can be AAV2 ITRs. Methods for using rAAV vectors are described, for example, in Tal et al., J. Biomed. Sci. 7:279 (2000), and Monahan and Samulski, Gene Delivery 7:24 (2000), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

The nucleic acids and vectors described herein can be incorporated into a rAAV virion in order to facilitate introduction of the nucleic acid or vector into a cell. The capsid proteins of AAV compose the exterior, non-nucleic acid portion of the virion and are encoded by the AAV cap gene. The cap gene encodes three viral coat proteins, VP1, VP2 and VP3, which are required for virion assembly. The construction of rAAV virions has been described, for instance, in US 5,173,414; US 5,139,941; US 5,863,541; US 5,869,305; US 6,057,152; and US 6,376,237; as well as in Rabinowitz et al., J. Virol. 76:791 (2002) and Bowles et al., J. Virol. 77:423 (2003), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

rAAV virions useful in conjunction with the compositions and methods described herein include those derived from a variety of AAV serotypes including AAV1, AAV2, AAV2quad(Y-F), AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, rh10, rh39, rh43, rh74, Anc80, Anc80L65, DJ/8, DJ/9, 7m8, PHP.B, PHP.eb, and PHP.S. For targeting cochlear hair cells, AAV1, AAV2, AAV6, AAV9, Anc80, Anc80L65, DJ/9, 7m8, and PHP.B may be particularly useful. Serotypes evolved for transduction of the retina may also be used in the methods and compositions described herein. The first and second nucleic acid vectors (e.g., AAV vectors) in the compositions and methods described herein may have the same serotype or different serotypes. Construction and use of AAV vectors and AAV proteins of different serotypes are described, for instance, in Chao et al., Mol. Ther. 2:619 (2000); Davidson et al., Proc. Natl. Acad. Sci. USA 97:3428 (2000); Xiao et al., J. Virol. 72:2224 (1998); Halbert et al., J. Virol. 74:1524 (2000); Halbert et al., J. Virol. 75:6615 (2001); and Auricchio et al., Hum. Molec. Genet. 10:3075 (2001),

the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

Also useful in conjunction with the compositions and methods described herein are pseudotyped rAAV vectors. Pseudotyped vectors include AAV vectors of a given serotype (e.g., AAV9) pseudotyped with a capsid gene derived from a serotype other than the given serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, etc.). Techniques involving the construction and use of pseudotyped rAAV virions are known in the art and are described, for instance, in Duan et al., J. Virol. 75:7662 (2001); Halbert et al., J. Virol. 74:1524 (2000); Zolotukhin et al., Methods, 28:158 (2002); and Auricchio et al., Hum. Molec. Genet. 10:3075 (2001).

AAV virions that have mutations within the virion capsid may be used to infect particular cell types more effectively than non-mutated capsid virions. For example, suitable AAV mutants may have ligand insertion mutations for the facilitation of targeting AAV to specific cell types. The construction and characterization of AAV capsid mutants including insertion mutants, alanine screening mutants, and epitope tag mutants is described in Wu et al., J. Virol. 74:8635 (2000). Other rAAV virions that can be used in methods described herein include those capsid hybrids that are generated by molecular breeding of viruses as well as by exon shuffling. See, e.g., Soong et al., Nat. Genet., 25:436 (2000) and Kolman and Stemmer, Nat. Biotechnol. 19:423 (2001).

In some embodiments, the use of AAV vectors for delivering a functional OTOF isoform 5 protein requires the use of a dual vector system, in which the first member of the dual vector system encodes an N-terminal portion of an OTOF isoform 5 protein and the second member encodes a C-terminal portion of an OTOF isoform 5 protein such that, upon administration of the dual vector system to a cell, the polynucleotide sequences contained within the two vectors can join to form a single sequence that results in the production of a full-length OTOF isoform 5 protein.

In some embodiments, the first member of the dual vector system will also include, in 5' to 3' order, a first inverted terminal repeat ("ITR"); a promoter (e.g., a Myo15 promoter); a Kozak sequence; an N-terminal portion of an OTOF isoform 5 coding sequence; a splice donor sequence; an AP gene fragment (e.g., an AP head sequence); and a second ITR; and the second member of the dual vector system will include, in 5' to 3' order, a first ITR; an AP gene fragment (e.g., an AP head sequence); a splice acceptor sequence; a C-terminal portion of an OTOF isoform 5 coding sequence; a polyA sequence; and a second ITR. In some embodiments, the N-terminal portion of the OTOF isoform 5 coding sequence and the C-terminal portion of the OTOF isoform 5 coding sequence do not overlap and are joined in a cell (e.g., by recombination at the overlapping region (the AP gene fragment), or by concatemerization of the ITRs) to produce the full-length OTOF isoform 5 amino sequence as set forth in SEQ ID NO:1. In particular embodiments, the N-terminal portion of the OTOF isoform 5 coding sequence encodes amino acids 1-802 of SEQ ID NO:1 (SEQ ID NO: 58) and the C-terminal portion of the OTOF isoform 5 coding sequence encodes amino acids 803-1997 of SEQ ID NO:1 (SEQ ID NO: 59).

In some embodiments, the first member of the dual vector system includes the Myo15 promoter of SEQ ID NO:21 (also represented by nucleotides 235-1199 of SEQ ID NO:66) operably linked to nucleotides that encode the N-terminal 802 amino acids of the OTOF isoform 5 protein (amino acids 1-802 of SEQ ID NO:1), which are encoded by exons 1-20 of the native polynucleotide sequence encoding that protein. In certain embodiments, the nucleotide sequence that encodes the N-terminal amino acids of

the OTOF isoform 5 protein is nucleotides 1222-3627 of SEQ ID NO:66. In some embodiments, the nucleotide sequence that encodes the N-terminal amino acids of the OTOF isoform 5 protein is any nucleotide sequence that, by redundancy of the genetic code, encodes amino acids 1-802 of SEQ ID NO:1. The nucleotide sequences that encode the OTOF isoform 5 protein can be partially or fully codon-optimized for expression. In some embodiments, the first member of the dual vector system includes the Kozak sequence corresponding to nucleotides 1216-1225 of SEQ ID NO:66. In some embodiments, the first member of the dual vector system includes the splice donor sequence corresponding to nucleotides 3628-3711 of SEQ ID NO:66. In some embodiments, the first member of the dual vector system includes the AP head sequence corresponding to nucleotides 3718-4004 of SEQ ID NO:66. In particular

embodiments, the first member of the dual vector system includes nucleotides 235-4004 of SEQ ID NO:66 flanked on each of the 5' and 3' sides by an inverted terminal repeat. In some embodiments, the flanking inverted terminal repeats are any variant of AAV2 inverted terminal repeats that can be encapsidated by a plasmid that carries the AAV2 Rep gene. In certain embodiments, the 5' flanking inverted terminal repeat has a sequence corresponding to nucleotides 12-141 of SEQ ID NO:66 or a sequence having at least 80% sequence identity (at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) thereto; and the 3' flanking inverted terminal repeat has a sequence corresponding to nucleotides 4098-4227 of SEQ ID NO:66 or a sequence having at least 80% sequence identity (at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) thereto. It will be understood by those of skill in the art that, for any given pair of inverted terminal repeat sequences in a transfer plasmid that is used to create the viral vector (typically by transfecting cells with that plasmid together with other plasmids carrying the necessary AAV genes for viral vector formation) (e.g., any of SEQ ID NOs: 60, 62, 64, 65, 66, or 68), that the corresponding sequence in the viral vector can be altered due to the ITRs adopting a "flip" or "flop" orientation during recombination.

Thus, the sequence of the ITR in the transfer plasmid is not necessarily the same sequence that is found in the viral vector prepared therefrom. However, in some very specific embodiments, the first member of the dual vector system includes nucleotides 12-4227 of SEQ ID NO:66.

In some embodiments, the second member of the dual vector system includes nucleotides that encode the C-terminal 1195 amino acids of the OTOF isoform 5 protein (amino acids 803-1997 of SEQ ID NO:1) immediately followed by a stop codon. In certain embodiments, the nucleotide sequence that encodes the C-terminal amino acids of the OTOF isoform 5 protein is nucleotides 587-4174 of SEQ ID NO:67. In some embodiments, the nucleotide sequence that encodes the C-terminal amino acids of the OTOF isoform 5 protein is any nucleotide sequence that, by redundancy of the genetic code, encodes amino acids 803-1997 of SEQ ID NO:1. The nucleotide sequences that encode the OTOF isoform 5 protein can be partially or fully codon-optimized for expression. In some embodiments, the second member of the dual vector system includes the splice acceptor sequence corresponding to nucleotides 538-586 of SEQ ID NO:67. In some embodiments, the second member of the dual vector system includes the AP head sequence corresponding to nucleotides 229-515 of SEQ ID NO:67. In some embodiments, the second member of the dual vector system includes the poly(A) sequence corresponding to nucleotides 4217-4438 of SEQ ID NO:67. In particular embodiments, the second member of the dual vector system includes nucleotides 229-4438 of SEQ ID NO:67 flanked on each of the 5' and 3' sides by

an inverted terminal repeat. In some embodiments, the flanking inverted terminal repeats are any variant of AAV2 inverted terminal repeats that can be encapsidated by a plasmid that carries the AAV2 Rep gene. In certain embodiments, the 5' flanking inverted terminal repeat has a sequence corresponding to nucleotides 12-141 of SEQ ID NO:67 or a sequence having at least 80% sequence identity (at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) thereto; and the 3' flanking inverted terminal repeat has a sequence corresponding to nucleotides 4526-4655 of SEQ ID NO:67 or a sequence having at least 80% sequence identity (at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) thereto. It will be understood by those of skill in the art that, for any given pair of inverted terminal repeat sequences in a transfer plasmid that is used to create the viral vector (typically by transfecting cells with that plasmid together with other plasmids carrying the necessary AAV genes for viral vector formation) (e.g., any of SEQ ID NOs: 61, 63, or 67), that the corresponding sequence in the viral vector can be altered due to the ITRs adopting a "flip" or "flop" orientation during recombination. Thus, the sequence of the ITR in the transfer plasmid is not necessarily the same sequence that is found in the viral vector prepared therefrom. However, in some very specific embodiments, the first member of the dual vector system includes nucleotides 12-4655 of SEQ ID NO:67. In some embodiments, the dual vector system is an AAV1 dual vector system. In some embodiments, the dual vector system is an AAV9 dual vector system. In some embodiments, the dual vector system is an Anc80 dual vector system.

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Pharmaceutical compositions

The AAV vectors described herein may be incorporated into a vehicle for administration into a patient, such as a human patient suffering from sensorineural hearing loss or auditory neuropathy, as described herein. Pharmaceutical compositions containing vectors, such as viral vectors, that contain a polynucleotide encoding a portion of an OTOF isoform 5 protein can be prepared using methods known in the art.

Mixtures of the AAV vectors described herein may be prepared in water suitably mixed with one or more excipients, carriers, or diluents. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (described in US 5,466,468, the disclosure of which is incorporated herein by reference). In any case the formulation may be sterile and may be fluid to the extent that easy syringability exists. Formulations may be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol,

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phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

5 For example, a solution containing a pharmaceutical composition described herein may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one
10 dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. For local administration to the inner ear, the composition may be formulated to contain a synthetic perilymph solution. An exemplary synthetic perilymph solution includes 20-200 mM NaCl, 1-5 mM KCl, 0.1-10 mM CaCl_2 , 1-10 mM glucose,
15 and 2-50 mM HEPES, with a pH between about 6 and 9 and an osmolality of about 300 mOsm/kg. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations may meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biologics standards.

In some embodiments, both vectors in the OTOF dual vector system (e.g., the first or 5' vector
20 and the second or 3' vector) are mixed in a single formulation. The formulation may be a sterile, aqueous suspension. In some embodiments, both vectors are present in the formulation in an about 3:1 to about 1:3 ratio (e.g., 3:1 to 1:3, 2:1 to 1:2, or 1:1). In some embodiments, both vectors are present in the formulation in an approximately 1:1 ratio (vector genomes (vg)). For example, a suspension containing a titer of 4×10^{13} total vg/mL can contain an approximately equimolar ratio of 2×10^{13} vg/mL of the first (5')
25 vector and 2×10^{13} vg/mL of the second (3') vector. In another example, a suspension containing a titer of 3×10^{13} total vg/mL can contain an approximately equimolar ratio of 1.5×10^{13} vg/mL of the first (5') vector and 1.5×10^{13} vg/mL of the second (3') vector, or a suspension containing a titer of 7.3×10^{13} total vg/mL can contain an approximately equimolar ratio of 3.65×10^{13} vg/mL of the first (5') vector and 3.65×10^{13} vg/mL of the second (3') vector. In some embodiments, in addition to the AAV vectors, the
30 formulation contains 10 mM sodium phosphate or disodium phosphate, 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 and has a target of pH 7.3-7.4. In some embodiments, in addition to the AAV vectors, the formulation contains 10 mM sodium phosphate, 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 and has a pH of about 7.4. In some embodiments, in addition to the AAV vectors, the formulation contains 10 mM disodium phosphate, 180 mM sodium
35 chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 and has a pH of about 7.4. The formulation can be frozen prior to administration (e.g., at or below -65°C). If needed, the formulation can be diluted to the desired titer on the day of administration using a diluent that contains the same excipients at the same target concentration (and lacks the AAV vectors).

Methods of treatment

The compositions described herein may be administered to a subject with sensorineural hearing loss or auditory neuropathy by local administration to the inner ear (e.g., administration into the perilymph or endolymph, e.g., by injection or catheter insertion through the round window membrane, injection into a semicircular canal, by canalostomy, or by intratympanic or transtympanic injection, e.g., administration to a cochlear hair cell). If the compositions are administered by direct delivery to the inner ear, a second fenestration or vent hole may be added elsewhere in the inner ear (e.g., in a semicircular canal). In some embodiments, the composition is administered as an intracochlear injection via a catheter placed through the round window membrane of the cochlea. The intracochlear injection may also utilize a syringe and syringe pump in combination with the catheter (e.g., a syringe is attached to the catheter and the syringe and catheter combination are loaded onto the syringe pump). For intracochlear injection, a site of egress will be created by fenestrating the lateral semicircular canal. The surgical approach may be similar to that used to place cochlear implants. In some embodiments, the intracochlear injection is at a rate of 0.8-1 mL/hr (e.g., 0.9 mL/hr). The most suitable route for administration in any given case will depend on the particular composition administered, the patient, pharmaceutical formulation methods, administration methods (e.g., administration time and administration route), the patient's age, body weight, sex, severity of the disease being treated, the patient's diet, and the patient's excretion rate. Compositions may be administered once (e.g., once per ear to be treated). In some embodiments, the first and second nucleic acid vectors (e.g., AAV vectors) are administered simultaneously (e.g., in a single composition). The first and second nucleic acid vector can have the same serotype or different serotypes (e.g., AAV serotypes). For example, the first and second nucleic acid vectors can both have an AAV1 capsid, an Anc80 capsid, an AAV2 capsid, or an AAV9 capsid.

The surgical approach and administration method described herein can also be used for the administration of other nucleic acid vectors (e.g., AAV vectors) to the inner ear (e.g., into the perilymph) of a human subject (e.g., for treatment of sensorineural hearing loss, such as genetic hearing loss or acquired hearing loss, or for the treatment of vestibular dysfunction, such as vertigo, dizziness, imbalance (e.g., loss of balance or a balance disorder), oscillopsia, or bilateral vestibulopathy). For example, the traditional surgical procedure for cochlear implant surgery can be used to access the middle ear, which involves a mastoidectomy and opening the facial recess. Openings can then be made in the round window membrane of the cochlea (e.g., for insertion of a catheter) and in a second location in the inner ear (e.g., a fenestration or vent hole to serve as a site of egress, such as a fenestration in a semicircular canal) and the nucleic acid vector can be infused into the perilymph via a catheter placed through the round window membrane. In some embodiments, the second opening (i.e., fenestration or vent hole) is made in the lateral semicircular canal. In some embodiments, the second opening (i.e., fenestration or vent hole) is made in the posterior semicircular canal. In some embodiments, the second opening (i.e., fenestration or vent hole) is made in the superior semicircular canal. A syringe and/or syringe pump can be used in combination with the catheter for the administration (e.g., a syringe is attached to the catheter and the syringe and catheter combination are loaded onto the syringe pump). The nucleic acid vector (e.g., a solution containing the nucleic acid vector) can be administered at a rate of 0.5-1 mL/hr (e.g., 0.6-1 mL/hr, 0.7-1 mL/hr, 0.8-1 mL/hr, or 0.9-1 mL/hour, such as 0.9 mL/hr). For the treatment of genetic hearing loss or vestibular dysfunction (i.e., hearing loss or vestibular dysfunction associated with a

genetic mutation), the nucleic acid vector may contain a polynucleotide encoding a functional protein that is deficient in the subject (e.g., a polynucleotide encoding a functional (e.g., wild-type) protein that is absent or that does not function properly in the inner ear of the subject due to a genetic mutation associated with the hearing loss or vestibular dysfunction) or a polynucleotide encoding an expression product designed to correct a genetic mutation in the subject (e.g., correct a genetic mutation associated with the hearing loss or vestibular dysfunction via gene editing). For treatment of acquired hearing loss (e.g., noise-induced hearing loss, age-related hearing loss, disease or infection-related hearing loss, head trauma-related hearing loss, or ototoxic drug-induced hearing loss; e.g., hearing loss associated with damage to or loss of cochlear hair cells) the nucleic acid vector may contain a polynucleotide encoding an expression product that induces or increases cochlear hair cell regeneration, increases cochlear supporting cell numbers, prevents or reduces cochlear hair cell damage, prevents or reduces cochlear hair cell death, increases cochlear hair cell maturation, improves cochlear hair cell function, or promotes or increases cochlear hair cell survival. For treatment of vestibular dysfunction associated with damage to or loss of vestibular cells (e.g., damage to or loss of vestibular cells, such as vestibular hair cells or supporting cells, related to disease or infection, head trauma, ototoxic drugs (e.g., vestibulotoxic drugs), or aging) the nucleic acid vector may contain a polynucleotide encoding an expression product that induces or increases vestibular hair cell regeneration, increases vestibular supporting cell numbers, prevents or reduces vestibular hair cell damage, prevents or reduces vestibular hair cell death, increases vestibular hair cell maturation, improves vestibular hair cell function, or promotes or increases vestibular hair cell survival.

Subjects that may be treated as described herein are subjects having or at risk of developing sensorineural hearing loss or auditory neuropathy. The compositions and methods described herein can be used to treat subjects having a mutation in OTOF (e.g., a mutation that reduces OTOF function or expression, or an OTOF mutation associated with sensorineural hearing loss), subjects having a family history of autosomal recessive sensorineural hearing loss or deafness (e.g., a family history of OTOF-related hearing loss), or subjects whose OTOF mutational status and/or OTOF activity level is unknown. In some embodiments, a subject to be treated as described herein has or has been diagnosed as having a biallelic mutation in OTOF (e.g., a likely pathogenic or pathogenic mutation). The methods described herein may include a step of screening a subject for a mutation in OTOF (e.g., a biallelic likely pathogenic or pathogenic mutation) prior to treatment with or administration of the compositions described herein. In some embodiments, prior to treatment with a composition described herein, the subject is also screened for mutations in other hearing loss-related genes. A subject can be screened for an OTOF mutation or a mutation in another hearing loss-related gene using standard methods known to those of skill in the art (e.g., genetic testing).

Subjects that may be treated as described herein include those for whom it has been determined that minimal benefit has been provided by amplification of the ear to be treated with the OTOF dual vector system. In some embodiments, the subject is deemed to meet the criteria for cochlear implantation in one or both ears prior to treatment initiation. In some embodiments, the subject has not previously received a cochlear implant in the ear(s) to be treated with an OTOF dual vector system. In some embodiments, the subject is diagnosed as having profound sensorineural hearing loss prior to treatment. The subject may be diagnosed as having profound sensorineural hearing loss based on behavioral and physiologic

measurements of inner ear function, such as having an absence of an ABR neural signal in response to a click stimulus at or below 85 decibels normalized Hearing Level (dB nHL) in the ear(s) to be treated with an OTOF dual vector system or having ≥ 90 dB HL as assessed by ABR. In some embodiments, outer hair cell presence is confirmed prior to treatment based on detectable otoacoustic emissions (OAEs). For example, the subject may have distortion product otoacoustic emission (DPOAE) present with ≥ 6 dB signal-to-noise ratio at ≥ 3 frequencies in a distortion product (DP) Gram measured from 1 to 8 kHz in the ear(s) to be treated with an OTOF dual vector system. In some embodiments, outer hair cell presence is confirmed via presence of the cochlear microphonic in the ear(s) to be treated with an OTOF dual vector system (e.g., in subjects older than 24 months of age). In some embodiments, the subject has behavioral open-set word detection scores of $< 30\%$ in the ear(s) to be treated with an OTOF dual vector system or has behavioral open-set word detection scores consistent with the speech criteria from the recommended cochlear implant label. In some embodiments, the subject has no evidence from measures of hearing loss that show dependence on body temperature. In some embodiments, the subject does not have a history or presence of other permanent or untreatable hearing loss conditions. In some embodiments, the subject's inner ear anatomy is compatible with the route of administration (e.g., for intracochlear injection, the subject does not have an enlarged cochlear or vestibular aqueduct). In some embodiments, the subject does not have a history or presence of treatment with ototoxic drugs. In some embodiments, the subject does not have a history of risk factor(s) for auditory neuropathy not caused by biallelic OTOF mutations (e.g., prematurity, low birth weight, hyperbilirubinemia, neonatal intensive care unit admission, and/or low Apgar scores).

In some embodiments, the subject to be treated with an OTOF dual vector system is a pediatric subject (a subject under 18 years of age). In some embodiments, the pediatric subject is at least 7 years of age (i.e., at least 7 years of age and under 18 years of age). In some embodiments, the subject is older than 24 months of age and under 18 years of age. In some embodiments, the subject is older than 24 months of age and younger than 7 years of age. In some embodiments, the subject is 24 months of age or younger (e.g., 24 months of age or younger, 18 months of age or younger, 12 months of age or younger, or 6 months of age or younger).

The methods described herein may also include a step of assessing hearing in a subject prior to treatment with or administration of the compositions described herein. Hearing can be assessed using standard tests, such as audiometry, ABR, electrocochleography (ECOG), and otoacoustic emissions. In some embodiments, the method further includes performing ABR measurements prior to treatment and after treatment initiation (e.g., prior to treatment and 1 day, 1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, 24 weeks, 36 weeks, 48 weeks, one year, 72 weeks, 2 years, 3 years, 4 years, and/or 5 years after administration of an OTOF dual vector system). In some embodiments, the method further includes performing behavioral audiometry prior to treatment and after treatment initiation (e.g., prior to treatment and 1 day, 1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, 24 weeks, 36 weeks, 48 weeks, one year, 72 weeks, 2 years, 3 years, 4 years, and/or 5 years after administration of an OTOF dual vector system), such as SAT/Speech Detection Threshold (SDT) audiometry, SRT/Speech Reception Threshold (SRT) audiometry, Pure tone air- and bone-conduction audiometry, visual reinforcement audiometry (VRA), and/or conditioned play audiometry (CPA). In some embodiments, the method further includes performing one or more assessments or questionnaires prior to treatment and after treatment initiation (e.g., prior to

treatment and 1 day, 1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, 24 weeks, 36 weeks, 48 weeks, one year, 72 weeks, 2 years, 3 years, 4 years, and/or 5 years after administration of an OTOF dual vector system), such as the Auditory Skills Checklist (ASC), Open & Closed (O&C) set task, Early Speech Perception (ESP) test, Pediatric Speech Intelligibility (PSI) test, Lexical Neighborhood Test (LNT)/Multisyllabic Lexical Neighborhood Test (MLNT), Consonant-Nucleus-Consonant (CNC) test, Bamford-Kowal-Bench (BKB) sentence test, LittEARS® Auditory Questionnaire, MacArthur-Bates Communicative Development Inventories (MB-CDI) Words and Gestures, Quality of Life—Cochlear Implant (QoL-CI), Pediatric Quality of Life Inventory (PedsQL™), Hearing Environments and Reflection on Quality of Life (HEAR-QL)-26, HEAR-QL-28, The Health Utilities Index 3 (HUI-3), Vanderbilt Fatigue Scales (VFS), or AzBio test (e.g., a pediatric AzBio test). The method may also include performing hearing assessments (e.g., DPOAE assessment, otoscopy, and tympanometry) and/or vestibular assessments (e.g., cervical vestibular-evoked myogenic potential, dizziness handicap inventory, pediatric vestibular symptom questionnaire, age and stages questionnaire, and video head impulse test) prior to treatment and/or after treatment initiation (e.g., prior to treatment and 1 day, 1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, 24 weeks, 36 weeks, 48 weeks, one year, 72 weeks, 2 years, 3 years, 4 years, and/or 5 years after administration of an OTOF dual vector system). The compositions and methods described herein may also be administered as a preventative treatment to patients at risk of developing hearing loss or auditory neuropathy, e.g., patients who have a family history of inherited hearing loss or patients carrying an OTOF mutation who do not yet exhibit hearing loss or impairment.

Treatment may include administration of a composition containing the nucleic acid vectors (e.g., AAV vectors) described herein in various unit doses. Each unit dose will ordinarily contain a predetermined quantity of the therapeutic composition. The quantity to be administered, and the particular route of administration and formulation, are within the skill of those in the clinical arts. A unit dose need not be administered as a single injection but may include continuous infusion over a set period of time.

Dosing may be performed using a syringe pump to control infusion rate in order to minimize damage to the cochlea. In cases in which the nucleic acid vectors are AAV vectors (e.g., AAV1, AAV2, AAV2quad(Y-F), AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, rh10, rh39, rh43, rh74, Anc80, Anc80L65, DJ/8, DJ/9, 7m8, PHP.B, PHP.eb, or PHP.S vectors), the AAV vectors may have a titer of, for example, from about 1×10^{13} vector genomes (vg)/mL to about 1×10^{14} vg/mL (e.g., 1×10^{13} vg/mL, 2×10^{13} VG/mL, 3×10^{13} vg/mL, 4×10^{13} vg /mL, 5×10^{13} VG/mL, 6×10^{13} vg /mL, 7×10^{13} vg/mL, 8×10^{13} vg/mL, 9×10^{13} vg/mL, 1×10^{14} vg /mL, e.g., 1.0×10^{13} vg/mL to 5.0×10^{13} vg/mL, 2.0×10^{13} vg/mL to 5.0×10^{13} vg/mL, 3.0×10^{13} vg/mL to 5.0×10^{13} vg/mL, 4.0×10^{13} vg/mL to 5.0×10^{13} vg/mL, 5.0×10^{13} vg/mL to 1.0×10^{14} vg/mL, 5.0×10^{13} vg/mL to 9.0×10^{13} vg/mL, 5.0×10^{13} vg/mL to 8.0×10^{13} vg/mL, 5.0×10^{13} vg/mL to 7.0×10^{13} vg/mL, or 5.0×10^{13} vg/mL to 6.0×10^{13} vg/mL) in a volume of 200 μ L to 250 μ L (e.g., 200 μ L, 210 μ L, 220 μ L, 230 μ L, 240 μ L, or 250 μ L, e.g., 200-225 μ L, 225-250 μ L, 200-210 μ L, 210-220 μ L, 220-230 μ L, 230-240 μ L, or 240-250 μ L). In some embodiments, the AAV vectors have a titer of about 3×10^{13} vg/mL to about 8×10^{13} vg/mL (e.g., 3.0×10^{13} vg/mL to 8.0×10^{13} vg/mL, 3.0×10^{13} vg/mL to 7.5×10^{13} vg/mL, 3.0×10^{13} vg/mL to 7.0×10^{13} vg/mL, 3.0×10^{13} vg/mL to 6.5×10^{13} vg/mL, 3.0×10^{13} vg/mL to 6.0×10^{13} vg/mL, 3.0×10^{13} vg/mL to 5.5×10^{13} vg/mL, 3.0×10^{13} vg/mL to 5.0×10^{13} vg/mL, 3.0×10^{13} vg/mL to 4.5×10^{13} vg/mL, 3.0×10^{13} vg/mL to 4.0×10^{13} vg/mL, 3.5×10^{13} vg/mL to 8.0×10^{13} vg/mL, 4.0×10^{13} vg/mL to 8.0×10^{13} vg/mL, 4.5×10^{13} vg/mL to 8.0×10^{13} vg/mL, 5.0×10^{13} vg/mL to 8.0×10^{13}

vg/mL, 5.5×10^{13} vg/mL to 8.0×10^{13} vg/mL, 6.0×10^{13} vg/mL to 8.0×10^{13} vg/mL, 6.5×10^{13} vg/mL to 8.0×10^{13} vg/mL, or 7.0×10^{13} vg/mL to 8.0×10^{13} vg/mL) in a volume of 225 μ L to 250 μ L (e.g., 225 μ L, 230 μ L, 235 μ L, 240 μ L, 245 μ L, or 250 μ L, e.g., 225-235 μ L, 230-240 μ L, 235-245 μ L, or 240-250 μ L). In some embodiments, the AAV vectors have a titer of about 3×10^{13} VG/mL or 7.3×10^{13} VG/mL in a volume of 240 μ L. The AAV vectors may be administered to the subject at a dose of about 2×10^{12} VG/ear to about 2.5×10^{13} VG/ear (e.g., 2×10^{12} VG/ear to 5×10^{12} VG/ear, 5×10^{12} VG/ear to 8×10^{12} VG/ear, 7×10^{12} VG/ear to 2×10^{13} VG/ear, 8×10^{12} VG/ear to 2.5×10^{13} VG/ear, e.g., 2×10^{12} VG/ear, 2.25×10^{12} VG/ear, 2.5×10^{12} VG/ear, 2.75×10^{12} VG/ear, 3×10^{12} VG/ear, 3.25×10^{12} VG/ear, 3.5×10^{12} VG/ear, 3.75×10^{12} VG/ear, 4×10^{12} VG/ear, 4.25×10^{12} VG/ear, 4.5×10^{12} VG/ear, 4.75×10^{12} VG/ear, 5×10^{12} VG/ear, 5.25×10^{12} VG/ear, 5.5×10^{12} VG/ear, 5.75×10^{12} VG/ear, 6×10^{12} VG/ear, 6.25×10^{12} VG/ear, 6.5×10^{12} VG/ear, 6.75×10^{12} VG/ear, 7×10^{12} VG/ear, 7.2×10^{12} VG/ear, 7.5×10^{12} VG/ear, 7.75×10^{12} VG/ear, 8×10^{12} VG/ear, 8.25×10^{12} VG/ear, 8.5×10^{12} VG/ear, 8.75×10^{12} VG/ear, 9×10^{12} VG/ear, 9.25×10^{12} VG/ear, 9.5×10^{12} VG/ear, 9.75×10^{12} VG/ear, 1×10^{13} VG/ear, 1.25×10^{13} VG/ear, 1.5×10^{13} VG/ear, 1.75×10^{13} VG/ear, 2×10^{13} VG/ear, 2.25×10^{13} VG/ear, or 2.5×10^{13} VG/ear). In some embodiments, the AAV vectors are administered to the subject at a dose of about 7.2×10^{12} VG/ear to about 1.75×10^{13} VG/ear (e.g., a dose of 7.2×10^{12} VG/ear or 1.75×10^{13} VG/ear).

The methods described herein may also include a step of administering antibiotics for prophylaxis for post-operative infection (e.g., before administration of the OTOF dual vector system) and/or administering a corticosteroid as prophylaxis against inflammatory and immunological responses (e.g., after administration of the OTOF dual vector system). For example, the day after administration of the dual vector system, a subject may begin a four-week treatment with a corticosteroid (e.g., prednisone or equivalent). In one embodiment, the subject may receive 1 mg/kg corticosteroid daily for the first two weeks post-treatment, 0.5 mg/kg corticosteroid daily for the third week post-treatment, and 0.25 mg/kg daily for the fourth week post-treatment. The total dose of corticosteroid is not to exceed 60 mg/day and may be weight adjusted as needed. If a pre-operative corticosteroid is administered (IV) as part of standard of care for cochlear implant surgery, the prophylactic corticosteroid dose on Day 0 may be adjusted so the daily corticosteroid dose does not exceed 1 mg/kg of prednisone equivalent dose; the Day 0 prophylactic corticosteroid may not be required if the pre-operative corticosteroid dose is ≥ 1 mg/kg of prednisone equivalent dose. If a pre-operative corticosteroid is not administered, the initial prophylactic corticosteroid dose may be spaced appropriately after the surgery and may be initiated the night before surgery.

The compositions described herein are administered in an amount sufficient to improve hearing, increase WT OTOF expression (e.g., expression of OTOF isoform 5 in a cochlear hair cell, e.g., an inner hair cell), or increase OTOF function. In some embodiments, the methods described herein instate hearing (e.g., instate more natural hearing and a more natural developmental trajectory) in profoundly deaf pediatric subjects by restoring the natural physiology of IHC transduction. Hearing may be evaluated using standard hearing tests (e.g., audiometry, ABR, electrocochleography (ECOG), and otoacoustic emissions) and may be improved by 5% or more (e.g., 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) compared to hearing measurements obtained prior to treatment. In some embodiments, the compositions are administered in an amount sufficient to improve the subject's ability to understand speech. In some embodiments, the methods described herein result in a change in

intensity threshold (decibels normalized hearing level (dB nHL)) across frequency domains as assessed using ABR (e.g., a change indicating improved hearing). In some embodiments, the methods described herein result in a change in intensity thresholds (decibel hearing level (dB HL)) across frequency domains and/or a change in speech awareness threshold (SAT) in the treated ear(s) (e.g., changes indicating improved hearing, e.g., assessed using behavioral audiometry such as SAT/SDT audiometry, SRT audiometry, pure tone air- and bone-conduction audiometry, VRA, or CPA). In some embodiments, methods described herein result in a change (e.g., an improvement) in score in one or more assessments or questionnaires after OTOF dual vector administration, such as a change (e.g., a change indicative of improved hearing) in score in the ASC, O & C set task, ESP test, PSI test, LNT/MLNT, CNC test, BKB sentence test, LittleEARS® Auditory Questionnaire, MB-CDI Words and Gestures, QoL-CI, PedsQL™, HEAR-QL-26, HEAR-QL-28, HUI-3, VFS, or AzBio test (e.g., a pediatric AzBio test). The compositions described herein may also be administered in an amount sufficient to slow or prevent the development or progression of sensorineural hearing loss or auditory neuropathy (e.g., in subjects who carry a mutation in OTOF or have a family history of autosomal recessive hearing loss but do not exhibit hearing impairment, or in subjects exhibiting mild to moderate hearing loss). OTOF expression may be evaluated using immunohistochemistry, Western blot analysis, quantitative real-time PCR, or other methods known in the art for detection protein or mRNA, and may be increased by 5% or more (e.g., 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) compared to OTOF expression prior to administration of the compositions described herein. OTOF expression may also be assessed by detecting antibodies to the therapeutic OTOF transgene in serum from treated subjects. OTOF function may be evaluated directly (e.g., using electrophysiological methods or imaging methods to assess exocytosis) or indirectly based on hearing tests, and may be increased by 5% or more (e.g., 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) compared to OTOF function prior to administration of the compositions described herein. These effects may occur, for example, within 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 12 weeks, 15 weeks, 20 weeks, 24 weeks, 25 weeks, 36 weeks, 48 weeks, one year, or more, following administration of the compositions described herein. The patient may be evaluated 1 month, 2 months, 3 months, 4 months, 5 months, 6 months or more following administration of the composition depending on the dose and route of administration used for treatment. Depending on the outcome of the evaluation, the patient may receive additional treatments.

Kits

The compositions described herein can be provided in a kit for use in treating sensorineural hearing loss or auditory neuropathy (e.g., hearing loss associated with a biallelic mutation in OTOF). Compositions may include nucleic acid vectors (e.g., AAV vectors) described herein (e.g., a first nucleic acid vector containing a polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein and a second nucleic acid vector containing a polynucleotide that encodes a C-terminal portion of an OTOF isoform 5 protein), optionally packaged in an AAV virus capsid (e.g., an AAV1 capsid, an Anc80 capsid, an AAV2 capsid, or an AAV9 capsid). The kit can further include a package insert that instructs a

user of the kit, such as a physician, to perform the methods described herein. The kit may optionally include a syringe, a syringe pump, a catheter, or other device for administering the composition.

Examples

5 The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used, made, and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

10 **Example 1. Administration of a composition containing a dual hybrid vector system that expresses OTOF isoform 5 to a subject under two years of age with profound genetic hearing loss**

 An eight-month-old female subject with profound congenital bilateral hearing loss and auditory neuropathy with present OAEs (i.e., the subject was identified as having detectable otoacoustic emissions) and biallelic mutations in the OTOF gene (pathogenic OTOF variant c.2887C>T
15 (p.Arg963Ter) / c.2676+1G>T) was screened, qualified, and enrolled in a Phase 1/2 study. The subject was 10 months old at dosing and received an intracochlear injection of a composition containing an OTOF dual hybrid vector system in one ear. The composition contained a first AAV1 vector containing a Myo15 promoter (SEQ ID NO: 21) operably linked to exons 1-20 of a polynucleotide encoding a human OTOF isoform 5 protein (SEQ ID NO: 56), a splice donor sequence 3' of the polynucleotide sequence,
20 and an AP recombinogenic region (SEQ ID NO: 51) 3' of the splice donor sequence, and a second AAV1 vector containing an AP recombinogenic region (SEQ ID NO: 51), a splice acceptor sequence 3' of the recombinogenic region, a polynucleotide 3' of the splice acceptor sequence that contains exons 21-45 and 47 of a polynucleotide encoding a human OTOF isoform 5 protein (SEQ ID NO: 57), and a bGH poly(A) sequence. The composition containing the OTOF dual hybrid AAV vector system was
25 administered by a single, unilateral intracochlear injection in the right ear at an amount of 7.2×10^{12} VG (3×10^{13} vg/mL in a volume of 240 μ L). The surgical approach used to access the middle ear was similar to that used for cochlear implant surgery and involved a mastoidectomy and opening the facial recess. The composition was administered via insertion of a catheter through the round window membrane into the inner ear perilymph for direct infusion. A fenestration was also made in the lateral semicircular canal (FIG.
30 5). A cochlear implant was placed in the contralateral (left) ear during the same procedure. The subject received intraoperative antibiotics. The subject also received perioperative steroids and oral steroids (1 mg/kg/d) for two weeks that were tapered over two weeks. The perioperative period was uneventful, and the subject was discharged on day 1 post-operation. Following administration of the composition containing the OTOF dual hybrid vector system, the subject was assessed for safety and tolerability
35 (including adverse events (AEs) and effects on balance and vestibular function (assessed by cervical vestibular evoked myogenic potential)) and efficacy (pure tone audiometry, auditory brainstem response, and hearing questionnaires) from Baseline (prior to dosing) to Week 12.

 At planned follow-ups, the subject experienced improvements in auditory responses through Week 6 compared to Baseline, per auditory brainstem response (ABR) and behavioral (pure tone)
40 audiometry. ABR, a clinically accepted physiologic measure of hearing sensitivity, is often absent in those with classic otoferlin-related hearing loss and was absent in both ears of the subject at Baseline. The

subject also had no detectable hearing by behavioral pure tone audiogram at Baseline. At Week 6, ABR showed a response between 80-90 dB in the treated ear in an incomplete test (the subject woke up while the test was ongoing) despite the presence of some electric interference. Behavioral testing at Week 6 showed similar results consistent with Week 4 showing thresholds in the 80 dB range; however, the results are considered less reliable than the previous results due to lack of cooperation of the subject, potential learning effect or fatigue from repeated testing, and an ongoing upper airway infection. The subject's parents reported possible response to vocalization and clear response to loud sounds in keeping with Week 4. DPOAEs (a measure of outer hair cell function) were similar to Week 4 but testing conditions were not ideal (abnormal tympanogram due to subject's nasal congestion). Results from Week 4 and Week 6 are early indicators of a positive auditory response. There were no concerning safety signals through Week 6 following treatment and notably no treatment-emergent adverse events related to the OTOF dual hybrid vector system. ABR and behavioral (pure tone) audiometry results are shown in FIGS. 1A-1D, FIG. 2, and FIG. 3. Behavioral audiometry results are also provided below in Table 5.

Table 5: Audiogram (behavioral pure tone testing)

Baseline									
Right Ear - Audiogram (air)					Left Ear - Audiogram				
	250	500	1000	2000	4000	500 Hz	1000	2000	4000
		Hz	Hz	Hz	Hz		Hz	Hz	Hz
Threshold (dBHL)- Air		NR	NR	NR	NR				
Max stimulation (air)		100	100	100	95				
Bone									
Week 4									
Right Ear - Audiogram (air)					Left Ear - Audiogram				
	500	1000	2000	4000	500 Hz	1000	2000	4000	
	Hz	Hz	Hz	Hz	Hz	Hz	Hz	Hz	
Threshold (dBHL)- Air	95	85	85	80	NR110	NR110	NR110	NR110	
Bone									
Week 6									
Right Ear - Audiogram (air)					Left Ear - Audiogram				
	500	1000	2000	4000	500 Hz	1000	2000	4000	
	Hz	Hz	Hz	Hz	Hz	Hz	Hz	Hz	
Threshold (dBHL)- Air	100	95	90	85	NR 110	NR 110	NR 110	NR 110	
Bone	NR 60	NR 70	NR 75	NR 80	DNT	DNT	DNT	DNT	

The subject was evaluated again at Week 12 and continued to show improvements in auditory responses compared to Baseline. Results from a behavioral pure tone audiogram are shown in FIG. 4. As shown in FIG. 4, the subject had profound hearing impairment at Baseline in the treated ear. At Week 4,

the treated ear responded to multiple pitches. At Week 12, the treated ear exhibited responses in the range of moderate sensorineural hearing loss, indicating that the subject's hearing had markedly improved from initial profound sensorineural hearing loss. At Week 12 from Baseline, an improvement of 55 dB HL in hearing thresholds with air conduction versus Baseline (absent thresholds at 100 dB HL, maximum air conduction intensity tested, 250–4000 Hz) was observed by pure tone audiometry in the treated ear (FIG. 6). No improvement was seen in the untreated ear with cochlear implant with the cochlear implant turned off (FIG. 6). A positive ABR wave V amplitude response (thresholds of 40–80 dB, no response was elicited at 100 dB at Baseline) at Week 12 was also reported in the treated ear (FIG. 7). No improvement in hearing intensity was observed in the untreated ear when compared with the treated ear (FIG. 7). An improvement in the subject's global auditory skill development was observed at Week 12 from Baseline, according to parental reports and as indicated by the LittEARS auditory questionnaire outcomes (FIG. 8). This is in line with normative data for patients with cochlear implants. The development of auditory skills was also observed, and parents reported more natural vocalizations (subject's voice less "screechy") when the cochlear implant was turned off. The parents and investigator indicated that the subject could hear sounds when the opposite ear cochlear implant was turned off, indicating improved hearing acuity in the treated ear.

Through Week 12 after treatment, the study drug was well-tolerated and no dose-limiting toxicities and no adverse events (AEs) related to the OTOF dual hybrid vector system were reported, including absence of vestibular manifestations. Seven Grade 1 AEs were reported after dosing (upper respiratory tract infection, hand/foot/mouth rash, white blood cells in urine, reduced distortion product otoacoustic emissions (considered not related to study drug), vomiting, and elevated alanine transaminase), all of which were unrelated to treatment with the OTOF dual hybrid vector system. One serious AE (possible mastoiditis in ear with cochlear implant; Grade 2) was also reported and was considered unrelated to the OTOF dual hybrid vector system. Cervical vestibular evoked myogenic potential (cVEMP) responses were present and were similar to Baseline (FIG. 9). No observable nystagmus or overt motor behavioral changes were reported.

Example 2. Administration of a composition containing dual hybrid vectors that express OTOF isoform 5 to a subject with sensorineural hearing loss

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with sensorineural hearing loss (e.g., sensorineural hearing loss associated with a biallelic mutation in OTOF) so as to improve or restore hearing. In one example, a physician of skill in the art can administer to the human patient a composition containing a first AAV vector (e.g., an AAV1 vector) containing a Myo15 promoter (e.g., SEQ ID NO: 19, 21, 22, 31, or 32) operably linked to exons 1-20 of a polynucleotide encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1, e.g., a polynucleotide having the sequence of SEQ ID NO: 56), a splice donor sequence 3' of the polynucleotide sequence, and an AP recombinogenic region (e.g., an AP gene fragment, any one of SEQ ID NOs: 48-53, e.g., SEQ ID NO: 51) 3' of the splice donor sequence, and a second AAV vector (e.g., an AAV1 vector) containing an AP recombinogenic region (an AP gene fragment, any one of SEQ ID NOs: 48-53, e.g., SEQ ID NO: 51), a splice acceptor sequence 3' of the recombinogenic region, a polynucleotide 3' of the splice acceptor sequence that contains exons 21-45 and 47 of a polynucleotide

encoding an OTOF isoform 5 protein (e.g., human OTOF isoform 5, e.g., SEQ ID NO: 1, e.g., a polynucleotide having the sequence of SEQ ID NO: 57), and a bGH poly(A) sequence. The composition containing the dual hybrid AAV vectors may be administered to the patient, for example, by intracochlear injection of an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of the OTOF dual vector system in a volume of 200-250 μ L (e.g., an amount of 3×10^{13} vg/mL or 7.3×10^{13} vg/mL in a volume of 240 μ L), to treat sensorineural hearing loss.

Following administration of the composition to a patient, a practitioner of skill in the art can monitor the expression of OTOF, and the patient's improvement in response to the therapy, by a variety of methods. For example, a physician can monitor the patient's hearing by performing standard tests, such as audiometry, ABR, and measuring otoacoustic emissions following administration of the composition. A finding that the patient exhibits improved hearing (e.g., improved ABR and/or pure tone audiometry) in one or more of the tests following administration of the composition compared to hearing test results prior to administration of the composition indicates that the patient is responding favorably to the treatment.

Exemplary embodiments of the invention are described in the enumerated paragraphs below.

- E1. A method of treating a human subject having biallelic otoferlin (OTOF) mutations, the method comprising administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of an OTOF dual vector system in a volume of 200-250 μ L by intracochlear injection, wherein the OTOF dual vector system comprises:
a first adeno-associated virus (AAV) vector comprising a Myo15 promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an Otoferlin (OTOF) isoform 5 protein, a splice donor sequence positioned 3' of the first coding polynucleotide, and a first recombinogenic region positioned 3' of the splice donor sequence; and
a second AAV vector comprising a second recombinogenic region, a splice acceptor sequence positioned 3' of the second recombinogenic region, a second coding polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence, and a poly(A) sequence positioned 3' of the second coding polynucleotide;
wherein the first coding polynucleotide and the second coding polynucleotide that encode the OTOF isoform 5 protein do not overlap, wherein neither the first nor second AAV vector encodes the full-length OTOF isoform 5 protein, and wherein the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to about 1:3 (e.g., 3:1 to 1:3, 2:1 to 1:2, or 1:1).
- E2. The method of E1, wherein the first AAV vector and the second AAV vector comprise an AAV1 capsid.
- E3. The method of E1 or E2, wherein the Myo15 promoter comprises a first region having at least 85% sequence identity to SEQ ID NO: 7 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 9 and/or SEQ ID NO: 10 operably linked to a second region having at least 85% sequence identity to SEQ ID NO: 8 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 14 and/or SEQ ID NO: 15, wherein the first region and the second region are directly fused or joined by a linker comprising one to one hundred nucleotides.

- E4. The method of E3, wherein the first region comprises the sequence of SEQ ID NO: 7.
- E5. The method of E4, wherein the first region consists of the sequence of SEQ ID NO: 7.
- E6. The method of E3, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 9.
- 5 E7. The method of E3, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 10.
- E8. The method of E3, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 9 and the sequence of SEQ ID NO: 10.
- E9. The method of E8, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of
10 SEQ ID NO: 11.
- E10. The method of E8, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 12.
- E11. The method of E8, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 13.
- 15 E12. The method of E8, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 33.
- E13. The method of any one of E3-E12, wherein the second region comprises the sequence of SEQ ID NO: 8.
- E14. The method of E13, wherein the second region consists of the sequence of SEQ ID NO: 8.
- 20 E15. The method of any one of E3-E12, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 14.
- E16. The method of any one of E3-E12, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 15.
- E17. The method of any one of E3-E12, wherein the functional portion of SEQ ID NO: 8 comprises the
25 sequence of SEQ ID NO: 14 and the sequence of SEQ ID NO: 15.
- E18. The method of E17, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 16.
- E19. The method of E17, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 17.
- 30 E20. The method of E17, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 18.
- E21. The method of any one of E3-E12, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 34.
- E22. The method of any one of E3-E12, wherein the functional portion of SEQ ID NO: 8 comprises the
35 sequence of SEQ ID NO: 35.
- E23. The method of any one of E3-E12, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 34 and the sequence of SEQ ID NO: 35.
- E24. The method of E23, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 38.
- 40 E25. The method of E3, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 19.
- E26. The method of E25, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 19

- E27. The method of E3, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 21.
- E28. The method of E27, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 21.
- E29. The method of E3, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 22.
- E30. The method of E29, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 22.
- 5 E31. The method of E3, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 42.
- E32. The method of E31, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 42.
- E33. The method of E3, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 43.
- E34. The method of E33, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 43.
- E35. The method of E1 or E2, wherein the Myo15 promoter comprises a first region having at least
- 10 85% sequence identity to SEQ ID NO: 23 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 25 operably linked to a second region having at least 85% sequence identity to SEQ ID NO: 24 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 26 and/or SEQ ID NO: 27, wherein the first region and the second region are directly fused or joined by linker comprising one to four hundred nucleotides between
- 15 the first region and the second region.
- E36. The method of E35, wherein the first region comprises the sequence of SEQ ID NO: 23.
- E37. The method of E36, wherein the first region consists of the sequence of SEQ ID NO: 23.
- E38. The method of E35, wherein the functional portion of SEQ ID NO: 23 comprises the sequence of SEQ ID NO: 25.
- 20 E39. The method of any one of E35-E38, wherein the second region comprises the sequence of SEQ ID NO: 24.
- E40. The method of E39, wherein the second region consists of the sequence of SEQ ID NO: 24.
- E41. The method of any one of E35-E38, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 26.
- 25 E42. The method of any one of E35-E38, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 27.
- E43. The method of any one of E35-E38, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 26 and the sequence of SEQ ID NO: 27.
- E44. The method of E43, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of
- 30 SEQ ID NO: 28.
- E45. The method of E43, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 29.
- E46. The method of E43, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 30.
- 35 E47. The method of E35, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 31.
- E48. The method of E47, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 31.
- E49. The method of E35, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 32.
- E50. The method of E49, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 32.
- E51. The method of E1 or E2, wherein the Myo15 promoter comprises a sequence having at least
- 40 85% sequence identity to SEQ ID NO: 34.

- E52. The method of E1 or E2, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 38.
- E53. The method of E1 or E2, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 39.
- 5 E54. The method of E1 or E2, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 40.
- E55. A method of treating a human subject having biallelic OTOF mutations (e.g., a biallelic likely pathogenic or pathogenic OTOF mutation), the method comprising administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of an OTOF dual vector system in a volume of 200-250 μ L by intracochlear injection, wherein the OTOF dual vector system comprises:
- 10 a first AAV vector comprising a ubiquitous promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein, a splice donor sequence positioned 3' of the first coding polynucleotide, and a first recombinogenic region positioned 3' of the splice donor sequence; and
- 15 a second AAV vector comprising a second recombinogenic region, a splice acceptor sequence positioned 3' of the second recombinogenic region, a second coding polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence, and a poly(A) sequence positioned 3' of the second coding polynucleotide;
- 20 wherein the first coding polynucleotide and the second coding polynucleotide do not overlap, wherein neither the first nor second AAV vector encodes the full-length OTOF isoform 5 protein, and wherein the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to about 1:3 (e.g., 3:1 to 1:3, 2:1 to 1:2, or 1:1).
- E56. The method of E55, wherein the ubiquitous promoter is selected from the group consisting of a CAG promoter, a cytomegalovirus (CMV) promoter, a truncated CMV-chicken β -actin promoter (smCBA promoter), a chicken β -actin promoter, a CB7 promoter, a hybrid CMV enhancer/human β -actin promoter, or a human β -actin promoter.
- 25 E57. The method of E56, wherein the ubiquitous promoter is the smCBA promoter.
- E58. The method of E57, wherein the smCBA promoter comprises the sequence of SEQ ID NO: 44.
- 30 E59. The method of E58, wherein the smCBA promoter consists of the sequence of SEQ ID NO: 44.
- E60. The method of any one of E55-E59, wherein the first AAV vector and the second AAV vector comprise an Anc80 capsid, an AAV2 capsid, an AAVquad(Y-F) capsid, an AAV8 capsid, an AAV9 capsid, an AAV1 capsid, an AAV6 capsid, a DJ/9 capsid, a 7m8 capsid, an Anc80L65 capsid, or a PHP.B capsid.
- 35 E61. A method of improving hearing in a human subject, the method comprising selecting a subject having biallelic OTOF mutations (e.g., a biallelic likely pathogenic or pathogenic OTOF mutation) and administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of an OTOF dual vector system in a volume of 200-250 μ L by intracochlear injection, wherein the OTOF dual vector system comprises:
- 40 a first AAV vector comprising a first ITR sequence; a promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein; a splice donor

- sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and
- a second AAV vector comprising a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding
- 5 polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence;
- wherein the first coding polynucleotide and the second coding polynucleotide that encode the OTOF isoform 5 protein do not overlap, wherein neither the first nor second AAV vector encodes
- 10 the full-length OTOF isoform 5 protein, and wherein the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to 1:3 (e.g., 3:1 to 1:3, 2:1 to 1:2, or 1:1).
- E62. The method of E61, wherein the promoter is a Myo15 promoter.
- E63. The method of E62, wherein the Myo15 promoter comprises a first region having at least 85% sequence identity to SEQ ID NO: 7 or a functional portion or derivative thereof comprising the
- 15 sequence of SEQ ID NO: 9 and/or SEQ ID NO: 10 operably linked to a second region having at least 85% sequence identity to SEQ ID NO: 8 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 14 and/or SEQ ID NO: 15, wherein the first region and the second region are directly fused or joined by a linker comprising one to one hundred nucleotides.
- 20 E64. The method of E63, wherein the first region comprises the sequence of SEQ ID NO: 7.
- E65. The method of E64, wherein the first region consists of the sequence of SEQ ID NO: 7.
- E66. The method of E63, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 9.
- E67. The method of E63, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of
- 25 SEQ ID NO: 10.
- E68. The method of E63, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 9 and the sequence of SEQ ID NO: 10.
- E69. The method of E68, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 11.
- 30 E70. The method of E68, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 12.
- E71. The method of E68, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of SEQ ID NO: 13.
- E72. The method of E68, wherein the functional portion of SEQ ID NO: 7 comprises the sequence of
- 35 SEQ ID NO: 33.
- E73. The method of any one of E63-E72, wherein the second region comprises the sequence of SEQ ID NO: 8.
- E74. The method of E73, wherein the second region consists of the sequence of SEQ ID NO: 8.
- E75. The method of any one of E63-E72, wherein the functional portion of SEQ ID NO: 8 comprises
- 40 the sequence of SEQ ID NO: 14.

- E76. The method of any one of E63-E72, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 15.
- E77. The method of any one of E63-E72, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 14 and the sequence of SEQ ID NO: 15.
- 5 E78. The method of E77, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 16.
- E79. The method of E77, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 17.
- 10 E80. The method of E77, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 18.
- E81. The method of any one of E63-E72, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 34.
- E82. The method of any one of E63-E72, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 35.
- 15 E83. The method of any one of E63-E72, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 34 and the sequence of SEQ ID NO: 35.
- E84. The method of E83, wherein the functional portion of SEQ ID NO: 8 comprises the sequence of SEQ ID NO: 38.
- E85. The method of E63, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 19.
- 20 E86. The method of E85, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 19.
- E87. The method of E63, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 21.
- E88. The method of E87, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 21.
- E89. The method of E63, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 22.
- E90. The method of E89, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 22.
- 25 E91. The method of 63, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 42.
- E92. The method of E91, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 42.
- E93. The method of E63, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 43.
- E94. The method of E93, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 43.
- 30 E95. The method of E62, wherein the Myo15 promoter comprises a first region having at least 85% sequence identity to SEQ ID NO: 23 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 25 operably linked to a second region having at least 85% sequence identity to SEQ ID NO: 24 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 26 and/or SEQ ID NO: 27, wherein the first region and the second region are directly fused or joined by linker comprising one to four hundred nucleotides between the first region and
- 35 the second region.
- E96. The method of E95, wherein the first region comprises the sequence of SEQ ID NO: 23.
- E97. The method of E96, wherein the first region consists of the sequence of SEQ ID NO: 23.
- E98. The method of E95, wherein the functional portion of SEQ ID NO: 23 comprises the sequence of SEQ ID NO: 25.
- 40 E99. The method of any one of E95-E98, wherein the second region comprises the sequence of SEQ ID NO: 24.

- E100. The method of E99, wherein the second region consists of the sequence of SEQ ID NO: 24.
- E101. The method of any one of E95-E98, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 26.
- 5 E102. The method of any one of E95-E98, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 27.
- E103. The method of any one of E95-E98, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 26 and the sequence of SEQ ID NO: 27.
- E104. The method of E103, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 28.
- 10 E105. The method of E103, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 29.
- E106. The method of E103, wherein the functional portion of SEQ ID NO: 24 comprises the sequence of SEQ ID NO: 30.
- E107. The method of E95, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 31.
- 15 E108. The method of E107, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 31.
- E109. The method of E95, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 32.
- E110. The method of E109, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 32.
- E111. The method of E62, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 34.
- 20 E112. The method of E62, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 38.
- E113. The method of E62, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 39.
- E114. The method of E62, wherein the Myo15 promoter comprises a sequence having at least 85% sequence identity to SEQ ID NO: 40.
- 25 E115. The method of E61, wherein the promoter is a ubiquitous promoter.
- E116. The method of E115, wherein the ubiquitous promoter is selected from the group consisting of a CAG promoter, a cytomegalovirus (CMV) promoter, a truncated CMV-chicken β -actin promoter (smCBA promoter), a chicken β -actin promoter, a CB7 promoter, a hybrid CMV enhancer/human β -actin promoter, or a human β -actin promoter.
- 30 E117. The method of E116, wherein the ubiquitous promoter is the smCBA promoter.
- E118. The method of E117, wherein the smCBA promoter comprises the sequence of SEQ ID NO: 44.
- E119. The method of E118, wherein the smCBA promoter consists of the sequence of SEQ ID NO: 44.
- 35 E120. The method of any one of E61-E119, wherein the first AAV vector and the second AAV vector comprise an Anc80 capsid, an AAV2 capsid, an AAVquad(Y-F) capsid, an AAV8 capsid, an AAV9 capsid, an AAV1 capsid, an AAV6 capsid, a DJ/9 capsid, a 7m8 capsid, an Anc80L65 capsid, or a PHP.B capsid.
- E121. The method of E120, wherein the first AAV vector and the second AAV vector comprise an AAV1 capsid.

- E122. The method of any one of E61-E121, wherein the subject has or is identified as having profound sensorineural hearing loss (e.g., the method comprises selecting a subject having biallelic OTOF mutations and profound sensorineural hearing loss).
- 5 E123. The method of any one of E61-E122, wherein the subject has or is identified as having present outer hair cell function (e.g., the method comprises selecting a subject having biallelic OTOF mutations and present outer hair cell function or selecting a subject having biallelic OTOF mutations, profound sensorineural hearing loss, and present outer hair cell function.).
- E124. The method of E123, wherein the subject is determined to have present outer hair cell function based on detectable otoacoustic emissions and/or a present cochlear microphonic.
- 10 E125. The method of any one of E1-E124, wherein the first and second recombinogenic regions are the same.
- E126. The method of any one of E1-E125, wherein the first recombinogenic region and/or the second recombinogenic region is an AK recombinogenic region (e.g., an AK recombinogenic region comprising or consisting of the sequence of SEQ ID NO: 47).
- 15 E127. The method of any one of E1-E125, wherein the first recombinogenic region and/or the second recombinogenic region is an AP gene fragment.
- E128. The method of E127, wherein the AP gene fragment comprises or consists of the sequence of any one of SEQ ID NOs: 48-53.
- E129. The method of E128, wherein the AP gene fragment comprises the sequence of SEQ ID NO: 51.
- 20 E130. The method of E129, wherein the AP gene fragment consists of the sequence of SEQ ID NO: 51.
- E131. The method of any one of E1-E130, wherein each of the first and second coding polynucleotides encode about half of the OTOF isoform 5 protein sequence.
- E132. The method of any one of E1-E131, wherein the first and second coding polynucleotides are divided at an OTOF exon boundary.
- 25 E133. The method of E132, wherein the first and second coding polynucleotides are divided at the OTOF exon 20/exon 21 boundary.
- E134. The method of any one of E1-E132, wherein the first coding polynucleotide consists of exons 1-20 of a polynucleotide encoding the OTOF isoform 5 protein and the second coding polynucleotide consists of exons 21-45 and 47 of a polynucleotide encoding the OTOF isoform 5 protein.
- 30 E135. The method of E132, wherein the first and second coding polynucleotides are divided at the OTOF exon 21/exon 22 boundary.
- E136. The method of any one of E1-E132, wherein the first coding polynucleotide consists of exons 1-21 of a polynucleotide encoding the OTOF isoform 5 protein and the second coding polynucleotide consists of exons 22-45 and 47 of a polynucleotide encoding the OTOF isoform 5 protein.
- 35 E137. The method of any one of E1-E136, wherein the first and second coding polynucleotides that encode the OTOF isoform 5 protein do not comprise introns.
- E138. The method of any one of E1-E137, wherein the OTOF isoform 5 protein is a human OTOF isoform 5 protein.
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- E139. The method of any one of E1-E138, wherein the OTOF isoform 5 protein comprises the sequence of SEQ ID NO: 1 or a variant thereof having one or more conservative amino acid substitutions.
- 5 E140. The method of E139, wherein no more than 10% of the amino acids in the OTOF isoform 5 protein variant are conservative amino acid substitutions.
- E141. The method of E139, wherein the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 1.
- E142. The method of any one of E1-E139 and E141, wherein the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 2 or SEQ ID NO: 3.
- 10 E143. The method of any one of E1-E134 and E137-E142, wherein the first coding polynucleotide encodes amino acids 1-802 of SEQ ID NO: 1 and the second coding polynucleotide encodes amino acids 803-1997 of SEQ ID NO: 1.
- E144. The method of any one of E1-E134 and E137-E143, wherein the N-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 58 or a variant thereof having one or
- 15 more conservative amino acid substitutions.
- E145. The method of E144, wherein no more than 10% (e.g., 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or fewer) of the amino acids in the N-terminal portion of the OTOF isoform 5 protein variant are conservative amino acid substitutions.
- E146. The method of E144, wherein the N-terminal portion of the OTOF isoform 5 protein consists of
- 20 the sequence of SEQ ID NO: 58.
- E147. The method of any one of E1-E134, E137-E144, and E146, wherein the N-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 56.
- E148. The method of any one of E1-E134 and E137-E147, wherein the C-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 59 or a variant thereof having one or
- 25 more conservative amino acid substitutions.
- E149. The method of E148, wherein no more than 10% (e.g., 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or fewer) of the amino acids in the C-terminal portion of the OTOF isoform 5 protein variant are conservative amino acid substitutions.
- E150. The method of E148, wherein the C-terminal portion of the OTOF isoform 5 protein consists of
- 30 the sequence of SEQ ID NO: 59.
- E151. The method of any one of E1-E134, E137-E148, and E150, wherein the C-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 57.
- E152. The method of any one of E1-E151, wherein the first vector comprises a first inverted terminal repeat (ITR) sequence 5' of the promoter and a second ITR sequence 3' of the recombinogenic
- 35 region, and the second vector comprises a first ITR sequence 5' of the recombinogenic region and a second ITR sequence 3' of the poly(A) sequence.
- E153. The method of E152, wherein the ITRs in the first vector and second vector are AAV2 ITRs or have at least 80% sequence identity (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) to
- 40 AAV2 ITRs.

- E154. The method of any one of E1-E153, wherein the splice donor sequence in the first vector comprises the sequence of SEQ ID NO: 54.
- E155. The method of E154, wherein the splice donor sequence in the first vector consists of the sequence of SEQ ID NO: 54.
- 5 E156. The method of any one of E1-E155, wherein the splice acceptor sequence in the second vector comprises the sequence of SEQ ID NO: 55.
- E157. The method of E156, wherein the splice acceptor sequence in the second vector consists of the sequence of SEQ ID NO: 55.
- 10 E158. The method of any one of E1-E157, wherein the first AAV vector comprises a Kozak sequence 3' of the promoter and 5' of the first coding polynucleotide that encodes the N-terminal portion of the OTOF isoform 5 protein.
- E159. The method of E1 or E62, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 2272 to 6041 of SEQ ID NO: 60.
- E160. The method of E1, E62, or E159, wherein the first AAV vector comprises a polynucleotide
15 sequence comprising the sequence of nucleotides 2049 to 6264 of SEQ ID NO: 60.
- E161. The method of E160, wherein the first AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 2049 to 6264 of SEQ ID NO: 60.
- E162. The method of E1 or E62, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 182 to 3949 of SEQ ID NO: 62.
- 20 E163. The method of E1, E62, or E162, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 19 to 4115 of SEQ ID NO: 62.
- E164. The method of E163, wherein the first AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 19 to 4115 of SEQ ID NO: 62.
- E165. The method of E55 or E115, wherein the first AAV vector comprises a polynucleotide sequence
25 comprising the sequence of positions 2267 to 6014 of SEQ ID NO: 64.
- E166. The method of E55, E115, or E165, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of positions 2049 to 6237 of SEQ ID NO: 64.
- E167. The method of E166, wherein the first AAV vector comprises a polynucleotide sequence consisting of the sequence of positions 2049 to 6237 of SEQ ID NO: 64.
- 30 E168. The method of E55 or E115, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of positions 177 to 3924 of SEQ ID NO: 65.
- E169. The method of E55, E115, or E168, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of positions 19 to 4090 of SEQ ID NO: 65.
- E170. The method of E169, wherein the first AAV vector comprises a polynucleotide sequence
35 consisting of the sequence of positions 19 to 4090 of SEQ ID NO: 65.
- E171. The method of any one of E1, E55, E62, E115, E159-E161, and E165-E167, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 2267 to 6476 of SEQ ID NO: 61.
- 40 E172. The method of any one of E1, E55, E62, E115, E159-E161, E165-E167, and E171, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 2049 to 6693 of SEQ ID NO: 61.

- 5 E173. The method of any one of E172, wherein the second AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 2049 to 6693 of SEQ ID NO: 61.
- E174. The method of any one of E1, E55, E62, E115, E162-E164, and E168-E170, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 187 to 4396 of SEQ ID NO: 63.
- E175. The method of any one of E1, E55, E62, E115, E162-E164, E168-E170, and E174, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 19 to 4589 of SEQ ID NO: 63.
- 10 E176. The method of E175, wherein the second AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 19 to 4589 of SEQ ID NO: 63.
- E177. The method of E1 or E62, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 235 to 4004 of SEQ ID NO: 66.
- E178. The method of E1, E62, or E177, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 12 to 4227 of SEQ ID NO: 66.
- 15 E179. The method of E178, wherein the first AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 12 to 4227 of SEQ ID NO: 66.
- E180. The method of E55 or E115, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 230 to 3977 of SEQ ID NO: 68.
- E181. The method of E55, E115, or E180, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 12 to 4200 of SEQ ID NO: 68.
- 20 E182. The method of E181, wherein the first AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 12 to 4200 of SEQ ID NO: 68.
- E183. The method of any one of E1, E55, E62, E115, and E177-E182, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 229 to 4438 of SEQ ID NO: 67.
- 25 E184. The method of any one of E1, E55, E62, E115, and E177-E183, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 12 to 4655 of SEQ ID NO: 67.
- E185. The method of E184, wherein the second AAV vector comprises a polynucleotide sequence consisting of the sequence of nucleotides 12 to 4655 of SEQ ID NO: 67.
- 30 E186. The method of any one of E1-E185, wherein the subject is less than 18 years of age.
- E187. The method of E186, wherein the subject is at least 7 years of age.
- E188. The method of E186, wherein the subject is older than 24 months of age and younger than 7 years of age.
- 35 E189. The method of E186, wherein the subject is 24 months of age or younger.
- E190. The method of any one of E1-E189, wherein the subject has or is identified as having profound sensorineural hearing loss (e.g., based on behavioral and/or physiological measurements of inner ear function, such as the absence of an ABR neural signal in response to a click stimulus at or below 85 dB normalized hearing level in the ear(s) to be treated or a ≥ 90 dB hearing level in the ear(s) to be treated).
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- E191. The method of any one of E1-E190, wherein the subject has or is identified as having behavioral open-set word detection scores consistent with the criteria from a cochlear implant label (e.g., has behavioral open-set word detection scores of < 30% in the ear(s) to be treated).
- 5 E192. The method of any one of E1-E191, wherein the subject has or is identified as having congenital auditory neuropathy.
- E193. The method of any one of E1-E192, wherein the subject has or is identified as having detectable otoacoustic emissions (e.g., presence of otoacoustic emissions (≥ 6 dB signal-to-noise ratio) at ≥ 3 frequencies from 1 to 8 kHz in the ear(s) to be treated).
- 10 E194. The method of any one of E1-E193, wherein the subject has or is identified as having a present cochlear microphonic in the ear(s) to be treated.
- E195. The method of any one of E1-E194, wherein the administering to an inner ear comprises intracochlear injection via a catheter placed through the round window membrane of the cochlea.
- E196. The method of any one of E1-E195, wherein the administering further comprises creating a fenestration in the lateral semicircular canal.
- 15 E197. The method of any one of E1-E196, wherein the injection is performed using a syringe and syringe pump.
- E198. The method of any one of E1-E197, wherein the administering is at a rate of 0.8-1 mL/hr (e.g., 0.9 mL/hr).
- E199. The method of any one of E1-E198, wherein the administering occurs once per ear.
- 20 E200. The method of any one of E1-E199, wherein the first vector and the second vector are administered at a ratio of about 1:1.
- E201. The method of any one of E1-E200, wherein the dual vector system is formulated as an aqueous suspension.
- E202. The method of E201, wherein the first vector and second vector are matched in titer and mixed at
25 an approximately equal ratio.
- E203. The method of E201 or E202, wherein the suspension comprises 10 mM sodium phosphate or disodium phosphate (also called sodium phosphate dibasic and having the formula Na_2HPO_4), 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 at a pH of about 7.4.
- 30 E204. The method of E203, wherein the suspension comprises 10 mM sodium phosphate.
- E205. The method of E203, wherein the suspension comprises 10 mM disodium phosphate.
- E206. The method of any one of E1-E205, wherein the method further comprises administering a corticosteroid to the subject for the first four weeks after the administering of the OTOF dual vector system.
- 35 E207. The method of E206, wherein the method comprises administering 1 mg/kg of the corticosteroid daily during the first two weeks after the administering of the OTOF dual vector system, administering 0.5 mg/kg daily during the third week after the administering of the OTOF dual vector system, and administering 0.25 mg/kg daily during the fourth week after the administering of the OTOF dual vector system.
- 40 E208. The method of any one of E1-E207, wherein the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μL .

- E209. The method of E208, wherein the OTOF dual vector system is administered in an amount of 2.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
- E210. The method of E209, wherein the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
- 5 E211. The method of E210, wherein the OTOF dual vector system is administered in an amount of 4.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
- E212. The method of any one of E208-E211, wherein the OTOF dual vector system is administered in a volume of 210-250 μ L.
- E213. The method of E212, wherein the OTOF dual vector system is administered in a volume of 220-
10 250 μ L.
- E214. The method of E213, wherein the OTOF dual vector system is administered in a volume of 230-250 μ L.
- E215. The method of E214, wherein the OTOF dual vector system is administered in a volume of 240-250 μ L.
- 15 E216. The method of E215, wherein the OTOF dual vector system is administered in a volume of 240 μ L.
- E217. The method of E216, wherein the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL in a volume of 240 μ L.
- E218. The method of any one of E1-E207, wherein the OTOF dual vector system is administered in an
20 amount of 5.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 200-250 μ L.
- E219. The method of E218, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 9.0×10^{13} vg/mL in a volume of 200-250 μ L.
- E220. The method of E219, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 8.0×10^{13} vg/mL in a volume of 200-250 μ L.
- 25 E221. The method of E220, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 7.0×10^{13} vg/mL in a volume of 200-250 μ L.
- E222. The method of E221, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 6.0×10^{13} vg/mL in a volume of 200-250 μ L.
- E223. The method of any one of E218-E222, wherein the OTOF dual vector system is administered in a
30 volume of 210-250 μ L.
- E224. The method of E223, wherein the OTOF dual vector system is administered in a volume of 220-250 μ L.
- E225. The method of E224, wherein the OTOF dual vector system is administered in a volume of 230-250 μ L.
- 35 E226. The method of E225, wherein the OTOF dual vector system is administered in a volume of 240-250 μ L.
- E227. The method of E226, wherein the OTOF dual vector system is administered in a volume of 240 μ L.
- E228. The method of E227, wherein the OTOF dual vector system is administered in an amount of 7.3×10^{13} vg/mL in a volume of 240 μ L.
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- E229. The method of any one of E1-E228, wherein the method further comprises identifying the subject as having a mutation in OTOF (e.g., a biallelic likely pathogenic or pathogenic OTOF mutation) prior to administering the dual vector system.
- 5 E230. The method of any one of E1-E229, wherein the subject has or is identified as having Deafness, Autosomal Recessive 9 (DFNB9).
- E231. The method of any one of E1-E230, wherein the method further comprises evaluating the hearing of the subject prior to administering the dual vector system (e.g., the method further comprises identifying the subject as having profound sensorineural hearing loss prior to administering the dual vector system).
- 10 E232. The method of any one of E1-E231, wherein the method further comprises identifying the subject as having present outer hair cell function prior to administering the dual vector system.
- E233. The method of any one of E1-E232, wherein the method increases OTOF expression in a cochlear hair cell.
- E234. The method of E233, wherein the cochlear hair cell is an inner hair cell.
- 15 E235. The method of any one of E1-E234, wherein the method further comprises evaluating the hearing of the subject after administering the dual vector system.
- E236. The method of any one of E1-E235, wherein the method prevents or reduces hearing loss, delays the development of hearing loss, slows the progression of hearing loss, improves hearing, improves speech discrimination, or improves hair cell function.
- 20 E237. The method of any one of E1-E236, wherein the method improves one or more parameters selected from the subject's auditory brainstem response (ABR), behavioral audiometry, and score in one or more hearing questionnaires or behavioral tasks.
- E238. The method of E237, wherein the hearing questionnaires and behavioral tasks include one or more of the Auditory Skills Checklist, Open & Closed set task, Early Speech Perception test, Pediatric Speech Intelligibility test, Lexical Neighborhood Test Multisyllabic Lexical Neighborhood Test, Consonant-Nucleus-Consonant test, Bamford-Kowal-Bench sentence test, LittleEARS® Auditory Questionnaire, MacArthur-Bates Communicative Development Inventories Words and Gestures, Quality of Life–Cochlear Implant, Pediatric Quality of Life Inventory, Hearing Environments and Reflection on Quality of Life (HEAR-QL)-26, HEAR-QL-28, The Health Utilities
- 25 Index 3, Vanderbilt Fatigue Scales, and AzBio test.
- E239. The method of E238, wherein the method improves the subject's score in the LittleEARS® Auditory Questionnaire.
- E240. The method of E237, wherein the method improves hearing thresholds by at least 55 decibels hearing level with air conduction as assessed by behavioral pure tone audiometry.
- 35 E241. The method of E237, wherein the method results in a positive ABR wave V amplitude response
- E242. A method of administering a nucleic acid vector to the inner ear of a human subject, the method comprising creating an opening in the round window membrane of the cochlea and an opening in a second location in the inner ear and inserting a catheter through the opening in the round window membrane to infuse the nucleic acid vector into the perilymph.
- 40 E243. The method of E242, wherein the opening made in the second location in the inner ear is made in a semicircular canal.

- E244. The method of E243, wherein the semicircular canal is a lateral semicircular canal.
- E245. The method of E243, wherein the semicircular canal is a posterior semicircular canal.
- E246. The method of E243, wherein the semicircular canal is a superior semicircular canal.
- 5 E247. The method of any one of E242-E246, wherein a surgical procedure is performed to access the middle ear before creating the opening in the round window membrane and lateral semicircular canal.
- E248. The method of E247, wherein the surgical procedure involves a mastoidectomy and opening a facial recess.
- E249. The method of E247 or E248, wherein the surgical procedure is a standard surgical procedure for
10 cochlear implant surgery.
- E250. The method of any one of E242-E249, wherein a syringe and/or a syringe pump are used in combination with the catheter for said administering.
- E251. The method of any one of E242-E250, wherein the nucleic acid vector (e.g., a solution containing the nucleic acid vector) is administered at a rate of 0.5-1 mL/hr (e.g., 0.6-1 mL/hr, 0.7-1 mL/hr,
15 0.8-1 mL/hr, or 0.9-1 mL/hour, such as 0.9 mL/hr).
- E252. The method of any one of E242-E251, wherein the nucleic acid vector is a viral vector.
- E253. The method of E252, wherein the viral vector is a lentivirus vector, an adenovirus vector, or an adeno-associated virus (AAV) vector.
- E254. The method of E253, wherein the viral vector is an AAV vector.
- 20 E255. The method of any one of E242-E254, wherein the human subject has or is at risk of developing sensorineural hearing loss.
- E256. The method of E255, wherein the hearing loss is genetic hearing loss.
- E257. The method of E256, wherein the genetic hearing loss is autosomal dominant hearing loss, autosomal recessive hearing loss, or X-linked hearing loss.
- 25 E258. The method of E256 or E257, wherein the nucleic acid vector comprises a polynucleotide encoding a protein that is absent or dysfunctional in the subject (e.g., a protein that is not produced or that does not function properly in the inner ear of the subject due to a genetic mutation associated with the hearing loss, e.g., the polynucleotide encodes a functional protein that is deficient in the subject due to a genetic mutation associated with the hearing loss) or a
30 polynucleotide encoding an expression product designed to correct a genetic mutation in the subject (e.g., correct a genetic mutation associated with the hearing loss via gene editing).
- E259. The method of E255, wherein the hearing loss is acquired hearing loss.
- E260. The method of E259 wherein the acquired hearing loss is noise-induced hearing loss, age-related hearing loss, disease or infection-related hearing loss, head trauma-related hearing loss, or
35 ototoxic drug-induced hearing loss.
- E261. The method of E259 or E260, wherein the nucleic acid vector comprises a polynucleotide encoding an expression product that induces or increases cochlear hair cell regeneration, increases cochlear supporting cell numbers, prevents or reduces cochlear hair cell damage, prevents or reduces cochlear hair cell death, increases cochlear hair cell maturation, improves
40 cochlear hair cell function, or promotes or increases cochlear hair cell survival.

- E262. The method of any one of E242-E254, wherein the human subject has or is at risk of developing vestibular dysfunction.
- E263. The method of E262, wherein the vestibular dysfunction is vertigo, dizziness, imbalance (e.g., loss of balance or a balance disorder), oscillopsia, or bilateral vestibulopathy.
- 5 E264. The method of E262 or E263, wherein the vestibular dysfunction is associated with a genetic mutation.
- E265. The method of E264, wherein the nucleic acid vector comprises a polynucleotide encoding a protein that is absent or dysfunctional in the subject (e.g., a protein that is not produced or that does not function properly in the inner ear of the subject due to the genetic mutation associated with the vestibular dysfunction, e.g., the polynucleotide encodes a functional protein that is
10 deficient in the subject due to the genetic mutation) or a polynucleotide encoding an expression product designed to correct a genetic mutation in the subject (e.g., correct a genetic mutation associated with the vestibular dysfunction via gene editing).
- E266. The method of E262 or E263, wherein the vestibular dysfunction is associated with damage to or loss of vestibular cells (e.g., vestibular hair cells or supporting cells, such as damage to or loss of vestibular cells, related to disease or infection, head trauma, ototoxic drugs (e.g., vestibulotoxic drugs), or aging).
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- E267. The method of E266, wherein the nucleic acid vector comprises a polynucleotide encoding an expression product that induces or increases vestibular hair cell regeneration, increases vestibular supporting cell numbers, prevents or reduces vestibular hair cell damage, prevents or reduces vestibular hair cell death, increases vestibular hair cell maturation, improves vestibular hair cell function, or promotes or increases vestibular hair cell survival.
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- E268. An aqueous suspension comprising an OTOF dual vector system, 10 mM sodium phosphate or disodium phosphate, 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 at a pH of 7.4, wherein the OTOF dual vector system comprises:
25 a first AAV vector comprising a first ITR sequence; a promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and
30 a second AAV vector comprising a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence;
35 wherein the first coding polynucleotide and the second coding polynucleotide that encode the OTOF isoform 5 protein do not overlap, and wherein neither the first nor second AAV vector encodes the full-length OTOF isoform 5 protein.
- E269. The suspension of E268, wherein the suspension comprises 10 mM sodium phosphate.
- E270. The suspension of E268, wherein the suspension comprises 10 mM disodium phosphate.
- 40 E271. The suspension of any one of E268-E270, wherein the ratio of the first vector to the second vector is about 3:1 to about 1:3.

- E272. The suspension of any one of E268-E271, wherein the first vector and second vector are matched in titer and mixed at an approximately equal ratio.
- E273. The suspension of E272, wherein the ratio of the first vector to the second vector is about 1:1.
- 5 E274. The suspension of any one of E268-E273, wherein the suspension has a titer of 1×10^{13} vg/mL to 1×10^{14} vg/mL.
- E275. The suspension of E274, wherein the suspension has a titer of 1×10^{13} vg/mL to 5×10^{13} vg/mL.
- E276. The suspension of E275, wherein the suspension has a titer of 1×10^{13} vg/mL to 4×10^{13} vg/mL.
- E277. The suspension of E276, wherein the suspension has a titer of 2×10^{13} vg/mL to 4×10^{13} vg/mL.
- E278. The suspension of E277, wherein the suspension has a titer of 3×10^{13} vg/mL.
- 10 E279. The suspension of E274, wherein the suspension has a titer of 5×10^{13} vg/mL to 1×10^{14} vg/mL.
- E280. The suspension of E279, wherein the suspension has a titer of 5×10^{13} vg/mL to 9×10^{13} vg/mL.
- E281. The suspension of E280, wherein the suspension has a titer of 5×10^{13} vg/mL to 8×10^{13} vg/mL.
- E282. The suspension of E281, wherein the suspension has a titer of 6×10^{13} vg/mL to 8×10^{13} vg/mL.
- E283. The suspension of E282, wherein the suspension has a titer of 7.3×10^{13} vg/mL.
- 15 E284. The suspension of any one of E268-E283, wherein the suspension is formulated for intracochlear injection.
- E285. The suspension of any one of E268-E284, wherein the promoter is a Myo15 promoter (e.g., a Myo15 promoter described hereinabove, such as a Myo15 promoter having at least 85% sequence identity to any one of SEQ ID NOs: 19, 21, 22, 31, 32, 34, 38, 39, 40, 42, or 43).
- 20 E286. The suspension of any one of E268-E284, wherein the promoter is a ubiquitous promoter.
- E287. The suspension of E286, wherein the ubiquitous promoter is selected from the group consisting of a CAG promoter, a cytomegalovirus (CMV) promoter, a truncated CMV-chicken β -actin promoter (smCBA promoter), a chicken β -actin promoter, a CB7 promoter, a hybrid CMV enhancer/human β -actin promoter, or a human β -actin promoter.
- 25 E288. The suspension of E287, wherein the ubiquitous promoter is the smCBA promoter.
- E289. The suspension of E288, wherein the smCBA promoter comprises the sequence of SEQ ID NO: 44.
- E290. The suspension of any one of E268-E289, wherein the first AAV vector and the second AAV vector comprise an Anc80 capsid, an AAV2 capsid, an AAVquad(Y-F) capsid, an AAV8 capsid,
- 30 an AAV9 capsid, an AAV1 capsid, an AAV6 capsid, a DJ/9 capsid, a 7m8 capsid, an Anc80L65 capsid, or a PHP.B capsid.
- E291. The suspension of E290, wherein the first AAV vector and the second AAV vector comprise an AAV1 capsid.
- E292. The suspension of any one of E268-E291, wherein the first and second recombinogenic regions are the same.
- 35 E293. The suspension of any one of E268-E292, wherein the first recombinogenic region and/or the second recombinogenic region is an AK recombinogenic region (e.g., an AK recombinogenic region comprising or consisting of the sequence of SEQ ID NO: 47).
- E294. The suspension of any one of E268-E292, wherein the first recombinogenic region and/or the
- 40 second recombinogenic region is an AP gene fragment (e.g., an AP gene fragment comprising or consisting of the sequence of any one of SEQ ID NOs: 48-53).

- E295. The suspension of any one of E268-E294, wherein each of the first and second coding polynucleotides encode about half of the OTOF isoform 5 protein sequence.
- E296. The suspension of any one of E268-E295, wherein the first and second coding polynucleotides are divided at an OTOF exon boundary.
- 5 E297. The suspension of E296, wherein the first and second coding polynucleotides are divided at the OTOF exon 20/exon 21 boundary.
- E298. The suspension of any one of E268-E296, wherein the first coding polynucleotide consists of exons 1-20 of a polynucleotide encoding the OTOF isoform 5 protein and the second coding polynucleotide consists of exons 21-45 and 47 of a polynucleotide encoding the OTOF isoform 5 protein.
- 10 E299. The suspension of E296, wherein the first and second coding polynucleotides are divided at the OTOF exon 21/exon 22 boundary.
- E300. The suspension of any one of E268-E296, wherein the first coding polynucleotide consists of exons 1-21 of a polynucleotide encoding the OTOF isoform 5 protein and the second coding polynucleotide consists of exons 22-45 and 47 of a polynucleotide encoding the OTOF isoform 5 protein.
- 15 E301. The suspension of any one of E268-E300, wherein the first and second coding polynucleotides that encode the OTOF isoform 5 protein do not comprise introns.
- E302. The suspension of any one of E268-E301, wherein the OTOF isoform 5 protein is a human OTOF isoform 5 protein.
- 20 E303. The suspension of any one of E268-E302, wherein the OTOF isoform 5 protein comprises the sequence of SEQ ID NO: 1 or a variant thereof having one or more conservative amino acid substitutions.
- E304. The suspension of E303, wherein no more than 10% of the amino acids in the OTOF isoform 5 protein variant are conservative amino acid substitutions.
- 25 E305. The suspension of E303, wherein the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 1.
- E306. The suspension of any one of E268-E303 and E305, wherein the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 2 or SEQ ID NO: 3.
- 30 E307. The suspension of any one of E268-E298 and E301-E306, wherein the first coding polynucleotide encodes amino acids 1-802 of SEQ ID NO: 1 and the second coding polynucleotide encodes amino acids 803-1997 of SEQ ID NO: 1.
- E308. The suspension of any one of E268-E298 and E301-E307, wherein the N-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 58 or a variant thereof having one or more conservative amino acid substitutions.
- 35 E309. The suspension of any one of E268-E298 and E301-E308, wherein the N-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 56.
- E310. The suspension of any one of E268-E298 and E301-E309, wherein the C-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 59 or a variant thereof having one or more conservative amino acid substitutions.
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- 5 E311. The suspension of any one of E268-E298 and E301-E310, wherein the C-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 57.
- E312. The suspension of any one of E268-E311, wherein the first vector comprises a first inverted terminal repeat (ITR) sequence 5' of the promoter and a second ITR sequence 3' of the recombino-
5 genic region, and the second vector comprises a first ITR sequence 5' of the recombino-
genic region and a second ITR sequence 3' of the poly(A) sequence.
- E313. The suspension of E312, wherein the ITRs in the first vector and second vector are AAV2 ITRs or have at least 80% sequence identity (e.g., at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,
88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity) to
10 AAV2 ITRs.
- E314. The suspension of any one of E268-E313, wherein the first AAV vector comprises a Kozak sequence 3' of the promoter and 5' of the first coding polynucleotide that encodes the N-terminal portion of the OTOF isoform 5 protein.
- E315. A kit comprising the suspension of any one of E268-E314.
- 15 E316. The kit of E315, further comprising one or more of a syringe, syringe pump, and catheter.
- E317. A syringe comprising the suspension of any one of E268-E314.

Other Embodiments

- 20 Various modifications and variations of the described invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention. Other embodiments are in the claims.

Claims

1. A method of treating a human subject having biallelic otoferlin (OTOF) mutations, the method comprising administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of an OTOF dual vector system in a volume of 200-250 μ L by intracochlear injection, wherein the OTOF dual vector system comprises:

a first adeno-associated virus (AAV) vector comprising a first inverted terminal repeat (ITR) sequence; a Myosin 15 (Myo15) promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and

a second AAV vector comprising a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence;

wherein the first coding polynucleotide and the second coding polynucleotide that encode the OTOF isoform 5 protein do not overlap, wherein neither the first nor second AAV vector encodes the full-length OTOF isoform 5 protein, and wherein the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to about 1:3.

2. A method of improving hearing in a human subject, the method comprising selecting a subject having biallelic OTOF mutations and administering to an inner ear of the subject an amount of 1×10^{13} vg/mL to 1×10^{14} vg/mL of an OTOF dual vector system in a volume of 200-250 μ L by intracochlear injection, wherein the OTOF dual vector system comprises:

a first AAV vector comprising a first ITR sequence; a Myo15 promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and

a second AAV vector comprising a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence;

wherein the first coding polynucleotide and the second coding polynucleotide that encode the OTOF isoform 5 protein do not overlap, wherein neither the first nor second AAV vector encodes the full-length OTOF isoform 5 protein, and wherein the first AAV vector and the second AAV vector are administered at a ratio of about 3:1 to 1:3.

3. The method of claim 1 or 2, wherein the first AAV vector and the second AAV vector comprise an AAV1 capsid.

4. The method of any one of claims 1-3, wherein the Myo15 promoter comprises a first region having at least 85% sequence identity to SEQ ID NO: 7 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 9 and/or SEQ ID NO: 10 operably linked to a second region having at least 85% sequence identity to SEQ ID NO: 8 or a functional portion or derivative thereof comprising the sequence of SEQ ID NO: 14 and/or SEQ ID NO: 15.
5. The method of any one of claims 1-4, wherein the Myo15 promoter comprises the sequence of SEQ ID NO: 21.
6. The method of claim 5, wherein the Myo15 promoter consists of the sequence of SEQ ID NO: 21.
7. The method of any one of claims 1-6, wherein the first recombinogenic region and the second recombinogenic region are each an AP gene fragment.
8. The method of claim 7, wherein the AP gene fragment comprises the sequence of any one of SEQ ID NOs: 48-53.
9. The method of claim 8, wherein the AP gene fragment consists of the sequence of any one of SEQ ID NOs: 48-53.
10. The method of claim 8, wherein the AP gene fragment comprises the sequence of SEQ ID NO: 51.
11. The method of claim 10, wherein the AP gene fragment consists of the sequence of SEQ ID NO: 51.
12. The method of any one of claims 1-11, wherein the OTOF isoform 5 protein comprises the sequence of SEQ ID NO: 1 or a variant thereof having one or more conservative amino acid substitutions.
13. The method of claim 12, wherein no more than 10% of the amino acids in the OTOF isoform 5 protein variant are conservative amino acid substitutions.
14. The method of claim 12, wherein the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 1.
15. The method of any one of claims 1-12 and 14, wherein the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 2.
16. The method of any one of claims 1-12 and 14, wherein the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 3.

17. The method of any one of claims 1-16, wherein the first coding polynucleotide encodes amino acids 1-802 of SEQ ID NO: 1 and the second coding polynucleotide encodes amino acids 803-1997 of SEQ ID NO: 1.
18. The method of any one of claims 1-17, wherein the N-terminal portion of the OTOF isoform 5 protein comprises the sequence of SEQ ID NO: 58 or a variant thereof having one or more conservative amino acid substitutions.
19. The method of claim 18, wherein no more than 10% of the amino acids in the N-terminal portion of the OTOF isoform 5 protein variant are conservative amino acid substitutions.
20. The method of claim 18, wherein the N-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 58.
21. The method of any one of claims 1-18 and 20, wherein the N-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 56.
22. The method of any one of claims 1-21, wherein the C-terminal portion of the OTOF isoform 5 protein comprises the sequence of SEQ ID NO: 59 or a variant thereof having one or more conservative amino acid substitutions.
23. The method of claim 22, wherein no more than 10% of the amino acids in the C-terminal portion of the OTOF isoform 5 protein variant are conservative amino acid substitutions.
24. The method of claim 22, wherein the C-terminal portion of the OTOF isoform 5 protein consists of the sequence of SEQ ID NO: 59.
25. The method of any one of claims 1-22 and 24, wherein the C-terminal portion of the OTOF isoform 5 protein is encoded by the sequence of SEQ ID NO: 57.
26. The method of any one of claims 1-25, wherein the ITRs in the first vector and second vector have at least 80% sequence identity to AAV2 ITRs.
27. The method of any one of claims 1-26, wherein the poly(A) sequence is a bovine growth hormone (bGH) poly(A) sequence.
28. The method of any one of claims 1-27, wherein the splice donor sequence in the first vector comprises the sequence of SEQ ID NO: 54.
29. The method of claim 28, wherein the splice donor sequence in the first vector consists of the sequence of SEQ ID NO: 54.

30. The method of any one of claims 1-29, wherein the splice acceptor sequence in the second vector comprises the sequence of SEQ ID NO: 55.
31. The method of claim 30, wherein the splice acceptor sequence in the second vector consists of the sequence of SEQ ID NO: 55.
32. The method of any one of claims 1-31, wherein the first AAV vector comprises a Kozak sequence 3' of the Myo15 promoter and 5' of the first coding polynucleotide that encodes the N-terminal portion of the OTOF isoform 5 protein.
33. The method of claim 1, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 235 to 4004 of SEQ ID NO: 66.
34. The method of claim 1 or 33, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 229 to 4438 of SEQ ID NO: 67.
35. The method of claim 1, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 2272 to 6041 of SEQ ID NO: 60.
36. The method of claim 1, wherein the first AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 182 to 3949 of SEQ ID NO: 62.
37. The method of claim 1 or 35, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 2267 to 6476 of SEQ ID NO: 61.
38. The method of claim 1 or 36, wherein the second AAV vector comprises a polynucleotide sequence comprising the sequence of nucleotides 187 to 4396 of SEQ ID NO: 63.
39. The method of any one of claims 1-38, wherein the subject is less than 18 years of age.
40. The method of claim 39, wherein the subject is at least 7 years of age.
41. The method of claim 39, wherein the subject is older than 24 months of age and younger than 7 years of age.
42. The method of claim 39, wherein the subject is 24 months of age or younger.
43. The method of any one of claims 1-42, wherein the subject has profound sensorineural hearing loss.

44. The method of any one of claims 1-43, wherein the subject has behavioral open-set word detection scores of < 30% in the ear(s) to be treated.
45. The method of any one of claims 1-44, wherein the subject has congenital auditory neuropathy.
46. The method of any one of claims 1-45, wherein the subject has present outer hair cell function.
47. The method of any one of claims 1-46, wherein the subject has detectable otoacoustic emissions.
48. The method of any one of claims 1-47, wherein a cochlear microphonic is present in the ear(s) to be treated.
49. The method of any one of claims 1-48, wherein the administering to the inner ear comprises intracochlear injection via insertion of a catheter through the round window membrane into the inner ear perilymph.
50. The method of any one of claims 1-49, wherein the administering further comprises creating a fenestration in the lateral semicircular canal.
51. The method of any one of claims 1-50, wherein the injection is performed using a syringe and syringe pump.
52. The method of any one of claims 1-51, wherein the administering is at a rate of 0.9 mL/hr.
53. The method of any one of claims 1-52, wherein the administering occurs once per ear.
54. The method of any one of claims 1-53, wherein the first vector and the second vector are administered at a ratio of about 1:1.
55. The method of any one of claims 1-54, wherein the dual vector system is formulated as an aqueous suspension.
56. The method of claim 55, wherein the first vector and second vector are matched in titer and mixed at an approximately equal ratio.
57. The method of claim 55 or 56, wherein the suspension comprises 10 mM sodium phosphate or disodium phosphate, 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 at a pH of 7.4.
58. The method of claim 57, wherein the suspension comprises 10 mM sodium phosphate.

59. The method of claim 57, wherein the suspension comprises 10 mM disodium phosphate.
60. The method of any one of claims 1-59, wherein the method further comprises administering a corticosteroid to the subject for the first four weeks after the administering of the OTOF dual vector system.
61. The method of claim 60, wherein the method comprises administering 1 mg/kg of the corticosteroid daily during the first two weeks after the administering of the OTOF dual vector system, administering 0.5 mg/kg daily during the third week after the administering of the OTOF dual vector system, and administering 0.25 mg/kg daily during the fourth week after the administering of the OTOF dual vector system.
62. The method of any one of claims 1-61, wherein the OTOF dual vector system is administered in an amount of 1.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
63. The method of claim 62, wherein the OTOF dual vector system is administered in an amount of 2.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
64. The method of claim 63, wherein the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
65. The method of claim 64, wherein the OTOF dual vector system is administered in an amount of 4.0×10^{13} vg/mL to 5.0×10^{13} vg/mL in a volume of 200-250 μ L.
66. The method of claim 63, wherein the OTOF dual vector system is administered in an amount of 3.0×10^{13} vg/mL in a volume of 240 μ L.
67. The method of any one of claims 1-61, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 1.0×10^{14} vg/mL in a volume of 200-250 μ L.
68. The method of claim 67, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 9.0×10^{13} vg/mL in a volume of 200-250 μ L.
69. The method of claim 68, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 8.0×10^{13} vg/mL in a volume of 200-250 μ L.
70. The method of claim 69, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 7.0×10^{13} vg/mL in a volume of 200-250 μ L.
71. The method of claim 70, wherein the OTOF dual vector system is administered in an amount of 5.0×10^{13} vg/mL to 6.0×10^{13} vg/mL in a volume of 200-250 μ L.

72. The method of claim 69, wherein the OTOF dual vector system is administered in an amount of 7.3×10^{13} vg/mL in a volume of 240 μ L.

73. The method of any one of claims 1-72, wherein the method improves one or more parameters selected from the subject's auditory brainstem response (ABR), behavioral audiometry, and score in one or more hearing questionnaires or behavioral tasks.

74. The method of claim 73, wherein the hearing questionnaires and behavioral tasks include one or more of the Auditory Skills Checklist, Open & Closed set task, Early Speech Perception test, Pediatric Speech Intelligibility test, Lexical Neighborhood Test Multisyllabic Lexical Neighborhood Test, Consonant-Nucleus-Consonant test, Bamford-Kowal-Bench sentence test, LittlEARS® Auditory Questionnaire, MacArthur-Bates Communicative Development Inventories Words and Gestures, Quality of Life–Cochlear Implant, Pediatric Quality of Life Inventory, Hearing Environments and Reflection on Quality of Life (HEAR-QL)-26, HEAR-QL-28, The Health Utilities Index 3, Vanderbilt Fatigue Scales, and AzBio test.

75. The method of claim 74, wherein the method improves the subject's score in the LittlEARS® Auditory Questionnaire.

76. The method of claim 73, wherein the method improves hearing thresholds by at least 55 decibels hearing level with air conduction as assessed by behavioral pure tone audiometry.

77. The method of claim 73, wherein the method results in a positive ABR wave V amplitude response.

78. An aqueous suspension comprising an OTOF dual vector system, 10 mM sodium phosphate or disodium phosphate, 180 mM sodium chloride, 5% (w/v) sucrose, and 0.001% (w/v) poloxamer 188 at a pH of 7.4, wherein the OTOF dual vector system comprises:

a first AAV vector comprising a first ITR sequence; a Myosin 15 (Myo15) promoter operably linked to a first coding polynucleotide that encodes an N-terminal portion of an OTOF isoform 5 protein; a splice donor sequence positioned 3' of the first coding polynucleotide; a recombinogenic region positioned 3' of the splice donor sequence; and a second ITR sequence; and

a second AAV vector comprising a first ITR sequence; a second recombinogenic region; a splice acceptor sequence positioned 3' of the second recombinogenic region; a second coding polynucleotide that encodes a C-terminal portion of the OTOF isoform 5 protein positioned 3' of the splice acceptor sequence; a poly(A) sequence positioned 3' of the second coding polynucleotide; and a second ITR sequence;

wherein the first coding polynucleotide and the second coding polynucleotide that encode the OTOF isoform 5 protein do not overlap, and wherein neither the first nor second AAV vector encodes the full-length OTOF isoform 5 protein.

79. The suspension of claim 78, wherein the suspension comprises 10 mM sodium phosphate.

80. The suspension of claim 78, wherein the suspension comprises 10 mM disodium phosphate.
81. The suspension of any one of claims 78-80, wherein the ratio of the first vector to the second vector is about 3:1 to about 1:3.
82. The suspension of any one of claims 78-81, wherein the first vector and second vector are matched in titer and mixed at an approximately equal ratio.
83. The suspension of claim 82, wherein the ratio of the first vector to the second vector is about 1:1.
84. The suspension of any one of claims 78-83, wherein the suspension has a titer of 1×10^{13} vg/mL to 1×10^{14} vg/mL.
85. The suspension of claim 84, wherein the suspension has a titer of 1×10^{13} vg/mL to 5×10^{13} vg/mL.
86. The suspension of claim 85, wherein the suspension has a titer of 3×10^{13} vg/mL.
87. The suspension of claim 84, wherein the suspension has a titer of 5×10^{13} vg/mL to 1×10^{14} vg/mL.
88. The suspension of claim 87, wherein the suspension has a titer of 7.3×10^{13} vg/mL.
89. The suspension of any one of claims 78-88, wherein the suspension is formulated for intracochlear injection.
90. A kit comprising the suspension of any one of claims 78-89.
91. The kit of claim 90, further comprising one or more of a syringe, syringe pump, and catheter.
92. A syringe comprising the suspension of any one of claims 78-89.