CRISPR AND AAV STRATEGIES FOR X-LINKED JUVENILE RETINOSCHISIS THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of US Application No. 62/932,608, filed November 8, 2019, which is herein incorporated by reference in its entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS WEB

[0002] The Sequence Listing written in file 694232SEQLIST.txt is 1.22 megabytes, was created on November 6, 2020, and is hereby incorporated by reference.

BACKGROUND

[0003] The *RS1* gene encodes a highly conserved extracellular protein involved in the cellular organization of the retina. It is assembled and secreted from photoreceptors and bipolar cells as a homo-oligomeric protein complex. Over 200 mutations have been detected in *RS1,* many leading to early onset of macular degeneration due to a non-functional protein or the absence of protein secretion. Lack of functional *Rs1* expression causes schisis within the retinal layers leading to the early and progressive vision loss associated with X-linked juvenile retinoschisis (XLRS). Although there have been gene therapy clinical trials for XLRS, the trials did not meet their endpoints. New strategies are needed for treating XLRS.

SUMMARY

[0004] Nucleic acid constructs and compositions that allow insertion of a retinoschisin coding sequence into a target genomic locus such as an endogenous *RS1* locus and/or expression of the retinoschisin coding sequence are provided. The nucleic acid constructs and compositions can be used in methods for integration into a target genomic locus and/or expression in a cell or in methods of treating X-linked juvenile retinoschisis.

[0005] In one aspect, provided are bidirectional nucleic acid constructs for integration into a target genomic locus. Some such nucleic acid constructs comprise: (a) a first segment comprising a first coding sequence for a first retinoschisin protein or fragment thereof; and (b) a second

segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or fragment thereof. In some such constructs, the second segment is located 3' (i.e., downstream) of the first segment.

[0006] In some such constructs, the first retinoschisin protein or fragment thereof is a human retinoschisin protein or fragment thereof, the second retinoschisin protein or fragment thereof is a human retinoschisin protein or fragment thereof, or both the first retinoschisin protein or fragment thereof and the second retinoschisin protein or fragment thereof are a human retinoschisin protein or fragment thereof. In some such constructs, the first coding sequence comprises, consists essentially of, or consists of complementary DNA (cDNA), the second coding sequence comprises, consists essentially of, or consists of cDNA, or both the first coding sequence and the second coding sequence comprise, consist essentially of, or consist of cDNA. In some such constructs, the first coding sequence comprises, consists essentially of, or consists of exons 2-6 of human *RS1* or degenerate variants thereof, the second coding sequence comprises, consists essentially of, or consists of exons 2-6 of human *RS1* or degenerate variants thereof, or both the first coding sequence and the second coding sequence comprise, consist essentially of, or consist of exons 2-6 of human *RS1* or degenerate variants thereof.

[0007] In some such constructs, the first segment comprises a fragment or portion of the first intron of human *RS1* located 5' (i.e., upstream) of the first coding sequence, and/or the second segment comprises a reverse complement of a fragment or portion of the second intron of human *RS1* located 3' (i.e., downstream) of the reverse complement of the second coding sequence.

[0008] In some such constructs, the first retinoschisin protein or fragment thereof is identical to the second retinoschisin protein or fragment thereof. In some such constructs, the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence. In some such constructs, the second segment has at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% complementarity to the first segment. In some such constructs, the second segment has less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, less than about 90%, less than about 95%,

less than about 97%, or less than about 99% complementarity to the first segment. In some such constructs, the reverse complement of the second coding sequence is: (a) not substantially complementary to the first coding sequence; (b) not substantially complementary to a fragment of the first coding sequence; (c) highly complementary to the first coding sequence; (d) highly complementary to the fragment of the first coding sequence; (e) at least about 60%, at least about 70%, at least about 80%, or at least about 90% identical to the reverse complement of the first coding sequence; (f) about 50% to about 80% identical to the reverse complement of the first coding sequence; or (g) about 60% to about 100% identical to the reverse complement of the first coding sequence.

[0009] In some such constructs, the first segment is linked to the second segment by a linker. Optionally, the linker is about 5 to about 2000 nucleotides in length.

[0010] In some such constructs, the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence. Optionally, the first polyadenylation signal sequence is different from the second polyadenylation signal sequence.

[0011] In some such constructs, the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or fragment thereof or the second retinoschisin protein or fragment thereof. In some such constructs, the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence. Optionally, the first splice acceptor site is from an *RS1* gene, the second splice acceptor site is from an *RS1* gene, or both the first splice acceptor site and the second splice acceptor site are from an *RS1* gene. Optionally, the first splice acceptor site is from intron 1 of human *RS1*, the second splice acceptor site is from intron 1 of human *RS1*, or both the first acceptor site and the second splice acceptor site are from intron 1 of human *RS1*.

[0012] In some such constructs, the nucleic acid construct does not comprise a homology arm. In some such constructs, the nucleic acid construct comprises homology arms. In some such constructs, the nucleic acid construct is single-stranded. In some such constructs, the nucleic acid construct is double-stranded. In some such constructs, the nucleic acid construct comprises DNA.

[0013] In some such constructs, the first coding sequence is codon-optimized for expression in a host cell, the second coding sequence is codon-optimized for expression in the host cell, or both the first coding sequence and the second coding sequence are codon-optimized for expression in the host cell. In some such constructs, the nucleic acid construct comprises one or more of the following terminal structures: hairpin, loop, inverted terminal repeat (ITR), or toroid. Optionally, the nucleic acid construct comprises ITRs.

[0014] In some such constructs, the first retinoschisin protein or fragment thereof and/or the second retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 5. In some such constructs, the first coding sequence and/or the second coding sequence comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 8 or 9. In some such constructs, the first coding sequence comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 8, and the second coding sequence comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 9. In some such constructs, the nucleic acid construct comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 46 or 47.

[0015] In some such constructs, the second segment is located 3' of the first segment, both the first retinoschisin protein or fragment thereof and the second retinoschisin protein or fragment thereof are a human retinoschisin protein or fragment thereof, the first retinoschisin protein or fragment thereof is identical to the second retinoschisin protein or fragment thereof, both the first coding sequence and the second coding sequence comprise complementary DNA

(cDNA) comprising exons 2-6 of human *RS1* or degenerate variants thereof, the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence, the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence, the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence, the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or fragment thereof or the second retinoschisin protein or fragment thereof, and the nucleic acid construct does not comprise a homology arm.

[0016] In another aspect, provided are vectors comprising any of the above bidirectional nucleic acid constructs. Some such vectors are viral vectors. Optionally, the vector is an adeno-associated virus (AAV) vector. Optionally, the AAV comprises a single-stranded genome (ssAAV). Optionally, the AAV comprises a self-complementary genome (scAAV). Optionally, the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, or AAV7m8.

[0017] Some such vectors do not comprise a promoter that drives expression of the first retinoschisin protein or fragment thereof or the second retinoschisin protein or fragment thereof. Some such vectors do not comprise a homology arm. Some such vectors do comprise homology arms.

[0018] In another aspect, provided are lipid nanoparticles comprising any of the above bidirectional nucleic acid constructs.

[0019] In another aspect, provided are cells comprising any of the above bidirectional nucleic acid constructs. Some such cells are *in vitro*. Some such cells are *in vivo*. Some such cells are mammalian cells. Some such cells are human cells. Some such cells are retinal cells.

[0020] Some such cells express the first retinoschisin protein or fragment thereof or the second retinoschisin protein or fragment thereof. In some such cells, the nucleic acid construct is genomically integrated at the target genomic locus. In some such cells, the target genomic locus is an endogenous *RS1* locus. Optionally, the nucleic acid construct is genomically integrated in intron 1 of the endogenous *RS1* locus. Optionally, endogenous *RS1* exon 1 splices into the first coding sequence or the second coding sequence of the nucleic acid construct. Optionally, the

modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4.

[0021] In some such cells, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. Optionally, the integration of the nucleic acid construct into the endogenous *RS1* locus reduces or eliminates expression of the endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or fragment thereof encoded by the nucleic acid construct. Optionally, the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis, and expression of the genomically integrated nucleic acid construct reduces or eliminates expression of the mutated *RS1* gene.

[0022] In another aspect, provided are nucleic acid constructs for homology-independent targeted integration into a target genomic locus. Some such nucleic acid constructs comprise a coding sequence for a retinoschisin protein or fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent. Also provided are nucleic acid constructs for homologous recombination with a target locus. Some such nucleic acid constructs comprise a coding sequence for a retinoschisin protein or fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a target sequence for a nuclease agent. Optionally, each homology arm is between about 25 nucleotides and about 2.5 kb in length.

[0023] In some such constructs for homology-independent targeted integration, the nuclease target sequence in the nucleic acid construct is identical to a nuclease target sequence for integration into the target genomic locus, wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

[0024] In some such constructs, the retinoschisin protein or fragment thereof is a human retinoschisin protein or fragment thereof. In some such constructs, the coding sequence for the retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of

complementary DNA (cDNA). In some such constructs, the coding sequence for the retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of exons 2-6 of human *RS1* or degenerate variants thereof.

[0025] In some such constructs, the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence. In some such constructs, the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or fragment thereof. In some such constructs, the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence. In some such constructs, the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence. Optionally, the splice acceptor site is from an *RS1* gene. Optionally, the splice acceptor site is from intron 1 of human *RS1*.

[0026] Some such constructs are single-stranded. Some such constructs are double-stranded. Some such constructs comprise DNA. In some such constructs, the coding sequence is codon-optimized for expression in a host cell.

[0027] In some such constructs, the construct comprises one or more of the following terminal structures: hairpin, loop, inverted terminal repeat (ITR), or toroid. Optionally, the nucleic acid construct comprising the coding sequence and the nuclease target sequences is flanked by ITRs.

[0028] In some such constructs, the nuclease agent is a Cas protein and a guide RNA, and the nuclease target sequence is a guide RNA target sequence. Optionally, the guide RNA target sequence is an inverted guide RNA target sequence. Optionally, the Cas protein is Cas9.

[0029] In some such constructs, the retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 5. In some such constructs, the coding sequence for the retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 8 or 9. In some such constructs, the nucleic acid construct comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least

about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 45.

[0030] In some such constructs, the nucleic acid construct is the nucleic acid construct for homology-independent targeted integration into the target genomic locus, the retinoschisin protein or fragment thereof is a human retinoschisin protein or fragment thereof, the coding sequence for the retinoschisin protein or fragment thereof comprises complementary DNA (cDNA) comprising exons 2-6 of human *RS1* or degenerate variants thereof, the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or fragment thereof, the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, and the nuclease target sequence in the nucleic acid construct is identical to a nuclease target sequence for integration into the target genomic locus, wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

[0031] In some such constructs, the nucleic acid construct is the nucleic acid construct for homologous recombination with the target genomic locus, the retinoschisin protein or fragment thereof is a human retinoschisin protein or fragment thereof, the coding sequence for the retinoschisin protein or fragment thereof comprises complementary DNA (cDNA) comprising exons 2-6 of human *RS1* or degenerate variants thereof, the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or fragment thereof, the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, and each homology arm is between about 25 nucleotides and about 2.5 kb in length.

[0032] In another aspect, provided are vectors comprising any of the above nucleic acid constructs for homology-independent targeted integration. Some such vectors are viral vectors. Some such vectors are adeno-associated virus (AAV) vectors. Optionally, the AAV comprises a single-stranded genome (ssAAV). Optionally, the AAV comprises a self-complementary genome (scAAV). Optionally, the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, or AAV7m8.

[0033] In some such vectors, the vector does not comprise a promoter that drives expression

of the retinoschisin protein or fragment thereof. In some such vectors, the vector does not comprise a homology arm.

[0034] In another aspect, provided are lipid nanoparticles comprising any of the above nucleic acid constructs for homology-independent targeted integration.

[0035] In another aspect, provided are cells comprising any of the above nucleic acid constructs for homology-independent targeted integration. Some such cells are *in vitro*. Some such cells are *in vivo*. Some such cells are mammalian cells. Some such cells are human cells. Some such cells are retinal cells.

[0036] In some such cells, the cell expresses the retinoschisin protein or fragment thereof. In some such cells, the nucleic acid construct is genomically integrated at the target genomic locus. Optionally, the target genomic locus is an endogenous *RS1* locus. Optionally, the nucleic acid construct is genomically integrated in intron 1 of the endogenous *RS1* locus. Optionally, endogenous *RS1* exon 1 splices into the coding sequence for the retinoschisin protein or fragment thereof in the nucleic acid construct. Optionally, the modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4.

[0037] In some such cells, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. Optionally, the integration of the nucleic acid construct into the endogenous *RS1* locus reduces or eliminates expression of the endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or fragment thereof encoded by the nucleic acid construct. Optionally, the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis, and expression of the genomically integrated nucleic acid construct reduces or eliminates expression of the mutated *RS1* gene.

[0038] In another aspect, provided are compositions for use in expressing retinoschisin in a cell or for use in integrating a coding sequence for a retinoschisin protein or fragment thereof into a target genomic locus in a cell. Some such compositions comprise: (a) a nucleic acid construct comprising the coding sequence for the retinoschisin protein or fragment thereof for integration into the target genomic locus; and (b) a nuclease agent or a nucleic acid encoding the

nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus.

[0039] Some such compositions comprise: (a) any of the above nucleic acid constructs for homology-independent targeted integration; and (b) a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus. Optionally, the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct. Optionally, the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

[0040] Some such compositions comprise: (a) any of the above bidirectional nucleic acid constructs; and (b) a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus.

[0041] In some such compositions, the target genomic locus is in an *RS1* gene. Optionally, the nuclease target sequence in the target genomic locus is in the first intron in the *RS1* gene. Optionally, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. Optionally, the integration of the nucleic acid construct into the endogenous *RS1* locus in the cell reduces or eliminates expression of the endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or fragment thereof encoded by the nucleic acid construct.

[0042] In some such compositions, the nuclease agent is a Cas protein and a guide RNA, and the nuclease target sequence is a guide RNA target sequence. Optionally, the Cas protein is Cas9. Optionally, the composition comprises the guide RNA and a messenger RNA encoding the Cas protein. Optionally, the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle. Optionally, the composition comprises a DNA encoding the Cas protein and a DNA encoding the guide RNA. Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors. Optionally, the one or more viral vectors are adeno-associated virus (AAV) viral vectors. Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in a single viral vector (e.g., a single AAV vector). Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in a separate viral vectors (e.g., a separate AAV vectors).

[0043] In some such compositions, the nucleic acid construct is in a viral vector. Optionally, the viral vector is an adeno-associated virus (AAV) viral vector. Optionally, the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, or AAV7m8.

[0044] Also provided are compositions comprising a guide RNA or a DNA encoding the guide RNA, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence in an *RS1* gene, and wherein the guide RNA binds to a Cas protein and targets the Cas protein to the guide RNA target sequence in the *RS1* gene.

[0045] In some such compositions or compositions for use, the composition further comprises the Cas protein or a nucleic acid encoding the Cas protein. Optionally, the Cas protein is a Cas9 protein. Optionally, the Cas protein is derived from a *Streptococcus pyogenes* Cas9 protein. In some such compositions or compositions for use, the composition comprises the Cas protein in the form of a protein.

[0046] In some such compositions or compositions for use, the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises a DNA encoding the Cas protein, optionally wherein the composition comprises the DNA encoding the guide RNA. Optionally, the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises a DNA encoding the Cas protein, wherein the composition comprises the DNA encoding the guide RNA, and wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors. Optionally, the one or more viral vectors are adeno-associated virus (AAV) viral vectors. Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in a single viral vector (e.g., a single AAV vector). Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in a separate viral vectors (e.g., a separate AAV vectors). Optionally, the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, or AAV7m8.

[0047] In some such compositions or compositions for use, the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises a messenger RNA encoding the Cas protein, optionally wherein the composition comprises the guide RNA in the form of RNA. In some such compositions or compositions for use, the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises a messenger RNA encoding the Cas protein, wherein the composition comprises the guide RNA in the form of RNA, and wherein the guide RNA and the messenger RNA encoding the Cas protein are in a

lipid nanoparticle.

[0048] In some such compositions or compositions for use, the messenger RNA encoding the Cas protein comprises at least one modification. Optionally, the messenger RNA encoding the Cas protein is modified to comprise a modified uridine at one or more or all uridine positions. Optionally, the modified uridine is pseudouridine. Optionally, the messenger RNA encoding the Cas protein is fully substituted with pseudouridine. In some such compositions or compositions for use, the messenger RNA encoding the Cas protein comprises a 5' cap. In some such compositions or compositions for use, the messenger RNA encoding the Cas protein comprises a poly(A) tail. In some such compositions or compositions for use, the messenger RNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 6243 or 6245.

[0049] In some such compositions or compositions for use, the nucleic acid encoding the Cas protein is codon-optimized for expression in a mammalian cell or a human cell. In some such compositions or compositions for use, the Cas protein comprises the sequence set forth in SEQ ID NO: 27, 6242, or 6246.

[0050] In some such compositions or compositions for use, the guide RNA target sequence is in an intron of the *RS1* gene. Optionally, the intron is the first intron of the *RS1* gene.

[0051] In some such compositions or compositions for use, the *RS1* gene is a human *RS1* gene.

[0052] In some such compositions or compositions for use, the DNA-targeting segment comprises: (a) at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence set forth in any one of SEQ ID NOS: 3148-6241; (b) at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence set forth in any one of SEQ ID NOS: 3148-4989; (c) at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351; (d) at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304; or (e) at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence set forth in any one of SEQ ID NOS: 4990-6241.

[0053] In some such compositions or compositions for use, the DNA-targeting segment is: (a) at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in any one of SEQ

ID NOS: 3148-6241; (b) at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in any one of SEQ ID NOS: 3148-4989; (c) at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351; (d) at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304; or (e) at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in any one of SEQ ID NOS: 4990-6241.

[0054] In some such compositions or compositions for use, the DNA-targeting segment comprises, consists essentially of, or consists of the sequence set forth in: (a) any one of SEQ ID NOS: 3148-6241; (b) any one of SEQ ID NOS: 3148-4989; (c) any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351; (d) any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304; or (e) any one of SEQ ID NOS: 4990-6241.

[0055] In some such compositions or compositions for use, the DNA-targeting segment comprises at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304.

[0056] In some such compositions or compositions for use, the DNA-targeting segment is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304.

[0057] In some such compositions or compositions for use, the DNA-targeting segment comprises, consists essentially of, or consist of the sequence set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304.

[0058] In some such compositions or compositions for use, the composition comprises the guide RNA in the form of RNA. In some such compositions or compositions for use, the composition comprises the DNA encoding the guide RNA.

[0059] In some such compositions or compositions for use, the guide RNA comprises at least one modification. In some such compositions or compositions for use, the at least one modification comprises a 2'-O-methyl-modified nucleotide. In some such compositions or

compositions for use, the at least one modification comprise a phosphorothioate bond between nucleotides. In some such compositions or compositions for use, the at least one modification comprise a modification at one or more of the first five nucleotides at the 5' end of the guide RNA. In some such compositions or compositions for use, the at least one modification comprises a modification at one or more of the last five nucleotides at the 3' end of the guide RNA. In some such compositions or compositions for use, the at least one modification comprises phosphorothioate bonds between the first four nucleotides at the 5' end of the guide RNA. In some such compositions or compositions for use, the at least one modification comprises phosphorothioate bonds between the last four nucleotides at the 3' end of the guide RNA. In some such compositions or compositions for use, the at least one modification comprises 2'-O-methyl-modified nucleotides at the first three nucleotides at the 5' end of the guide RNA. In some such compositions or compositions for use, the at least one modification comprises 2'-O-methyl-modified nucleotides at the last three nucleotides at the 3' end of the guide RNA. In some such compositions or compositions for use, the at least one modification comprises: (i) phosphorothioate bonds between the first four nucleotides at the 5' end of the guide RNA; (ii) phosphorothioate bonds between the last four nucleotides at the 3' end of the guide RNA; (iii) 2'-O-methyl-modified nucleotides at the first three nucleotides at the 5' end of the guide RNA; and (iv) 2'-O-methyl-modified nucleotides at the last three nucleotides at the 3' end of the guide RNA. In some such compositions or compositions for use, the guide RNA comprises the modified nucleotides of SEQ ID NO: 44.

[0060] In some such compositions or compositions for use, the guide RNA is a single guide RNA (sgRNA). Optionally, the guide RNA comprises, consists essentially of, or consists of the sequence set forth in any one of SEQ ID NOS: 33-39 and 53. In some such compositions or compositions for use, the guide RNA is a dual guide RNA (dgRNA) comprising two separate RNA molecules comprising a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA). Optionally, the crRNA comprises the sequence set forth in any one of SEQ ID NOS: 29 and 52. Optionally, the tracrRNA comprises the sequence set forth in any one of SEQ ID NOS: 30-32.

[0061] In some such compositions or compositions for use, the composition is associated with a lipid nanoparticle, optionally wherein the composition comprises the guide RNA. In some such compositions or compositions for use, the DNA encoding the guide RNA is in a viral vector. In some such compositions or compositions for use, the viral vector is an adeno-

associated virus (AAV) viral vector. Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in a single viral vector (e.g., a single AAV vector). Optionally, the DNA encoding the Cas protein and the DNA encoding the guide RNA are in a separate viral vectors (e.g., a separate AAV vectors). Optionally, the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, or AAV7m8.

[0062] In some such compositions or compositions for use, the composition is a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

[0063] In some such compositions or compositions for use, the composition further comprises a second guide RNA or a DNA encoding the second guide RNA, wherein the second guide RNA comprises a DNA-targeting segment that targets a second guide RNA target sequence in the *RS1* gene, and wherein the second guide RNA binds to the Cas protein and targets the Cas protein to the second guide RNA target sequence in the *RS1* gene.

[0064] Also provided are cells comprising any of the above compositions or compositions for use. Optionally, the cell is *in vitro*. Optionally, the cell is *in vivo*. Some such cells are mammalian cells. Optionally, the cell is a human cell. Optionally, the cell is a retinal cell.

[0065] In some such cells, the cell expresses the first retinoschisin protein or fragment thereof or the second retinoschisin protein or fragment thereof. In some such cells, the nucleic acid construct is genomically integrated at the target genomic locus. Optionally, the target genomic locus is an endogenous *RS1* locus. Optionally, the nucleic acid construct is genomically integrated in intron 1 of the endogenous *RS1* locus. Optionally, endogenous *RS1* exon 1 splices into the first coding sequence or the second coding sequence of the nucleic acid construct. Optionally, the modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4.

[0066] In some such cells, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. Optionally, the integration of the nucleic acid construct into the endogenous *RS1* locus reduces or eliminates expression of the endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or fragment thereof encoded by the nucleic acid construct.

Optionally, the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis, and expression of the genomically integrated nucleic acid construct reduces or eliminates expression of the mutated *RS1* gene.

[0067] In another aspect, provided are methods of integrating a coding sequence for a retinoschisin protein or fragment thereof into a target genomic locus and expressing the retinoschisin protein or fragment thereof in a cell. Some such methods comprise administering any of the above nucleic acid constructs, vectors, lipid nanoparticles, or compositions to the cell, wherein the coding sequence is integrated into the target genomic locus and the retinoschisin protein or fragment thereof is expressed in the cell. Optionally, the cell is a mammalian cell. Optionally, the cell is a human cell. Optionally, the cell is a retinal cell. Optionally, the cell is *in vitro*. Optionally, the cell is *in vivo*. Optionally, the cell is a retinal cell *in vivo*, and the administering comprises subretinal injection or intravitreal injection.

[0068] In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered simultaneously. In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered sequentially in any order. Optionally, the nucleic acid construct is administered prior to the nuclease agent or the nucleic acid encoding the nuclease agent. Optionally, the nucleic acid construct is administered subsequent to the nuclease agent or the nucleic acid encoding the nuclease agent. Optionally, the time between the sequential administration is about 2 hours to about 48 hours. In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered in the same delivery vehicle. In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered in different delivery vehicles.

[0069] In some such methods, the target genomic locus is in an endogenous *RS1* gene. Optionally, the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene. Optionally, the nucleic acid construct is genomically integrated in intron 1 of the endogenous *RS1* locus, and wherein endogenous *RS1* exon 1 splices into the coding sequence for the retinoschisin protein or fragment thereof in the nucleic acid construct. Optionally, the modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about

90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4. In some such methods, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. Optionally, the integration of the nucleic acid construct into the endogenous *RS1* locus reduces or eliminates expression of the endogenous retinoschisin protein from the endogenous *RS1* locus and replaces it with expression of the retinoschisin protein or fragment thereof encoded by the nucleic acid construct.

[0070] In another aspect, provided are methods of treating a subject with X-linked juvenile retinoschisis. Some such methods can comprise administering any of the above nucleic acid constructs, vectors, lipid nanoparticles, or compositions to the subject, wherein the nucleic acid construct is integrated into and expressed from the target genomic locus in one or more retinal cells of the subject, and wherein a therapeutically effective level of retinoschisin expression is achieved in the subject. In some such methods, the subject is a human. In some such methods, the subject has an endogenous *RS1* gene comprising at least one mutation associated with or that causes X-linked juvenile retinoschisis. Optionally, the mutation is a R141C mutation. In some such methods, the administering comprises subretinal injection or intravitreal injection. In some such methods, integration of the nucleic acid construct results in retinal structural restoration.

[0071] In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered simultaneously. In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered sequentially in any order. Optionally, the nucleic acid construct is administered prior to the nuclease agent or the nucleic acid encoding the nuclease agent. Optionally, the nucleic acid construct is administered subsequent to the nuclease agent or the nucleic acid encoding the nuclease agent. Optionally, the time between the sequential administration is about 2 hours to about 48 hours. In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered in the same delivery vehicle. In some such methods, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent are administered in different delivery vehicles.

[0072] In some such methods, the target genomic locus is in an endogenous *RS1* gene.

Optionally, the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene. Optionally, the nucleic acid construct is genomically integrated in intron 1 of the endogenous *RS1* locus, and wherein endogenous *RS1* exon 1 splices into the coding sequence for the retinoschisin protein or fragment thereof in the nucleic acid construct. Optionally, the modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4. In some such methods, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. Optionally, the integration of the nucleic acid construct into the endogenous *RS1* locus reduces or eliminates expression of the endogenous retinoschisin protein from the endogenous *RS1* locus and replaces it with expression of the retinoschisin protein or fragment thereof encoded by the nucleic acid construct.

[0073] In another aspect, provided are methods of modifying an *RS1* gene in a cell. Some such methods comprise administering to the cell any of the above compositions comprising the guide RNA or the DNA encoding the guide RNA and the Cas protein or the nucleic acid encoding the Cas protein, wherein the guide RNA binds to the Cas protein and targets the Cas protein to the guide RNA target sequence in the *RS1* gene, and the Cas protein cleaves the guide RNA target sequence. In some such methods, the cell is a mammalian cell. Optionally, the cell is a human cell. Optionally, the cell is a retinal cell. Optionally, the cell is *in vitro*. Optionally, the cell is *in vivo*. In some such methods, the cell is a retinal cell, and the administering comprises subretinal injection or intravitreal injection. In some such methods, the guide RNA target sequence is in the first intron in the *RS1* gene.

BRIEF DESCRIPTION OF THE FIGURES

[0074] **Figure 1** (not to scale) shows a schematic of the murine *Rs1* locus, including the location of the R141C mutation associated with X-linked juvenile retinoschisis (XLRS) and the insertion site for a nucleic acid construct comprising exons 2-6 of human *RS1*.

[0075] **Figure 2** shows an alignment of mouse retinoschisin, human retinoschisin, human retinoschisin with an R141C mutation, mouse retinoschisin with an R141C mutation, and a

mouse/human retinoschisin hybrid expressed upon integration of a nucleic acid construct comprising exons 2-6 of human *RS1* into intron 1 of the mouse *Rs1* locus.

[0076] **Figure 3A** (not to scale) shows a schematic of a bidirectional nucleic acid construct comprising a first segment comprising a splice acceptor (A), exons 2-6 of human *RS1*, and bovine growth hormone (bGH) polyA and a second segment comprising the reverse complement of SV40 polyA, the reverse complement of exons 2-6 of human *RS1*, and the reverse complement of a splice acceptor (A). The bidirectional construct also comprises a U6 promoter operably linked to a sequence encoding a guide RNA targeting intron 1 of the murine *Rs1* locus in between the two human *RS1* segments. The horizontal arrows flanking the star represent the next generation targeted resequencing amplicons designed for NGS. A bidirectional ssAAV construct is shown at the top, and a bidirectional scAAV construct is shown at the bottom.

[0077] **Figure 3B** (not to scale) shows a schematic for a homology-independent targeted integration nucleic acid construct comprising a splice acceptor (A), exons 2-6 of human *RS1*, and a polyA sequence. The construct also comprises a U6 promoter operably linked to a sequence encoding a guide RNA targeting intron 1 of the murine *Rs1* locus downstream of the human *RS1* segment. The horizontal arrows flanking the star represent the next generation targeted resequencing amplicons designed for NGS.

[0078] **Figure 4** shows scoring of retinal cavities shown in optical coherence tomography (OCT) scans in eyes from $Rosa^{Cas9/+}$; $Rs1^{R141C/Y}$ mice injected with *RS1* viral vector version 1, *RS1* viral vector version 2, or *RS1* viral vector version 3. A score of 1 was assigned if there were 1-4 cavities on at least one individual image. A score of 2 was assigned if there were ≥ 4 cavities on at least one individual image, but the cavities were not fused. A score of 3 was assigned if there were fused cavities on at least one individual image. A score of 4 was assigned if there were fused cavities on at least one individual image and the retina was stretched. The average scores for each treatment group were compared with a control group containing the pooled non-treated eyes by nonparametric Kruskal–Wallis one-way analysis of variance and Post hoc test Dunn's multiple comparisons test.

[0079] **Figure 5** shows NGS results from mouse retina samples in eyes from $Rosa^{Cas9/+}$; $Rs1^{R141C/Y}$ mice injected with *RS1* viral vector version 1 (pssAAV mhRS1-sgu), *RS1* viral vector version 2 (pscAAV rs1_tandem), or *RS1* viral vector version 3 (pssAAV hRs1_HITI). Read counts for the four expected sequence variants are shown: (1) WT mouse, the mouse reference

sequence not containing the R141C mutant; (2) mutant mouse, the mouse reference sequence harboring the R141C mutant; (3) humanized transcript 1, the human reference sequence; and (4) humanized transcript 2, the mouse codon-optimized human reference sequence. Next generation targeted resequencing amplicons were designed for the regions depicted in Figure 3 (horizontal arrows). mRNA from the mouse retinas was used to generate cDNA to act as a template for next generation sequencing (NGS) amplification.

[0080] **Figures 6A** and **6B** show NGS results from mouse retina samples in eyes from *Rosa*$^{Cas9/+}$; *Rs1*$^{R141C/Y}$ mice injected with *RS1* viral vector version 1 (mhRS1-sgu), *RS1* viral vector version 2 (pscAAV_rs1_tandem), or *RS1* viral vector version 3 (hRs1_cDNA HITI). For these NGS results, a separate amplicon was used to amplify the *Rs1* intron 1 guide RNA target sequence. Reads that matched the mouse reference sequence or contained non-homologous end joining were quantified to assess how often the guide RNA cut without making an insertion.

[0081] **Figures 7A-7C** show NGS results from mouse retina samples in eyes from *Rosa*$^{Cas9/+}$; *Rs1*$^{R141C/Y}$ mice injected with *RS1* viral vector version 1 (pssAAV mhRS1-sgu; **Figure 7A**), *RS1* viral vector version 2 (pscAAV rs1_tandem; **Figure 7B**), or *RS1* viral vector version 3 (pssAAV hRs1_HITI; **Figure 7C**). Read counts for the four expected sequence variants are shown: (1) WT mouse, the mouse reference sequence not containing the R141C mutant; (2) mutant mouse, the mouse reference sequence harboring the R141C mutant; (3) humanized transcript 1, the human reference sequence; and (4) humanized transcript 2, the mouse codon-optimized human reference sequence. Next generation targeted resequencing amplicons were designed for the regions depicted in Figure 3 (horizontal arrows). mRNA from the mouse retinas was used to generate cDNA to act as a template for next generation sequencing (NGS) amplification. Reads that matched the mouse reference sequence or contained non-homologous end joining were quantified to assess how often the guide RNA cut without making an insertion.

[0082] **Figures 8A** and **8B** show RT-qPCR results from human retinoblastoma cells treated with *RS1* viral vector version 1 (pssAAV mhRS1-sgu; **Figure 8A**) or *RS1* viral vector version 2 (pscAAV rs1_tandem; **Figure 8B**) two hours before treatment with lipid nanoparticles formulated with Cas9 mRNA and one of six guide RNAs targeted to human *RS1* intron 1. Delta Ct values are shown (the lower the number, the higher the expression). "Ho" refers to human reference sequence, and "Mo" refers to human reference sequence codon-optimized for mouse expression.

[0083] **Figures 9A** and **9B** show RT-qPCR results from human retinoblastoma cells treated with *RS1* viral vector version 1 (pssAAV mhRS1-sgu; **Figure 9A**) or *RS1* viral vector version 2 (pscAAV rs1_tandem; **Figure 9B**) two hours after treatment with lipid nanoparticles formulated with Cas9 mRNA and one of six guide RNAs targeted to human *RS1* intron 1. Delta Ct values are shown (the lower the number, the higher the expression). "Ho" refers to human reference sequence, and "Mo" refers to human reference sequence codon-optimized for mouse expression.

[0084] **Figure 10** shows a schematic for a nucleic acid construct for homologous recombination comprising a splice acceptor (A), exons 2-6 of human *RS1*, and a polyA sequence. The construct also comprises a U6 promoter operably linked to a sequence encoding a guide RNA targeting intron 1 of the murine *Rs1* locus downstream of the human *RS1* segment. The construct also comprises upstream and downstream homology arms (HA). The horizontal arrows flanking the star represent the next generation targeted resequencing amplicons designed for NGS.

DEFINITIONS

[0085] The terms "protein," "polypeptide," and "peptide," used interchangeably herein, include polymeric forms of amino acids of any length, including coded and non-coded amino acids and chemically or biochemically modified or derivatized amino acids. The terms also include polymers that have been modified, such as polypeptides having modified peptide backbones. The term "domain" refers to any part of a protein or polypeptide having a particular function or structure.

[0086] The terms "nucleic acid" and "polynucleotide," used interchangeably herein, include polymeric forms of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, or analogs or modified versions thereof. They include single-, double-, and multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, and polymers comprising purine bases, pyrimidine bases, or other natural, chemically modified, biochemically modified, non-natural, or derivatized nucleotide bases.

[0087] The term "genomically integrated" refers to a nucleic acid that has been introduced into a cell such that the nucleotide sequence integrates into the genome of the cell. Any protocol may be used for the stable incorporation of a nucleic acid into the genome of a cell.

[0088] The term "expression vector" or "expression construct" or "expression cassette" refers to a recombinant nucleic acid containing a desired coding sequence operably linked to appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host cell or organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, as well as other sequences. Eukaryotic cells are generally known to utilize promoters, enhancers, and termination and polyadenylation signals, although some elements may be deleted and other elements added without sacrificing the necessary expression.

[0089] The term "viral vector" refers to a recombinant nucleic acid that includes at least one element of viral origin and includes elements sufficient for or permissive of packaging into a viral vector particle. The vector and/or particle can be utilized for the purpose of transferring DNA, RNA, or other nucleic acids into cells either *ex vivo* or *in vivo*. Numerous forms of viral vectors are known.

[0090] The term "isolated" with respect to proteins, nucleic acids, and cells includes proteins, nucleic acids, and cells that are relatively purified with respect to other cellular or organism components that may normally be present *in situ*, up to and including a substantially pure preparation of the protein, nucleic acid, or cell. The term "isolated" may include proteins and nucleic acids that have no naturally occurring counterpart or proteins or nucleic acids that have been chemically synthesized and are thus substantially uncontaminated by other proteins or nucleic acids. The term "isolated" may include proteins, nucleic acids, or cells that have been separated or purified from most other cellular components or organism components with which they are naturally accompanied (e.g., but not limited to, other cellular proteins, nucleic acids, or cellular or extracellular components).

[0091] The term "wild type" includes entities having a structure and/or activity as found in a normal (as contrasted with mutant, diseased, altered, or so forth) state or context. Wild type genes and polypeptides often exist in multiple different forms (e.g., alleles).

[0092] The term "endogenous sequence" refers to a nucleic acid sequence that occurs naturally within a cell or animal. For example, an endogenous *RS1* sequence of an animal refers to a native *RS1* sequence that naturally occurs at the *RS1* locus in the animal.

[0093] "Exogenous" molecules or sequences include molecules or sequences that are not normally present in a cell in that form. Normal presence includes presence with respect to the

particular developmental stage and environmental conditions of the cell. An exogenous molecule or sequence, for example, can include a mutated version of a corresponding endogenous sequence within the cell, such as a humanized version of the endogenous sequence, or can include a sequence corresponding to an endogenous sequence within the cell but in a different form (i.e., not within a chromosome). In contrast, endogenous molecules or sequences include molecules or sequences that are normally present in that form in a particular cell at a particular developmental stage under particular environmental conditions.

[0094] The term "heterologous" when used in the context of a nucleic acid or a protein indicates that the nucleic acid or protein comprises at least two segments that do not naturally occur together in the same molecule. For example, the term "heterologous," when used with reference to segments of a nucleic acid or segments of a protein, indicates that the nucleic acid or protein comprises two or more sub-sequences that are not found in the same relationship to each other (e.g., joined together) in nature. As one example, a "heterologous" region of a nucleic acid vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid vector could include a coding sequence flanked by sequences not found in association with the coding sequence in nature. Likewise, a "heterologous" region of a protein is a segment of amino acids within or attached to another peptide molecule that is not found in association with the other peptide molecule in nature (e.g., a fusion protein, or a protein with a tag). Similarly, a nucleic acid or protein can comprise a heterologous label or a heterologous secretion or localization sequence.

[0095] "Codon optimization" takes advantage of the degeneracy of codons, as exhibited by the multiplicity of three-base pair codon combinations that specify an amino acid, and generally includes a process of modifying a nucleic acid sequence for enhanced expression in particular host cells by replacing at least one codon of the native sequence with a codon that is more frequently or most frequently used in the genes of the host cell while maintaining the native amino acid sequence. For example, a nucleic acid encoding a Cas9 protein can be modified to substitute codons having a higher frequency of usage in a given prokaryotic or eukaryotic cell, including a bacterial cell, a yeast cell, a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell, a hamster cell, or any other host cell, as compared to the naturally occurring nucleic acid sequence. Codon usage tables are readily available, for example,

at the "Codon Usage Database." These tables can be adapted in a number of ways. *See* Nakamura et al. (2000) *Nucleic Acids Research* 28:292, herein incorporated by reference in its entirety for all purposes. Computer algorithms for codon optimization of a particular sequence for expression in a particular host are also available (*see, e.g.*, Gene Forge).

[0096] The term "locus" refers to a specific location of a gene (or significant sequence), DNA sequence, polypeptide-encoding sequence, or position on a chromosome of the genome of an organism. For example, an "*RS1* locus" may refer to the specific location of an *RS1* gene, *RS1* DNA sequence, retinoschisin-encoding sequence, or *RS1* position on a chromosome of the genome of an organism that has been identified as to where such a sequence resides. An "*RS1* locus" may comprise a regulatory element of an *RS1* gene, including, for example, an enhancer, a promoter, 5' and/or 3' untranslated region (UTR), or a combination thereof.

[0097] The term "gene" refers to DNA sequences in a chromosome that may contain, if naturally present, at least one coding and at least one non-coding region. The DNA sequence in a chromosome that codes for a product (e.g., but not limited to, an RNA product and/or a polypeptide product) can include the coding region interrupted with non-coding introns and sequence located adjacent to the coding region on both the 5' and 3' ends such that the gene corresponds to the full-length mRNA (including the 5' and 3' untranslated sequences). Additionally, other non-coding sequences including regulatory sequences (e.g., but not limited to, promoters, enhancers, and transcription factor binding sites), polyadenylation signals, internal ribosome entry sites, silencers, insulating sequence, and matrix attachment regions may be present in a gene. These sequences may be close to the coding region of the gene (e.g., but not limited to, within 10 kb) or at distant sites, and they influence the level or rate of transcription and translation of the gene.

[0098] The term "allele" refers to a variant form of a gene. Some genes have a variety of different forms, which are located at the same position, or genetic locus, on a chromosome. A diploid organism has two alleles at each genetic locus. Each pair of alleles represents the genotype of a specific genetic locus. Genotypes are described as homozygous if there are two identical alleles at a particular locus and as heterozygous if the two alleles differ.

[0099] A "promoter" is a regulatory region of DNA usually comprising a TATA box capable of directing RNA polymerase II to initiate RNA synthesis at the appropriate transcription initiation site for a particular polynucleotide sequence. A promoter may additionally comprise

other regions which influence the transcription initiation rate. The promoter sequences disclosed herein modulate transcription of an operably linked polynucleotide. A promoter can be active in one or more of the cell types disclosed herein (e.g., a eukaryotic cell, a non-human mammalian cell, a human cell, a rodent cell, a pluripotent cell, a one-cell stage embryo, a differentiated cell, or a combination thereof). A promoter can be, for example, a constitutively active promoter, a conditional promoter, an inducible promoter, a temporally restricted promoter (e.g., a developmentally regulated promoter), or a spatially restricted promoter (e.g., a cell-specific or tissue-specific promoter). Examples of promoters can be found, for example, in WO 2013/176772, herein incorporated by reference in its entirety for all purposes.

[00100] A constitutive promoter is one that is active in all tissues or particular tissues at all developing stages. Examples of constitutive promoters include the human cytomegalovirus immediate early (hCMV), mouse cytomegalovirus immediate early (mCMV), human elongation factor 1 alpha (hEF1α), mouse elongation factor 1 alpha (mEF1α), mouse phosphoglycerate kinase (PGK), chicken beta actin hybrid (CAG or CBh), SV40 early, and beta 2 tubulin promoters.

[00101] Examples of inducible promoters include, for example, chemically regulated promoters and physically-regulated promoters. Chemically regulated promoters include, for example, alcohol-regulated promoters (e.g., an alcohol dehydrogenase (alcA) gene promoter), tetracycline-regulated promoters (e.g., a tetracycline-responsive promoter, a tetracycline operator sequence (tetO), a tet-On promoter, or a tet-Off promoter), steroid regulated promoters (e.g., a rat glucocorticoid receptor, a promoter of an estrogen receptor, or a promoter of an ecdysone receptor), or metal-regulated promoters (e.g., a metalloprotein promoter). Physically regulated promoters include, for example temperature-regulated promoters (e.g., a heat shock promoter) and light-regulated promoters (e.g., a light-inducible promoter or a light-repressible promoter).

[00102] Tissue-specific promoters can be, for example, neuron-specific promoters, glia-specific promoters, muscle cell-specific promoters, heart cell-specific promoters, kidney cell-specific promoters, bone cell-specific promoters, endothelial cell-specific promoters, or immune cell-specific promoters (e.g., a B cell promoter or a T cell promoter).

[00103] Developmentally regulated promoters include, for example, promoters active only during an embryonic stage of development, or only in an adult cell.

[00104] "Operable linkage" or being "operably linked" includes juxtaposition of two or more components (e.g., a promoter and another sequence element) such that both components function normally and allow the possibility that at least one of the components can mediate a function that is exerted upon at least one of the other components. For example, a promoter can be operably linked to a coding sequence if the promoter controls the level of transcription of the coding sequence in response to the presence or absence of one or more transcriptional regulatory factors. Operable linkage can include such sequences being contiguous with each other or acting *in trans* (e.g., a regulatory sequence can act at a distance to control transcription of the coding sequence).

[00105] "Complementarity" of nucleic acids means that a 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 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 Tm (melting temperature) of hybridized strands, or by empirical determination of Tm by using routine methods. Tm 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 Tm, formation of a hybridization complex is favored, whereas at a temperature above the Tm, melting or separation of the strands in the hybridization complex is favored. Tm may be estimated for a nucleic acid having a known G+C content in an aqueous 1 M NaCl solution by using, e.g., Tm=81.5+0.41(% G+C), although other known Tm computations consider nucleic acid structural characteristics.

[00106] Hybridization requires that the two nucleic acids contain complementary sequences, although mismatches between bases are possible. The conditions appropriate for hybridization between two nucleic acids depend on the length of the nucleic acids and the degree of

complementation, variables which are well known. The greater the degree of complementation between two nucleotide sequences, the greater the value of the melting temperature (Tm) for hybrids of nucleic acids having those sequences. For hybridizations between nucleic acids with short stretches of complementarity (e.g. complementarity over 35 or fewer, 30 or fewer, 25 or fewer, 22 or fewer, 20 or fewer, or 18 or fewer nucleotides) the position of mismatches becomes important (*see* Sambrook et al., supra, 11.7-11.8). Typically, the length for a hybridizable nucleic acid is at least about 10 nucleotides. Illustrative minimum lengths for a hybridizable nucleic acid include at least about 15 nucleotides, at least about 20 nucleotides, at least about 22 nucleotides, at least about 25 nucleotides, and at least about 30 nucleotides. Furthermore, the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the region of complementation and the degree of complementation.

[00107] The sequence of polynucleotide need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, a polynucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure). A polynucleotide (e.g., gRNA) can comprise at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, a gRNA in which 18 of 20 nucleotides are complementary to a target region, and would therefore specifically hybridize, would represent 90% complementarity. In this example, the remaining noncomplementary nucleotides may be clustered or interspersed with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides.

[00108] Percent complementarity between particular stretches of nucleic acid sequences within nucleic acids can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; Zhang and Madden (1997) *Genome Res.* 7:649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489).

[00109] The methods and compositions provided herein employ a variety of different components. Some components throughout the description can have active variants and

fragments. Such components include, for example, Cas proteins, CRISPR RNAs, tracrRNAs, and guide RNAs. Biological activity for each of these components is described elsewhere herein. The term "functional" refers to the innate ability of a protein or nucleic acid (or a fragment or variant thereof) to exhibit a biological activity or function. Such biological activities or functions can include, for example, the ability of a Cas protein to bind to a guide RNA and to a target DNA sequence. The biological functions of functional fragments or variants may be the same or may in fact be changed (e.g., with respect to their specificity or selectivity or efficacy) in comparison to the original molecule, but with retention of the molecule's basic biological function.

[00110] The term "variant" refers to a nucleotide sequence differing from the sequence most prevalent in a population (e.g., by one nucleotide) or a protein sequence different from the sequence most prevalent in a population (e.g., by one amino acid).

[00111] The term "fragment," when referring to a protein, means a protein that is shorter or has fewer amino acids than the full-length protein. The term "fragment," when referring to a nucleic acid, means a nucleic acid that is shorter or has fewer nucleotides than the full-length nucleic acid. A fragment can be, for example, when referring to a protein fragment, an N-terminal fragment (i.e., removal of a portion of the C-terminal end of the protein), a C-terminal fragment (i.e., removal of a portion of the N-terminal end of the protein), or an internal fragment (i.e., removal of a portion of an internal portion of the protein).

[00112] "Sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences refers to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins, residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well known. Typically, this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution

is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

[00113] "Percentage of sequence identity" includes the value determined by comparing two optimally aligned sequences (greatest number of perfectly matched residues) over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. Unless otherwise specified (e.g., the shorter sequence includes a linked heterologous sequence), the comparison window is the full length of the shorter of the two sequences being compared.

[00114] Unless otherwise stated, sequence identity/similarity values include the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. "Equivalent program" includes any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

[00115] The term "conservative amino acid substitution" refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine, or leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, or between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine, or histidine for another, or the substitution of one acidic residue such as

aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, or methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue. Typical amino acid categorizations are summarized in **Table 1** below.

[00116] Table 1. Amino Acid Categorizations.

Alanine	Ala	A	Nonpolar	Neutral	1.8
Arginine	Arg	R	Polar	Positive	-4.5
Asparagine	Asn	N	Polar	Neutral	-3.5
Aspartic acid	Asp	D	Polar	Negative	-3.5
Cysteine	Cys	C	Nonpolar	Neutral	2.5
Glutamic acid	Glu	E	Polar	Negative	-3.5
Glutamine	Gln	Q	Polar	Neutral	-3.5
Glycine	Gly	G	Nonpolar	Neutral	-0.4
Histidine	His	H	Polar	Positive	-3.2
Isoleucine	Ile	I	Nonpolar	Neutral	4.5
Leucine	Leu	L	Nonpolar	Neutral	3.8
Lysine	Lys	K	Polar	Positive	-3.9
Methionine	Met	M	Nonpolar	Neutral	1.9
Phenylalanine	Phe	F	Nonpolar	Neutral	2.8
Proline	Pro	P	Nonpolar	Neutral	-1.6
Serine	Ser	S	Polar	Neutral	-0.8
Threonine	Thr	T	Polar	Neutral	-0.7
Tryptophan	Trp	W	Nonpolar	Neutral	-0.9
Tyrosine	Tyr	Y	Polar	Neutral	-1.3
Valine	Val	V	Nonpolar	Neutral	4.2

[00117] A "homologous" sequence (e.g., nucleic acid sequence) includes a sequence that is either identical or substantially similar to a known reference sequence, such that it is, for example, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the known reference sequence. Homologous sequences can include, for example, orthologous sequence and paralogous sequences. Homologous genes, for example, typically descend from a common ancestral DNA sequence, either through a speciation event (orthologous genes) or a genetic duplication event (paralogous genes). "Orthologous" genes include genes in different species that evolved from a common ancestral gene by

speciation. Orthologs typically retain the same function in the course of evolution. "Paralogous" genes include genes related by duplication within a genome. Paralogs can evolve new functions in the course of evolution.

[00118] The term "*in vitro*" includes artificial environments and to processes or reactions that occur within an artificial environment (e.g., a test tube or an isolated cell or cell line). The term "*in vivo*" includes natural environments (e.g., a cell or organism or body) and to processes or reactions that occur within a natural environment. The term "*ex vivo*" includes cells that have been removed from the body of an individual and processes or reactions that occur within such cells.

[00119] Repair in response to double-strand breaks (DSBs) occurs principally through two conserved DNA repair pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). *See* Kasparek & Humphrey (2011) *Seminars in Cell & Dev. Biol.* 22:886-897, herein incorporated by reference in its entirety for all purposes. Likewise, repair of a target nucleic acid mediated by an exogenous donor nucleic acid can include any process of exchange of genetic information between the two polynucleotides.

[00120] The term "recombination" includes any process of exchange of genetic information between two polynucleotides and can occur by any mechanism. Recombination can occur via homology directed repair (HDR) or homologous recombination (HR). HDR or HR includes a form of nucleic acid repair that can require nucleotide sequence homology, uses a "donor" molecule as a template for repair of a "target" molecule (i.e., the one that experienced the double-strand break), and leads to transfer of genetic information from the donor to target. Without wishing to be bound by any particular theory, such transfer can involve mismatch correction of heteroduplex DNA that forms between the broken target and the donor, and/or synthesis-dependent strand annealing, in which the donor is used to resynthesize genetic information that will become part of the target, and/or related processes. In some cases, the donor polynucleotide, a portion of the donor polynucleotide, a copy of the donor polynucleotide, or a portion of a copy of the donor polynucleotide integrates into the target DNA. *See* Wang et al. (2013) *Cell* 153:910-918; Mandalos et al. (2012) *PLOS ONE* 7:e45768:1-9; and Wang et al. (2013) *Nat Biotechnol.* 31:530-532, each of which is herein incorporated by reference in its entirety for all purposes.

[00121] Non-homologous end joining (NHEJ) includes the repair of double-strand breaks in a nucleic acid by direct ligation of the break ends to one another or to an exogenous sequence without the need for a homologous template. Ligation of non-contiguous sequences by NHEJ can often result in deletions, insertions, or translocations near the site of the double-strand break. For example, NHEJ can also result in the targeted integration of an exogenous donor nucleic acid through direct ligation of the break ends with the ends of the exogenous donor nucleic acid (i.e., NHEJ-based capture). Such NHEJ-mediated targeted integration can be preferred for insertion of an exogenous donor nucleic acid when homology directed repair (HDR) pathways are not readily usable (e.g., in non-dividing cells, primary cells, and cells which perform homology-based DNA repair poorly). In addition, in contrast to homology-directed repair, knowledge concerning large regions of sequence identity flanking the cleavage site is not needed, which can be beneficial when attempting targeted insertion into organisms that have genomes for which there is limited knowledge of the genomic sequence. The integration can proceed via ligation of blunt ends between the exogenous donor nucleic acid and the cleaved genomic sequence, or via ligation of sticky ends (i.e., having 5' or 3' overhangs) using an exogenous donor nucleic acid that is flanked by overhangs that are compatible with those generated by a nuclease agent in the cleaved genomic sequence. *See, e.g.*, US 2011/020722, WO 2014/033644, WO 2014/089290, and Maresca et al. (2013) *Genome Res.* 23(3):539-546, each of which is herein incorporated by reference in its entirety for all purposes. If blunt ends are ligated, target and/or donor resection may be needed to generation regions of microhomology needed for fragment joining, which may create unwanted alterations in the target sequence.

[00122] Compositions or methods "comprising" or "including" one or more recited elements may include other elements not specifically recited. For example, a composition that "comprises" or "includes" a protein may contain the protein alone or in combination with other ingredients. The transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified elements recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising."

[00123] "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur and that the description includes instances in which the event or circumstance occurs and instances in which the event or circumstance does not.

[00124] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[00125] Unless otherwise apparent from the context, the term "about" encompasses values ± 5 of a stated value.

[00126] The term "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[00127] The term "or" refers to any one member of a particular list and also includes any combination of members of that list.

[00128] The singular forms of the articles "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a protein" or "at least one protein" can include a plurality of proteins, including mixtures thereof.

[00129] Statistically significant means $p \leq 0.05$.

DETAILED DESCRIPTION

I. Overview

[00130] X-linked juvenile retinoschisis (XLRS) is juvenile-onset macular degeneration caused by mutations in retinoschisin (*RS1*). The *RS1* gene encodes a 24 kDa discoidin-domain-containing protein that is secreted as a homo-oligomeric complex. Gene mutations of *RS1* lead to either a non-functional protein or the absence of protein secretion, which causes schisis or splitting within the retinal layers leading to early and progressive vision loss. More than 200 different mutations in the *RS1* gene are known to cause XLRS. Forty percent of the disease-causing mutations are nonsense or frameshift mutations that are predicted to result in the absence of a full-length retinoschisin protein. Fifty percent of the disease-causing mutations are missense mutations that allow for production of full-length mutant protein. Most of these are in the discoidin domain and result in misfolded protein retained in ER.

[00131] Because XLRS is a recessive disease caused by the loss in retinoschisin function, gene replacement therapy is as a potential treatment for this disease. Furthermore, because

retinoschisin functions as an extracellular protein, beneficial treatment is not necessarily restricted to the transfected cell expressing the replacement gene but can encompass a wider area due to the spread of the secreted protein from the site of expression.

[00132] Provided herein are nucleic acid constructs and compositions that allow insertion of a retinoschisin coding sequence into a target genomic locus such as an endogenous *RS1* locus and/or expression of the retinoschisin coding sequence. The nucleic acid constructs and compositions can be used in methods for integration into a target genomic locus and/or expression in a cell or in methods of treating X-linked juvenile retinoschisis. Also provided are nuclease agents (e.g., targeting an endogenous *RS1* locus) or nucleic acids encoding nuclease agents to facilitate integration of the nucleic acid constructs into a target genomic locus such as an endogenous *RS1* locus.

[00133] Integration of the nucleic acid construct into an endogenous *RS1* locus, such as intron 1 of *RS1*, can prevent transcription of the endogenous *RS1* gene downstream of the integration site. The integration of the nucleic acid construct into the endogenous *RS1* locus can reduce or eliminate expression of the endogenous retinoschisin protein (e.g., an endogenous retinoschisin protein with an XLRS-causing mutation) and replace it with expression of the retinoschisin protein or fragment or variant thereof encoded by the nucleic acid construct (e.g., retinoschisin without an XLRS-causing mutation). In one example, integration of the nucleic acid construct into the endogenous *RS1* locus reduces expression of the endogenous retinoschisin protein. In another example, integration of the nucleic acid construct into the endogenous *RS1* locus eliminates expression of the endogenous retinoschisin protein. In this way, integration of the nucleic acid construct can simultaneously knock out the endogenous *RS1* gene (e.g., an endogenous *RS1* gene comprising one or more mutations associated with or that cause XLRS, such as R141C) and knock in a replacement retinoschisin coding sequence (e.g., a replacement retinoschisin coding sequence that does not comprise mutations associated with or that cause XLRS).

II. Nucleic Acid Constructs Comprising a Retinoschisin Coding Sequence for Integration into and Expression from a Target Genomic Locus

[00134] Provided herein are nucleic acid constructs (i.e., exogenous donor nucleic acids) comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or

variant thereof) for integration into and expression from a target genomic locus. The nucleic acid constructs can be isolated nucleic acid constructs.

[00135] Retinoschisin (X-linked juvenile retinoschisis protein) is a protein required for normal structure and function of the retina. An exemplary human retinoschisin protein is assigned UniProt Accession No. O15537 and has the sequence set forth in SEQ ID NO: 2. Orthologs in other species are also known. For example, an exemplary mouse retinoschisin protein is assigned UniProt Accession No. Q9Z1L4 and has the sequence set forth in SEQ ID NO: 1. Retinoschisin is encoded by the *RS1* gene (also known as XLRS1). The human *RS1* gene contains six separate exons interspaced by five introns. The human *RS1* gene is assigned NCBI GeneID 6247. The mouse *Rs1* gene is assigned NCBI GeneID 20147. An exemplary coding sequence for human *RS1* is assigned CCDS ID CCDS14187.1 and is set forth in SEQ ID NO: 6. Mutations in retinoschisin cause X-linked juvenile retinoschisis (XLRS), a vitreo-retinal dystrophy characterized by macular pathology and by splitting of the superficial layer of the retina. The nucleic acid constructs disclosed herein can be used in methods to treat XLRS as described in more detail elsewhere herein.

[00136] The functional domains of RS1 are a signal peptide (SP), RS1, and the discoidin domains. The signal sequence guides the translocation of nascent RS1 from the endoplasmic reticulum (the site of synthesis) to external leaflet of the plasma membrane, during which signal sequence is cleaved by signal peptidase to generate mature protein with characteristic RS1 and a highly conserved discoidin domain. The different subdomains of RS1 signal sequence are the positively charged N region at the amino terminal end which mediates translocation, the hydrophobic core (H) required for targeting and membrane insertion and a polar "C" region that determines the site of recognition and cleavage by signal peptidase. RS1 is prominently expressed by the retinal photoreceptor and bipolar cells and is also in the pineal gland.

[00137] The retinoschisin coding sequence included in the nucleic acid constructs disclosed herein can be a coding sequence for a full-length retinoschisin protein or a fragment or variant thereof. In one example, the retinoschisin coding sequence included in the nucleic acid construct does not include the first exon of *RS1*. For example, the retinoschisin coding sequence included in the nucleic acid construct can include exons 2-6 of an *RS1* gene or variants or degenerate variants thereof. As one example, a cDNA fragment comprising exons 2-6 of an *RS1* gene can comprise the sequence set forth in SEQ ID NO: 8. Although each of the 64 codons is specific for

only one amino acid or stop signal, the genetic code is degenerate (i.e., redundant) because a single amino acid may be coded for by more than one codon. A degenerate variant of a gene encodes the same protein but uses at least one different codon. The retinoschisin coding sequence in the nucleic acid construct can comprise complementary DNA (cDNA) without intervening introns, or the nucleic acid construct can comprise one or more introns separating exons in the retinoschisin coding sequence. For example, the nucleic acid construct can comprise a sequence corresponding to the *RS1* genomic locus having both exons and introns.

[00138] The retinoschisin coding sequence can be from any organism. For example, the retinoschisin coding sequence can be mammalian, non-human mammalian, rodent, mouse, rat, or human or variants thereof. Alternatively, the retinoschisin coding sequence can be chimeric (e.g., part mouse and part human). In a specific example, the retinoschisin coding sequence is a human retinoschisin coding sequence.

[00139] The retinoschisin coding sequence can be codon-optimized for efficient translation into retinoschisin in a particular cell or organism. As one example, a codon-optimized version of exons 2-6 of human *RS1* is set forth in SEQ ID NO: 9. For example, the nucleic acid can be modified to substitute codons having a higher frequency of usage in a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell, or any other host cell of interest.

[00140] The retinoschisin coding sequence can encode a wild type retinoschisin protein or a fragment or variant thereof. Likewise, the retinoschisin coding sequence can be a wild type coding sequence or variant thereof. In one example, the retinoschisin coding sequence does not comprise any mutations associated with or that cause X-linked juvenile retinoschisis. Alternatively, the retinoschisin coding sequence can comprise one or more mutations associated with or that cause X-linked juvenile retinoschisis (e.g., R141C).

[00141] The nucleic acid construct can further comprise one or more *RS1* introns or fragments or variants thereof (e.g., one or more human *RS1* introns or fragments or variants thereof). For example, the nucleic acid construct can comprise *RS1* intron 1 or a fragment or variant thereof. The *RS1* intron or the fragment or variant thereof can comprise a splice acceptor site or a fragment thereof. Examples of fragments of *RS1* intron 1 are set forth in SEQ ID NOS: 15 and 16. In one specific example, the nucleic acid construct can comprise *RS1* intron 1 or a fragment or variant thereof located 5' of exons 2-6 of *RS1* (e.g., upstream of a cDNA sequence comprising, consisting essentially of, or consisting of exons 2-6 of *RS1*).

[00142] The nucleic acid constructs can further comprise one or more splice acceptor sites. Examples of sequences (e.g., intronic sequences) and reverse complements thereof comprising splice acceptor sites are set forth in SEQ ID NOS: 15-21. For example, the nucleic acid construct can comprise a splice acceptor site located 5' of the retinoschisin coding sequence. In a specific example, the retinoschisin coding sequence comprises, consists essentially of, or consists of exons 2-6 of *RS1* (e.g., exons 2-6 of human *RS1*), and the splice acceptor site is the splice acceptor site from intron 1 of *RS1* (e.g., human *RS1*) used in splicing *RS1* exon 1 to *RS1* exon 2. The term splice acceptor site refers to a nucleic acid sequence at a 3' intron/exon boundary that can be recognized and bound by splicing machinery.

[00143] The nucleic acid constructs disclosed herein can also comprise post-transcriptional regulatory elements, such as the woodchuck hepatitis virus post-transcriptional regulatory element.

[00144] The nucleic acid constructs can further comprise one or more polyadenylation signal sequences. Examples of polyadenylation signal sequences, or sequences comprising polyadenylation signal sequences, or reverse complements thereof are set forth in SEQ ID NOS: 22-25. For example, the nucleic acid construct can comprise a polyadenylation signal sequence located 3' of the retinoschisin coding sequence. Any suitable polyadenylation signal sequence can be used. The term polyadenylation signal sequence refers to any sequence that directs termination of transcription and addition of a poly-A tail to the mRNA transcript. In eukaryotes, transcription terminators are recognized by protein factors, and termination is followed by polyadenylation, a process of adding a poly(A) tail to the mRNA transcripts in presence of the poly(A) polymerase. The mammalian poly(A) signal typically consists of a core sequence, about 45 nucleotides long, that may be flanked by diverse auxiliary sequences that serve to enhance cleavage and polyadenylation efficiency. The core sequence consists of a highly conserved upstream element (AATAAA or AAUAAA) in the mRNA, referred to as a poly A recognition motif or poly A recognition sequence), recognized by cleavage and polyadenylation-specificity factor (CPSF), and a poorly defined downstream region (rich in Us or Gs and Us), bound by cleavage stimulation factor (CstF). Examples of transcription terminators that can be used include, for example, the human growth hormone (HGH) polyadenylation signal, the simian virus 40 (SV40) late polyadenylation signal, the rabbit beta-globin polyadenylation signal, the bovine growth hormone (BGH) polyadenylation signal, the phosphoglycerate kinase (PGK)

polyadenylation signal, an AOX1 transcription termination sequence, a CYC1 transcription termination sequence, or any transcription termination sequence known to be suitable for regulating gene expression in eukaryotic cells.

[00145] The nucleic acid constructs can also comprise a polyadenylation signal sequence upstream of the retinoschisin coding sequence. The polyadenylation signal sequence upstream of the retinoschisin coding sequence can be flanked by recombinase recognition sites recognized by a site-specific recombinase. In some constructs, the recombinase recognition sites also flank a selection cassette comprising, for example, the coding sequence for a drug resistance protein. In other constructs, the recombinase recognition sites do not flank a selection cassette. The polyadenylation signal sequence prevents transcription and expression of the protein or RNA encoded by the coding sequence. However, upon exposure to the site-specific recombinase, the polyadenylation signal sequence will be excised, and the protein or RNA can be expressed.

[00146] Such a configuration can enable tissue-specific expression or developmental-stage-specific expression in animals comprising the retinoschisin coding sequence if the polyadenylation signal sequence is excised in a tissue-specific or developmental-stage-specific manner. Excision of the polyadenylation signal sequence in a tissue-specific or developmental-stage-specific manner can be achieved if an animal comprising the nucleic acid construct further comprises a coding sequence for the site-specific recombinase operably linked to a tissue-specific or developmental-stage-specific promoter. The polyadenylation signal sequence will then be excised only in those tissues or at those developmental stages, enabling tissue-specific expression or developmental-stage-specific expression. In one example, the retinoschisin or fragment or variant thereof encoded by the nucleic acid constructs can be expressed in an eye-specific or retinal-cell-specific manner.

[00147] Site-specific recombinases include enzymes that can facilitate recombination between recombinase recognition sites, where the two recombination sites are physically separated within a single nucleic acid or on separate nucleic acids. Examples of recombinases include Cre, Flp, and Dre recombinases. One example of a Cre recombinase gene is Crei, in which two exons encoding the Cre recombinase are separated by an intron to prevent its expression in a prokaryotic cell. Such recombinases can further comprise a nuclear localization signal to facilitate localization to the nucleus (e.g., NLS-Crei). Recombinase recognition sites include nucleotide sequences that are recognized by a site-specific recombinase and can serve as a

substrate for a recombination event. Examples of recombinase recognition sites include FRT, FRT11, FRT71, attp, att, rox, and lox sites such as loxP, lox511, lox2272, lox66, lox71, loxM2, and lox5171.

[00148] The nucleic acid constructs can further comprise a promoter operably linked to the retinoschisin coding sequence. The retinoschisin coding sequence in the nucleic acid construct can be operably linked to any suitable promoter for expression *in vivo* within an animal or *in vitro* within an isolated cell. The promoter can be a constitutively active promoter (e.g., a CAG promoter or a U6 promoter), a conditional promoter, an inducible promoter, a temporally restricted promoter (e.g., a developmentally regulated promoter), or a spatially restricted promoter (e.g., a cell-specific or tissue-specific promoter). Such promoters are well-known and are discussed elsewhere herein. Promoters that can be used in an expression construct include promoters active, for example, in one or more of a eukaryotic cell, a human cell, a non-human cell, a mammalian cell, a non-human mammalian cell, a rodent cell, a mouse cell, a rat cell, a hamster cell, a rabbit cell, a pluripotent cell, an eye cell, a retinal cell, an embryonic stem (ES) cell, or a zygote. In a specific example, the promoter is active in an eye cell or a retinal cell.

[00149] Alternatively, some nucleic acid constructs do not comprise a promoter operably linked to the retinoschisin coding sequence (e.g., some nucleic acid constructs are promoterless constructs). Such nucleic acid constructs can be designed, for example, to be operably linked to an endogenous promoter at a target genomic locus (e.g., an endogenous *RS1* promoter at an endogenous *RS1* locus) upon integration into the target genomic locus.

[00150] Any target genomic locus capable of expressing a gene can be used, such as a safe harbor locus (safe harbor gene) or an endogenous *RS1* locus. Interactions between integrated exogenous DNA and a host genome can limit the reliability and safety of integration and can lead to overt phenotypic effects that are not due to the targeted genetic modification but are instead due to unintended effects of the integration on surrounding endogenous genes. For example, randomly inserted transgenes can be subject to position effects and silencing, making their expression unreliable and unpredictable. Likewise, integration of exogenous DNA into a chromosomal locus can affect surrounding endogenous genes and chromatin, thereby altering cell behavior and phenotypes. Safe harbor loci include chromosomal loci where transgenes or other exogenous nucleic acid inserts can be stably and reliably expressed in all tissues of interest without overtly altering cell behavior or phenotype (i.e., without any deleterious effects on the

host cell). *See, e.g.*, Sadelain et al. (2012) *Nat. Rev. Cancer* 12:51-58, herein incorporated by reference in its entirety for all purposes. For example, the safe harbor locus can be one in which expression of the inserted gene sequence is not perturbed by any read-through expression from neighboring genes. For example, safe harbor loci can include chromosomal loci where exogenous DNA can integrate and function in a predictable manner without adversely affecting endogenous gene structure or expression. Safe harbor loci can include extragenic regions or intragenic regions such as, for example, loci within genes that are non-essential, dispensable, or able to be disrupted without overt phenotypic consequences.

[00151] Such safe harbor loci can offer an open chromatin configuration in all tissues and can be ubiquitously expressed during embryonic development and in adults. *See, e.g.*, Zambrowicz et al. (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94:3789-3794, herein incorporated by reference in its entirety for all purposes. In addition, the safe harbor loci can be targeted with high efficiency, and safe harbor loci can be disrupted with no overt phenotype. Examples of safe harbor loci include albumin, CCR5, HPRT, AAVS1, and Rosa26. *See, e.g.*, US Patent Nos. 7,888,121; 7,972,854; 7,914,796; 7,951,925; 8,110,379; 8,409,861; 8,586,526; and US Patent Publication Nos. 2003/0232410; 2005/0208489; 2005/0026157; 2006/0063231; 2008/0159996; 2010/00218264; 2012/0017290; 2011/0265198; 2013/0137104; 2013/0122591; 2013/0177983; 2013/0177960; and 2013/0122591, each of which is herein incorporated by reference in its entirety for all purposes.

[00152] The target genomic locus can also be an endogenous *RS1* locus, such as an endogenous *RS1* locus comprising one or more mutations associated with or that cause XLRS (e.g., an R141C mutation in the encoded retinoschisin protein). Integration of the nucleic acid construct into the endogenous *RS1* locus can, in some cases, prevent transcription of the endogenous *RS1* gene downstream of the integration site. The integration of the nucleic acid construct into the endogenous *RS1* locus can reduce or eliminate expression of the endogenous retinoschisin protein and replace it with expression of the retinoschisin protein or fragment or variant thereof encoded by the nucleic acid construct. In one example, integration of the nucleic acid construct into the endogenous *RS1* locus reduces expression of the endogenous retinoschisin protein. In another example, integration of the nucleic acid construct into the endogenous *RS1* locus eliminates expression of the endogenous retinoschisin protein. In this way, integration of the nucleic acid construct can simultaneously knock out the endogenous *RS1* gene (e.g., an

endogenous *RS1* gene comprising one or more mutations associated with or that cause XLRS) and knock in a replacement retinoschisin coding sequence (e.g., a replacement retinoschisin coding sequence that does not comprise mutations associated with or that cause XLRS).

[00153] The nucleic acid construct can be integrated into any part of the target genomic locus. For example, the nucleic acid construct can be inserted into an intron or an exon of a target genomic locus or can replace one or more introns and/or exons of a target genomic locus. In a specific example, the nucleic acid construct can be integrated into an intron of the target genomic locus, such as the first intron of the target genomic locus (e.g., *RS1* intron 1). Expression cassettes integrated into a target genomic locus can be operably linked to an endogenous promoter at the target genomic locus (e.g., the endogenous *RS1* promoter) or can be operably linked to an exogenous promoter that is heterologous to the target genomic locus (e.g., a CMV promoter).

[00154] The nucleic acid constructs can comprise deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), they can be single-stranded or double-stranded, and they can be in linear or circular form. For example, the nucleic acid construct can be a single-stranded oligodeoxynucleotide (ssODN). *See, e.g.*, Yoshimi et al. (2016) *Nat. Commun.* 7:10431, herein incorporated by reference in its entirety for all purposes. The nucleic acid constructs can be naked nucleic acids or can be delivered by vectors, such as AAV vectors. In a specific example, the nucleic acid construct can be delivered via AAV and can be capable of insertion into an endogenous *RS1* locus by non-homologous end joining (e.g., the nucleic acid construct can be one that does not comprise homology arms). If introduced in linear form, the ends of the nucleic acid construct (e.g., donor sequence) can be protected (e.g., from exonucleolytic degradation) by well-known methods. For example, one or more dideoxynucleotide residues can be added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides can be ligated to one or both ends. *See, e.g.*, Chang et al. (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:4959-4963 and Nehls et al. (1996) *Science* 272:886-889, each of which is herein incorporated by reference in its entirety for all purposes. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

[00155] An exemplary nucleic acid construct is between about 50 nucleotides to about 5 kb in length or between about 50 nucleotides to about 3 kb in length. Alternatively, a nucleic acid construct can be between about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, about 2 kb to about 2.5 kb, about 2.5 kb to about 3 kb, about 3 kb to about 3.5 kb, about 3.5 kb to about 4 kb, about 4 kb to about 4.5 kb, or about 4.5 kb to about 5 kb in length. Alternatively, a nucleic acid construct can be, for example, no more than 5 kb, 4.5 kb, 4 kb, 3.5 kb, 3 kb, or 2.5 kb in length.

[00156] Integration of a nucleic acid construct at a target genomic locus can result in addition of a nucleic acid sequence of interest to the target genomic locus or replacement of a nucleic acid sequence of interest at the target genomic locus (i.e., deletion and insertion). Some nucleic acid constructs are designed for insertion of a nucleic acid construct at a target genomic locus without any corresponding deletion at the target genomic locus. Other nucleic acid constructs are designed to delete a nucleic acid sequence of interest at a target genomic locus and replace it with a nucleic acid construct.

[00157] The nucleic acid construct or the corresponding nucleic acid at the target genomic locus being deleted and/or replaced can be various lengths. An exemplary nucleic acid construct or corresponding nucleic acid at the target genomic locus being deleted and/or replaced is between about 1 nucleotide to about 5 kb in length or is between about 1 nucleotide to about 3 kb nucleotides in length. For example, a nucleic acid construct or a corresponding nucleic acid at the target genomic locus being deleted and/or replaced can be between about 1 to about 100, about 100 to about 200, about 200 to about 300, about 300 to about 400, about 400 to about 500, about 500 to about 600, about 600 to about 700, about 700 to about 800, about 800 to about 900, or about 900 to about 1,000 nucleotides in length. Likewise, a nucleic acid construct or a corresponding nucleic acid at the target genomic locus being deleted and/or replaced can be between about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, about 2 kb to about 2.5 kb, about 2.5 kb to about 3 kb, about 3 kb to about 3.5 kb, about 3.5 kb to about 4 kb, about 4 kb to about 4.5 kb, about 4.5 kb to about 5 kb in length, or longer.

[00158] The nucleic acid construct or the corresponding nucleic acid at the target genomic locus being deleted and/or replaced can be a coding region such as an exon; a non-coding region such as an intron, an untranslated region, or a regulatory region (e.g., a promoter, an enhancer, or a transcriptional repressor-binding element), or any combination thereof.

[00159] The nucleic acid constructs can, in some cases, comprise one or more of the following terminal structures: hairpin, loop, inverted terminal repeat (ITR), or toroid. For example, the nucleic acid construct can comprise ITRs.

[00160] Some such nucleic acid constructs can modify the target genomic locus (e.g., but not limited to, an endogenous *RS1* locus) following cleavage or nicking of the target genomic locus with a nuclease agent such as a Cas protein. The nucleic acid construct can be designed to repair the cleaved or nicked locus via non-homologous end joining (NHEJ)-mediated ligation or homology-directed repair. Optionally, repair with the nucleic acid construct removes or disrupts the nuclease target sequence so that alleles that have been targeted cannot be re-targeted by the nuclease agent.

[00161] Some nucleic acid constructs comprise homology arms. The homology arms can be symmetrical (e.g., each 40 nucleotides or each 60 nucleotides in length), or they can be asymmetrical (e.g., one homology arm or complementary region that is 36 nucleotides in length, and one homology arm or complementary region that is 91 nucleotides in length). Other nucleic acid constructs do not comprise homology arms.

[00162] Some nucleic acid constructs disclosed herein comprise homology arms. The homology arms can flank the retinoschisin coding sequence. For ease of reference, the homology arms are referred to herein as 5' and 3' (i.e., upstream and downstream) homology arms. This terminology relates to the relative position of the homology arms to a nucleic acid insert (e.g., the retinoschisin coding sequence) within a nucleic acid construct. The 5' and 3' homology arms correspond to regions within the target genomic locus, which are referred to herein as "5' target sequence" and "3' target sequence," respectively.

[00163] A homology arm and a target sequence "correspond" or are "corresponding" to one another when the two regions share a sufficient level of sequence identity to one another to act as substrates for a homologous recombination reaction. The term "homology" includes DNA sequences that are either identical or share sequence identity to a corresponding sequence. The sequence identity between a given target sequence and the corresponding homology arm found in the nucleic acid construct can be any degree of sequence identity that allows for homologous recombination to occur. For example, the amount of sequence identity shared by the homology arm of the nucleic acid construct (or a fragment thereof) and the target sequence (or a fragment thereof) can be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, such that the sequences undergo homologous recombination. Moreover, a corresponding region of homology between the homology arm and the corresponding target sequence can be of any length that is sufficient to promote homologous recombination. Exemplary homology arms are between about 25 nucleotides to about 2.5 kb in length, are between about 25 nucleotides to about 1.5 kb in length, or are between about 25 to about 500 nucleotides in length. For example, a given homology arm (or each of the homology arms) and/or corresponding target sequence can comprise corresponding regions of homology that are between about 25 to about 30, about 30 to about 40, about 40 to about 50, about 50 to about 60, about 60 to about 70, about 70 to about 80, about 80 to about 90, about 90 to about 100, about 100 to about 150, about 150 to about 200, about 200 to about 250, about 250 to about 300, about 300 to about 350, about 350 to about 400, about 400 to about 450, or about 450 to about 500 nucleotides in length, such that the homology arms have sufficient homology to undergo homologous recombination with the corresponding target sequences within the target nucleic acid. Alternatively, a given homology arm (or each homology arm) and/or corresponding target sequence can comprise corresponding regions of homology that are between about 0.5 kb to about 1 kb, about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, or about 2 kb to about 2.5 kb in length. For example, the homology arms can each be about 750 nucleotides in length. In another example, the homology arms can each be about 150 to about 750, about 200 to about 700, about 250 to about 650, about 300 to about 600, about 350 to about 550, about 400 to about 500, about 150 to about 450, about 200 to about 450, about 250 to about 450, about 300 to about 450, about 350 to about 450, about 400 to about 450, about 450 to about 500, about 450 to about 550, about 450 to about 600, about 450 to about 650, about 450 to about 700, about 450 to about 750, or about 450 nucleotides in length. In another example, the homology arms can each be about 500 to about 1300, about 550 to about 1250, about 600 to about 1200, about 650 to about 1150, about 700 to about 1100, about 750 to about 1050, about 800 to about 1000, about 850 to about 950, about 500 to about 900, about 550 to about 900, about 600 to about 900, about 650 to about 900, about 700 to about 900, about 750 to about 900, about 800 to about 900, about 850 to about 900, about 900 to about 950, about 900 to about 1000, about 900 to about 1050, about 900 to about 1100, about 900 to about 1150, about 900 to about 1200, about 900 to about 1250, about 900 to about 1300, or about 900 nucleotides in length. In another example, the homology arms

can each be about 1500 to about 2100, about 1550 to about 2050, about 1600 to about 2000, about 1650 to about 1950, about 1700 to about 1900, about 1750 to about 1850, about 1500 to about 1800, about 1550 to about 1800, about 1600 to about 1800, about 1650 to about 1800, about 1700 to about 1800, about 1750 to about 1800, about 1800 to about 1850, about 1800 to about 1900, about 1800 to about 1950, about 1800 to about 2000, about 1800 to about 2050, about 1800 to about 2100, or about 1800 nucleotides. In another example, each homology arm is no more than about 450 nucleotides, no more than about 900 nucleotides, or no more than about 1800 nucleotides. In another example, each homology arm is at least about 450 nucleotides, at least about 900 nucleotides, or at least about 1800 nucleotides. The homology arms can be symmetrical (each about the same size in length), or they can be asymmetrical (one longer than the other).

[00164] When a CRISPR/Cas system or other nuclease agent is used in combination with an nucleic acid construct disclosed herein, the 5' and 3' target sequences can be located in sufficient proximity to the nuclease cleavage site (e.g., within sufficient proximity to a guide RNA target sequence) so as to promote the occurrence of a homologous recombination event between the target sequences and the homology arms upon a single-strand break (nick) or double-strand break at the nuclease cleavage site or nuclease cleavage site. The term "nuclease cleavage site" includes a DNA sequence at which a nick or double-strand break is created by a nuclease agent (e.g., a Cas9 protein complexed with a guide RNA). The target sequences within the targeted locus that correspond to the 5' and 3' homology arms of the nucleic acid construct are "located in sufficient proximity" to a nuclease cleavage site if the distance is such as to promote the occurrence of a homologous recombination event between the 5' and 3' target sequences and the homology arms upon a single-strand break or double-strand break at the nuclease cleavage site. Thus, the target sequences corresponding to the 5' and/or 3' homology arms of the nucleic acid construct can be, for example, within at least 1 nucleotide of a given nuclease cleavage site or within at least 10 nucleotides to about 1,000 nucleotides of a given nuclease cleavage site. As an example, the nuclease cleavage site can be immediately adjacent to at least one or both of the target sequences.

[00165] The spatial relationship of the target sequences that correspond to the homology arms of the nucleic acid construct and the nuclease cleavage site can vary. For example, target

sequences can be located 5' to the nuclease cleavage site, target sequences can be located 3' to the nuclease cleavage site, or the target sequences can flank the nuclease cleavage site.

[00166] Other nucleic acid constructs do not comprise any homology arms. Such nucleic acid constructs can be capable of insertion by non-homologous end joining. For example, such nucleic acid constructs can be inserted into a blunt end double-strand break following cleavage with a nuclease agent. In a specific example, the nucleic acid construct acid can be delivered via AAV and can be capable of insertion into a target genomic locus by non-homologous end joining (e.g., the nucleic acid construct can be one that does not comprise homology arms).

[00167] In a specific example, the nucleic acid construct can be inserted via homology-independent targeted integration. For example, the retinoschisin coding sequence in the nucleic acid construct can be flanked on each side by a target site for a nuclease agent (e.g., the same target site as in the target genomic locus, and the same nuclease agent being used to cleave the target site in the target genomic locus). The nuclease agent can then cleave the target sites flanking the retinoschisin coding sequence. In a specific example, the nucleic acid construct is delivered AAV-mediated delivery, and cleavage of the target sites flanking the retinoschisin coding sequence can remove the inverted terminal repeats (ITRs) of the AAV. In some methods, the target site in the target genomic locus (e.g., a gRNA target sequence including the flanking protospacer adjacent motif) is no longer present if the retinoschisin coding sequence is inserted into the target genomic locus in the correct orientation but it is reformed if the retinoschisin coding sequence is inserted into the target genomic locus in the opposite orientation. This can help ensure that the retinoschisin coding sequence is inserted in the correct orientation for expression.

[00168] In one exemplary nucleic acid construct for homology-independent targeted integration into a target genomic locus, the retinoschisin protein or fragment thereof is a human retinoschisin protein or fragment thereof, the coding sequence for the retinoschisin protein or fragment thereof comprises complementary DNA (cDNA) comprising exons 2-6 of human *RS1* or degenerate variants thereof, the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or fragment thereof, the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, and the nuclease target sequence in the nucleic acid construct is identical to a nuclease target sequence for

integration into the target genomic locus, wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

[00169] In one exemplary nucleic acid construct for homology-independent targeted integration into a target genomic locus, the retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 5. In one exemplary nucleic acid construct for homology-independent targeted integration into a target genomic locus, the coding sequence for the retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 6, 8, or 9 or degenerate variants thereof. In one exemplary nucleic acid construct for homology-independent targeted integration into a target genomic locus, the nucleic acid construct comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 45.

[00170] Other nucleic acid constructs can have short single-stranded regions at the 5' end and/or the 3' end that are complementary to one or more overhangs created by nuclease-agent-mediated cleavage at the target genomic locus. For example, some nucleic acid constructs have short single-stranded regions at the 5' end and/or the 3' end that are complementary to one or more overhangs created by nuclease-mediated cleavage at 5' and/or 3' target sequences at the target genomic locus. Some such nucleic acid constructs have a complementary region only at the 5' end or only at the 3' end. For example, some such nucleic acid constructs have a complementary region only at the 5' end complementary to an overhang created at a 5' target sequence at the target genomic locus or only at the 3' end complementary to an overhang created at a 3' target sequence at the target genomic locus. Other such nucleic acid constructs have complementary regions at both the 5' and 3' ends. For example, other such nucleic acid

constructs have complementary regions at both the 5' and 3' ends (e.g., complementary to first and second overhangs, respectively) generated by nuclease-mediated cleavage at the target genomic locus. For example, if the nucleic acid construct is double-stranded, the single-stranded complementary regions can extend from the 5' end of the top strand of the nucleic acid construct and the 5' end of the bottom strand of the donor nucleic acid, creating 5' overhangs on each end. Alternatively, the single-stranded complementary region can extend from the 3' end of the top strand of the nucleic acid construct and from the 3' end of the bottom strand of the template, creating 3' overhangs.

[00171] The complementary regions can be of any length sufficient to promote ligation between the nucleic acid construct and the target nucleic acid. Exemplary complementary regions are between about 1 to about 5 nucleotides in length, between about 1 to about 25 nucleotides in length, or between about 5 to about 150 nucleotides in length. For example, a complementary region can be at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. Alternatively, the complementary region can be about 5 to about 10, about 10 to about 20, about 20 to about 30, about 30 to about 40, about 40 to about 50, about 50 to about 60, about 60 to about 70, about 70 to about 80, about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 130 to about 140, about 140 to about 150 nucleotides in length, or longer.

[00172] Such complementary regions can be complementary to overhangs created by two pairs of nickases. Two double-strand breaks with staggered ends can be created by using first and second nickases that cleave opposite strands of DNA to create a first double-strand break, and third and fourth nickases that cleave opposite strands of DNA to create a second double-strand break. For example, a Cas protein can be used to nick first, second, third, and fourth guide RNA target sequences corresponding with first, second, third, and fourth guide RNAs. The first and second guide RNA target sequences can be positioned to create a first cleavage site such that the nicks created by the first and second nickases on the first and second strands of DNA create a double-strand break (i.e., the first cleavage site comprises the nicks within the first and second guide RNA target sequences). Likewise, the third and fourth guide RNA target sequences can be positioned to create a second cleavage site such that the nicks created by the third and fourth nickases on the first and second strands of DNA create a double-strand break (i.e., the second cleavage site comprises the nicks within the third and fourth guide RNA target sequences). The

nicks within the first and second guide RNA target sequences and/or the third and fourth guide RNA target sequences can be off-set nicks that create overhangs. The offset window can be, for example, at least about 5 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp or more. *See* Ran et al. (2013) *Cell* 154:1380-1389; Mali et al. (2013) *Nat. Biotechnol.* 31:833-838; and Shen et al. (2014) *Nat. Methods* 11:399-404, each of which is herein incorporated by reference in its entirety for all purposes. In such cases, a double-stranded nucleic acid construct can be designed with single-stranded complementary regions that are complementary to the overhangs created by the nicks within the first and second guide RNA target sequences and by the nicks within the third and fourth guide RNA target sequences. Such nucleic acid construct can then be inserted by non-homologous-end-joining-mediated ligation.

[00173] Some of the nucleic acid constructs disclosed herein are bidirectional constructs that can be inserted into and expressed from the target genomic locus in either orientation. Such nucleic acid constructs can comprise a first segment comprising a first coding sequence for a first retinoschisin protein or fragment or variant thereof and a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or fragment or variant thereof. The second segment can be, for example, located 3' of the first segment in the nucleic acid construct.

[00174] The first segment and the second segment can be linked directly together or can be linked by a linker such as a peptide linker. The peptide linker can be any suitable length. For example, the linker can be between about 5 to about 2000 nucleotides in length. As an example, the linker sequence can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 500, 1000, 1500, 2000, or more nucleotides in length.

[00175] In some bidirectional constructs, the first retinoschisin protein or fragment or variant thereof is identical to the second retinoschisin protein or fragment or variant thereof. In other bidirectional constructs, the first retinoschisin protein or fragment or variant thereof is different from the second retinoschisin protein or fragment or variant thereof.

[00176] In some bidirectional constructs, the codon usage in the first coding sequence is the same as the codon usage in the second coding sequence. In other bidirectional constructs, the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence in order to reduce hairpin formation. Such a reverse complement forms base pairs with

fewer than all nucleotides of the coding sequence in the first segment, but it can optionally encode the same polypeptide.

[00177] The second segment can have any percentage of complementarity to the first segment. For example, the second segment sequence can have at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% complementarity to the first segment. As another example, the second segment sequence can have less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, less than about 90%, less than about 95%, less than about 97%, or less than about 99% complementarity to the first segment. The reverse complement of the second coding sequence can be, in some nucleic acid constructs, not substantially complementary (e.g., not more than 70% complementary) to the first coding sequence, not substantially complementary to a fragment of the first coding sequence, highly complementary (e.g., at least 90% complementary) to the first coding sequence, highly complementary to a fragment of the first coding sequence, about 50% to about 80% identical to the reverse complement of the first coding sequence, or about 60% to about 100% identical to the reverse complement of the first coding sequence.

[00178] The bidirectional constructs can, in some cases, comprise one or more (e.g., two) polyadenylation signal sequences. In some bidirectional constructs, the first segment can comprise a first polyadenylation signal sequence. In some bidirectional constructs, the first segment can comprise a second polyadenylation signal sequence. In some bidirectional constructs, the first segment can comprise a first polyadenylation signal sequence, and the second segment can comprise a second polyadenylation signal sequence (e.g., a reverse complement of a polyadenylation signal sequence). In some bidirectional constructs, the first segment can comprise a first polyadenylation signal sequence located 3' of the first coding sequence. In some bidirectional constructs, the second segment can comprise a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence. In some bidirectional constructs, the first segment can comprise a first polyadenylation signal sequence located 3' of the first coding sequence, and the second

segment can comprise a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence. The first and second polyadenylation signal sequences can be the same or different. In one example, the first and second polyadenylation signals are different.

[00179] The bidirectional constructs can, in some cases, can comprise one or more (e.g., two) splice acceptor sites. In some bidirectional constructs, the first segment can comprise a first splice acceptor site. In some bidirectional constructs, the first segment can comprise a second splice acceptor site. In some bidirectional constructs, the first segment can comprise a first splice acceptor site, and the second segment can comprise a second splice acceptor site (e.g., a reverse complement of a splice acceptor site). In some bidirectional constructs, the first segment comprises a first splice acceptor site located 5' of the first coding sequence. In some bidirectional constructs, the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence. In some bidirectional constructs, the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence. The first and second splice acceptor sites can be the same or different. In one example, the first and second splice acceptor sites are different. The first and/or the second splice acceptor site can be from an *RS1* gene (e.g., from intron 1 of an *RS1* gene) such as a human *RS1* gene.

[00180] Some bidirectional constructs can comprise a promoter that drives expression of the first retinoschisin protein or fragment or variant thereof and/or the reverse complement of a promoter that drives expression of the second retinoschisin protein or fragment or variant thereof. Alternatively, a bidirectional construct can be a construct that does not comprise a promoter that drives expression of the first retinoschisin protein or fragment or variant thereof or the second retinoschisin protein or fragment or variant thereof (i.e., a promoterless construct).

[00181] One or both of the coding sequences can be codon-optimized for expression in a host cell. In some bidirectional constructs, only one of the coding sequences is codon-optimized. In some bidirectional constructs, the first coding sequence is codon-optimized. In some bidirectional constructs, the second coding sequence is codon-optimized. In some bidirectional constructs, both coding sequences are codon-optimized.

[00182] In an exemplary bidirectional construct, the second segment is located 3' of the first segment, both the first retinoschisin protein or fragment thereof and the second retinoschisin protein or fragment thereof are a human retinoschisin protein or fragment thereof, the first retinoschisin protein or fragment thereof is identical to the second retinoschisin protein or fragment thereof, both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) comprising exons 2-6 of human *RS1* or degenerate variants thereof, the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence, the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence, the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence, the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or fragment thereof or the second retinoschisin protein or fragment thereof, and optionally the nucleic acid construct does not comprise a homology arm.

[00183] In an exemplary bidirectional construct, the first retinoschisin protein or fragment thereof and/or the second retinoschisin protein or fragment thereof comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 5. In an exemplary bidirectional construct, the first coding sequence and/or the second coding sequence comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 6, 8, or 9 or degenerate variants thereof. In an exemplary bidirectional construct, the first coding sequence comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 8, and the second coding sequence comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about

92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 9. In an exemplary bidirectional construct, the nucleic acid construct comprises, consists essentially of, or consists of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 46 or 47.

[00184] The nucleic acid constructs can include modifications or sequences that provide for additional desirable features (e.g., modified or regulated stability; tracking or detecting with a fluorescent label; a binding site for a protein or protein complex; and so forth). Nucleic acid constructs can comprise one or more fluorescent labels, purification tags, epitope tags, or a combination thereof. For example, a nucleic acid construct can comprise one or more fluorescent labels (e.g., fluorescent proteins or other fluorophores or dyes), such as at least 1, at least 2, at least 3, at least 4, or at least 5 fluorescent labels. Exemplary fluorescent labels include fluorophores such as fluorescein (e.g., 6-carboxyfluorescein (6-FAM)), Texas Red, HEX, Cy3, Cy5, Cy5.5, Pacific Blue, 5-(and-6)-carboxytetramethylrhodamine (TAMRA), and Cy7. A wide range of fluorescent dyes are available commercially for labeling oligonucleotides (e.g., from Integrated DNA Technologies). Such fluorescent labels (e.g., internal fluorescent labels) can be used, for example, to detect a nucleic acid construct that has been directly integrated into a cleaved target nucleic acid having protruding ends compatible with the ends of the nucleic acid construct. The label or tag can be at the 5' end, the 3' end, or internally within the nucleic acid construct. For example, a nucleic acid construct can be conjugated at 5' end with the IR700 fluorophore from Integrated DNA Technologies (5'IRDYE®700).

[00185] The nucleic acid construct can also comprise a conditional allele. The conditional allele can be a multifunctional allele, as described in US 2011/0104799, herein incorporated by reference in its entirety for all purposes. For example, the conditional allele can comprise: (a) an actuating sequence in sense orientation with respect to transcription of a target gene; (b) a drug selection cassette (DSC) in sense or antisense orientation; (c) a nucleotide sequence of interest (NSI) in antisense orientation; and (d) a conditional by inversion module (COIN, which utilizes an exon-splitting intron and an invertible gene-trap-like module) in reverse orientation. *See, e.g.*, US 2011/0104799. The conditional allele can further comprise recombinable units that recombine upon exposure to a first recombinase to form a conditional allele that (i) lacks the

actuating sequence and the DSC; and (ii) contains the NSI in sense orientation and the COIN in antisense orientation. *See, e.g.*, US 2011/0104799.

[00186] Nucleic acid constructs can also comprise a polynucleotide encoding a selection marker. Alternatively, the nucleic acid constructs can lack a polynucleotide encoding a selection marker. The selection marker can be contained in a selection cassette. Optionally, the selection cassette can be a self-deleting cassette. *See, e.g.,* US 8,697,851 and US 2013/0312129, each of which is herein incorporated by reference in its entirety for all purposes. As an example, the self-deleting cassette can comprise a Crei gene (comprises two exons encoding a Cre recombinase, which are separated by an intron) operably linked to a mouse *Prm1* promoter and a neomycin resistance gene operably linked to a human ubiquitin promoter. By employing the *Prm1* promoter, the self-deleting cassette can be deleted specifically in male germ cells of F0 animals. Exemplary selection markers include neomycin phosphotransferase (neo^r), hygromycin B phosphotransferase (hyg^r), puromycin-N-acetyltransferase ($puro^r$), blasticidin S deaminase (bsr^r), xanthine/guanine phosphoribosyl transferase (gpt), or herpes simplex virus thymidine kinase (HSV-k), or a combination thereof. The polynucleotide encoding the selection marker can be operably linked to a promoter active in a cell being targeted. Examples of promoters are described elsewhere herein.

[00187] The nucleic acid construct can also comprise a reporter gene. Exemplary reporter genes include those encoding luciferase, β-galactosidase, green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), enhanced yellow fluorescent protein (eYFP), blue fluorescent protein (BFP), enhanced blue fluorescent protein (eBFP), DsRed, ZsGreen, MmGFP, mPlum, mCherry, tdTomato, mStrawberry, J-Red, mOrange, mKO, mCitrine, Venus, YPet, Emerald, CyPet, Cerulean, T-Sapphire, and alkaline phosphatase. Such reporter genes can be operably linked to a promoter active in a cell being targeted. Examples of promoters are described elsewhere herein.

[00188] The nucleic acid construct can also comprise one or more expression cassettes or deletion cassettes. A given cassette can comprise one or more of a nucleotide sequence of interest, a polynucleotide encoding a selection marker, and a reporter gene, along with various regulatory components that influence expression. Examples of selectable markers and reporter genes that can be included are discussed in detail elsewhere herein.

[00189] The nucleic acid construct can comprise a nucleic acid flanked with site-specific recombination target sequences. Alternatively, the nucleic acid construct can comprise one or more site-specific recombination target sequences. Although the entire nucleic acid construct can be flanked by such site-specific recombination target sequences, any region or individual polynucleotide of interest within the nucleic acid construct can also be flanked by such sites. Site-specific recombination target sequences, which can flank the nucleic acid construct or any polynucleotide of interest in the nucleic acid construct can include, for example, loxP, lox511, lox2272, lox66, lox71, loxM2, lox5171, FRT, FRT11, FRT71, attp, att, FRT, rox, or a combination thereof. In one example, the site-specific recombination sites flank a polynucleotide encoding a selection marker and/or a reporter gene contained within the nucleic acid construct. Following integration of the nucleic acid construct at a targeted locus, the sequences between the site-specific recombination sites can be removed.

[00190] Nucleic acid constructs can also comprise one or more restriction sites for restriction endonucleases (i.e., restriction enzymes), which include Type I, Type II, Type III, and Type IV endonucleases. Type I and Type III restriction endonucleases recognize specific recognition sites, but typically cleave at a variable position from the nuclease binding site, which can be hundreds of base pairs away from the cleavage site (recognition site). In Type II systems the restriction activity is independent of any methylase activity, and cleavage typically occurs at specific sites within or near to the binding site. Most Type II enzymes cut palindromic sequences, however Type IIa enzymes recognize non-palindromic recognition sites and cleave outside of the recognition site, Type IIb enzymes cut sequences twice with both sites outside of the recognition site, and Type IIs enzymes recognize an asymmetric recognition site and cleave on one side and at a defined distance of about 1-20 nucleotides from the recognition site. Type IV restriction enzymes target methylated DNA. Restriction enzymes are further described and classified, for example in the REBASE database (webpage at rebase.neb.com; Roberts et al., (2003) *Nucleic Acids Res.* 31:418-420; Roberts et al., (2003) *Nucleic Acids Res.* 31:1805-1812; and Belfort et al. (2002) in Mobile DNA II, pp. 761-783, Eds. Craigie et al., (ASM Press, Washington, DC)).

[00191] The nucleic acid constructs disclosed herein can also comprise additional coding sequences. For example, some nucleic acid constructs disclosed herein can comprise a sequence encoding a guide RNA that targets the target genomic locus (e.g., that targets *RS1*, such as intron

1 of *RS1*). The sequence encoding the guide RNA can be operably linked to a promoter, such as a U6 promoter. In some nucleic acid constructs, the guide RNA expression cassette is located 3' (downstream) of the retinoschisin coding sequence(s). In some bidirectional nucleic acid constructs, the guide RNA expression cassette is located between the first segment and the second segment.

III. Vectors Comprising Nucleic Acid Constructs

[00192] Also provided herein are vectors comprising the nucleic acid constructs (i.e., exogenous donor nucleic acids) comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus. Also provided herein are vectors comprising the nucleic acids encoding nuclease agents (e.g., targeting an endogenous *RS1* locus) disclosed elsewhere herein. Also provided herein are vectors comprising the nucleic acid constructs and/or nucleic acids encoding nuclease agents (e.g., targeting an endogenous *RS1* locus) disclosed elsewhere herein (e.g., vectors comprising the nucleic acid construct and a DNA encoding a guide RNA). A vector can comprise additional sequences such as, for example, replication origins, promoters, and genes encoding antibiotic resistance. Some such vectors comprise homology arms corresponding to target sites in the target genomic locus. Other such vectors do not comprise any homology arms.

[00193] Some vectors may be circular. Alternatively, the vector may be linear. The vector can be in the packaged for delivered via a lipid nanoparticle, liposome, non-lipid nanoparticle, or viral capsid. Non-limiting exemplary vectors include plasmids, phagemids, cosmids, artificial chromosomes, minichromosomes, transposons, viral vectors, and expression vectors.

[00194] The vectors can be, for example, viral vectors such as adeno-associated virus (AAV) vectors. The AAV may be any suitable serotype and may be a single-stranded AAV (ssAAV) or a self-complementary AAV (scAAV). Other exemplary viruses/viral vectors include retroviruses, adenoviruses, vaccinia viruses, poxviruses, and herpes simplex viruses. The viruses can infect dividing cells, non-dividing cells, or both dividing and non-dividing cells. The viruses can integrate into the host genome or alternatively do not integrate into the host genome. Such viruses can also be engineered to have reduced immunity. The viruses can be replication-competent or can be replication-defective (e.g., defective in one or more genes necessary for additional rounds of virion replication and/or packaging). Viruses can cause transient expression,

long-lasting expression (e.g., at least 1 week, 2 weeks, 1 month, 2 months, or 3 months), or permanent expression (e.g., of Cas9 and/or gRNA). Exemplary viral titers (e.g., AAV titers) include 10^{12}, 10^{13}, 10^{14}, 10^{15}, and 10^{16} vector genomes/mL. Exemplary viral titers (e.g., AAV titers) include about 10^{12}, about 10^{13}, about 10^{14}, about 10^{15}, and about 10^{16} vector genomes (vg)/mL, or between about 10^{12} to about 10^{16}, between about 10^{12} to about 10^{15}, between about 10^{12} to about 10^{14}, between about 10^{12} to about 10^{13}, between about 10^{13} to about 10^{16}, between about 10^{14} to about 10^{16}, between about 10^{15} to about 10^{16}, or between about 10^{13} to about 10^{15} vg/mL. Other exemplary viral titers (e.g., AAV titers) include about 10^{12}, about 10^{13}, about 10^{14}, about 10^{15}, and about 10^{16} vector genomes (vg)/kg of body weight, or between about 10^{12} to about 10^{16}, between about 10^{12} to about 10^{15}, between about 10^{12} to about 10^{14}, between about 10^{12} to about 10^{13}, between about 10^{13} to about 10^{16}, between about 10^{14} to about 10^{16}, between about 10^{15} to about 10^{16}, or between about 10^{13} to about 10^{15} vg/kg of body weight.

[00195] The ssDNA AAV genome consists of two open reading frames, Rep and Cap, flanked by two inverted terminal repeats that allow for synthesis of the complementary DNA strand. When constructing an AAV transfer plasmid, the transgene is placed between the two ITRs, and Rep and Cap can be supplied *in trans*. In addition to Rep and Cap, AAV can require a helper plasmid containing genes from adenovirus. These genes (E4, E2a, and VA) mediate AAV replication. For example, the transfer plasmid, Rep/Cap, and the helper plasmid can be transfected into HEK293 cells containing the adenovirus gene E1+ to produce infectious AAV particles. Alternatively, the Rep, Cap, and adenovirus helper genes may be combined into a single plasmid. Similar packaging cells and methods can be used for other viruses, such as retroviruses.

[00196] Multiple serotypes of AAV have been identified. These serotypes differ in the types of cells they infect (i.e., their tropism), allowing preferential transduction of specific cell types. Serotypes for photoreceptor cells include AAV2, AAV5, and AAV8. Serotypes for retinal pigment epithelium tissue include AAV1, AAV2, AAV4, AAV5, and AAV8. In a specific example, the AAV vector comprising the nucleic acid construct can be AAV2, AAV5, or AAV8.

[00197] Tropism can be further refined through pseudotyping, which is the mixing of a capsid and a genome from different viral serotypes. For example AAV2/5 indicates a virus containing the genome of serotype 2 packaged in the capsid from serotype 5. Use of pseudotyped viruses can improve transduction efficiency, as well as alter tropism. Hybrid capsids derived from

different serotypes can also be used to alter viral tropism. For example, AAV-DJ contains a hybrid capsid from eight serotypes and displays high infectivity across a broad range of cell types *in vivo*. AAV-DJ8 is another example that displays the properties of AAV-DJ but with enhanced brain uptake. AAV serotypes can also be modified through mutations. Examples of mutational modifications of AAV2 include Y444F, Y500F, Y730F, and S662V. Examples of mutational modifications of AAV3 include Y705F, Y731F, and T492V. Examples of mutational modifications of AAV6 include S663V and T492V. Other pseudotyped/modified AAV variants include AAV2/1, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV2.5, AAV8.2, and AAV/SASTG. In a specific example, the AAV is AAV7m8, an AAV variant that mediates highly efficient delivery to all retinal layers and to photoreceptors. *See, e.g.*, Dalkara et al. (2013) *Sci. Transl. Med.* 5:189ra76, herein incorporated by reference in its entirety for all purposes.

[00198] To accelerate transgene expression, self-complementary AAV (scAAV) variants can be used. Because AAV depends on the cell's DNA replication machinery to synthesize the complementary strand of the AAV's single-stranded DNA genome, transgene expression may be delayed. To address this delay, scAAV containing complementary sequences that are capable of spontaneously annealing upon infection can be used, eliminating the requirement for host cell DNA synthesis. However, single-stranded AAV (ssAAV) vectors can also be used.

[00199] To increase packaging capacity, longer transgenes may be split between two AAV transfer plasmids, the first with a 3' splice donor and the second with a 5' splice acceptor. Upon co-infection of a cell, these viruses form concatemers, are spliced together, and the full-length transgene can be expressed. Although this allows for longer transgene expression, expression is less efficient. Similar methods for increasing capacity utilize homologous recombination. For example, a transgene can be divided between two transfer plasmids but with substantial sequence overlap such that co-expression induces homologous recombination and expression of the full-length transgene.

IV. Lipid Nanoparticles Comprising Nucleic Acid Constructs

[00200] Also provided herein are lipid nanoparticles comprising the nucleic acid constructs (i.e., exogenous donor nucleic acids) comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus. Also provided herein are lipid nanoparticles comprising the nucleic acids

encoding nuclease agents (e.g., targeting an endogenous *RS1* locus) disclosed elsewhere herein. Also provided herein are lipid nanoparticles comprising the nucleic acid constructs and the nucleic acids encoding nuclease agents (e.g., targeting an endogenous *RS1* locus) disclosed elsewhere herein.

[00201] Lipid formulations can protect biological molecules from degradation while improving their cellular uptake. Lipid nanoparticles are particles comprising a plurality of lipid molecules physically associated with each other by intermolecular forces. These include microspheres (including unilamellar and multilamellar vesicles, e.g., liposomes), a dispersed phase in an emulsion, micelles, or an internal phase in a suspension. Such lipid nanoparticles can be used to encapsulate one or more nucleic acids or proteins for delivery. Formulations which contain cationic lipids are useful for delivering polyanions such as nucleic acids. Other lipids that can be included are neutral lipids (i.e., uncharged or zwitterionic lipids), anionic lipids, helper lipids that enhance transfection, and stealth lipids that increase the length of time for which nanoparticles can exist *in vivo*. Examples of suitable cationic lipids, neutral lipids, anionic lipids, helper lipids, and stealth lipids can be found in WO 2016/010840 A1 and WO 2017/173054 A1, each of which is herein incorporated by reference in its entirety for all purposes. An exemplary lipid nanoparticle can comprise a cationic lipid and one or more other components. In one example, the other component can comprise a helper lipid such as cholesterol. In another example, the other components can comprise a helper lipid such as cholesterol and a neutral lipid such as DSPC. In another example, the other components can comprise a helper lipid such as cholesterol, an optional neutral lipid such as DSPC, and a stealth lipid such as S010, S024, S027, S031, or S033.

[00202] The LNP may contain one or more or all of the following: (i) a lipid for encapsulation and for endosomal escape; (ii) a neutral lipid for stabilization; (iii) a helper lipid for stabilization; and (iv) a stealth lipid. *See, e.g.*, Finn et al. (2018) *Cell Rep.* 22(9):2227-2235 and WO 2017/173054 A1, each of which is herein incorporated by reference in its entirety for all purposes. In certain LNPs, the cargo can further comprise a nuclease agent. In certain LNPs, the cargo can further comprise a guide RNA or a nucleic acid encoding a guide RNA. In certain LNPs, the cargo can further comprise an mRNA encoding a Cas nuclease, such as Cas9, and a guide RNA or a nucleic acid encoding a guide RNA. In certain LNPs, the cargo can include an mRNA encoding a Cas nuclease, such as Cas9, a guide RNA or a nucleic acid encoding a guide

RNA, and the nucleic acid construct.

[00203] The lipid for encapsulation and endosomal escape can be a cationic lipid. The lipid can also be a biodegradable lipid, such as a biodegradable ionizable lipid. One example of a suitable lipid is Lipid A or LP01, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. *See, e.g.*, Finn et al. (2018) *Cell Rep.* 22(9):2227-2235 and WO 2017/173054 A1, each of which is herein incorporated by reference in its entirety for all purposes. Another example of a suitable lipid is Lipid B, which is ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate), also called ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate). Another example of a suitable lipid is Lipid C, which is 2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1,3-diyl(9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate). Another example of a suitable lipid is Lipid D, which is 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl 3-octylundecanoate. Other suitable lipids include heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (also known as Dlin-MC3-DMA (MC3))).

[00204] Some such lipids suitable for use in the LNPs described herein are biodegradable *in vivo*. For example, LNPs comprising such a lipid include those where at least 75% of the lipid is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. As another example, at least 50% of the LNP is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days.

[00205] Such lipids may be ionizable depending upon the pH of the medium they are in. For example, in a slightly acidic medium, the lipids may be protonated and thus bear a positive charge. Conversely, in a slightly basic medium, such as, for example, blood where pH is approximately 7.35, the lipids may not be protonated and thus bear no charge. In some embodiments, the lipids may be protonated at a pH of at least about 9, 9.5, or 10. The ability of such a lipid to bear a charge is related to its intrinsic pKa. For example, the lipid may, independently, have a pKa in the range of from about 5.8 to about 6.2.

[00206] Neutral lipids function to stabilize and improve processing of the LNPs. Examples of suitable neutral lipids include a variety of neutral, uncharged or zwitterionic lipids. Examples of

neutral phospholipids suitable for use in the present disclosure include, but are not limited to, 5-heptadecylbenzene-1,3-diol (resorcinol), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), phosphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine (PLPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauryloylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DBPC), 1-stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine, and combinations thereof. For example, the neutral phospholipid may be selected from the group consisting of distearoylphosphatidylcholine (DSPC) and dimyristoyl phosphatidyl ethanolamine (DMPE).

[00207] Helper lipids include lipids that enhance transfection. The mechanism by which the helper lipid enhances transfection can include enhancing particle stability. In certain cases, the helper lipid can enhance membrane fusogenicity. Helper lipids include steroids, sterols, and alkyl resorcinols. Examples of suitable helper lipids suitable include cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. In one example, the helper lipid may be cholesterol or cholesterol hemisuccinate.

[00208] Stealth lipids include lipids that alter the length of time the nanoparticles can exist in vivo. Stealth lipids may assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. Stealth lipids may modulate pharmacokinetic properties of the LNP. Suitable stealth lipids include lipids having a hydrophilic head group linked to a lipid moiety.

[00209] The hydrophilic head group of stealth lipid can comprise, for example, a polymer moiety selected from polymers based on PEG (sometimes referred to as poly(ethylene oxide)), poly(oxazoline), poly(vinyl alcohol), poly(glycerol), poly(N- vinylpyrrolidone), polyaminoacids,

and poly N-(2-hydroxypropyl)methacrylamide. The term PEG means any polyethylene glycol or other polyalkylene ether polymer. In certain LNP formulations, the PEG, is a PEG-2K, also termed PEG 2000, which has an average molecular weight of about 2,000 daltons. *See, e.g.,* WO 2017/173054 A1, herein incorporated by reference in its entirety for all purposes.

[00210] The lipid moiety of the stealth lipid may be derived, for example, from diacylglycerol or diacylglycamide, including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C4 to about C40 saturated or unsaturated carbon atoms, wherein the chain may comprise one or more functional groups such as, for example, an amide or ester. The dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups.

[00211] As one example, the stealth lipid may be selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG), PEG-dipalmitoylglycerol, PEG-distearoylglycerol (PEG-DSPE), PEG-dilaurylglycamide, PEG- dimyristylglycamide, PEG-dipalmitoylglycamide, and PEG-distearoylglycamide, PEG- cholesterol (l-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-sn- glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k- DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE), 1,2-distearoyl-sn-glycerol, methoxypoly ethylene glycol (PEG2k-DSG), poly(ethylene glycol)-2000-dimethacrylate (PEG2k-DMA), and 1,2- distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA). In one particular example, the stealth lipid may be PEG2k-DMG.

[00212] The LNPs can comprise different respective molar ratios of the component lipids in the formulation. The mol-% of the CCD lipid may be, for example, from about 30 mol-% to about 60 mol-%, from about 35 mol-% to about 55 mol-%, from about 40 mol-% to about 50 mol-%, from about 42 mol-% to about 47 mol-%, or about 45%. The mol-% of the helper lipid may be, for example, from about 30 mol-% to about 60 mol-%, from about 35 mol-% to about 55 mol-%, from about 40 mol-% to about 50 mol-%, from about 41 mol-% to about 46 mol-%, or about 44 mol-%. The mol-% of the neutral lipid may be, for example, from about 1 mol-% to about 20 mol-%, from about 5 mol-% to about 15 mol-%, from about 7 mol-% to about 12 mol-%, or about 9 mol-%. The mol-% of the stealth lipid may be, for example, from about 1 mol-%

to about 10 mol-%, from about 1 mol-% to about 5 mol-%, from about 1 mol-% to about 3 mol-%, about 2 mol-%, or about 1 mol-%.

[00213] The LNPs can have different ratios between the positively charged amine groups of the biodegradable lipid (N) and the negatively charged phosphate groups (P) of the nucleic acid to be encapsulated. This may be mathematically represented by the equation N/P. For example, the N/P ratio may be from about 0.5 to about 100, from about 1 to about 50, from about 1 to about 25, from about 1 to about 10, from about 1 to about 7, from about 3 to about 5, from about 4 to about 5, about 4, about 4.5, or about 5.

[00214] In some LNPs, the cargo can comprise Cas mRNA and gRNA. The Cas mRNA and gRNAs can be in different ratios. For example, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid ranging from about 25:1 to about 1:25, ranging from about 10:1 to about 1:10, ranging from about 5:1 to about 1:5, or about 1:1. Alternatively, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid from about 1:1 to about 1:5, or about 10:1. Alternatively, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid of about 1:10, 25:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, or 1:25. Alternatively, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid of from about 1:1 to about 1:2. In specific examples, the ratio of Cas mRNA to gRNA can be about 1:1 or about 1:2.

[00215] Exemplary dosing of LNPs includes about 0.1, about 0.25, about 0.3, about 0.5, about 1, about 2, about 3, about 4, about 5, about 6, about 8, or about 10 mg/kg body weight (mpk) or about 0.1 to about 10, about 0.25 to about 10, about 0.3 to about 10, about 0.5 to about 10, about 1 to about 10, about 2 to about 10, about 3 to about 10, about 4 to about 10, about 5 to about 10, about 6 to about 10, about 8 to about 10, about 0.1 to about 8, about 0.1 to about 6, about 0.1 to about 5, about 0.1 to about 4, about 0.1 to about 3, about 0.1 to about 2, about 0.1 to about 1, about 0.1 to about 0.5, about 0.1 to about 0.3, about 0.1 to about 0.25, about 0.25 to about 8, about 0.3 to about 6, about 0.5 to about 5, about 1 to about 5, or about 2 to about 3 mg/kg body weight with respect to total RNA (Cas9 mRNA and gRNA) cargo content. Such LNPs can be administered, for example, intravenously. In one example, LNP doses between about 0.01 mg/kg and about 10 mg/kg, between about 0.1 and about 10 mg/kg, or between about 0.01 and about 0.3 mg/kg can be used. For example, LNP doses of about 0.01, about 0.03, about 0.1, about 0.3, about 1, about 3, or about 10 mg/kg can be used. Additional exemplary dosing of LNPs includes about 0.1, about 0.25, about 0.3, about 0.5, about 1, about 2, about 3, about 4, about 5, about 6,

about 8, or about 10 mg/kg (mpk) body weight or about 0.1 to about 10, about 0.25 to about 10, about 0.3 to about 10, about 0.5 to about 10, about 1 to about 10, about 2 to about 10, about 3 to about 10, about 4 to about 10, about 5 to about 10, about 6 to about 10, about 8 to about 10, about 0.1 to about 8, about 0.1 to about 6, about 0.1 to about 5, about 0.1 to about 4, about 0.1 to about 3, about 0.1 to about 2, about 0.1 to about 1, about 0.1 to about 0.5, about 0.1 to about 0.3, about 0.1 to about 0.25, about 0.25 to about 8, about 0.3 to about 6, about 0.5 to about 5, about 1 to about 5, or about 2 to about 3 mg/kg body weight with respect to total RNA (Cas9 mRNA and gRNA) cargo content. Such LNPs can be administered, for example, intravenously. In one example, LNP doses between about 0.01 mg/kg and about 10 mg/kg, between about 0.1 and about 10 mg/kg, or between about 0.01 and about 0.3 mg/kg can be used. For example, LNP doses of about 0.01, about 0.03, about 0.1, about 0.3, about 0.5, about 1, about 2, about 3, or about 10 mg/kg can be used. In another example, LNP doses between about 0.5 and about 10, between about 0.5 and about 5, between about 0.5 and about 3, between about 1 and about 10, between about 1 and about 5, between about 1 and about 3, or between about 1 and about 2 mg/kg can be used.

V. Compositions Comprising Nucleic Acid Constructs and/or a Nuclease Agent or a Nucleic Acid Encoding a Nuclease Agent

[00216] Also provided herein are compositions comprising the nucleic acid constructs comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus, vectors, or lipid nanoparticles disclosed herein and a nuclease agent or a nucleic acid encoding the nuclease agent. Also provided herein are compositions comprising the nucleic acid constructs comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus, vectors, or lipid nanoparticles disclosed herein. Also provided herein are compositions comprising a nuclease agent or a nucleic acid encoding the nuclease agent (e.g., wherein the nuclease agent targets a *RS1* gene or locus) or vectors or lipid nanoparticles comprising the nuclease agent or the nucleic acid encoding the nuclease agent. Such compositions can be, for example, for use in expressing retinoschisin in a cell or for use in integrating a coding sequence for a retinoschisin protein or fragment or variant thereof into a target genomic locus in a cell. Such compositions can also be,

for example, for use in treating a subject with X-linked juvenile retinoschisis (XLRS). Such compositions can comprise a nucleic acid construct comprising the coding sequence for the retinoschisin protein or fragment thereof for integration into the target genomic locus (or a vector or lipid nanoparticle comprising the nucleic acid construct) and a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus. The nuclease agent can be a CRISPR/Cas system (e.g., a Cas protein and a guide RNA) or any other suitable nuclease agent. Examples of suitable nuclease agents are provided below.

A. CRISPR/Cas Systems

[00217] The methods and compositions disclosed herein can utilize Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) systems or components of such systems to modify a genome within a cell (e.g., an *RS1* locus). CRISPR/Cas systems include transcripts and other elements involved in the expression of, or directing the activity of, Cas genes. A CRISPR/Cas system can be, for example, a type I, a type II, a type III system, or a type V system (e.g., subtype V-A or subtype V-B). The methods and compositions disclosed herein can employ CRISPR/Cas systems by utilizing CRISPR complexes (comprising a guide RNA (gRNA) complexed with a Cas protein) for site-directed binding or cleavage of nucleic acids.

[00218] CRISPR/Cas systems used in the compositions and methods disclosed herein can be non-naturally occurring. A "non-naturally occurring" system includes anything indicating the involvement of the hand of man, such as one or more components of the system being altered or mutated from their naturally occurring state, being at least substantially free from at least one other component with which they are naturally associated in nature or being associated with at least one other component with which they are not naturally associated. For example, some CRISPR/Cas systems employ non-naturally occurring CRISPR complexes comprising a gRNA and a Cas protein that do not naturally occur together, employ a Cas protein that does not occur naturally, or employ a gRNA that does not occur naturally.

1. Cas Proteins

[00219] Cas proteins generally comprise at least one RNA recognition or binding domain that can interact with guide RNAs. Cas proteins can also comprise nuclease domains (e.g., DNase domains or RNase domains), DNA-binding domains, helicase domains, protein-protein interaction domains, dimerization domains, and other domains. Some such domains (e.g., DNase domains) can be from a native Cas protein. Other such domains can be added to make a modified Cas protein. A nuclease domain possesses catalytic activity for nucleic acid cleavage, which includes the breakage of the covalent bonds of a nucleic acid molecule. Cleavage can produce blunt ends or staggered ends, and it can be single-stranded or double-stranded. For example, a wild type Cas9 protein will typically create a blunt cleavage product. Alternatively, a wild type Cpf1 protein (e.g., FnCpf1) can result in a cleavage product with a 5-nucleotide 5' overhang, with the cleavage occurring after the 18th base pair from the PAM sequence on the non-targeted strand and after the 23rd base on the targeted strand. A Cas protein can have full cleavage activity to create a double-strand break at a target genomic locus (e.g., a double-strand break with blunt ends), or it can be a nickase that creates a single-strand break at a target genomic locus.

[00220] Examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, and homologs or modified versions thereof.

[00221] An exemplary Cas protein is a Cas9 protein or a protein derived from a Cas9 protein. Cas9 proteins are from a type II CRISPR/Cas system and typically share four key motifs with a conserved architecture. Motifs 1, 2, and 4 are RuvC-like motifs, and motif 3 is an HNH motif. Exemplary Cas9 proteins are from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Staphylococcus aureus*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*,

Polaromonas naphthalenivorans, *Polaromonas sp.*, *Crocosphaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor becscii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter sp.*, *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrotoga mobilis*, *Thermosipho africanus*, *Acaryochloris marina*, *Neisseria meningitidis*, or *Campylobacter jejuni*. Additional examples of the Cas9 family members are described in WO 2014/131833, herein incorporated by reference in its entirety for all purposes. Cas9 from *S. pyogenes* (SpCas9) (e.g., assigned UniProt accession number Q99ZW2) is an exemplary Cas9 protein. An exemplary SpCas9 protein sequence is set forth in SEQ ID NO: 27 (encoded by the DNA sequence set forth in SEQ ID NO: 26). An exemplary SpCas9 cDNA sequence is set forth in SEQ ID NO: 28. Smaller Cas9 proteins (e.g., Cas9 proteins whose coding sequences are compatible with the maximum AAV packaging capacity when combined with a guide RNA coding sequence and regulatory elements for the Cas9 and guide RNA, such as SaCas9 and CjCas9 and Nme2Cas9) are other exemplary Cas9 proteins. For example, Cas9 from *S. aureus* (SaCas9) (e.g., assigned UniProt accession number J7RUA5) is another exemplary Cas9 protein. Likewise, Cas9 from *Campylobacter jejuni* (CjCas9) e.g., (assigned UniProt accession number Q0P897) is another exemplary Cas9 protein. *See, e.g.*, Kim et al. (2017) *Nat. Commun.* 8:14500, herein incorporated by reference in its entirety for all purposes. SaCas9 is smaller than SpCas9, and CjCas9 is smaller than both SaCas9 and SpCas9. Cas9 from *Neisseria meningitidis* (Nme2Cas9) is another exemplary Cas9 protein. *See, e.g.*, Edraki et al. (2019) *Mol. Cell* 73(4):714-726, herein incorporated by reference in its entirety for all purposes. Cas9 proteins from *Streptococcus thermophilus* (e.g., *Streptococcus thermophilus* LMD-9 Cas9 encoded by the CRISPR1 locus (St1Cas9) or *Streptococcus thermophilus* Cas9 from the CRISPR3 locus (St3Cas9)) are other exemplary Cas9 proteins. Cas9 from *Francisella novicida* (FnCas9) or the RHA *Francisella novicida* Cas9 variant that recognizes an alternative PAM (E1369R/E1449H/R1556A substitutions) are other exemplary Cas9 proteins. These and other exemplary Cas9 proteins are reviewed, e.g., in Cebrian-Serrano

and Davies (2017) *Mamm. Genome* 28(7):247-261, herein incorporated by reference in its entirety for all purposes. Examples of Cas9 coding sequences, Cas9 mRNAs, and Cas9 protein sequences are provided in WO 2013/176772, WO 2014/065596, WO 2016/106121, and WO 2019/067910, each of which is herein incorporated by reference in its entirety for all purposes. Specific examples of ORFs and Cas9 amino acid sequences are provided in Table 30 at paragraph [0449] WO 2019/067910, and specific examples of Cas9 mRNAs and ORFs are provided in paragraphs [0214]-[0234] of WO 2019/067910. As one example, a Cas9 protein can comprise, consist essentially of, or consist of the sequence set forth in SEQ ID NO: 6242. Such a Cas9 protein can be encoded by a mRNA comprising, consisting essentially of, or consisting of SEQ ID NO: 6243. As another example, a Cas9 protein can comprise, consist essentially of, or consist of the sequence set forth in SEQ ID NO: 6246. Such a Cas9 protein can be encoded by a mRNA comprising, consisting essentially of, or consisting of SEQ ID NO: 6245.

[00222] Another example of a Cas protein is a Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) protein. Cpf1 is a large protein (about 1300 amino acids) that contains a RuvC-like nuclease domain homologous to the corresponding domain of Cas9 along with a counterpart to the characteristic arginine-rich cluster of Cas9. However, Cpf1 lacks the HNH nuclease domain that is present in Cas9 proteins, and the RuvC-like domain is contiguous in the Cpf1 sequence, in contrast to Cas9 where it contains long inserts including the HNH domain. *See, e.g.*, Zetsche et al. (2015) *Cell* 163(3):759-771, herein incorporated by reference in its entirety for all purposes. Exemplary Cpf1 proteins are from *Francisella tularensis 1*, *Francisella tularensis subsp. novicida*, *Prevotella albensis*, *Lachnospiraceae bacterium MC2017 1*, *Butyrivibrio proteoclasticus*, *Peregrinibacteria bacterium GW2011_GWA2_33_10*, *Parcubacteria bacterium GW2011_GWC2_44_17*, *Smithella sp. SCADC*, *Acidaminococcus sp. BV3L6*, *Lachnospiraceae bacterium MA2020*, *Candidatus Methanoplasma termitum*, *Eubacterium eligens*, *Moraxella bovoculi 237*, *Leptospira inadai*, *Lachnospiraceae bacterium ND2006*, *Porphyromonas crevioricanis 3*, *Prevotella disiens*, and *Porphyromonas macacae*. Cpf1 from *Francisella novicida* U112 (FnCpf1; assigned UniProt accession number A0Q7Q2) is an exemplary Cpf1 protein.

[00223] Cas proteins can be wild type proteins (i.e., those that occur in nature), modified Cas proteins (i.e., Cas protein variants), or fragments of wild type or modified Cas proteins. Cas proteins can also be active variants or fragments with respect to catalytic activity of wild type or

modified Cas proteins. Active variants or fragments with respect to catalytic activity can comprise at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the wild type or modified Cas protein or a portion thereof, wherein the active variants retain the ability to cut at a desired cleavage site and hence retain nick-inducing or double-strand-break-inducing activity. Assays for nick-inducing or double-strand-break-inducing activity are known and generally measure the overall activity and specificity of the Cas protein on DNA substrates containing the cleavage site.

[00224] Cas proteins can be modified to increase or decrease one or more of nucleic acid binding affinity, nucleic acid binding specificity, and enzymatic activity. Cas proteins can also be modified to change any other activity or property of the protein, such as stability. For example, one or more nuclease domains of the Cas protein can be modified, deleted, or inactivated, or a Cas protein can be truncated to remove domains that are not essential for the function of the protein or to optimize (e.g., enhance or reduce) the activity of or a property of the Cas protein.

[00225] One example of a modified Cas protein is the modified SpCas9-HF1 protein, which is a high-fidelity variant of *Streptococcus pyogenes* Cas9 harboring alterations (N497A/R661A/Q695A/Q926A) designed to reduce non-specific DNA contacts. *See, e.g.*, Kleinstiver et al. (2016) *Nature* 529(7587):490-495, herein incorporated by reference in its entirety for all purposes. Another example of a modified Cas protein is the modified eSpCas9 variant (K848A/K1003A/R1060A) designed to reduce off-target effects. *See, e.g.*, Slaymaker et al. (2016) *Science* 351(6268):84-88, herein incorporated by reference in its entirety for all purposes. Other SpCas9 variants include K855A and K810A/K1003A/R1060A. These and other modified Cas proteins are reviewed, e.g., in Cebrian-Serrano and Davies (2017) *Mamm. Genome* 28(7):247-261, herein incorporated by reference in its entirety for all purposes. Another example of a modified Cas9 protein is xCas9, which is a SpCas9 variant that can recognize an expanded range of PAM sequences. *See, e.g.*, Hu et al. (2018) *Nature* 556:57-63, herein incorporated by reference in its entirety for all purposes.

[00226] Cas proteins can comprise at least one nuclease domain, such as a DNase domain. For example, a wild type Cpf1 protein generally comprises a RuvC-like domain that cleaves both strands of target DNA, perhaps in a dimeric configuration. Cas proteins can also comprise at least two nuclease domains, such as DNase domains. For example, a wild type Cas9 protein generally comprises a RuvC-like nuclease domain and an HNH-like nuclease domain. The RuvC

and HNH domains can each cut a different strand of double-stranded DNA to make a double-stranded break in the DNA. *See, e.g.*, Jinek et al. (2012) *Science* 337:816-821, herein incorporated by reference in its entirety for all purposes.

[00227] One or more or all of the nuclease domains can be deleted or mutated so that they are no longer functional or have reduced nuclease activity. For example, if one of the nuclease domains is deleted or mutated in a Cas9 protein, the resulting Cas9 protein can be referred to as a nickase and can generate a single-strand break within a double-stranded target DNA but not a double-strand break (i.e., it can cleave the complementary strand or the non-complementary strand, but not both). If both of the nuclease domains are deleted or mutated, the resulting Cas protein (e.g., Cas9) will have a reduced ability to cleave both strands of a double-stranded DNA (e.g., a nuclease-null or nuclease-inactive Cas protein, or a catalytically dead Cas protein (dCas)). If none of the nuclease domains is deleted or mutated in a Cas9 protein, the Cas9 protein will retain double-strand-break-inducing activity. An example of a mutation that converts Cas9 into a nickase is a D10A (aspartate to alanine at position 10 of Cas9) mutation in the RuvC domain of Cas9 from *S. pyogenes*. Likewise, H939A (histidine to alanine at amino acid position 839), H840A (histidine to alanine at amino acid position 840), or N863A (asparagine to alanine at amino acid position N863) in the HNH domain of Cas9 from *S. pyogenes* can convert the Cas9 into a nickase. Other examples of mutations that convert Cas9 into a nickase include the corresponding mutations to Cas9 from *S. thermophilus*. *See, e.g.*, Sapranauskas et al. (2011) *Nucleic Acids Res.* 39(21):9275-9282 and WO 2013/141680, each of which is herein incorporated by reference in its entirety for all purposes. Such mutations can be generated using methods such as site-directed mutagenesis, PCR-mediated mutagenesis, or total gene synthesis. Examples of other mutations creating nickases can be found, for example, in WO 2013/176772 and WO 2013/142578, each of which is herein incorporated by reference in its entirety for all purposes. If all of the nuclease domains are deleted or mutated in a Cas protein (e.g., both of the nuclease domains are deleted or mutated in a Cas9 protein), the resulting Cas protein (e.g., Cas9) will have a reduced ability to cleave both strands of a double-stranded DNA (e.g., a nuclease-null or nuclease-inactive Cas protein). One specific example is a D10A/H840A *S. pyogenes* Cas9 double mutant or a corresponding double mutant in a Cas9 from another species when optimally aligned with *S. pyogenes* Cas9. Another specific example is a D10A/N863A *S. pyogenes* Cas9

double mutant or a corresponding double mutant in a Cas9 from another species when optimally aligned with *S. pyogenes* Cas9.

[00228] Examples of inactivating mutations in the catalytic domains of xCas9 are the same as those described above for SpCas9. Examples of inactivating mutations in the catalytic domains of *Staphylococcus aureus* Cas9 proteins are also known. For example, the *Staphylococcus aureus* Cas9 enzyme (SaCas9) may comprise a substitution at position N580 (e.g., N580A substitution) and a substitution at position D10 (e.g., D10A substitution) to generate a nuclease-inactive Cas protein. *See, e.g.*, WO 2016/106236, herein incorporated by reference in its entirety for all purposes. Examples of inactivating mutations in the catalytic domains of Nme2Cas9 are also known (e.g., combination of D16A and H588A). Examples of inactivating mutations in the catalytic domains of St1Cas9 are also known (e.g., combination of D9A, D598A, H599A, and N622A). Examples of inactivating mutations in the catalytic domains of St3Cas9 are also known (e.g., combination of D10A and N870A). Examples of inactivating mutations in the catalytic domains of CjCas9 are also known (e.g., combination of D8A and H559A). Examples of inactivating mutations in the catalytic domains of FnCas9 and RHA FnCas9 are also known (e.g., N995A).

[00229] Examples of inactivating mutations in the catalytic domains of Cpf1 proteins are also known. With reference to Cpf1 proteins from *Francisella novicida* U112 (FnCpf1), *Acidaminococcus* sp. BV3L6 (AsCpf1), *Lachnospiraceae bacterium* ND2006 (LbCpf1), and *Moraxella bovoculi* 237 (MbCpf1 Cpf1), such mutations can include mutations at positions 908, 993, or 1263 of AsCpf1 or corresponding positions in Cpf1 orthologs, or positions 832, 925, 947, or 1180 of LbCpf1 or corresponding positions in Cpf1 orthologs. Such mutations can include, for example one or more of mutations D908A, E993A, and D1263A of AsCpf1 or corresponding mutations in Cpf1 orthologs, or D832A, E925A, D947A, and D1180A of LbCpf1 or corresponding mutations in Cpf1 orthologs. *See, e.g.*, US 2016/0208243, herein incorporated by reference in its entirety for all purposes.

[00230] Cas proteins can also be operably linked to heterologous polypeptides as fusion proteins. For example, a Cas protein can be fused to a cleavage domain or an epigenetic modification domain. *See* WO 2014/089290, herein incorporated by reference in its entirety for all purposes. Cas proteins can also be fused to a heterologous polypeptide providing increased or

decreased stability. The fused domain or heterologous polypeptide can be located at the N-terminus, the C-terminus, or internally within the Cas protein.

[00231] As one example, a Cas protein can be fused to one or more heterologous polypeptides that provide for subcellular localization. Such heterologous polypeptides can include, for example, one or more nuclear localization signals (NLS) such as the monopartite SV40 NLS and/or a bipartite alpha-importin NLS for targeting to the nucleus, a mitochondrial localization signal for targeting to the mitochondria, an ER retention signal, and the like. *See, e.g.*, Lange et al. (2007) *J. Biol. Chem.* 282(8):5101-5105, herein incorporated by reference in its entirety for all purposes. Such subcellular localization signals can be located at the N-terminus, the C-terminus, or anywhere within the Cas protein. An NLS can comprise a stretch of basic amino acids and can be a monopartite sequence or a bipartite sequence. Optionally, a Cas protein can comprise two or more NLSs, including an NLS (e.g., an alpha-importin NLS or a monopartite NLS) at the N-terminus and an NLS (e.g., an SV40 NLS or a bipartite NLS) at the C-terminus. A Cas protein can also comprise two or more NLSs at the N-terminus and/or two or more NLSs at the C-terminus.

[00232] A Cas protein may, for example, be fused with 1-10 NLSs (e.g., fused with 1-5 NLSs or fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the Cas protein sequence. It may also be inserted within the Cas protein sequence. Alternatively, the Cas protein may be fused with more than one NLS. For example, the Cas protein may be fused with 2, 3, 4, or 5 NLSs. In a specific example, the Cas protein may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (e.g., two SV40 NLSs) or different. For example, the Cas protein can be fused to two SV40 NLS sequences linked at the carboxy terminus. Alternatively, the Cas protein may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In other examples, the Cas protein may be fused with 3 NLSs or with no NLS. The NLS may be a monopartite sequence, such as, e.g., the SV40 NLS, PKKKRKV (SEQ ID NO: 49) or PKKKRRV (SEQ ID NO: 50). The NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 51). In a specific example, a single PKKKRKV (SEQ ID NO: 49) NLS may be linked at the C-terminus of the Cas protein. One or more linkers are optionally included at the fusion site.

[00233] Cas proteins can also be operably linked to a cell-penetrating domain or protein transduction domain. For example, the cell-penetrating domain can be derived from the HIV-1

TAT protein, the TLM cell-penetrating motif from human hepatitis B virus, MPG, Pep-1, VP22, a cell penetrating peptide from Herpes simplex virus, or a polyarginine peptide sequence. *See, e.g.*, WO 2014/089290 and WO 2013/176772, each of which is herein incorporated by reference in its entirety for all purposes. The cell-penetrating domain can be located at the N-terminus, the C-terminus, or anywhere within the Cas protein.

[00234] Cas proteins can also be operably linked to a heterologous polypeptide for ease of tracking or purification, such as a fluorescent protein, a purification tag, or an epitope tag. Examples of fluorescent proteins include green fluorescent proteins (e.g., GFP, GFP-2, tagGFP, turboGFP, eGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreenl), yellow fluorescent proteins (e.g., YFP, eYFP, Citrine, Venus, YPet, PhiYFP, ZsYellowl), blue fluorescent proteins (e.g., eBFP, eBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (e.g., eCFP, Cerulean, CyPet, AmCyanl, Midoriishi-Cyan), red fluorescent proteins (e.g., mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedl, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred), orange fluorescent proteins (e.g., mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato), and any other suitable fluorescent protein. Examples of tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein, thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, hemagglutinin (HA), nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, histidine (His), biotin carboxyl carrier protein (BCCP), and calmodulin.

[00235] Cas proteins can also be tethered to labeled nucleic acids or donor sequences. Such tethering (i.e., physical linking) can be achieved through covalent interactions or noncovalent interactions, and the tethering can be direct (e.g., through direct fusion or chemical conjugation, which can be achieved by modification of cysteine or lysine residues on the protein or intein modification) or can be achieved through one or more intervening linkers or adapter molecules such as streptavidin or aptamers. *See, e.g.*, Pierce et al. (2005) *Mini Rev. Med. Chem.* 5(1):41-55; Duckworth et al. (2007) *Angew. Chem. Int. Ed. Engl.* 46(46):8819-8822; Schaeffer and Dixon (2009) *Australian J. Chem.* 62(10):1328-1332; Goodman et al. (2009) *Chembiochem.* 10(9):1551-1557; and Khatwani et al. (2012) *Bioorg. Med. Chem.* 20(14):4532-4539, each of which is herein incorporated by reference in its entirety for all purposes. Noncovalent strategies

for synthesizing protein-nucleic acid conjugates include biotin-streptavidin and nickel-histidine methods. Covalent protein-nucleic acid conjugates can be synthesized by connecting appropriately functionalized nucleic acids and proteins using a wide variety of chemistries. Some of these chemistries involve direct attachment of the oligonucleotide to an amino acid residue on the protein surface (e.g., a lysine amine or a cysteine thiol), while other more complex schemes require post-translational modification of the protein or the involvement of a catalytic or reactive protein domain. Methods for covalent attachment of proteins to nucleic acids can include, for example, chemical cross-linking of oligonucleotides to protein lysine or cysteine residues, expressed protein-ligation, chemoenzymatic methods, and the use of photoaptamers. The labeled nucleic acid or donor sequence can be tethered to the C-terminus, the N-terminus, or to an internal region within the Cas protein. In one example, the labeled nucleic acid or donor sequence is tethered to the C-terminus or the N-terminus of the Cas protein. Likewise, the Cas protein can be tethered to the 5' end, the 3' end, or to an internal region within the labeled nucleic acid or donor sequence. That is, the labeled nucleic acid or donor sequence can be tethered in any orientation and polarity. For example, the Cas protein can be tethered to the 5' end or the 3' end of the labeled nucleic acid or donor sequence.

[00236] Cas proteins can be provided in any form. For example, a Cas protein can be provided in the form of a protein, such as a Cas protein complexed with a gRNA. Alternatively, a Cas protein can be provided in the form of a nucleic acid encoding the Cas protein, such as an RNA (e.g., messenger RNA (mRNA)) or DNA. Optionally, the nucleic acid encoding the Cas protein can be codon optimized for efficient translation into protein in a particular cell or organism. For example, the nucleic acid encoding the Cas protein can be modified to substitute codons having a higher frequency of usage in a bacterial cell, a yeast cell, a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell, or any other host cell of interest, as compared to the naturally occurring polynucleotide sequence. When a nucleic acid encoding the Cas protein is introduced into the cell, the Cas protein can be transiently, conditionally, or constitutively expressed in the cell.

[00237] Cas proteins provided as mRNAs can be modified for improved stability and/or immunogenicity properties. The modifications may be made to one or more nucleosides within the mRNA. Examples of chemical modifications to mRNA nucleobases include pseudouridine, 1-methyl-pseudouridine, and 5-methyl-cytidine. For example, capped and polyadenylated Cas

mRNA containing N1-methyl pseudouridine can be used. Likewise, Cas mRNAs can be modified by depletion of uridine using synonymous codons.

[00238] Nucleic acids encoding Cas proteins can be stably integrated in the genome of a cell and operably linked to a promoter active in the cell. Alternatively, nucleic acids encoding Cas proteins can be operably linked to a promoter in an expression construct. Expression constructs include any nucleic acid constructs capable of directing expression of a gene or other nucleic acid sequence of interest (e.g., a Cas gene) and which can transfer such a nucleic acid sequence of interest to a target cell. For example, the nucleic acid encoding the Cas protein can be in a vector comprising a DNA encoding a gRNA. Alternatively, it can be in a vector or plasmid that is separate from the vector comprising the DNA encoding the gRNA. Promoters that can be used in an expression construct include promoters active, for example, in one or more of a eukaryotic cell, a human cell, a non-human cell, a mammalian cell, a non-human mammalian cell, a rodent cell, a mouse cell, a rat cell, a pluripotent cell, an embryonic stem (ES) cell, an adult stem cell, a developmentally restricted progenitor cell, an induced pluripotent stem (iPS) cell, or a one-cell stage embryo. Such promoters can be, for example, conditional promoters, inducible promoters, constitutive promoters, or tissue-specific promoters. Optionally, the promoter can be a bidirectional promoter driving expression of both a Cas protein in one direction and a guide RNA in the other direction. Such bidirectional promoters can consist of (1) a complete, conventional, unidirectional Pol III promoter that contains 3 external control elements: a distal sequence element (DSE), a proximal sequence element (PSE), and a TATA box; and (2) a second basic Pol III promoter that includes a PSE and a TATA box fused to the 5′ terminus of the DSE in reverse orientation. For example, in the H1 promoter, the DSE is adjacent to the PSE and the TATA box, and the promoter can be rendered bidirectional by creating a hybrid promoter in which transcription in the reverse direction is controlled by appending a PSE and TATA box derived from the U6 promoter. *See, e.g.*, US 2016/0074535, herein incorporated by references in its entirety for all purposes. Use of a bidirectional promoter to express genes encoding a Cas protein and a guide RNA simultaneously allow for the generation of compact expression cassettes to facilitate delivery.

[00239] Different promoters can be used to drive Cas expression or Cas9 expression. In some methods, small promoters are used so that the Cas or Cas9 coding sequence can fit into an AAV construct. For example, Cas or Cas9 and one or more gRNAs (e.g., 1 gRNA or 2 gRNAs or 3

gRNAs or 4 gRNAs) can be delivered via LNP-mediated delivery (e.g., in the form of RNA) or adeno-associated virus (AAV)-mediated delivery (e.g., AAV2-mediated delivery, AAV5-mediated delivery, AAV8-mediated delivery, or AAV7m8-mediated delivery). For example, the nuclease agent can be CRISPR/Cas9, and a Cas9 mRNA and a gRNA targeting an endogenous *RS1* locus (e.g., intron 1 of *RS1*) can be delivered via LNP-mediated delivery, or a DNA encoding Cas9 and a DNA encoding a gRNA targeting an endogenous *RS1* locus (e.g., intron 1 of *RS1*) can be delivered via AAV-mediated delivery. The Cas or Cas9 and the gRNA(s) can be delivered in a single AAV or via two separate AAVs. For example, a first AAV can carry a Cas or Cas9 expression cassette, and a second AAV can carry a gRNA expression cassette. Similarly, a first AAV can carry a Cas or Cas9 expression cassette, and a second AAV can carry two or more gRNA expression cassettes. Alternatively, a single AAV can carry a Cas or Cas9 expression cassette (e.g., Cas or Cas9 coding sequence operably linked to a promoter) and a gRNA expression cassette (e.g., gRNA coding sequence operably linked to a promoter). Similarly, a single AAV can carry a Cas or Cas9 expression cassette (e.g., Cas or Cas9 coding sequence operably linked to a promoter) and two or more gRNA expression cassettes (e.g., gRNA coding sequences operably linked to promoters). Different promoters can be used to drive expression of the gRNA, such as a U6 promoter or the small tRNA Gln. Likewise, different promoters can be used to drive Cas9 expression. For example, small promoters are used so that the Cas9 coding sequence can fit into an AAV construct. Similarly, small Cas9 proteins (e.g., SaCas9 or CjCas9 are used to maximize the AAV packaging capacity).

[00240] Cas proteins provided as mRNAs can be modified for improved stability and/or immunogenicity properties. The modifications may be made to one or more nucleosides within the mRNA. Examples of chemical modifications to mRNA nucleobases include pseudouridine, 1-methyl-pseudouridine, and 5-methyl-cytidine. mRNA encoding Cas proteins can also be capped. The cap can be, for example, a cap 1 structure in which the +1 ribonucleotide is methylated at the 2'O position of the ribose. The capping can, for example, give superior activity *in vivo* (e.g., by mimicking a natural cap), can result in a natural structure that reduce stimulation of the innate immune system of the host (e.g., can reduce activation of pattern recognition receptors in the innate immune system). mRNA encoding Cas proteins can also be polyadenylated (to comprise a poly(A) tail). mRNA encoding Cas proteins can also be modified to include pseudouridine (e.g., can be fully substituted with pseudouridine). As another example,

capped and polyadenylated Cas mRNA containing N1-methyl pseudouridine can be used. As another example, Cas mRNA fully substituted with pseudouridine can be used (i.e., all standard uracil residues are replaced with pseudouridine, a uridine isomer in which the uracil is attached with a carbon-carbon bond rather than nitrogen-carbon). Likewise, Cas mRNAs can be modified by depletion of uridine using synonymous codons. For example, capped and polyadenylated Cas mRNA fully substituted with pseudouridine can be used.

[00241] Cas mRNAs can comprise a modified uridine at least at one, a plurality of, or all uridine positions. The modified uridine can be a uridine modified at the 5 position (e.g., with a halogen, methyl, or ethyl). The modified uridine can be a pseudouridine modified at the 1 position (e.g., with a halogen, methyl, or ethyl). The modified uridine can be, for example, pseudouridine, N1-methyl-pseudouridine, 5-methoxyuridine, 5-iodouridine, or a combination thereof. In some examples, the modified uridine is 5-methoxyuridine. In some examples, the modified uridine is 5-iodouridine. In some examples, the modified uridine is pseudouridine. In some examples, the modified uridine is N1-methyl-pseudouridine. In some examples, the modified uridine is a combination of pseudouridine and N1-methyl-pseudouridine. In some examples, the modified uridine is a combination of pseudouridine and 5-methoxyuridine. In some examples, the modified uridine is a combination of N1-methyl pseudouridine and 5-methoxyuridine. In some examples, the modified uridine is a combination of 5-iodouridine and N1-methyl-pseudouridine. In some examples, the modified uridine is a combination of pseudouridine and 5-iodouridine. In some examples, the modified uridine is a combination of 5-iodouridine and 5-methoxyuridine.

[00242] Cas mRNAs disclosed herein can also comprise a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, e.g., with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first nucleotide of the 5'-to-3' chain of the mRNA (i.e., the first cap-proximal nucleotide). In Cap0, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-hydroxyl. In Cap1, the riboses of the first and second transcribed nucleotides of the mRNA comprise a 2'-methoxy and a 2'-hydroxyl, respectively. In Cap2, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-methoxy. *See, e.g.,* Katibah et al. (2014) *Proc. Natl. Acad. Sci. U.S.A.* 111(33):12025-30 and Abbas et al. (2017) *Proc. Natl. Acad. Sci. U.S.A.* 114(11):E2106-E2115, each of which is herein incorporated by reference in its

entirety for all purposes. Most endogenous higher eukaryotic mRNAs, including mammalian mRNAs such as human mRNAs, comprise Cap1 or Cap2. Cap0 and other cap structures differing from Cap1 and Cap2 may be immunogenic in mammals, such as humans, due to recognition as non-self by components of the innate immune system such as IFIT-1 and IFIT-5, which can result in elevated cytokine levels including type I interferon. Components of the innate immune system such as IFIT-1 and IFIT-5 may also compete with eIF4E for binding of an mRNA with a cap other than Cap1 or Cap2, potentially inhibiting translation of the mRNA.

[00243] A cap can be included co-transcriptionally. For example, ARCA (anti-reverse cap analog; Thermo Fisher Scientific Cat. No. AM8045) is a cap analog comprising a 7-methylguanine 3'-methoxy-5'-triphosphate linked to the 5' position of a guanine ribonucleotide which can be incorporated in vitro into a transcript at initiation. ARCA results in a Cap0 cap in which the 2' position of the first cap-proximal nucleotide is hydroxyl. *See, e.g.,* Stepinski et al. (2001) *RNA* 7:1486-1495, herein incorporated by reference in its entirety for all purposes.

[00244] CleanCap™ AG (m7G(5')ppp(5')(2'OMeA)pG; TriLink Biotechnologies Cat. No. N-7113) or CleanCap™ GG (m7G(5')ppp(5')(2'OMeG)pG; TriLink Biotechnologies Cat. No. N-7133) can be used to provide a Cap1 structure co-transcriptionally. 3'-O-methylated versions of CleanCap™ AG and CleanCap™ GG are also available from TriLink Biotechnologies as Cat. Nos. N-7413 and N-7433, respectively.

[00245] Alternatively, a cap can be added to an RNA post-transcriptionally. For example, Vaccinia capping enzyme is commercially available (New England Biolabs Cat. No. M2080S) and has RNA triphosphatase and guanylyltransferase activities, provided by its D1 subunit, and guanine methyltransferase, provided by its D12 subunit. As such, it can add a 7-methylguanine to an RNA, so as to give Cap0, in the presence of S-adenosyl methionine and GTP. *See, e.g.,* Guo and Moss (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:4023-4027 and Mao and Shuman (1994) *J. Biol. Chem.* 269:24472-24479, each of which is herein incorporated by reference in its entirety for all purposes.

[00246] Cas mRNAs can further comprise a poly-adenylated (poly-A or poly(A) or poly-adenine) tail. The poly-A tail can, for example, comprise at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 adenines, and optionally up to 300 adenines. For example, the poly-A tail can comprise 95, 96, 97, 98, 99, or 100 adenine nucleotides.

2. Guide RNAs

[00247] A "guide RNA" or "gRNA" is an RNA molecule that binds to a Cas protein (e.g., Cas9 protein) and targets the Cas protein to a specific location within a target DNA. Guide RNAs can comprise two segments: a "DNA-targeting segment" (also called "guide sequence") and a "protein-binding segment." "Segment" includes a section or region of a molecule, such as a contiguous stretch of nucleotides in an RNA. Some gRNAs, such as those for Cas9, can comprise two separate RNA molecules: an "activator-RNA" (e.g., tracrRNA) and a "targeter-RNA" (e.g., CRISPR RNA or crRNA). Other gRNAs are a single RNA molecule (single RNA polynucleotide), which can also be called a "single-molecule gRNA," a "single-guide RNA," or an "sgRNA." *See, e.g.*, WO 2013/176772, WO 2014/065596, WO 2014/089290, WO 2014/093622, WO 2014/099750, WO 2013/142578, and WO 2014/131833, each of which is herein incorporated by reference in its entirety for all purposes. A guide RNA can refer to either a CRISPR RNA (crRNA) or the combination of a crRNA and a trans-activating CRISPR RNA (tracrRNA). The crRNA and tracrRNA can be associated as a single RNA molecule (single guide RNA or sgRNA) or in two separate RNA molecules (dual guide RNA or dgRNA). For Cas9, for example, a single-guide RNA can comprise a crRNA fused to a tracrRNA (e.g., via a linker). For Cpf1, for example, only a crRNA is needed to achieve binding to and/or cleavage of a target sequence. The terms "guide RNA" and "gRNA" include both double-molecule (i.e., modular) gRNAs and single-molecule gRNAs. In some of the methods and compositions disclosed herein, a gRNA is a *S. pyogenes* Cas9 gRNA or an equivalent thereof. In some of the methods and compositions disclosed herein, a gRNA is a *S. aureus* Cas9 gRNA or an equivalent thereof.

[00248] An exemplary two-molecule gRNA comprises a crRNA-like ("CRISPR RNA" or "targeter-RNA" or "crRNA" or "crRNA repeat") molecule and a corresponding tracrRNA-like ("trans-activating CRISPR RNA" or "activator-RNA" or "tracrRNA") molecule. A crRNA comprises both the DNA-targeting segment (single-stranded) of the gRNA and a stretch of nucleotides (i.e., the crRNA tail) that forms one half of the dsRNA duplex of the protein-binding segment of the gRNA. An example of a crRNA tail, located downstream (3') of the DNA-targeting segment, comprises, consists essentially of, or consists of GUUUUAGAGCUAUGCU (SEQ ID NO: 29) or GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 52). Any of the

DNA-targeting segments disclosed herein can be joined to the 5' end of SEQ ID NO: 29 or 52 to form a crRNA.

[00249] A corresponding tracrRNA (activator-RNA) comprises a stretch of nucleotides that forms the other half of the dsRNA duplex of the protein-binding segment of the gRNA. A stretch of nucleotides of a crRNA are complementary to and hybridize with a stretch of nucleotides of a tracrRNA to form the dsRNA duplex of the protein-binding domain of the gRNA. As such, each crRNA can be said to have a corresponding tracrRNA. Exemplary tracrRNA sequences comprise, consist essentially of, or consist of AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUU (SEQ ID NO: 30), AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUUUU (SEQ ID NO: 31), or GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 32).

[00250] In systems in which both a crRNA and a tracrRNA are needed, the crRNA and the corresponding tracrRNA hybridize to form a gRNA. In systems in which only a crRNA is needed, the crRNA can be the gRNA. The crRNA additionally provides the single-stranded DNA-targeting segment that hybridizes to the complementary strand of a target DNA. If used for modification within a cell, the exact sequence of a given crRNA or tracrRNA molecule can be designed to be specific to the species in which the RNA molecules will be used. *See, e.g.*, Mali et al. (2013) *Science* 339(6121):823-826; Jinek et al. (2012) *Science* 337(6096):816-821; Hwang et al. (2013) *Nat. Biotechnol.* 31(3):227-229; Jiang et al. (2013) *Nat. Biotechnol.* 31(3):233-239; and Cong et al. (2013) *Science* 339(6121):819-823, each of which is herein incorporated by reference in its entirety for all purposes.

[00251] The DNA-targeting segment (crRNA) of a given gRNA comprises a nucleotide sequence that is complementary to a sequence on the complementary strand of the target DNA, as described in more detail below. The DNA-targeting segment of a gRNA interacts with the target DNA in a sequence-specific manner via hybridization (i.e., base pairing). As such, the nucleotide sequence of the DNA-targeting segment may vary and determines the location within the target DNA with which the gRNA and the target DNA will interact. The DNA-targeting segment of a subject gRNA can be modified to hybridize to any desired sequence within a target

DNA. Naturally occurring crRNAs differ depending on the CRISPR/Cas system and organism but often contain a targeting segment of between 21 to 72 nucleotides length, flanked by two direct repeats (DR) of a length of between 21 to 46 nucleotides (*see, e.g.*, WO 2014/131833, herein incorporated by reference in its entirety for all purposes). In the case of *S. pyogenes*, the DRs are 36 nucleotides long and the targeting segment is 30 nucleotides long. The 3' located DR is complementary to and hybridizes with the corresponding tracrRNA, which in turn binds to the Cas protein.

[00252] The DNA-targeting segment can have, for example, a length of at least about 12, at least about 15, at least about 17, at least about 18, at least about 19, at least about 20, at least about 25, at least about 30, at least about 35, or at least about 40 nucleotides. Such DNA-targeting segments can have, for example, a length from about 12 to about 100, from about 12 to about 80, from about 12 to about 50, from about 12 to about 40, from about 12 to about 30, from about 12 to about 25, or from about 12 to about 20 nucleotides. For example, the DNA targeting segment can be from about 15 to about 25 nucleotides (e.g., from about 17 to about 20 nucleotides, or about 17, 18, 19, or 20 nucleotides). *See, e.g.*, US 2016/0024523, herein incorporated by reference in its entirety for all purposes. For Cas9 from *S. pyogenes*, a typical DNA-targeting segment is between 16 and 20 nucleotides in length or between 17 and 20 nucleotides in length. For Cas9 from *S. aureus*, a typical DNA-targeting segment is between 21 and 23 nucleotides in length. For Cpf1, a typical DNA-targeting segment is at least 16 nucleotides in length or at least 18 nucleotides in length.

[00253] In one example, the DNA-targeting segment can be about 20 nucleotides in length. However, shorter and longer sequences can also be used for the targeting segment (e.g., 15-25 nucleotides in length, such as 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length). The degree of identity between the DNA-targeting segment and the corresponding guide RNA target sequence (or degree of complementarity between the DNA-targeting segment and the other strand of the guide RNA target sequence) can be, for example, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. The DNA-targeting segment and the corresponding guide RNA target sequence can contain one or more mismatches. For example, the DNA-targeting segment of the guide RNA and the corresponding guide RNA target sequence can contain 1-4, 1-3, 1-2, 1, 2, 3, or 4 mismatches (e.g., where the total length of the guide RNA target sequence is at least 17, at least

18, at least 19, or at least 20 or more nucleotides). For example, the DNA-targeting segment of the guide RNA and the corresponding guide RNA target sequence can contain 1-4, 1-3, 1-2, 1, 2, 3, or 4 mismatches where the total length of the guide RNA target sequence 20 nucleotides.

[00254] As one example, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment (i.e., guide sequence) comprising, consisting essentially of, or consisting of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Alternatively, a guide RNA targeting an *RSI* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a

sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-6241. Examples of such guide sequences are set forth in **Tables 2 and 3**.

[00255] The guide RNA can target a human *RS1* gene. As one example, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment (i.e., guide sequence) comprising, consisting essentially of, or consisting of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989. Alternatively, a guide RNA

targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-4989.

[00256] The guide RNA can target a human *RS1* gene and be selected to avoid off-target effects. As one example, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment (i.e., guide sequence) comprising, consisting essentially of, or consisting of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a

DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351.

[00257] The guide RNA can target a human *RS1* gene. As one example, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment (i.e., guide sequence) comprising, consisting essentially of, or consisting of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at

least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304. Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304.

[00258] The guide RNA can target a mouse *Rs1* gene. As one example, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment (i.e., guide sequence) comprising, consisting essentially of, or consisting of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS:

4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981). Alternatively, a guide RNA targeting an *RS1* gene can comprise a DNA-targeting segment comprising, consisting essentially of, or consisting of a sequence that differs by no more than 3, no more than 2, or no more than 1 nucleotide from at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the sequence (DNA-targeting segment) set forth in any one of SEQ ID NOS: 4990-6241 (e.g., SEQ ID NO: 5477 or 5981).

[00259] TracrRNAs can be in any form (e.g., full-length tracrRNAs or active partial tracrRNAs) and of varying lengths. They can include primary transcripts or processed forms. For example, tracrRNAs (as part of a single-guide RNA or as a separate molecule as part of a two-molecule gRNA) may comprise, consist essentially of, or consist of all or a portion of a wild type tracrRNA sequence (e.g., about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild type tracrRNA sequence). Examples of wild type tracrRNA sequences from *S. pyogenes* include 171-nucleotide, 89-nucleotide, 75-nucleotide, and 65-nucleotide versions. *See, e.g.*, Deltcheva et al. (2011) *Nature* 471(7340):602-607; WO 2014/093661, each of which is herein incorporated by reference in its entirety for all purposes. Examples of tracrRNAs within single-guide RNAs (sgRNAs) include the tracrRNA segments found within +48, +54, +67, and +85 versions of sgRNAs, where "+n" indicates that up to the +n nucleotide of wild type tracrRNA is included in the sgRNA. *See* US 8,697,359, herein incorporated by reference in its entirety for all purposes.

[00260] The percent complementarity between the DNA-targeting segment of the guide RNA and the complementary strand of the target DNA can be at least 60% (e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or 100%). The percent complementarity between the DNA-targeting segment

and the complementary strand of the target DNA can be at least 60% over about 20 contiguous nucleotides. As an example, the percent complementarity between the DNA-targeting segment and the complementary strand of the target DNA can be 100% over the 14 contiguous nucleotides at the 5' end of the complementary strand of the target DNA and as low as 0% over the remainder. In such a case, the DNA-targeting segment can be considered to be 14 nucleotides in length. As another example, the percent complementarity between the DNA-targeting segment and the complementary strand of the target DNA can be 100% over the seven contiguous nucleotides at the 5' end of the complementary strand of the target DNA and as low as 0% over the remainder. In such a case, the DNA-targeting segment can be considered to be 7 nucleotides in length. In some guide RNAs, at least 17 nucleotides within the DNA-targeting segment are complementary to the complementary strand of the target DNA. For example, the DNA-targeting segment can be 20 nucleotides in length and can comprise 1, 2, or 3 mismatches with the complementary strand of the target DNA. In one example, the mismatches are not adjacent to the region of the complementary strand corresponding to the protospacer adjacent motif (PAM) sequence (i.e., the reverse complement of the PAM sequence) (e.g., the mismatches are in the 5' end of the DNA-targeting segment of the guide RNA, or the mismatches are at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 base pairs away from the region of the complementary strand corresponding to the PAM sequence).

[00261] The protein-binding segment of a gRNA can comprise two stretches of nucleotides that are complementary to one another. The complementary nucleotides of the protein-binding segment hybridize to form a double-stranded RNA duplex (dsRNA). The protein-binding segment of a subject gRNA interacts with a Cas protein, and the gRNA directs the bound Cas protein to a specific nucleotide sequence within target DNA via the DNA-targeting segment.

[00262] Single-guide RNAs can comprise a DNA-targeting segment and a scaffold sequence (i.e., the protein-binding or Cas-binding sequence of the guide RNA). For example, such guide RNAs can have a 5' DNA-targeting segment joined to a 3' scaffold sequence. Exemplary scaffold sequences comprise, consist essentially of, or consist of:
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCU (version 1; SEQ ID NO: 33);
GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACUUGAAAAAGUGGCACCGAGUCGGUGC (version 2; SEQ ID NO: 34);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGC (version 3; SEQ ID NO: 35); GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU AUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (version 4; SEQ ID NO: 36);GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (version 5; SEQ ID NO: 37); GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCUUUU (version 6; SEQ ID NO: 38); GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU (version 7; SEQ ID NO: 39); or GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGG CACCGAGUCGGUGC (version 8; SEQ ID NO: 53). In some guide sgRNAs, the four terminal U residues of version 6 are not present. In some sgRNAs, only 1, 2, or 3 of the four terminal U residues of version 6 are present. Guide RNAs targeting any of the guide RNA target sequences disclosed herein can include, for example, a DNA-targeting segment on the 5' end of the guide RNA fused to any of the exemplary guide RNA scaffold sequences on the 3' end of the guide RNA. That is, any of the DNA-targeting segments disclosed herein can be joined to the 5' end of any one of the above scaffold sequences to form a single guide RNA (chimeric guide RNA).

[00263] Guide RNAs can include modifications or sequences that provide for additional desirable features (e.g., modified or regulated stability; subcellular targeting; tracking with a fluorescent label; a binding site for a protein or protein complex; and the like). That is, guide RNAs can include one or more modified nucleosides or nucleotides, or one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. Examples of such modifications include, for example, a 5' cap (e.g., a 7-methylguanylate cap (m7G)); a 3' polyadenylated tail (i.e., a 3' poly(A) tail); a riboswitch sequence (e.g., to allow for regulated stability and/or regulated accessibility by proteins and/or protein complexes); a stability control sequence; a sequence that forms a dsRNA duplex (i.e., a hairpin); a modification or sequence that targets the RNA to a subcellular location (e.g., nucleus, mitochondria, chloroplasts, and the like); a modification or sequence that provides for tracking (e.g., direct conjugation to a fluorescent molecule,

conjugation to a moiety that facilitates fluorescent detection, a sequence that allows for fluorescent detection, and so forth); a modification or sequence that provides a binding site for proteins (e.g., proteins that act on DNA, including transcriptional activators, transcriptional repressors, DNA methyltransferases, DNA demethylases, histone acetyltransferases, histone deacetylases, and the like); and combinations thereof. Other examples of modifications include engineered stem loop duplex structures, engineered bulge regions, engineered hairpins 3' of the stem loop duplex structure, or any combination thereof. *See, e.g.*, US 2015/0376586, herein incorporated by reference in its entirety for all purposes. A bulge can be an unpaired region of nucleotides within the duplex made up of the crRNA-like region and the minimum tracrRNA-like region. A bulge can comprise, on one side of the duplex, an unpaired 5′-XXXY-3′ where X is any purine and Y can be a nucleotide that can form a wobble pair with a nucleotide on the opposite strand, and an unpaired nucleotide region on the other side of the duplex.

[00264] Unmodified nucleic acids can be prone to degradation. Exogenous nucleic acids can also induce an innate immune response. Modifications can help introduce stability and reduce immunogenicity. Guide RNAs can comprise modified nucleosides and modified nucleotides including, for example, one or more of the following: (1) alteration or replacement of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (2) alteration or replacement of a constituent of the ribose sugar such as alteration or replacement of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (3) replacement (e.g., wholesale replacement) of the phosphate moiety with dephospho linkers (an exemplary backbone modification); (4) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (5) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (6) modification of the 3' end or 5' end of the oligonucleotide (e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap, or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification)); and (7) modification ore replacement of the sugar (an exemplary sugar modification). Other possible guide RNA modifications include modifications of or replacement of uracils or poly-uracil tracts. *See, e.g.*, WO 2015/048577 and US 2016/0237455, each of which is herein incorporated by reference in its entirety for all purposes. Similar modifications can be made to Cas-encoding nucleic acids, such

as Cas mRNAs. For example, Cas mRNAs can be modified by depletion of uridine using synonymous codons.

[00265] Chemical modifications such at hose listed above can be combined to provide modified gRNAs and/or mRNAs comprising residues (nucleosides and nucleotides) that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In one example, every base of a gRNA is modified (e.g., all bases have a modified phosphate group, such as a phosphorothioate group). For example, all or substantially all of the phosphate groups of a gRNA can be replaced with phosphorothioate groups. Alternatively or additionally, a modified gRNA can comprise at least one modified residue at or near the 5' end. Alternatively or additionally, a modified gRNA can comprise at least one modified residue at or near the 3' end.

[00266] Some gRNAs comprise one, two, three or more modified residues. For example, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of the positions in a modified gRNA can be modified nucleosides or nucleotides.

[00267] Unmodified nucleic acids can be prone to degradation. Exogenous nucleic acids can also induce an innate immune response. Modifications can help introduce stability and reduce immunogenicity. Some gRNAs described herein can contain one or more modified nucleosides or nucleotides to introduce stability toward intracellular or serum-based nucleases. Some modified gRNAs described herein can exhibit a reduced innate immune response when introduced into a population of cells.

[00268] The gRNAs disclosed herein can comprise a backbone modification in which the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. The modification can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. Backbone modifications of the phosphate backbone can also include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[00269] Examples of modified phosphate groups include, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. The phosphorous atom in

an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms can render the phosphorous atom chiral. The stereogenic phosphorous atom can possess either the "R" configuration (Rp) or the "S" configuration (Sp). The backbone can also be modified by replacement of a bridging oxygen, (i.e., the oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at either linking oxygen or at both of the linking oxygens.

[00270] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications. In some embodiments, the charged phosphate group can be replaced by a neutral moiety. Examples of moieties which can replace the phosphate group can include, without limitation, e.g., methyl phosphonate, hydroxylamino, siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino.

[00271] Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include, without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[00272] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group (a sugar modification). For example, the 2' hydroxyl group (OH) can be modified (e.g., replaced with a number of different oxy or deoxy substituents. Modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion.

[00273] Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein "R" can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, e.g., H or optionally substituted alkyl, and n can be an integer from 0 to 20 (e.g., from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and

from 4 to 20). The 2' hydroxyl group modification can be 2'-O-Me. Likewise, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. The 2' hydroxyl group modification can include locked nucleic acids (LNA) in which the 2' hydroxyl can be connected, e.g., by a C_{1-6} alkylene or C_{1-6} heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, e.g., NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino) and aminoalkoxy, $O(CH_2)_n$-amino, (wherein amino can be, e.g., NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino). The 2' hydroxyl group modification can include unlocked nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. The 2' hydroxyl group modification can include the methoxyethyl group (MOE), ($OCH_2CH_2OCH_3$, e.g., a PEG derivative).

[00274] Deoxy 2' modifications can include hydrogen (i.e. deoxyribose sugars, e.g., at the overhang portions of partially dsRNA); halo (e.g., bromo, chloro, fluoro, or iodo); amino (wherein amino can be, e.g., NH2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); $NH(CH_2CH_2NH)_nCH_2CH_2$-amino (wherein amino can be, e.g., as described herein), -NHC(O)R (wherein R can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with e.g., an amino as described herein.

[00275] The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing e.g., arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar atoms. The modified nucleic acids can also include one or more sugars that are in the L form (e.g. L-nucleosides).

[00276] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C),

and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

[00277] In a dual guide RNA, each of the crRNA and the tracrRNA can contain modifications. Such modifications may be at one or both ends of the crRNA and/or tracrRNA. In a sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, and/or internal nucleosides may be modified, and/or the entire sgRNA may be chemically modified. Some gRNAs comprise a 5' end modification. Some gRNAs comprise a 3' end modification.

[00278] The guide RNAs disclosed herein can comprise one of the modification patterns disclosed in WO 2018/107028 Al, herein incorporated by reference in its entirety for all purposes. The guide RNAs disclosed herein can also comprise one of the structures/modification patterns disclosed in US 2017/0114334, herein incorporated by reference in its entirety for all purposes. The guide RNAs disclosed herein can also comprise one of the structures/modification patterns disclosed in WO 2017/136794, WO 2017/004279, US 2018/0187186, or US 2019/0048338, each of which is herein incorporated by reference in its entirety for all purposes.

[00279] As one example, nucleotides at the 5' or 3' end of a guide RNA can include phosphorothioate linkages (e.g., the bases can have a modified phosphate group that is a phosphorothioate group). For example, a guide RNA can include phosphorothioate linkages between the 2, 3, or 4 terminal nucleotides at the 5' or 3' end of the guide RNA. As another example, nucleotides at the 5' and/or 3' end of a guide RNA can have 2'-O-methyl modifications. For example, a guide RNA can include 2'-O-methyl modifications at the 2, 3, or 4 terminal nucleotides at the 5' and/or 3' end of the guide RNA (e.g., the 5' end). *See, e.g.*, WO 2017/173054 A1 and Finn et al. (2018) *Cell Rep.* 22(9):2227-2235, each of which is herein incorporated by reference in its entirety for all purposes. Other possible modifications are described in more detail elsewhere herein. In one specific example, the guide RNA includes 2'-O-methyl analogs and 3' phosphorothioate internucleotide linkages at the first three 5' and 3' terminal RNA residues. In another specific example, the guide RNA is modified such that all 2'OH groups that do not interact with the Cas9 protein are replaced with 2'-O-methyl analogs,

and the tail region of the guide RNA, which has minimal interaction with Cas9, is modified with 5' and 3' phosphorothioate internucleotide linkages. Additionally, the DNA-targeting segment can have 2'-fluoro modifications on some bases. *See, e.g.*, Yin et al. (2017) *Nat. Biotech.* 35(12):1179-1187, herein incorporated by reference in its entirety for all purposes. Other examples of modified guide RNAs are provided, e.g., in WO 2018/107028 A1, herein incorporated by reference in its entirety for all purposes. Such chemical modifications can, for example, provide greater stability and protection from exonucleases to guide RNAs, allowing them to persist within cells for longer than unmodified guide RNAs. Such chemical modifications can also, for example, protect against innate intracellular immune responses that can actively degrade RNA or trigger immune cascades that lead to cell death.

[00280] As one example, any of the guide RNAs described herein can comprise at least one modification. In one example, the at least one modification comprises a 2'-O-methyl (2'-O-Me) modified nucleotide, a phosphorothioate (PS) bond between nucleotides, a 2'-fluoro (2'-F) modified nucleotide, or a combination thereof. For example, the at least one modification can comprise a 2'-O-methyl (2'-O-Me) modified nucleotide. Alternatively or additionally, the at least one modification can comprise a phosphorothioate (PS) bond between nucleotides. Alternatively or additionally, the at least one modification can comprise a 2'-fluoro (2'-F) modified nucleotide. In one example, a guide RNA described herein comprises one or more 2'-O-methyl (2'-O-Me) modified nucleotides and one or more phosphorothioate (PS) bonds between nucleotides.

[00281] The modifications can occur anywhere in the guide RNA. As one example, the guide RNA comprises a modification at one or more of the first five nucleotides at the 5' end of the guide RNA, the guide RNA comprises a modification at one or more of the last five nucleotides of the 3' end of the guide RNA, or a combination thereof. For example, the guide RNA can comprise phosphorothioate bonds between the first four nucleotides of the guide RNA, phosphorothioate bonds between the last four nucleotides of the guide RNA, or a combination thereof. Alternatively or additionally, the guide RNA can comprise 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end of the guide RNA, can comprise 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end of the guide RNA, or a combination thereof.

[00282] In one example, a modified gRNA can comprise the following sequence: mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (SEQ ID NO: 44), where "N" may be any natural or non-natural nucleotide, and wherein the totality of N residues comprise an *RS1* DNA-targeting segment as described herein (e.g., the sequence set forth in SEQ ID NO: 44, wherein the N residues are replaced with the DNA-targeting segment of any one of SEQ ID NOS: 3148-6241, or any one of SEQ ID NOS: 3148-4989, or any one of SEQ ID NOS: 3148-3151, 3154-3186, 3188-3247, and 3249-4351, or any one of SEQ ID NOS: 3150, 3151, 3159, 3675, 4297, and 4304, or any one of SEQ ID NOS: 4990-6241 (e.g., 5477 or 5981). The terms "mA," "mC," "mU," and "mG" denote a nucleotide (A, C, U, and G, respectively) that has been modified with 2'-O-Me. The symbol "*" depicts a phosphorothioate modification. A phosphorothioate linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos. The terms A*, C*, U*, or G* denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a phosphorothioate bond. The terms "mA*," "mC*," "mU*," and "mG*" denote a nucleotide (A, C, U, and G, respectively) that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a phosphorothioate bond.

[00283] Another chemical modification that has been shown to influence nucleotide sugar rings is halogen substitution. For example, 2'-fluoro (2'-F) substitution on nucleotide sugar rings can increase oligonucleotide binding affinity and nuclease stability. Abasic nucleotides refer to those which lack nitrogenous bases. Inverted bases refer to those with linkages that are inverted from the normal 5' to 3' linkage (i.e., either a 5' to 5' linkage or a 3' to 3' linkage).

[00284] An abasic nucleotide can be attached with an inverted linkage. For example, an abasic nucleotide may be attached to the terminal 5' nucleotide via a 5' to 5' linkage, or an abasic nucleotide may be attached to the terminal 3' nucleotide via a 3' to 3' linkage. An inverted abasic nucleotide at either the terminal 5' or 3' nucleotide may also be called an inverted abasic end cap.

[00285] In one example, one or more of the first three, four, or five nucleotides at the 5' terminus, and one or more of the last three, four, or five nucleotides at the 3' terminus are modified. The modification can be, for example, a 2'-O-Me, 2'-F, inverted abasic nucleotide, phosphorothioate bond, or other nucleotide modification well known to increase stability and/or performance.

[00286] In another example, the first four nucleotides at the 5' terminus, and the last four nucleotides at the 3' terminus can be linked with phosphorothioate bonds.

[00287] In another example, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus can comprise a 2'-O-methyl (2'-O-Me) modified nucleotide. In another example, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-fluoro (2'-F) modified nucleotide. In another example, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise an inverted abasic nucleotide.

[00288] Guide RNAs can be provided in any form. For example, the gRNA can be provided in the form of RNA, either as two molecules (separate crRNA and tracrRNA) or as one molecule (sgRNA), and optionally in the form of a complex with a Cas protein. The gRNA can also be provided in the form of DNA encoding the gRNA. The DNA encoding the gRNA can encode a single RNA molecule (sgRNA) or separate RNA molecules (e.g., separate crRNA and tracrRNA). In the latter case, the DNA encoding the gRNA can be provided as one DNA molecule or as separate DNA molecules encoding the crRNA and tracrRNA, respectively.

[00289] When a gRNA is provided in the form of DNA, the gRNA can be transiently, conditionally, or constitutively expressed in the cell. DNAs encoding gRNAs can be stably integrated into the genome of the cell and operably linked to a promoter active in the cell. Alternatively, DNAs encoding gRNAs can be operably linked to a promoter in an expression construct. For example, the DNA encoding the gRNA can be in a vector comprising a heterologous nucleic acid, such as a nucleic acid encoding a Cas protein. Alternatively, it can be in a vector or a plasmid that is separate from the vector comprising the nucleic acid encoding the Cas protein. Promoters that can be used in such expression constructs include promoters active, for example, in one or more of a eukaryotic cell, a human cell, a non-human cell, a mammalian cell, a non-human mammalian cell, a rodent cell, a mouse cell, a rat cell, a pluripotent cell, an embryonic stem (ES) cell, an adult stem cell, a developmentally restricted progenitor cell, an

induced pluripotent stem (iPS) cell, or a one-cell stage embryo. Such promoters can be, for example, conditional promoters, inducible promoters, constitutive promoters, or tissue-specific promoters. Such promoters can also be, for example, bidirectional promoters. Specific examples of suitable promoters include an RNA polymerase III promoter, such as a human U6 promoter, a rat U6 polymerase III promoter, or a mouse U6 polymerase III promoter. In another example, the small tRNA Gln can be used to drive expression of a guide RNA.

[00290] Alternatively, gRNAs can be prepared by various other methods. For example, gRNAs can be prepared by *in vitro* transcription using, for example, T7 RNA polymerase (*see, e.g.*, WO 2014/089290 and WO 2014/065596, each of which is herein incorporated by reference in its entirety for all purposes). Guide RNAs can also be a synthetically produced molecule prepared by chemical synthesis. For example, a guide RNA can be chemically synthesized to include 2'-O-methyl analogs and 3' phosphorothioate internucleotide linkages at the first three 5' and 3' terminal RNA residues.

[00291] Guide RNAs (or nucleic acids encoding guide RNAs) can be in compositions comprising one or more guide RNAs (e.g., 1, 2, 3, 4, or more guide RNAs) and a carrier increasing the stability of the guide RNA (e.g., prolonging the period under given conditions of storage (e.g., -20°C, 4°C, or ambient temperature) for which degradation products remain below a threshold, such below 0.5% by weight of the starting nucleic acid or protein; or increasing the stability in vivo). Non-limiting examples of such carriers include poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules. Such compositions can further comprise a Cas protein, such as a Cas9 protein, or a nucleic acid encoding a Cas protein.

3. Guide RNA Target Sequences

[00292] Target DNAs for guide RNAs include nucleic acid sequences present in a DNA to which a DNA-targeting segment of a gRNA will bind, provided sufficient conditions for binding exist. Suitable DNA/RNA binding conditions include physiological conditions normally present in a cell. Other suitable DNA/RNA binding conditions (e.g., conditions in a cell-free system) are known in the art (*see, e.g.*, Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., Harbor Laboratory Press 2001), herein incorporated by reference in its entirety for all purposes). The strand of the target DNA that is complementary to and hybridizes with the gRNA can be

called the "complementary strand," and the strand of the target DNA that is complementary to the "complementary strand" (and is therefore not complementary to the Cas protein or gRNA) can be called "noncomplementary strand" or "template strand."

[00293] The target DNA includes both the sequence on the complementary strand to which the guide RNA hybridizes and the corresponding sequence on the non-complementary strand (e.g., adjacent to the protospacer adjacent motif (PAM)). Unless otherwise specified, the term "guide RNA target sequence" as used herein refers specifically to the sequence on the non-complementary strand corresponding to (i.e., the reverse complement of) the sequence to which the guide RNA hybridizes on the complementary strand. That is, the guide RNA target sequence refers to the sequence on the non-complementary strand adjacent to the PAM (e.g., upstream or 5' of the PAM in the case of Cas9). A guide RNA target sequence is equivalent to the DNA-targeting segment of a guide RNA, but with thymines instead of uracils. As one example, a guide RNA target sequence for an SpCas9 enzyme can refer to the sequence upstream of the 5'-NGG-3' PAM on the non-complementary strand. A guide RNA is designed to have complementarity to the complementary strand of a target DNA, where hybridization between the DNA-targeting segment of the guide RNA and the complementary strand of the target DNA promotes the formation of a CRISPR complex. Full complementarity is not necessarily required, provided that there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex. If a guide RNA is referred to herein as targeting a guide RNA target sequence, what is meant is that the guide RNA hybridizes to the complementary strand sequence of the target DNA that is the reverse complement of the guide RNA target sequence on the non-complementary strand.

[00294] A target DNA or guide RNA target sequence can comprise any polynucleotide, and can be located, for example, in the nucleus or cytoplasm of a cell or within an organelle of a cell, such as a mitochondrion or chloroplast. A target DNA or guide RNA target sequence can be any nucleic acid sequence endogenous or exogenous to a cell. The guide RNA target sequence can be a sequence coding a gene product (e.g., a protein) or a non-coding sequence (e.g., a regulatory sequence) or can include both.

[00295] Site-specific binding and cleavage of a target DNA by a Cas protein can occur at locations determined by both (i) base-pairing complementarity between the guide RNA and the complementary strand of the target DNA and (ii) a short motif, called the protospacer adjacent

motif (PAM), in the non-complementary strand of the target DNA. The PAM can flank the guide RNA target sequence. Optionally, the guide RNA target sequence can be flanked on the 3' end by the PAM (e.g., for Cas9). Alternatively, the guide RNA target sequence can be flanked on the 5' end by the PAM (e.g., for Cpf1). For example, the cleavage site of Cas proteins can be about 1 to about 10 or about 2 to about 5 base pairs (e.g., 3 base pairs) upstream or downstream of the PAM sequence (e.g., within the guide RNA target sequence). In the case of SpCas9, the PAM sequence (i.e., on the non-complementary strand) can be 5'-N_1GG-3', where N_1 is any DNA nucleotide, and where the PAM is immediately 3' of the guide RNA target sequence on the non-complementary strand of the target DNA. As such, the sequence corresponding to the PAM on the complementary strand (i.e., the reverse complement) would be 5'-CCN_2-3', where N_2 is any DNA nucleotide and is immediately 5' of the sequence to which the DNA-targeting segment of the guide RNA hybridizes on the complementary strand of the target DNA. In some such cases, N_1 and N_2 can be complementary and the N_1- N_2 base pair can be any base pair (e.g., N_1=C and N_2=G; N_1=G and N_2=C; N_1=A and N_2=T; or N_1=T, and N_2=A). In the case of Cas9 from *S. aureus*, the PAM can be NNGRRT or NNGRR, where N can A, G, C, or T, and R can be G or A. In the case of Cas9 from *C. jejuni*, the PAM can be, for example, NNNNACAC or NNNNRYAC, where N can be A, G, C, or T, and R can be G or A. In some cases (e.g., for FnCpf1), the PAM sequence can be upstream of the 5' end and have the sequence 5'-TTN-3'.

[00296] An example of a guide RNA target sequence is a 20-nucleotide DNA sequence immediately preceding an NGG motif recognized by an SpCas9 protein. For example, two examples of guide RNA target sequences plus PAMs are GN_{19}NGG (SEQ ID NO: 40) or N_{20}NGG (SEQ ID NO: 41). *See, e.g.*, WO 2014/165825, herein incorporated by reference in its entirety for all purposes. The guanine at the 5' end can facilitate transcription by RNA polymerase in cells. Other examples of guide RNA target sequences plus PAMs can include two guanine nucleotides at the 5' end (e.g., GGN_{20}NGG; SEQ ID NO: 42) to facilitate efficient transcription by T7 polymerase *in vitro*. *See, e.g.*, WO 2014/065596, herein incorporated by reference in its entirety for all purposes. Other guide RNA target sequences plus PAMs can have between 4-22 nucleotides in length of SEQ ID NOS: 40-42, including the 5' G or GG and the 3' GG or NGG. Yet other guide RNA target sequences plus PAMs can have between 14 and 20 nucleotides in length of SEQ ID NOS: 40-42.

[00297] Guide RNAs targeting an *RS1* gene can target, for example, the first intron of the *RS1* gene, or a sequence adjacent to the first intron of the *RS1* gene (e.g., in the first exon or the second exon of the *RS1* gene.

[00298] Formation of a CRISPR complex hybridized to a target DNA can result in cleavage of one or both strands of the target DNA within or near the region corresponding to the guide RNA target sequence (i.e., the guide RNA target sequence on the non-complementary strand of the target DNA and the reverse complement on the complementary strand to which the guide RNA hybridizes). For example, the cleavage site can be within the guide RNA target sequence (e.g., at a defined location relative to the PAM sequence). The "cleavage site" includes the position of a target DNA at which a Cas protein produces a single-strand break or a double-strand break. The cleavage site can be on only one strand (e.g., when a nickase is used) or on both strands of a double-stranded DNA. Cleavage sites can be at the same position on both strands (producing blunt ends; e.g. Cas9)) or can be at different sites on each strand (producing staggered ends (i.e., overhangs); e.g., Cpf1). Staggered ends can be produced, for example, by using two Cas proteins, each of which produces a single-strand break at a different cleavage site on a different strand, thereby producing a double-strand break. For example, a first nickase can create a single-strand break on the first strand of double-stranded DNA (dsDNA), and a second nickase can create a single-strand break on the second strand of dsDNA such that overhanging sequences are created. In some cases, the guide RNA target sequence or cleavage site of the nickase on the first strand is separated from the guide RNA target sequence or cleavage site of the nickase on the second strand by at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 250, 500, or 1,000 base pairs.

[00299] A guide RNA targeting an *RS1* gene, such as a human *RS1* gene, can target any desired location in the *RS1* gene. Guide RNAs targeting an *RS1* gene can target, for example, the first intron of the *RS1* gene, or a sequence adjacent to the first intron of the *RS1* gene (e.g., in the first exon or the second exon of the *RS1* gene. For example, a guide RNA target sequence can comprise any contiguous sequence in the *RS1* gene. The term *RS1* gene includes the genomic region encompassing the *RS1* regulatory promoters and enhancer sequences as well as the coding sequence. A guide RNA target sequence can comprise a coding sequence, a non-coding sequence (e.g., a regulatory element such as a promoter or enhancer region), or a combination thereof. As one example, a guide RNA target sequence can comprise a contiguous coding sequence in any of

the *RS1* coding exons. As one example, the guide RNA target sequence can be in exon 1 of the *RS1* gene. As another example, the guide RNA target sequence can be in exon 2 of the *RS1* gene. As another example, the guide RNA target sequence can be in exon 3 of the *RS1* gene. As another example, the guide RNA target sequence can be in exon 4 of the *RS1* gene. As another example, the guide RNA target sequence can be in exon 5 of the *RS1* gene. As another example, the guide RNA target sequence can be in exon 6 of the *RS1* gene. A guide RNA target sequence can also comprise a contiguous sequence in any of the *RS1* introns. As one example, the guide RNA target sequence can be in intron 1 of the *RS1* gene. As another example, the guide RNA target sequence can be in intron 2 of the *RS1* gene. As another example, the guide RNA target sequence can be in intron 3 of the *RS1* gene. As another example, the guide RNA target sequence can be in intron 4 of the *RS1* gene. As another example, the guide RNA target sequence can be in intron 5 of the *RS1* gene. As another example, the guide RNA target sequence can be in intron 6 of the *RS1* gene.

[00300] The guide RNA target sequence can also be selected to minimize off-target modification or avoid off-target effects (e.g., by avoiding two or fewer mismatches to off-target genomic sequences).

[00301] As one example, a guide RNA targeting an *RS1* gene can target the guide RNA target sequence set forth in any one of SEQ ID NOS: 54-3147. As another example, a guide RNA targeting an *RS1* gene can target at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the guide RNA target sequence set forth in any one of SEQ ID NOS: 54-3147. Examples of such guide RNA target sequences are set forth in **Tables 2 and 3**.

[00302] As one example, a guide RNA targeting a human *RS1* gene can target the guide RNA target sequence set forth in any one of SEQ ID NOS: 54-1895. As another example, a guide RNA targeting a human *RS1* gene can target at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the guide RNA target sequence set forth in any one of SEQ ID NOS: 54-1895.

[00303] As one example, a guide RNA targeting a human *RS1* gene can target the guide RNA target sequence set forth in any one of SEQ ID NOS: 54-57, 60-92, 94-153, and 155-1257. As another example, a guide RNA targeting a human *RS1* gene can target at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the guide RNA target sequence set forth in any one of SEQ ID NOS: 54-57, 60-92, 94-153, and 155-1257.

[00304] As one example, a guide RNA targeting a human *RS1* gene can target the guide RNA target sequence set forth in any one of SEQ ID NOS: 56, 57, 65, 581, 1203, and 1210. As another example, a guide RNA targeting a human *RS1* gene can target at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the guide RNA target sequence set forth in any one of SEQ ID NOS: 56, 57, 65, 581, 1203, and 1210.

[00305] As one example, a guide RNA targeting a mouse *Rs1* gene can target the guide RNA target sequence set forth in any one of SEQ ID NOS: 1896-3147 (e.g., SEQ ID NO: 2383 or 2887). As another example, a guide RNA targeting a mouse *Rs1* gene can target at least 17, at least 18, at least 19, or at least 20 contiguous nucleotides of the guide RNA target sequence set forth in any one of SEQ ID NOS: 1896-3147 (e.g., SEQ ID NO: 2383 or 2887).

[00306] **Table 2. Human *RS1* Intron 1 Guide RNA Target Sequences and Guide Sequences.**

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGGCCTTATGCATCTCCATC	54	TGG	-	GGGCCUUAUGCAUCUCCAUC	3148
TAATCAGCATATTATCCAGA	55	TGG	+	UAAUCAGCAUAUUAUCCAGA	3149
CCCTTTTGTACGTTTTTTAA	56	**TGG**	+	**CCCUUUUGUACGUUUUUUAA**	3150
CTGTATCTTCTTAGCCAGTA	57	**CGG**	+	**CUGUAUCUUCUUAGCCAGUA**	3151
GTTAACGCGTCATTTACATT	58	AGG	-	GUUAACGCGUCAUUUACAUU	3152
TACAATAAATTAGCCGGATG	59	TGG	-	UACAAUAAAUUAGCCGGAUG	3153
AAACTTTTAGGAGTCAAAGT	60	GGG	+	AAACUUUUAGGAGUCAAAGU	3154
AAAACTTTTAGGAGTCAAAG	61	TGG	+	AAAACUUUUAGGAGUCAAAG	3155
AATGTTAACAGTGTTGGCTT	62	TGG	+	AAUGUUAACAGUGUUGGCUU	3156
GTGGTGGCTGCAGGTGAGGG	63	TGG	+	GUGGUGGCUGCAGGUGAGGG	3157
TACTAAAATGTTAACAGTGT	64	TGG	+	UACUAAAAUGUUAACAGUGU	3158
AAAAAAGGACTTGACATGCA	65	**GGG**	+	**AAAAAAGGACUUGACAUGCA**	3159
GTCCCAGCTTCTCTTGAGGG	66	TGG	+	GUCCCAGCUUCUCUUGAGGG	3160
GTTAACAGTGTTGGCTTTGG	67	TGG	+	GUUAACAGUGUUGGCUUUGG	3161
GTTGGTGGTCACTGACAGTG	68	TGG	-	GUUGGUGGUCACUGACAGUG	3162
GAAACTAGTGGCTACCGTAC	69	TGG	-	GAAACUAGUGGCUACCGUAC	3163
AAAAAACGTACAAAAGGGGC	70	CGG	-	AAAAAACGUACAAAAGGGGC	3164
AGCCCCAGGCCATCAACCGG	71	CGG	+	AGCCCCAGGCCAUCAACCGG	3165
GGCAGCCAGACAGATGCTTC	72	TGG	-	GGCAGCCAGACAGAUGCUUC	3166
CTGGAGAACAAAAGCATTTT	73	TGG	-	CUGGAGAACAAAAGCAUUUU	3167
ATCTTAGATTTATGCAATGT	74	GGG	-	AUCUUAGAUUUAUGCAAUGU	3168
ACAAGTAGAATCAGAATTCT	75	TGG	+	ACAAGUAGAAUCAGAAUUCU	3169
AATATCGGCTCACTGCAACC	76	CGG	+	AAUAUCGGCUCACUGCAACC	3170
CCCTCAAGAGAAGCTGGGAC	77	TGG	-	CCCUCAAGAGAAGCUGGGAC	3171
TCTTAGGAGATGACGAGGTT	78	GGG	-	UCUUAGGAGAUGACGAGGUU	3172
AATAATAAAGAACTAGAGAA	79	AGG	+	AAUAAUAAAGAACUAGAGAA	3173
AAAAACGTACAAAAGGGGCC	80	GGG	-	AAAAACGUACAAAAGGGGCC	3174
GGCTGAGCAGGCGGAGAAGT	81	AGG	+	GGCUGAGCAGGCGGAGAAGU	3175
AGAGAAAGGAGAACAGTCTG	82	AGG	+	AGAGAAAGGAGAACAGUCUG	3176
CTTCTTTCTCTTTCACGAGA	83	GGG	+	CUUCUUUCUCUUUCACGAGA	3177
AATCTTAGATTTATGCAATG	84	TGG	-	AAUCUUAGAUUUAUGCAAUG	3178
CTGAGCAGGCGGAGAAGTAG	85	GGG	+	CUGAGCAGGCGGAGAAGUAG	3179
ACCTAGTGTTGACAAGGCTG	86	TGG	-	ACCUAGUGUUGACAAGGCUG	3180
TCCCAGCTTCTCTTGAGGGT	87	GGG	+	UCCCAGCUUCUCUUGAGGGU	3181
AATAATTCTGTTGGGACGAC	88	AGG	+	AAUAAUUCUGUUGGGACGAC	3182

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CCTATAAAGAGGACGTTTCC	89	TGG	-	CCUAUAAAGAGGACGUUUCC	3183
CCTTCTTTCTCTTTCACGAG	90	AGG	+	CCUUCUUUCUCUUUCACGAG	3184
CCCATCGAAGTACTCACCAT	91	GGG	+	CCCAUCGAAGUACUCACCAU	3185
GTGTCATATGTTGCTGGTGG	92	GGG	-	GUGUCAUAUGUUGCUGGUGG	3186
AATGTAAATGACGCGTTAAC	93	AGG	+	AAUGUAAAUGACGCGUUAAC	3187
GCCTTTGAGCCAAGTGGATT	94	TGG	+	GCCUUUGAGCCAAGUGGAUU	3188
GTCAGACTCTGTGCTGGCTG	95	AGG	-	GUCAGACUCUGUGCUGGCUG	3189
CCGTCAATAGCATTCCGGTT	96	TGG	-	CCGUCAAUAGCAUUCCGGUU	3190
CCATTAAAAAACGTACAAAA	97	GGG	-	CCAUUAAAAAACGUACAAAA	3191
GGGATTTGGGGAGGATGGGG	98	AGG	-	GGGAUUUGGGGAGGAUGGGG	3192
ACTGTGTCATATGTTGCTGG	99	TGG	-	ACUGUGUCAUAUGUUGCUGG	3193
TACAATGTTGTGGACCGGGC	100	TGG	-	UACAAUGUUGUGGACCGGGC	3194
GATAGAGTAGCAAACAAAAC	101	AGG	-	GAUAGAGUAGCAAACAAAAC	3195
GCTCTCAAAGGAGACTGAGA	102	AGG	+	GCUCUCAAAGGAGACUGAGA	3196
GGAAGAGCAAGGAGCTCCTG	103	AGG	-	GGAAGAGCAAGGAGCUCCUG	3197
TGTGTCATATGTTGCTGGTG	104	GGG	-	UGUGUCAUAUGUUGCUGGUG	3198
CTGAGAAGGTGTACCTAGAG	105	TGG	+	CUGAGAAGGUGUACCUAGAG	3199
GTCCGGGAGCCAGTTCCTGC	106	AGG	-	GUCCGGGAGCCAGUUCCUGC	3200
TTAGGAGATGACGAGGTTGG	107	GGG	-	UUAGGAGAUGACGAGGUUGG	3201
GGAGGAAGCAGAGCTCTCAA	108	AGG	+	GGAGGAAGCAGAGCUCUCAA	3202
AGTCATCAGCCTGGGAAACA	109	TGG	-	AGUCAUCAGCCUGGGAAACA	3203
TCCGGGAGCCAGTTCCTGCA	110	GGG	-	UCCGGGAGCCAGUUCCUGCA	3204
GAGCAAGGAGCTCCTGAGGC	111	TGG	-	GAGCAAGGAGCUCCUGAGGC	3205
TCTGGTACAATGTTGTGGAC	112	CGG	-	UCUGGUACAAUGUUGUGGAC	3206
CCTGGGAGTGTCTGACAACC	113	AGG	-	CCUGGGAGUGUCUGACAACC	3207
CCAGTTCCTGCAGGGCCTCG	114	TGG	-	CCAGUUCCUGCAGGGCCUCG	3208
TAGGGAATGGGCTGAGCAGG	115	CGG	+	UAGGGAAUGGGCUGAGCAGG	3209
AGCTTTCTGTCCCTGTGAGA	116	AGG	+	AGCUUUCUGUCCCUGUGAGA	3210
TCGTCTCCCTGCCTTCTCAC	117	AGG	-	UCGUCUCCCUGCCUUCUCAC	3211
CGAAGTACTCACCATGGGCA	118	TGG	+	CGAAGUACUCACCAUGGGCA	3212
AGGCCACTGGACCATGCCCA	119	TGG	-	AGGCCACUGGACCAUGCCCA	3213
GGCAGGGAGACGAGTCTAAT	120	GGG	+	GGCAGGGAGACGAGUCUAAU	3214
GGGCTTAGATTTATGCAATA	121	TGG	-	GGGCUUAGAUUUAUGCAAUA	3215
TTCAGCGAAGACAAGGGATT	122	TGG	-	UUCAGCGAAGACAAGGGAUU	3216
CATGGCCCACGAGGCCCTGC	123	AGG	+	CAUGGCCCACGAGGCCCUGC	3217
CAGTTCCTGCAGGGCCTCGT	124	GGG	-	CAGUUCCUGCAGGGCCUCGU	3218
CATTAAAAAACGTACAAAAG	125	GGG	-	CAUUAAAAAACGUACAAAAG	3219
AGGCAGGGAGACGAGTCTAA	126	TGG	+	AGGCAGGGAGACGAGUCUAA	3220
CTAACATCTGGATGAACCTG	127	AGG	-	CUAACAUCUGGAUGAACCUG	3221
CCAGTGGCCTGTCTTCTCCT	128	GGG	+	CCAGUGGCCUGUCUUCUCCU	3222
TATTACAAATACTGCCGCTA	129	TGG	+	UAUUACAAAUACUGCCGCUA	3223
GCAGGGAGACGAGTCTAATG	130	GGG	+	GCAGGGAGACGAGUCUAAUG	3224
CAATCTGCTTGAGAAACAGG	131	AGG	-	CAAUCUGCUUGAGAAACAGG	3225
GGCTTAGATTTATGCAATAT	132	GGG	-	GGCUUAGAUUUAUGCAAUAU	3226
ACACTCCCAGGGCTCACTCT	133	CGG	+	ACACUCCCAGGGCUCACUCU	3227
TATGGGCTCATTCTGCCCCT	134	TGG	-	UAUGGGCUCAUUCUGCCCCU	3228
CGAAGACAAGGGATTTGGGG	135	AGG	-	CGAAGACAAGGGAUUUGGGG	3229
AGCACAGAGTCTGACATTGA	136	GGG	+	AGCACAGAGUCUGACAUUGA	3230
GTCTTAGGAGATGACGAGGT	137	TGG	-	GUCUUAGGAGAUGACGAGGU	3231
GCTGAGCAGGCGGAGAAGTA	138	GGG	+	GCUGAGCAGGCGGAGAAGUA	3232
TTCCAATCTGCTTGAGAAAC	139	AGG	-	UUCCAAUCUGCUUGAGAAAC	3233
GAGATCATAATACATCTCAG	140	AGG	+	GAGAUCAUAAUACAUCUCAG	3234
GCCCATCGAAGTACTCACCA	141	TGG	+	GCCCAUCGAAGUACUCACCA	3235
CAGGCGGAGAAGTAGGGGTG	142	GGG	+	CAGGCGGAGAAGUAGGGGUG	3236
TTGAAACCCAGTCACCAATG	143	TGG	-	UUGAAACCCAGUCACCAAUG	3237
ACATAATCCAACTGAGAGTC	144	AGG	+	ACAUAAUCCAACUGAGAGUC	3238
GTGAGTGTACTGAGTCTCCG	145	AGG	-	GUGAGUGUACUGAGUCUCCG	3239
CAATGGAGTCACACAGCATG	146	TGG	+	CAAUGGAGUCACACAGCAUG	3240

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGGGTGGGGGGACGCATTTG	147	AGG	+	GGGGUGGGGGGACGCAUUUG	3241
AACTGAGAGTCAGGAAACCT	148	CGG	+	AACUGAGAGUCAGGAAACCU	3242
TGCTCACTTCCATAAGGATA	149	TGG	+	UGCUCACUUCCAUAAGGAUA	3243
ATTTGGGGAGGATGGGGAGG	150	AGG	-	AUUUGGGGAGGAUGGGGAGG	3244
CGGGCTGGCCACGGCAAGTG	151	TGG	-	CGGGCUGGCCACGGCAAGUG	3245
CTCAATGTCAGACTCTGTGC	152	TGG	-	CUCAAUGUCAGACUCUGUGC	3246
TGCAGACTATTAATTGCAAG	153	GGG	-	UGCAGACUAUUAAUUGCAAG	3247
ACAGGCGTGAGCCACGGGCC	154	TGG	+	ACAGGCGUGAGCCACGGGCC	3248
CATCAATTGTGCCAGGCCCG	155	TGG	-	CAUCAAUUGUGCCAGGCCCG	3249
CAGCACAGAGTCTGACATTG	156	AGG	+	CAGCACAGAGUCUGACAUUG	3250
GTTGCAGACTATTAATTGCA	157	AGG	-	GUUGCAGACUAUUAAUUGCA	3251
GCAAGTGTGGCAACAGGGTC	158	CGG	-	GCAAGUGUGGCAACAGGGUC	3252
TAGGGTCTTGGAGTTTTGAT	159	AGG	-	UAGGGUCUUGGAGUUUUGAU	3253
AAAGCCTCACATGGCCCACG	160	AGG	+	AAAGCCUCACAUGGCCCACG	3254
CCTCGAGGTGAGCGTGTGCT	161	TGG	-	CCUCGAGGUGAGCGUGUGCU	3255
GCAGGCGGAGAAGTAGGGGT	162	GGG	+	GCAGGCGGAGAAGUAGGGGU	3256
CATTTTTGTGGACAGTTGTG	163	TGG	+	CAUUUUUGUGGACAGUUGUG	3257
TTGCAGACTATTAATTGCAA	164	GGG	-	UUGCAGACUAUUAAUUGCAA	3258
CTGGTTGTCAGACACTCCCA	165	GGG	+	CUGGUUGUCAGACACUCCCA	3259
CAGCGAAGACAAGGGATTTG	166	GGG	-	CAGCGAAGACAAGGGAUUUG	3260
TCAGCGAAGACAAGGGATTT	167	GGG	-	UCAGCGAAGACAAGGGAUUU	3261
TGGATTTGAAAATAAGAAGT	168	TGG	+	UGGAUUUGAAAAUAAGAAGU	3262
AGGTTTAAAAATATTGGGTG	169	TGG	+	AGGUUUAAAAAUAUUGGGUG	3263
TTGGCCCAGGGTGGTGGCAG	170	TGG	-	UUGGCCCAGGGUGGUGGCAG	3264
ACAGAAAAAGCTGTCACCAA	171	AGG	-	ACAGAAAAAGCUGUCACCAA	3265
ACAAGGGATTTGGGGAGGAT	172	GGG	-	ACAAGGGAUUUGGGGAGGAU	3266
GGCAGCCCCAGGCCATCAAC	173	CGG	+	GGCAGCCCCAGGCCAUCAAC	3267
CAGGTTCCCAGCTGTACAAT	174	GGG	-	CAGGUUCCCAGCUGUACAAU	3268
AGGCGGAGAAGTAGGGGTGG	175	GGG	+	AGGCGGAGAAGUAGGGGUGG	3269
AGTGTGGTGGCTGCAGGTGA	176	GGG	+	AGUGUGGUGGCUGCAGGUGA	3270
AGCACATTGGGCAGACACAA	177	GGG	+	AGCACAUUGGGCAGACACAA	3271
CCACGAGGCCCTGCAGGAAC	178	TGG	+	CCACGAGGCCCUGCAGGAAC	3272
CGTCTCCCTGCCTTCTCACA	179	GGG	-	CGUCUCCCUGCCUUCUCACA	3273
AGATACTAATCCCATGTATG	180	AGG	+	AGAUACUAAUCCCAUGUAUG	3274
TTAGGCTTTGCCCTCATACA	181	TGG	-	UUAGGCUUUGCCCUCAUACA	3275
CTACTCGGGGGAGGCTGAAG	182	CGG	+	CUACUCGGGGGAGGCUGAAG	3276
ACCTCACTGCCTCAGCAGCG	183	TGG	+	ACCUCACUGCCUCAGCAGCG	3277
TTGAGGACCCAGGAGAAGAC	184	AGG	-	UUGAGGACCCAGGAGAAGAC	3278
CTTAGGAGATGACGAGGTTG	185	GGG	-	CUUAGGAGAUGACGAGGUUG	3279
GGCGGAGAAGTAGGGGTGGG	186	GGG	+	GGCGGAGAAGUAGGGGUGGG	3280
CTGGTACAATGTTGTGGACC	187	GGG	-	CUGGUACAAUGUUGUGGACC	3281
GGCAAAGCATTCTGGGCAGA	188	GGG	-	GGCAAAGCAUUCUGGGCAGA	3282
GAAAATTACGGTGACTGCTG	189	TGG	-	GAAAAUUACGGUGACUGCUG	3283
GGACCAACCTAGTGTTGACA	190	AGG	-	GGACCAACCUAGUGUUGACA	3284
GCTTTTGTTCTCCAGTTTGT	191	TGG	+	GCUUUUGUUCUCCAGUUUGU	3285
TGATTTAATCACCAACAAAC	192	TGG	-	UGAUUUAAUCACCAACAAAC	3286
ATTTAGCAATGTTTACTGGG	193	TGG	+	AUUUAGCAAUGUUUACUGGG	3287
GGCATTGAATCCAATATGTG	194	CGG	-	GGCAUUGAAUCCAAUAUGUG	3288
GTGTTACCATCAGAGATTGC	195	AGG	+	GUGUUACCAUCAGAGAUUGC	3289
GTAGGCCATTCAGTGCTTAC	196	TGG	+	GUAGGCCAUUCAGUGCUUAC	3290
TTTGAAAATAAGAAGTTGGC	197	TGG	+	UUUGAAAAUAAGAAGUUGGC	3291
CACCTCGTTCCGCCGGTTGA	198	TGG	-	CACCUCGUUCCGCCGGUUGA	3292
ACACGCTCACCTCGAGGGCT	199	TGG	+	ACACGCUCACCUCGAGGGCU	3293
GATATCCAGTAAGCACTGAA	200	TGG	-	GAUAUCCAGUAAGCACUGAA	3294
TGGATTTAGCAATGTTTACT	201	GGG	+	UGGAUUUAGCAAUGUUUACU	3295
TAAAGAGGACGTTTCCTGGC	202	CGG	-	UAAAGAGGACGUUUCCUGGC	3296
TTAATCTGTCTTTTGTCGTA	203	GGG	-	UUAAUCUGUCUUUUGUCGUA	3297
ATAGGAAATGCCTTACTGTG	204	AGG	-	AUAGGAAAUGCCUUACUGUG	3298

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CTTACTGGATATCTGAACCT	205	TGG	+	CUUACUGGAUAUCUGAACCU	3299
AGTAAACATTGCTAAATCCA	206	AGG	-	AGUAAACAUUGCUAAAUCCA	3300
GGGTTCTTGCCTTGGGGTAT	207	CGG	-	GGGUUCUUGCCUUGGGGUAU	3301
GTGAGGCTTTGTGGAACTTG	208	AGG	-	GUGAGGCUUUGUGGAACUUG	3302
ACATCTTACTACTACCACAT	209	TGG	+	ACAUCUUACUACUACCACAU	3303
CCACCAACACCCCTTGCCTT	210	TGG	+	CCACCAACACCCCUUGCCUU	3304
TTTTTGGGAGACCAAAGGCA	211	AGG	-	UUUUUGGGAGACCAAAGGCA	3305
TTTCATCCTCCACGCTGCTG	212	AGG	-	UUUCAUCCUCCACGCUGCUG	3306
TCAGAGCCACATGAAACTAG	213	TGG	-	UCAGAGCCACAUGAAACUAG	3307
TCCATTAAAAAACGTACAAA	214	AGG	-	UCCAUUAAAAAACGUACAAA	3308
AAAAAAAGGACTTGACATGC	215	AGG	+	AAAAAAAGGACUUGACAUGC	3309
GCTCCTGAGGCTGGAGCTAG	216	AGG	-	GCUCCUGAGGCUGGAGCUAG	3310
AGAAGGTGTACCTAGAGTGG	217	TGG	+	AGAAGGUGUACCUAGAGUGG	3311
GGTGCCAGCAGATTTGGGTC	218	TGG	+	GGUGCCAGCAGAUUUGGGUC	3312
GATACTAATCCCATGTATGA	219	GGG	+	GAUACUAAUCCCAUGUAUGA	3313
GAAATCCGAGAGTGAGCCCT	220	GGG	-	GAAAUCCGAGAGUGAGCCCU	3314
AAAACCCCTGCAATCTCTGA	221	TGG	-	AAAACCCCUGCAAUCUCUGA	3315
TAATAATATTTATATTATTC	222	AGG	-	UAAUAAUAUUUAUAUUAUUC	3316
AGAAAAGAAATGCAAGAAAT	223	GGG	+	AGAAAAGAAAUGCAAGAAAU	3317
TGAACTGTGTCATATGTTGC	224	TGG	-	UGAACUGUGUCAUAUGUUGC	3318
CAGAAAGCTTTCTGTATCTC	225	AGG	-	CAGAAAGCUUUCUGUAUCUC	3319
AGAAGCTGGGACTGGCTGTG	226	AGG	-	AGAAGCUGGGACUGGCUGUG	3320
CTTCTCTTGAGGGTGGGGAT	227	GGG	+	CUUCUCUUGAGGGUGGGGAU	3321
GCCCAGAATGCTTTGCCCAC	228	AGG	+	GCCCAGAAUGCUUUGCCCAC	3322
AGCAGGCGGAGAAGTAGGGG	229	TGG	+	AGCAGGCGGAGAAGUAGGGG	3323
TCTCCTCTAGCTCCAGCCTC	230	AGG	+	UCUCCUCUAGCUCCAGCCUC	3324
GGTTAAATGAAAGGGAAAGC	231	AGG	+	GGUUAAAUGAAAGGGAAAGC	3325
CTAAAGCGCGCCTTTCCTCA	232	TGG	+	CUAAAGCGCGCCUUUCCUCA	3326
AGAAGATGCTGGCTTGGCCC	233	AGG	-	AGAAGAUGCUGGCUUGGCCC	3327
CAGGAGCTCCTTGCTCTTCC	234	TGG	+	CAGGAGCUCCUUGCUCUUCC	3328
CTGTGTCATATGTTGCTGGT	235	GGG	-	CUGUGUCAUAUGUUGCUGGU	3329
TAGCACATTGGGCAGACACA	236	AGG	+	UAGCACAUUGGGCAGACACA	3330
GAAGCATATCACTTCTGCTA	237	GGG	-	GAAGCAUAUCACUUCUGCUA	3331
GTTGTGGACCGGGCTGGCCA	238	CGG	-	GUUGUGGACCGGGCUGGCCA	3332
TCCAGTGGCCTGTCTTCTCC	239	TGG	+	UCCAGUGGCCUGUCUUCUCC	3333
AATTTCCTGCTGTGACAACA	240	AGG	+	AAUUUCCUGCUGUGACAACA	3334
CAAGGGATTTGGGGAGGATG	241	GGG	-	CAAGGGAUUUGGGGAGGAUG	3335
AGGAAGTAGGGAACAGGGAC	242	TGG	-	AGGAAGUAGGGAACAGGGAC	3336
ACCTCCATCTCTATAAAAAT	243	AGG	+	ACCUCCAUCUCUAUAAAAAU	3337
TTACTTCTCTTTGGCTATGA	244	AGG	+	UUACUUCUCUUUGGCUAUGA	3338
TCCCACTGGCCCACGTTGCA	245	GGG	-	UCCCACUGGCCCACGUUGCA	3339
CAGCCATGTTGCTTCTCCTT	246	TGG	+	CAGCCAUGUUGCUUCUCCUU	3340
TATTTTTCTCCCTGTTTAGA	247	TGG	-	UAUUUUUCUCCCUGUUUAGA	3341
GGGCAAAGCATTCTGGGCAG	248	AGG	-	GGGCAAAGCAUUCUGGGCAG	3342
GAAGGAGGCGAGACACCTGT	249	GGG	-	GAAGGAGGCGAGACACCUGU	3343
ATTTCCTGCTGTGACAACAA	250	GGG	+	AUUUCCUGCUGUGACAACAA	3344
AAGAAAAGAAATGCAAGAAA	251	TGG	+	AAGAAAAGAAAUGCAAGAAA	3345
GCTTCTCTTGAGGGTGGGGA	252	TGG	+	GCUUCUCUUGAGGGUGGGGA	3346
ATTAATCTGTCTTTTGTCGT	253	AGG	-	AUUAAUCUGUCUUUUGUCGU	3347
ACAGGAGGAACGCACGCAGC	254	AGG	-	ACAGGAGGAACGCACGCAGC	3348
CCTGGTTGTCAGACACTCCC	255	AGG	+	CCUGGUUGUCAGACACUCCC	3349
AGGTCAACATCAATTGTGCC	256	AGG	-	AGGUCAACAUCAAUUGUGCC	3350
GACAAGGGATTTGGGGAGGA	257	TGG	-	GACAAGGGAUUUGGGGAGGA	3351
GGCTTAGATCTAAATACTCC	258	TGG	+	GGCUUAGAUCUAAAUACUCC	3352
GAGCACACAGCCTCACAGTA	259	AGG	+	GAGCACACAGCCUCACAGUA	3353
GTTACCATCAGAGATTGCAG	260	GGG	+	GUUACCAUCAGAGAUUGCAG	3354
TTGGATTTAGCAATGTTTAC	261	TGG	+	UUGGAUUUAGCAAUGUUUAC	3355
CTTGGAGTTTTGATAGGCAC	262	AGG	-	CUUGGAGUUUUGAUAGGCAC	3356

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ATTTAATAAAAGAGAAACCT	263	TGG	+	AUUUAAUAAAAGAGAAACCU	3357
TTTAATAAAAGAGAAACCTT	264	GGG	+	UUUAAUAAAAGAGAAACCUU	3358
ATTAAAAAGCAGGGAGAAGG	265	AGG	+	AUUAAAAAGCAGGGAGAAGG	3359
GAGTTCCAGAAGCATCTGTC	266	TGG	+	GAGUUCCAGAAGCAUCUGUC	3360
GAAGATGCTGGCTTGGCCCA	267	GGG	-	GAAGAUGCUGGCUUGGCCCA	3361
GCTAGAGGAGAGAGAGTCTT	268	AGG	-	GCUAGAGGAGAGAGAGUCUU	3362
CGAAGGAGGCGAGACACCTG	269	TGG	-	CGAAGGAGGCGAGACACCUG	3363
GACAAACAGCCTTTGTCTTC	270	AGG	-	GACAAACAGCCUUUGUCUUC	3364
TATTGGATTCAATGCCAATA	271	AGG	+	UAUUGGAUUCAAUGCCAAUA	3365
TCCCCACCCTCAAGAGAAGC	272	TGG	-	UCCCCACCCUCAAGAGAAGC	3366
TCACAGCAGAGTGGCCCTTT	273	TGG	+	UCACAGCAGAGUGGCCCUUU	3367
GATTGAGAGGGAATTGGAAC	274	TGG	-	GAUUGAGAGGGAAUUGGAAC	3368
TTTTGGGAGACCAAAGGCAA	275	GGG	-	UUUUGGGAGACCAAAGGCAA	3369
CCCAGCTTCTCTTGAGGGTG	276	GGG	+	CCCAGCUUCUCUUGAGGGUG	3370
GACTGCACTCACCTCGGAAC	277	CGG	+	GACUGCACUCACCUCGGAAC	3371
GGCAACAGGGTCCGGTTCCG	278	AGG	-	GGCAACAGGGUCCGGUUCCG	3372
TGACGAGGTTGGGGGTGTCC	279	GGG	-	UGACGAGGUUGGGGGUGUCC	3373
TAGGCTTTGCCCTCATACAT	280	GGG	-	UAGGCUUUGCCCUCAUACAU	3374
TGAAGCATATCACTTCTGCT	281	AGG	-	UGAAGCAUAUCACUUCUGCU	3375
AATCAGAATTCTTGGTTTCA	282	CGG	+	AAUCAGAAUUCUUGGUUUCA	3376
GATGTTCTAAAAATCCTTAT	283	TGG	-	GAUGUUCUAAAAAUCCUUAU	3377
AGACAAATGCTTTCTGCGGG	284	AGG	+	AGACAAAUGCUUUCUGCGGG	3378
TGTTACCATCAGAGATTGCA	285	GGG	+	UGUUACCAUCAGAGAUUGCA	3379
CCCGCCAAAGCGTTTTGCGC	286	AGG	+	CCCGCCAAAGCGUUUUGCGC	3380
CAGTTGTGTGGAAATATGAT	287	TGG	+	CAGUUGUGUGGAAAUAUGAU	3381
ACGATGTCCACAAAGAAGAC	288	AGG	-	ACGAUGUCCACAAAGAAGAC	3382
TTAGGCGACAGGCATATTCA	289	TGG	-	UUAGGCGACAGGCAUAUUCA	3383
GCTGGTTTAAAGCACTGATG	290	GGG	-	GCUGGUUUAAAGCACUGAUG	3384
CCAGGAAACGTCCTCTTTAT	291	AGG	+	CCAGGAAACGUCCUCUUUAU	3385
TCAGCCCATTCCCTATAAAG	292	AGG	-	UCAGCCCAUUCCCUAUAAAG	3386
AGAAAGCTTTCTGTATCTCA	293	GGG	-	AGAAAGCUUUCUGUAUCUCA	3387
ACTTATTAAATAAGTATGGA	294	TGG	-	ACUUAUUAAAUAAGUAUGGA	3388
ATTGGATTGCACCCAGGAAT	295	TGG	+	AUUGGAUUGCACCCAGGAAU	3389
TGCTTTCAGAGCCAATTCCT	296	GGG	-	UGCUUUCAGAGCCAAUUCCU	3390
TTCTGCCCCTTGGTCCAAAA	297	GGG	-	UUCUGCCCCUUGGUCCAAAA	3391
CAAATGGTGAGCTGAGGGAC	298	AGG	+	CAAAUGGUGAGCUGAGGGAC	3392
CCTTTAGCTTCCACCACTCT	299	AGG	-	CCUUUAGCUUCCACCACUCU	3393
AGGTCAACCTAGTTTAAATA	300	TGG	+	AGGUCAACCUAGUUUAAAUA	3394
TTGGTTGCCTGTCTTCTTTG	301	TGG	+	UUGGUUGCCUGUCUUCUUUG	3395
AGAAATCCGAGAGTGAGCCC	302	TGG	-	AGAAAUCCGAGAGUGAGCCC	3396
ACTGCAACCTTCCGCCCGCC	303	TGG	+	ACUGCAACCUUCCGCCCGCC	3397
ATCGATTGAACCCAGGCGGG	304	CGG	-	AUCGAUUGAACCCAGGCGGG	3398
TAGGCGACAGGCATATTCAT	305	GGG	-	UAGGCGACAGGCAUAUUCAU	3399
GGCGTGTTCCAGGAAGAGCA	306	AGG	-	GGCGUGUUCCAGGAAGAGCA	3400
CAAGCACACGCTCACCTCGA	307	GGG	+	CAAGCACACGCUCACCUCGA	3401
TTTATTATTATATGCATCAT	308	AGG	+	UUUAUUAUUAUAUGCAUCAU	3402
ATCTCAGGGTTCTTGCCTTG	309	GGG	-	AUCUCAGGGUUCUUGCCUUG	3403
CTTATTTAATAAGTTTTACA	310	TGG	+	CUUAUUUAAUAAGUUUUACA	3404
GGCAACCAATTTCTAACATC	311	TGG	-	GGCAACCAAUUUCUAACAUC	3405
AGAAGCAGTGGAGGGTAAAT	312	TGG	-	AGAAGCAGUGGAGGGUAAAU	3406
ATCACAGAAGCCATCTAAAC	313	AGG	+	AUCACAGAAGCCAUCUAAAC	3407
GCTGGCTTGGCCCAGGGTGG	314	TGG	-	GCUGGCUUGGCCCAGGGUGG	3408
TTTAGCAATGTTTACTGGGT	315	GGG	+	UUUAGCAAUGUUUACUGGGU	3409
CTGTTTGTTCAGATTCGTGT	316	TGG	+	CUGUUUGUUCAGAUUCGUGU	3410
ACTGAGAAAACTGCAGAAGC	317	AGG	-	ACUGAGAAAACUGCAGAAGC	3411
TAAATTAATTAAAATTGGCC	318	GGG	-	UAAAUUAAUUAAAAUUGGCC	3412
TCTCCAGGTGCCCTGCAACG	319	TGG	+	UCUCCAGGUGCCCUGCAACG	3413
TTCCCACTGGCCCACGTTGC	320	AGG	-	UUCCCACUGGCCCACGUUGC	3414

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TCGCTTAGCATATGTGGTTG	321	AGG	+	UCGCUUAGCAUAUGUGGUUG	3415
GATGCTTCTGGAACTCAAGG	322	GGG	-	GAUGCUUCUGGAACUCAAGG	3416
CAGCATGTGCCAAGCCCTCG	323	AGG	-	CAGCAUGUGCCAAGCCCUCG	3417
TCACAACAACTTCGGCCTCC	324	TGG	-	UCACAACAACUUCGGCCUCC	3418
ATCATCGCAGAAGGTTGCAT	325	TGG	-	AUCAUCGCAGAAGGUUGCAU	3419
GGCCTTTAACAGCACTTGAA	326	AGG	+	GGCCUUUAACAGCACUUGAA	3420
CGAGGCGGGCAAACCACTTG	327	AGG	-	CGAGGCGGGCAAACCACUUG	3421
TCTGAAGGGCCCTGTCAGTT	328	AGG	-	UCUGAAGGGCCCUGUCAGUU	3422
AGAAATATCTCAGCCTCTAA	329	AGG	-	AGAAAUAUCUCAGCCUCUAA	3423
GATGCTGGCTTGGCCCAGGG	330	TGG	-	GAUGCUGGCUUGGCCCAGGG	3424
CCACGGCAAGTGTGGCAACA	331	GGG	-	CCACGGCAAGUGUGGCAACA	3425
CCAAGCACACGCTCACCTCG	332	AGG	+	CCAAGCACACGCUCACCUCG	3426
CATCTTCACACCGCAATGTT	333	GGG	-	CAUCUUCACACCGCAAUGUU	3427
TTGTGTGGAAATATGATTGG	334	AGG	+	UUGUGUGGAAAUAUGAUUGG	3428
CTGCTTAGATTTTCTTGCTG	335	AGG	-	CUGCUUAGAUUUUCUUGCUG	3429
CTCCAGGTGCCCTGCAACGT	336	GGG	+	CUCCAGGUGCCCUGCAACGU	3430
GATGCATCCCATTGTACAGC	337	TGG	+	GAUGCAUCCCAUUGUACAGC	3431
GAAGGTTTCAGCGAAGACAA	338	GGG	-	GAAGGUUUCAGCGAAGACAA	3432
GTAGTGCCCACAGGATCTGC	339	GGG	-	GUAGUGCCCACAGGAUCUGC	3433
TCAGGTTCCCAGCTGTACAA	340	TGG	-	UCAGGUUCCCAGCUGUACAA	3434
TCCAAAATTGGATTGCACCC	341	AGG	+	UCCAAAAUUGGAUUGCACCC	3435
CTCCCTCTGGTACAATGTTG	342	TGG	-	CUCCCUCUGGUACAAUGUUG	3436
CACCTGTGGGCAAAGCATTC	343	TGG	-	CACCUGUGGGCAAAGCAUUC	3437
AGGACCATCCAGGGCAAATT	344	GGG	+	AGGACCAUCCAGGGCAAAUU	3438
AGTAACAAAAGTCATCAGCC	345	TGG	-	AGUAACAAAAGUCAUCAGCC	3439
CTGCAACCTTCCGCCCGCCT	346	GGG	+	CUGCAACCUUCCGCCCGCCU	3440
ACAGATGCTTCTGGAACTCA	347	AGG	-	ACAGAUGCUUCUGGAACUCA	3441
GGGCGGGCGGAAGGGGAAGA	348	AGG	-	GGGCGGGCGGAAGGGGAAGA	3442
CCCTGTTGCCACACTTGCCG	349	TGG	+	CCCUGUUGCCACACUUGCCG	3443
CGTTTCCTGGCCGGACGCAG	350	TGG	-	CGUUUCCUGGCCGGACGCAG	3444
CAGCCTTGTCAACACTAGGT	351	TGG	+	CAGCCUUGUCAACACUAGGU	3445
GGACCATCCAGGGCAAATTG	352	GGG	+	GGACCAUCCAGGGCAAAUUG	3446
GTAACAAAAGTCATCAGCCT	353	GGG	-	GUAACAAAAGUCAUCAGCCU	3447
ATGCATCCCATTGTACAGCT	354	GGG	+	AUGCAUCCCAUUGUACAGCU	3448
TCTCGCTGTGTTGCTCAGGG	355	TGG	+	UCUCGCUGUGUUGCUCAGGG	3449
ACTGGGGAAGTAGTGCCCAC	356	AGG	-	ACUGGGGAAGUAGUGCCCAC	3450
GCTGAAGCAGATACACCACA	357	TGG	-	GCUGAAGCAGAUACACCACA	3451
ACTGACAGCACCCAACATTG	358	CGG	+	ACUGACAGCACCCAACAUUG	3452
CCATCTTCACACCGCAATGT	359	TGG	-	CCAUCUUCACACCGCAAUGU	3453
TACAGTGGACACAAAAAAAG	360	TGG	+	UACAGUGGACACAAAAAAAG	3454
GAGTCTCGCTGTGTTGCTCA	361	GGG	+	GAGUCUCGCUGUGUUGCUCA	3455
ATTACTGACCACAGTTTCAA	362	AGG	-	AUUACUGACCACAGUUUCAA	3456
ATGTGCTAGATGATCCATAG	363	CGG	-	AUGUGCUAGAUGAUCCAUAG	3457
TATCCAGATGGAGATGCATA	364	AGG	+	UAUCCAGAUGGAGAUGCAUA	3458
TCTGAGGTACCATATCCTTA	365	TGG	-	UCUGAGGUACCAUAUCCUUA	3459
CAGGAAACGTCCTCTTTATA	366	GGG	+	CAGGAAACGUCCUCUUUAUA	3460
AATTGCCTTCACTTAGGCGT	367	TGG	+	AAUUGCCUUCACUUAGGCGU	3461
AGAGGGAGCCTTTGAAACTG	368	TGG	+	AGAGGGAGCCUUUGAAACUG	3462
GCGTGTGCTTGGCGTGTTCC	369	AGG	-	GCGUGUGCUUGGCGUGUUCC	3463
TTGAAAATAAGAAGTTGGCT	370	GGG	+	UUGAAAAUAAGAAGUUGGCU	3464
ATCTGCGGGCAGATTACGCG	371	TGG	-	AUCUGCGGGCAGAUUACGCG	3465
GTAAGCACTGAATGGCCTAC	372	AGG	-	GUAAGCACUGAAUGGCCUAC	3466
CAGGACCATCCAGGGCAAAT	373	TGG	+	CAGGACCAUCCAGGGCAAAU	3467
ATTCTGCCCCTTGGTCCAAA	374	AGG	-	AUUCUGCCCCUUGGUCCAAA	3468
ATGCATAAGGCCCATAGACA	375	TGG	+	AUGCAUAAGGCCCAUAGACA	3469
ATGCATCTTTCCCATGTCTA	376	TGG	-	AUGCAUCUUUCCCAUGUCUA	3470
TCCTGGGTGCAATCCAATTT	377	TGG	-	UCCUGGGUGCAAUCCAAUUU	3471
GCCTTTCCTCATGGGTTGCA	378	AGG	+	GCCUUUCCUCAUGGGUUGCA	3472

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AGAGCTGCCATTTGCGGGGC	379	GGG	-	AGAGCUGCCAUUUGCGGGGC	3473
ACACCCCAATTTGCCCTGGA	380	TGG	-	ACACCCCAAUUUGCCCUGGA	3474
TTTCGCTGTGTCTGTCTACC	381	AGG	-	UUUCGCUGUGUCUGUCUACC	3475
TTACAAAAATGATCCGGGCG	382	TGG	-	UUACAAAAAUGAUCCGGGCG	3476
CTGCTTTCAGAGCCAATTCC	383	TGG	-	CUGCUUUCAGAGCCAAUUCC	3477
CCCCACCCTCAAGAGAAGCT	384	GGG	-	CCCCACCCUCAAGAGAAGCU	3478
GGTTGCCCAGGGGCTGGGGG	385	AGG	-	GGUUGCCCAGGGGCUGGGGG	3479
GTCCACAACATTGTACCAGA	386	GGG	+	GUCCACAACAUUGUACCAGA	3480
ACCTGTGGGCAAAGCATTCT	387	GGG	-	ACCUGUGGGCAAAGCAUUCU	3481
TTCCAAGTAGCTCGGATCAC	388	AGG	+	UUCCAAGUAGCUCGGAUCAC	3482
CAGTGTGGTGGCTGCAGGTG	389	AGG	+	CAGUGUGGUGGCUGCAGGUG	3483
ATTTTCCCACATTATGTATA	390	TGG	+	AUUUUCCCACAUUAUGUAUA	3484
GGGATGAGCTCTTAAAAGAA	391	GGG	-	GGGAUGAGCUCUUAAAAGAA	3485
TAGAATTTAATATTCCAAAC	392	CGG	+	UAGAAUUUAAUAUUCCAAAC	3486
GAGGTTTCCTGACTCTCAGT	393	TGG	-	GAGGUUUCCUGACUCUCAGU	3487
AATCTAAGCAGACTCTTTCA	394	AGG	+	AAUCUAAGCAGACUCUUUCA	3488
TTTGGGAGACCAAAGGCAAG	395	GGG	-	UUUGGGAGACCAAAGGCAAG	3489
AATGTACCTTGCAACCCATG	396	AGG	-	AAUGUACCUUGCAACCCAUG	3490
AGTGAAATATTTCGATTATG	397	TGG	-	AGUGAAAUAUUUCGAUUAUG	3491
CAGATGCTTCTGGAACTCAA	398	GGG	-	CAGAUGCUUCUGGAACUCAA	3492
AGTAGTGCCCACAGGATCTG	399	CGG	-	AGUAGUGCCCACAGGAUCUG	3493
CTCCTGGGCCCTTGTTCCAC	400	TGG	-	CUCCUGGGCCCUUGUUCCAC	3494
TAATAAGTTTTACATGGCAC	401	AGG	+	UAAUAAGUUUUACAUGGCAC	3495
TCTGCTCAGAATTTCTCCTC	402	AGG	+	UCUGCUCAGAAUUUCUCCUC	3496
AGGAGGGGGCAAGGAGTAAA	403	AGG	-	AGGAGGGGGCAAGGAGUAAA	3497
TAGAGCTGCCATTTGCGGGG	404	CGG	-	UAGAGCUGCCAUUUGCGGGG	3498
ACAGTTTCAAAGGCTCCCTC	405	TGG	-	ACAGUUUCAAAGGCUCCCUC	3499
CGGTAGCCACTAGTTTCATG	406	TGG	+	CGGUAGCCACUAGUUUCAUG	3500
CCTTACACCCCAATTTGCCC	407	TGG	-	CCUUACACCCCAAUUUGCCC	3501
AAGAAATACAATAAATTAGC	408	CGG	-	AAGAAAUACAAUAAAUUAGC	3502
CTAAACCTGAGTGTAAAAAA	409	TGG	-	CUAAACCUGAGUGUAAAAAA	3503
CCAGTCCCAGCTTCTCTTGA	410	GGG	+	CCAGUCCCAGCUUCUCUUGA	3504
AGAAACGGAAGTCTGAGGCT	411	GGG	-	AGAAACGGAAGUCUGAGGCU	3505
CCCAGGAGAAGACAGGCCAC	412	TGG	-	CCCAGGAGAAGACAGGCCAC	3506
AAGTCGATCCGTGGTTGCCC	413	AGG	-	AAGUCGAUCCGUGGUUGCCC	3507
AAGAAACGGAAGTCTGAGGC	414	TGG	-	AAGAAACGGAAGUCUGAGGC	3508
GCCACGGCAAGTGTGGCAAC	415	AGG	-	GCCACGGCAAGUGUGGCAAC	3509
ATGACGAGGTTGGGGGTGTC	416	CGG	-	AUGACGAGGUUGGGGGUGUC	3510
AAAGCCTAATCACCTCCCAA	417	AGG	+	AAAGCCUAAUCACCUCCCAA	3511
GGTCTCCCAAAAAGAGGATC	418	TGG	+	GGUCUCCCAAAAAGAGGAUC	3512
TAGGTTTGATGGCCTGCTTC	419	AGG	+	UAGGUUUGAUGGCCUGCUUC	3513
CTTGGCGCTATACTAGGACT	420	GGG	-	CUUGGCGCUAUACUAGGACU	3514
GGAAACGCTACCATCAAATA	421	TGG	-	GGAAACGCUACCAUCAAAUA	3515
CCTACTGTGTGCCCTACCCT	422	GGG	+	CCUACUGUGUGCCCUACCCU	3516
CATCCCTCTGGCCCAGGGTA	423	GGG	-	CAUCCCUCUGGCCCAGGGUA	3517
TGGAGCATCCCTCTGGCCCA	424	GGG	-	UGGAGCAUCCCUCUGGCCCA	3518
TGTAGATGCTCTGCTTTTGA	425	TGG	+	UGUAGAUGCUCUGCUUUUGA	3519
GTAGTCCCAGCTACCATGGG	426	AGG	+	GUAGUCCCAGCUACCAUGGG	3520
GCTTGGCGCTATACTAGGAC	427	TGG	-	GCUUGGCGCUAUACUAGGAC	3521
AAAAAAACAAGTCTGGGAAA	428	TGG	+	AAAAAAACAAGUCUGGGAAA	3522
TGCCCCCTCCCTCCTGAAGC	429	TGG	-	UGCCCCCUCCCUCCUGAAGC	3523
CTAAAATTACAAAAATGATC	430	CGG	-	CUAAAAUUACAAAAAUGAUC	3524
TCTCTCTGCTCACTTCCATA	431	AGG	+	UCUCUCUGCUCACUUCCAUA	3525
TCCTCTTTTTGGGAGACCAA	432	AGG	-	UCCUCUUUUUGGGAGACCAA	3526
TCACAGAAGCCATCTAAACA	433	GGG	+	UCACAGAAGCCAUCUAAACA	3527
AATGTTTACTGGGTGGGTGG	434	AGG	+	AAUGUUUACUGGGUGGGUGG	3528
GCGCACATGTCTCTTAGCCT	435	AGG	-	GCGCACAUGUCUCUUAGCCU	3529
TGCTGGTTTAAAGCACTGAT	436	GGG	-	UGCUGGUUUAAAGCACUGAU	3530

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GCCTTTGGTCTCCCAAAAAG	437	AGG	+	GCCUUUGGUCUCCCAAAAAG	3531
CAGTGCCAGATCCTCTTTTT	438	GGG	-	CAGUGCCAGAUCCUCUUUUU	3532
CGGGCAGATTACGCGTGGCA	439	GGG	-	CGGGCAGAUUACGCGUGGCA	3533
GAGAGTCTTAGGAGATGACG	440	AGG	-	GAGAGUCUUAGGAGAUGACG	3534
AAGGGGCAAGCTAGTGATAC	441	TGG	+	AAGGGGCAAGCUAGUGAUAC	3535
GTGGAGTTTGGCCAGGCTGT	442	CGG	+	GUGGAGUUUGGCCAGGCUGU	3536
CCCACTCTTCTCCGACAGCC	443	TGG	-	CCCACUCUUCUCCGACAGCC	3537
ATATGGCACCTGGACACACA	444	AGG	-	AUAUGGCACCUGGACACACA	3538
GCATTTGTCTGTAGACAGCC	445	TGG	-	GCAUUUGUCUGUAGACAGCC	3539
GTTGCCCAGGGGCTGGGGGA	446	GGG	-	GUUGCCCAGGGGCUGGGGGA	3540
AGATGCTTCTGGAACTCAAG	447	GGG	-	AGAUGCUUCUGGAACUCAAG	3541
GCTGGATTCTGACTACACTG	448	GGG	-	GCUGGAUUCUGACUACACUG	3542
AAAAAAAAAGTCATTGTTCT	449	TGG	+	AAAAAAAAAGUCAUUGUUCU	3543
AGGCCTGGGGGTGGAGCCCG	450	AGG	-	AGGCCUGGGGGUGGAGCCCG	3544
ATTAATGTAGCAAAGTGGAC	451	AGG	-	AUUAAUGUAGCAAAGUGGAC	3545
GCCCTGCAACGTGGGCCAGT	452	GGG	+	GCCCUGCAACGUGGGCCAGU	3546
TTCTCTTACACCGCACATAT	453	TGG	+	UUCUCUUACACCGCACAUAU	3547
AAAAATAGGTTTAAAAATAT	454	TGG	+	AAAAAUAGGUUUAAAAAUAU	3548
CACAACAACTTCGGCCTCCT	455	GGG	-	CACAACAACUUCGGCCUCCU	3549
GCAAGGTTTTATTGAGTGGA	456	AGG	-	GCAAGGUUUUAUUGAGUGGA	3550
TATCTCAGGGTTCTTGCCTT	457	GGG	-	UAUCUCAGGGUUCUUGCCUU	3551
ATGTCTGGGAAGGCCGGGTG	458	TGG	+	AUGUCUGGGAAGGCCGGGUG	3552
TGCATCTTTCCCATGTCTAT	459	GGG	-	UGCAUCUUUCCCAUGUCUAU	3553
TGTGAAAGTGTTACTATTTT	460	AGG	-	UGUGAAAGUGUUACUAUUUU	3554
AAGAAAGAGAATAGATAGTA	461	TGG	-	AAGAAAGAGAAUAGAUAGUA	3555
CTAGCCTATGAGCTTTATGA	462	TGG	+	CUAGCCUAUGAGCUUUAUGA	3556
TCCACGCTGCTGAGGCAGTG	463	AGG	-	UCCACGCUGCUGAGGCAGUG	3557
TTTCTATGGCCAGCTTCAGG	464	AGG	+	UUUCUAUGGCCAGCUUCAGG	3558
GGGAAGGAACGCTCTATGGT	465	TGG	+	GGGAAGGAACGCUCUAUGGU	3559
GGGCAGATTACGCGTGGCAG	466	GGG	-	GGGCAGAUUACGCGUGGCAG	3560
CTTGCCATCATAAAGCTCAT	467	AGG	-	CUUGCCAUCAUAAAGCUCAU	3561
GAGTTTTGATAGGCACAGGA	468	TGG	-	GAGUUUUGAUAGGCACAGGA	3562
GCAAGAAATGGGTCCAAAAT	469	TGG	+	GCAAGAAAUGGGUCCAAAAU	3563
GGATGGGACAGAGCATCTCC	470	TGG	+	GGAUGGGACAGAGCAUCUCC	3564
GTTCCGCCGGTTGATGGCCT	471	GGG	-	GUUCCGCCGGUUGAUGGCCU	3565
TTTTTACACTCAGGTTTAGT	472	CGG	+	UUUUUACACUCAGGUUUAGU	3566
TGCCCTGCAACGTGGGCCAG	473	TGG	+	UGCCCUGCAACGUGGGCCAG	3567
TGGGCCAGTGGGAAGGTTTG	474	GGG	+	UGGGCCAGUGGGAAGGUUUG	3568
GACAAATGCTTTCTGCGGGA	475	GGG	+	GACAAAUGCUUUCUGCGGGA	3569
CAGATTACGCGTGGCAGGGG	476	TGG	-	CAGAUUACGCGUGGCAGGGG	3570
GCCCTGCAGGAACTGGCTCC	477	CGG	+	GCCCUGCAGGAACUGGCUCC	3571
TCTTTAGCAGGTAGAGTACC	478	TGG	+	UCUUUAGCAGGUAGAGUACC	3572
CACCTACCAAGCCCGACTCC	479	AGG	+	CACCUACCAAGCCCGACUCC	3573
CTCCTGGAGTCGGGCTTGGT	480	AGG	-	CUCCUGGAGUCGGGCUUGGU	3574
GATGCTTTTCTCCTGGAGTC	481	GGG	-	GAUGCUUUUCUCCUGGAGUC	3575
ACTGACCTTCCTAGGTTTGA	482	TGG	+	ACUGACCUUCCUAGGUUUGA	3576
GCAGGCCATCAAACCTAGGA	483	AGG	-	GCAGGCCAUCAAACCUAGGA	3577
CTCATCCCTGACTATCTGCT	484	AGG	+	CUCAUCCCUGACUAUCUGCU	3578
GTGGGCCAGTGGGAAGGTTT	485	GGG	+	GUGGGCCAGUGGGAAGGUUU	3579
AGAATCGATTGAACCCAGGC	486	GGG	-	AGAAUCGAUUGAACCCAGGC	3580
ACGTCCTCTTTATAGGGAAT	487	GGG	+	ACGUCCUCUUUAUAGGGAAU	3581
CTATTTGGCAATTTATACAA	488	AGG	-	CUAUUUGGCAAUUUAUACAA	3582
CAAAAATCAGCTGGGCTTTG	489	TGG	-	CAAAAAUCAGCUGGGCUUUG	3583
GGCTGTGAGGCAGTTTCTGA	490	AGG	-	GGCUGUGAGGCAGUUUCUGA	3584
CGTACAAAAGGGGCCGGGCG	491	CGG	-	CGUACAAAAGGGGCCGGGCG	3585
CTTCTTATTTTCAAATCCAA	492	TGG	-	CUUCUUAUUUUCAAAUCCAA	3586
CGTTCCGCCGGTTGATGGCC	493	TGG	-	CGUUCCGCCGGUUGAUGGCC	3587
ACTACTACCACATTGGTGAC	494	TGG	+	ACUACUACCACAUUGGUGAC	3588

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CACAAGGGGCTTGAGGACCC	495	AGG	-	CACAAGGGGCUUGAGGACCC	3589
TTATAGGGAATGGGCTGAGC	496	AGG	+	UUAUAGGGAAUGGGCUGAGC	3590
CTTTAGCAGGTAGAGTACCT	497	GGG	+	CUUUAGCAGGUAGAGUACCU	3591
CCTAGACACTGCCGTGGGTT	498	TGG	+	CCUAGACACUGCCGUGGGUU	3592
GCTGTGGGGCTCCAAACCCA	499	CGG	-	GCUGUGGGGCUCCAAACCCA	3593
CATAGCGGGTGAGTAACCAG	500	TGG	+	CAUAGCGGGUGAGUAACCAG	3594
CTCTTAGCCTAGGAAGATTC	501	AGG	-	CUCUUAGCCUAGGAAGAUUC	3595
TAAAGAAATTGCCTTCACTT	502	AGG	+	UAAAGAAAUUGCCUUCACUU	3596
TTCATCCAACGCCTAAGTGA	503	AGG	-	UUCAUCCAACGCCUAAGUGA	3597
TGGCTGGATTCTGACTACAC	504	TGG	-	UGGCUGGAUUCUGACUACAC	3598
CTACTACCACATTGGTGACT	505	GGG	+	CUACUACCACAUUGGUGACU	3599
TACTTCGATGGGCTATGCAG	506	GGG	-	UACUUCGAUGGGCUAUGCAG	3600
TGTCCAGGTGCCATATTTGA	507	TGG	+	UGUCCAGGUGCCAUAUUUGA	3601
TGCATAAGGCCCATAGACAT	508	GGG	+	UGCAUAAGGCCCAUAGACAU	3602
GCCCAGGGGCTGGGGGAGGG	509	GGG	-	GCCCAGGGGCUGGGGGAGGG	3603
ACCTTGCAACCCATGAGGAA	510	AGG	-	ACCUUGCAACCCAUGAGGAA	3604
ATTACGCGTGGCAGGGGTGG	511	GGG	-	AUUACGCGUGGCAGGGGUGG	3605
TGTGTGTGTTGGGTGGGAGT	512	GGG	-	UGUGUGUGUUGGGUGGGAGU	3606
ATCATAATACATCTCAGAGG	513	TGG	+	AUCAUAAUACAUCUCAGAGG	3607
CTACCATCCTATGGCCACCA	514	GGG	-	CUACCAUCCUAUGGCCACCA	3608
AACCTCTTTGGAAAACTATT	515	TGG	-	AACCUCUUUGGAAAACUAUU	3609
CCCCTTGTGAGCAGAGAGTG	516	AGG	+	CCCCUUGUGAGCAGAGAGUG	3610
CATGAACCTTGAGATAAGTG	517	AGG	-	CAUGAACCUUGAGAUAAGUG	3611
CAGAAAAACTTCACACTGCT	518	AGG	+	CAGAAAAACUUCACACUGCU	3612
GGCCCATTTCAGGAGAGAAA	519	GGG	+	GGCCCAUUUCAGGAGAGAAA	3613
TTAAAGCACTGATGGGGTGC	520	AGG	-	UUAAAGCACUGAUGGGGUGC	3614
GCATATTCTGGAAAACTTTT	521	AGG	+	GCAUAUUCUGGAAAACUUUU	3615
CTGGAGCATCCCTCTGGCCC	522	AGG	-	CUGGAGCAUCCCUCUGGCCC	3616
CTCCCCCCTCCCCCAGCCCC	523	TGG	+	CUCCCCCCUCCCCCAGCCCC	3617
TCCCCCCTCCCCCAGCCCCT	524	GGG	+	UCCCCCCUCCCCCAGCCCCU	3618
TCCGTGGTTGCCCAGGGGCT	525	GGG	-	UCCGUGGUUGCCCAGGGGCU	3619
ATCCGTGGTTGCCCAGGGGC	526	TGG	-	AUCCGUGGUUGCCCAGGGGC	3620
CGACAAAAGACAGATTAATG	527	AGG	+	CGACAAAAGACAGAUUAAUG	3621
AAGATTAGACTGACCTTCCT	528	AGG	+	AAGAUUAGACUGACCUUCCU	3622
CTTGCTGAGGAAAGAGGAGG	529	GGG	-	CUUGCUGAGGAAAGAGGAGG	3623
GCAGAGAGAGAAGGAACGAC	530	AGG	-	GCAGAGAGAGAAGGAACGAC	3624
CTCTGCTCACAAGGGGCTTG	531	AGG	-	CUCUGCUCACAAGGGGCUUG	3625
GACAAAAGACAGATTAATGA	532	GGG	+	GACAAAAGACAGAUUAAUGA	3626
AAGTATGCAATGAATGTCAA	533	TGG	-	AAGUAUGCAAUGAAUGUCAA	3627
CCCCAGCCCCTGGGCAACCA	534	CGG	+	CCCCAGCCCCUGGGCAACCA	3628
GTGTTACTATTTTAGGCGAC	535	AGG	-	GUGUUACUAUUUUAGGCGAC	3629
AAAGCAGAGCATCTACATCG	536	GGG	-	AAAGCAGAGCAUCUACAUCG	3630
GGGTCTTACTCTTGTCGCCC	537	AGG	-	GGGUCUUACUCUUGUCGCCC	3631
TAAAACTTATTAAATAAGTA	538	TGG	-	UAAAACUUAUUAAAUAAGUA	3632
ATCTGTGGGCAAGAAAAAGG	539	TGG	+	AUCUGUGGGCAAGAAAAAGG	3633
GAGTACCTGGGAGAGAGGGT	540	GGG	+	GAGUACCUGGGAGAGAGGGU	3634
CCTCACTCTCTGCTCACAAG	541	GGG	-	CCUCACUCUCUGCUCACAAG	3635
AGCTGGGAACCTGAAGACAA	542	AGG	+	AGCUGGGAACCUGAAGACAA	3636
GCGGGCAGATTACGCGTGGC	543	AGG	-	GCGGGCAGAUUACGCGUGGC	3637
AACACAAGGAAGTAGGGAAC	544	AGG	-	AACACAAGGAAGUAGGGAAC	3638
CTCACCAGACCCAAATCTGC	545	TGG	-	CUCACCAGACCCAAAUCUGC	3639
GAAGGGAGATGCTTTTCTCC	546	TGG	-	GAAGGGAGAUGCUUUUCUCC	3640
AAATGGAGTTTTGGTGGTGT	547	AGG	+	AAAUGGAGUUUUGGUGGUGU	3641
TGAAGCAGGCCATCAAACCT	548	AGG	-	UGAAGCAGGCCAUCAAACCU	3642
AGGTTTGATGGCCTGCTTCA	549	GGG	+	AGGUUUGAUGGCCUGCUUCA	3643
CACCTCTTCTTCCCTGAAGC	550	AGG	-	CACCUCUUCUUCCCUGAAGC	3644
ACAGTGCCAGATCCTCTTTT	551	TGG	-	ACAGUGCCAGAUCCUCUUUU	3645
CTGTTCATCAAGAGATGCAA	552	AGG	-	CUGUUCAUCAAGAGAUGCAA	3646

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGCTGGGGGAGGGGGGAGAC	553	AGG	-	GGCUGGGGGAGGGGGGAGAC	3647
ATAATCTGTGGGCAAGAAAA	554	AGG	+	AUAAUCUGUGGGCAAGAAAA	3648
TTATTATTATATGCATCATA	555	GGG	+	UUAUUAUUAUAUGCAUCAUA	3649
TAAACCTATTTTTATAGAGA	556	TGG	-	UAAACCUAUUUUUAUAGAGA	3650
ATCTGCTGGCACCTTGATCG	557	TGG	-	AUCUGCUGGCACCUUGAUCG	3651
TTGTTTCTATGGCCAGCTTC	558	AGG	+	UUGUUUCUAUGGCCAGCUUC	3652
GAGGTGAGTGCAGTCATCCG	559	AGG	-	GAGGUGAGUGCAGUCAUCCG	3653
AAAAGCAGAGCATCTACATC	560	GGG	-	AAAAGCAGAGCAUCUACAUC	3654
CAAGCTAGTGATACTGGAGG	561	GGG	+	CAAGCUAGUGAUACUGGAGG	3655
AGGATAGAGCTGCCATTTGC	562	GGG	-	AGGAUAGAGCUGCCAUUUGC	3656
AGATTACGCGTGGCAGGGGT	563	GGG	-	AGAUUACGCGUGGCAGGGGU	3657
AGGTGAGTGCAGTCATCCGA	564	GGG	-	AGGUGAGUGCAGUCAUCCGA	3658
CCTAGAGTGGTGGAAGCTAA	565	AGG	+	CCUAGAGUGGUGGAAGCUAA	3659
CGATAGACGCCGGCAGCCCC	566	AGG	+	CGAUAGACGCCGGCAGCCCC	3660
CCCCGCTGCTCAAACCTCTG	567	GGG	-	CCCCGCUGCUCAAACCUCUG	3661
ACTTTTCCTCACTTATCTCA	568	AGG	+	ACUUUUCCUCACUUAUCUCA	3662
AATCAGCTGGGCTTTGTGGC	569	AGG	-	AAUCAGCUGGGCUUUGUGGC	3663
GCCAGTCCCAGCTTCTCTTG	570	AGG	+	GCCAGUCCCAGCUUCUCUUG	3664
AGGGGGGAGACAGGGAGAAT	571	GGG	-	AGGGGGGAGACAGGGAGAAU	3665
GTGTGTGTGTTGGGTGGGAG	572	TGG	-	GUGUGUGUGUUGGGUGGGAG	3666
GGGAGGATCATGAGAGTTGG	573	AGG	-	GGGAGGAUCAUGAGAGUUGG	3667
AAGTGTCCTTCAAGGGGTCA	574	TGG	-	AAGUGUCCUUCAAGGGGUCA	3668
TACCATCCTATGGCCACCAG	575	GGG	-	UACCAUCCUAUGGCCACCAG	3669
CCTACCATCCTATGGCCACC	576	AGG	-	CCUACCAUCCUAUGGCCACC	3670
CCAGCTACCATGGGAGGCTG	577	AGG	+	CCAGCUACCAUGGGAGGCUG	3671
CTGATTCTTCCGATACCCCA	578	AGG	+	CUGAUUCUUCCGAUACCCCA	3672
CGTGGTTGCCCAGGGGCTGG	579	GGG	-	CGUGGUUGCCCAGGGGCUGG	3673
GAGGATAGAGCTGCCATTTG	580	CGG	-	GAGGAUAGAGCUGCCAUUUG	3674
AGTCTGAGGCTGGGGCAACT	581	**GGG**	-	**AGUCUGAGGCUGGGGCAACU**	3675
AGATGCTTTTCTCCTGGAGT	582	CGG	-	AGAUGCUUUUCUCCUGGAGU	3676
CCCATGGTGAGTACTTCGAT	583	GGG	-	CCCAUGGUGAGUACUUCGAU	3677
CAACAATTAATGAGAAAGTA	584	TGG	+	CAACAAUUAAUGAGAAAGUA	3678
CAATTTGCCCTGGATGGTCC	585	TGG	-	CAAUUUGCCCUGGAUGGUCC	3679
CGTAACATACCCAGTACCAC	586	TGG	+	CGUAACAUACCCAGUACCAC	3680
TCTTGCTGAGGAAAGAGGAG	587	GGG	-	UCUUGCUGAGGAAAGAGGAG	3681
GGTCCACAACATTGTACCAG	588	AGG	+	GGUCCACAACAUUGUACCAG	3682
TCATAATACATCTCAGAGGT	589	GGG	+	UCAUAAUACAUCUCAGAGGU	3683
AGGGATGAGCTCTTAAAAGA	590	AGG	-	AGGGAUGAGCUCUUAAAAGA	3684
CCCAGGGTAGGGCACACAGT	591	AGG	-	CCCAGGGUAGGGCACACAGU	3685
TACAGACAAATGCTTTCTGC	592	GGG	+	UACAGACAAAUGCUUUCUGC	3686
TAAAGCGCGCCTTTCCTCAT	593	GGG	+	UAAAGCGCGCCUUUCCUCAU	3687
AGTGGCCCTTTTGGACCAAG	594	GGG	+	AGUGGCCCUUUUGGACCAAG	3688
CCTCACTGAGAAGGTGATGT	595	TGG	-	CCUCACUGAGAAGGUGAUGU	3689
GGAGAAAACACAAGGAAGTA	596	GGG	-	GGAGAAAACACAAGGAAGUA	3690
AAGCTAGTGATACTGGAGGG	597	GGG	+	AAGCUAGUGAUACUGGAGGG	3691
CGTTGAGAGGAGTGCGCCGG	598	CGG	+	CGUUGAGAGGAGUGCGCCGG	3692
TCTGGGAAATGGAGTTTTGG	599	TGG	+	UCUGGGAAAUGGAGUUUUGG	3693
TTGCCCAGGGGCTGGGGGAG	600	GGG	-	UUGCCCAGGGGCUGGGGGAG	3694
GGGGCGAATGTTTACAGCTG	601	AGG	-	GGGGCGAAUGUUUACAGCUG	3695
TCAAAATGTCTGGGAAGGCC	602	GGG	+	UCAAAAUGUCUGGGAAGGCC	3696
TGGCCCATTTCAGGAGAGAA	603	AGG	+	UGGCCCAUUUCAGGAGAGAA	3697
TTCTCAGTATTTGGAAAGAC	604	TGG	+	UUCUCAGUAUUUGGAAAGAC	3698
GGGTGGGGAGGTCACAGAAC	605	AGG	-	GGGUGGGGAGGUCACAGAAC	3699
TTCTGGACAGTTCATTTCAA	606	TGG	+	UUCUGGACAGUUCAUUUCAA	3700
AGAAAGAAGGAAATTGCTTC	607	TGG	-	AGAAAGAAGGAAAUUGCUUC	3701
GCAAGGTGGTCTAACCTCTT	608	TGG	-	GCAAGGUGGUCUAACCUCUU	3702
TTCCGCCGGTTGATGGCCTG	609	GGG	-	UUCCGCCGGUUGAUGGCCUG	3703
GCCCATGGTGAGTACTTCGA	610	TGG	-	GCCCAUGGUGAGUACUUCGA	3704

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGGGCAAACATTGTGTATGA	611	CGG	+	GGGGCAAACAUUGUGUAUGA	3705
TAAGTATATTTACAGATTTG	612	TGG	+	UAAGUAUAUUUACAGAUUUG	3706
CCGTGGTTGCCCAGGGGCTG	613	GGG	-	CCGUGGUUGCCCAGGGGCUG	3707
AATCTGCCCGCAGATCCTGT	614	GGG	+	AAUCUGCCCGCAGAUCCUGU	3708
ACACAAGGAAGTAGGGAACA	615	GGG	-	ACACAAGGAAGUAGGGAACA	3709
GCCAGGCTGTCGGAGAAGAG	616	TGG	+	GCCAGGCUGUCGGAGAAGAG	3710
TTAATGTAGCAAAGTGGACA	617	GGG	-	UUAAUGUAGCAAAGUGGACA	3711
TGCAACGTGGGCCAGTGGGA	618	AGG	+	UGCAACGUGGGCCAGUGGGA	3712
AGCTCCCCAAACCTTCCCAC	619	TGG	-	AGCUCCCCAAACCUUCCCAC	3713
AGAGTGGCCCTTTTGGACCA	620	AGG	+	AGAGUGGCCCUUUUGGACCA	3714
ACTACATTAATGTAGCAAAG	621	TGG	-	ACUACAUUAAUGUAGCAAAG	3715
TGACTAGTCCCATGTGGTAT	622	TGG	-	UGACUAGUCCCAUGUGGUAU	3716
GATGCTATTAATAATTCTGT	623	TGG	+	GAUGCUAUUAAUAAUUCUGU	3717
CTGCCTCGGGCTCCACCCCC	624	AGG	+	CUGCCUCGGGCUCCACCCCC	3718
GGTTGATGGCCTGGGGCTGC	625	CGG	-	GGUUGAUGGCCUGGGGCUGC	3719
TAGTAAGATGTGGGGCCTTT	626	GGG	-	UAGUAAGAUGUGGGGCCUUU	3720
CAACTCCTGAATAGCACATT	627	GGG	+	CAACUCCUGAAUAGCACAUU	3721
CGTTAATCCATATTTAAACT	628	AGG	-	CGUUAAUCCAUAUUUAAACU	3722
GCTGGGGGAGGGGGGAGACA	629	GGG	-	GCUGGGGGAGGGGGGAGACA	3723
AGAAGGTTTCAGCGAAGACA	630	AGG	-	AGAAGGUUUCAGCGAAGACA	3724
GAAACGGAAGTCTGAGGCTG	631	GGG	-	GAAACGGAAGUCUGAGGCUG	3725
TCTACGGGAAGATATCCTGT	632	AGG	+	UCUACGGGAAGAUAUCCUGU	3726
GAAAAAAAAAAAAACAAGTC	633	TGG	+	GAAAAAAAAAAAAACAAGUC	3727
AGAAAAACTTCACACTGCTA	634	GGG	+	AGAAAAACUUCACACUGCUA	3728
TTCTTGCTGAGGAAAGAGGA	635	GGG	-	UUCUUGCUGAGGAAAGAGGA	3729
CAGGAATTGGCTCTGAAAGC	636	AGG	+	CAGGAAUUGGCUCUGAAAGC	3730
CAAAAGCAGAGCATCTACAT	637	CGG	-	CAAAAGCAGAGCAUCUACAU	3731
CCCGCTGCTCAAACCTCTGG	638	GGG	-	CCCGCUGCUCAAACCUCUGG	3732
CACACTTGCCGTGGCCAGCC	639	CGG	+	CACACUUGCCGUGGCCAGCC	3733
ACCTATTTTTATAGAGATGG	640	AGG	-	ACCUAUUUUUAUAGAGAUGG	3734
AGGAGGGAGGGGGCAGGAGC	641	AGG	+	AGGAGGGAGGGGGCAGGAGC	3735
GTCGATCCGTGGTTGCCCAG	642	GGG	-	GUCGAUCCGUGGUUGCCCAG	3736
CAAGTGGCTGGACGTGCAGA	643	AGG	+	CAAGUGGCUGGACGUGCAGA	3737
GGATTTCTCTTCTTTGTGAT	644	TGG	+	GGAUUUCUCUUCUUUGUGAU	3738
GCAATTAGGGTCTAGTTGGC	645	TGG	-	GCAAUUAGGGUCUAGUUGGC	3739
GGGGGGAGACAGGGAGAATG	646	GGG	-	GGGGGGAGACAGGGAGAAUG	3740
AGAGGGTGGGGCCTCAAAGC	647	AGG	+	AGAGGGUGGGGCCUCAAAGC	3741
CCATCCCACTGCCTGCTTTG	648	AGG	-	CCAUCCCACUGCCUGCUUUG	3742
AAGCAGGCAGTGGGATGGAG	649	AGG	+	AAGCAGGCAGUGGGAUGGAG	3743
CCCCAGAGGTTTGAGCAGCG	650	GGG	+	CCCCAGAGGUUUGAGCAGCG	3744
CAGGGCATGCAAGGGAGGTG	651	TGG	-	CAGGGCAUGCAAGGGAGGUG	3745
TTTTGTTCGTTATAGAATTT	652	TGG	-	UUUUGUUCGUUAUAGAAUUU	3746
AAATATGATTGGAGGATCAA	653	AGG	+	AAAUAUGAUUGGAGGAUCAA	3747
CTGCTGGTTTAAAGCACTGA	654	TGG	-	CUGCUGGUUUAAAGCACUGA	3748
GATTTTCTTGCTGAGGAAAG	655	AGG	-	GAUUUUCUUGCUGAGGAAAG	3749
CATTTGTCTGTAGACAGCCT	656	GGG	-	CAUUUGUCUGUAGACAGCCU	3750
ATCCGAGGGAGAGAAGATGC	657	TGG	-	AUCCGAGGGAGAGAAGAUGC	3751
CCAACATTGCGGTGTGAAGA	658	TGG	+	CCAACAUUGCGGUGUGAAGA	3752
GTAGTAAGATGTGGGGCCTT	659	TGG	-	GUAGUAAGAUGUGGGGCCUU	3753
TCAACTCCTGAATAGCACAT	660	TGG	+	UCAACUCCUGAAUAGCACAU	3754
AGAAAATAAATTAAAAAGCA	661	GGG	+	AGAAAAUAAAUUAAAAAGCA	3755
CCAAAGGCAAGGGGTGTTGG	662	TGG	-	CCAAAGGCAAGGGGUGUUGG	3756
TTGATGTTGACCTAACTGAC	663	AGG	+	UUGAUGUUGACCUAACUGAC	3757
CCTTCCGCCCGCCCCGCAAA	664	TGG	+	CCUUCCGCCCGCCCCGCAAA	3758
GCTGCCATTTGCGGGGCGGG	665	CGG	-	GCUGCCAUUUGCGGGGCGGG	3759
GGATAGAGCTGCCATTTGCG	666	GGG	-	GGAUAGAGCUGCCAUUUGCG	3760
AGCGAGGAACATCACACACT	667	GGG	+	AGCGAGGAACAUCACACACU	3761
AATATGATTGGAGGATCAAA	668	GGG	+	AAUAUGAUUGGAGGAUCAAA	3762

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ATTGAACCCAGGCGGGCGGA	669	AGG	-	AUUGAACCCAGGCGGGCGGA	3763
AAGTCTGAGGCTGGGGCAAC	670	TGG	-	AAGUCUGAGGCUGGGGCAAC	3764
GATTACGCGTGGCAGGGGTG	671	GGG	-	GAUUACGCGUGGCAGGGGUG	3765
TAATCTGCCCGCAGATCCTG	672	TGG	+	UAAUCUGCCCGCAGAUCCUG	3766
ATAGATAGTATGGAAAATTA	673	CGG	-	AUAGAUAGUAUGGAAAAUUA	3767
TGGAGAGGAGAGAAAGCCCC	674	TGG	+	UGGAGAGGAGAGAAAGCCCC	3768
AGAGGAGAGAAAGCCCCTGG	675	TGG	+	AGAGGAGAGAAAGCCCCUGG	3769
GGGCTATGCAGGGGCTGCAG	676	TGG	-	GGGCUAUGCAGGGGCUGCAG	3770
CCAGGGCAAATTGGGGTGTA	677	AGG	+	CCAGGGCAAAUUGGGGUGUA	3771
CGTGGGCCAGTGGGAAGGTT	678	TGG	+	CGUGGGCCAGUGGGAAGGUU	3772
TTGGTGATTAAATCATTGTG	679	TGG	+	UUGGUGAUUAAAUCAUUGUG	3773
TGTCTCTTGCTAGCTCAAAG	680	TGG	-	UGUCUCUUGCUAGCUCAAAG	3774
GGGCAAGCTAGTGATACTGG	681	AGG	+	GGGCAAGCUAGUGAUACUGG	3775
CTTCAATTAGGAAAAAATAA	682	GGG	+	CUUCAAUUAGGAAAAAAUAA	3776
TAGAAAATAAATTAAAAAGC	683	AGG	+	UAGAAAAUAAAUUAAAAAGC	3777
ACCTACTGTGTGCCCTACCC	684	TGG	+	ACCUACUGUGUGCCCUACCC	3778
GAGTGGCCCTTTTGGACCAA	685	GGG	+	GAGUGGCCCUUUUGGACCAA	3779
ATAATAAAGTGTCCTTCAAG	686	GGG	-	AUAAUAAAGUGUCCUUCAAG	3780
CTCCACCGTCAATAGCATTC	687	CGG	-	CUCCACCGUCAAUAGCAUUC	3781
ATGTTGCTGGTGGGGGTGCA	688	AGG	-	AUGUUGCUGGUGGGGGUGCA	3782
GTACTTCGATGGGCTATGCA	689	GGG	-	GUACUUCGAUGGGCUAUGCA	3783
CCCCAGCCATGCACAAAGAT	690	TGG	-	CCCCAGCCAUGCACAAAGAU	3784
AGTACCTGGGAGAGAGGGTG	691	GGG	+	AGUACCUGGGAGAGAGGGUG	3785
GTTTTGATAGGCACAGGATG	692	GGG	-	GUUUUGAUAGGCACAGGAUG	3786
TCTGTAACACACGCCTGCTG	693	GGG	+	UCUGUAACACACGCCUGCUG	3787
AATAGCAAAGTGGTTAAAGG	694	AGG	+	AAUAGCAAAGUGGUUAAAGG	3788
TATCATCAGAAAAGAAAGAA	695	AGG	+	UAUCAUCAGAAAAGAAAGAA	3789
GAATTTAAATTAATTAAAAT	696	TGG	-	GAAUUUAAAUUAAUUAAAAU	3790
CATATATTAGCAGAAACTGA	697	TGG	-	CAUAUAUUAGCAGAAACUGA	3791
GGTGGGGAGGTCACAGAACA	698	GGG	-	GGUGGGGAGGUCACAGAACA	3792
ACAAATGCTTTCTGCGGGAG	699	GGG	+	ACAAAUGCUUUCUGCGGGAG	3793
AGGGCCTCGTGGGCCATGTG	700	AGG	-	AGGGCCUCGUGGGCCAUGUG	3794
CCTGCGCAAAACGCTTTGGC	701	GGG	-	CCUGCGCAAAACGCUUUGGC	3795
CCGCTCCCCCACCCCACGAC	702	AGG	-	CCGCUCCCCCACCCCACGAC	3796
TAAGTACCATATACATAATG	703	TGG	-	UAAGUACCAUAUACAUAAUG	3797
GTGTGTTGGGTGGGAGTGGG	704	TGG	-	GUGUGUUGGGUGGGAGUGGG	3798
AAGAAAAAGGTGGACTGAAA	705	AGG	+	AAGAAAAAGGUGGACUGAAA	3799
CGTTTTTTAATGGATCCATG	706	TGG	+	CGUUUUUUAAUGGAUCCAUG	3800
AATTTGCCCTGGATGGTCCT	707	GGG	-	AAUUUGCCCUGGAUGGUCCU	3801
GGCTGGATTCTGACTACACT	708	GGG	-	GGCUGGAUUCUGACUACACU	3802
CTCTCCTGCGCAAAACGCTT	709	TGG	-	CUCUCCUGCGCAAAACGCUU	3803
ATCTCGGCTCACAACAACTT	710	CGG	-	AUCUCGGCUCACAACAACUU	3804
TTTTGATAGGCACAGGATGG	711	GGG	-	UUUUGAUAGGCACAGGAUGG	3805
AAAGTGGACAGGGCAGGGAG	712	AGG	-	AAAGUGGACAGGGCAGGGAG	3806
AAGAGGTGAAAGGAATTCGA	713	TGG	+	AAGAGGUGAAAGGAAUUCGA	3807
AGACCAAAGGCAAGGGGTGT	714	TGG	-	AGACCAAAGGCAAGGGGUGU	3808
AAGGTTGCATTGGACACTGC	715	TGG	-	AAGGUUGCAUUGGACACUGC	3809
ACCACACTGCTGTCTCTCCT	716	GGG	-	ACCACACUGCUGUCUCUCCU	3810
AATTAATATCGTTGATAAAT	717	AGG	+	AAUUAAUAUCGUUGAUAAAU	3811
ATGCTATTAATAATTCTGTT	718	GGG	+	AUGCUAUUAAUAAUUCUGUU	3812
AGGGCACCATCCAGCCTGTC	719	TGG	+	AGGGCACCAUCCAGCCUGUC	3813
TCATAGGCTAGTGTGTGTGT	720	TGG	-	UCAUAGGCUAGUGUGUGUGU	3814
GAAGATGGAAGGTCAGTGTT	721	GGG	+	GAAGAUGGAAGGUCAGUGUU	3815
CTGGGGGTGGAGCCCGAGGC	722	AGG	-	CUGGGGGUGGAGCCCGAGGC	3816
TTCTGTCCCTGTGAGAAGGC	723	AGG	+	UUCUGUCCCUGUGAGAAGGC	3817
GTATGTCACTGAAGAGAGTC	724	TGG	-	GUAUGUCACUGAAGAGAGUC	3818
AGTCGATCCGTGGTTGCCCA	725	GGG	-	AGUCGAUCCGUGGUUGCCCA	3819
CGGCGGAACGAGGTGGAGTT	726	TGG	+	CGGCGGAACGAGGUGGAGUU	3820

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TTGTTTAGAAATTATTTTAT	727	AGG	-	UUGUUUAGAAAUUAUUUUAU	3821
GAGGAGAAATTCTGAGCAGA	728	TGG	-	GAGGAGAAAUUCUGAGCAGA	3822
AAACATTGTGTATGACGGCG	729	GGG	+	AAACAUUGUGUAUGACGGCG	3823
TGGAGAAAACACAAGGAAGT	730	AGG	-	UGGAGAAAACACAAGGAAGU	3824
TGAAGATGGAAGGTCAGTGT	731	TGG	+	UGAAGAUGGAAGGUCAGUGU	3825
TTTAAAAATATTGGGTGTGG	732	TGG	+	UUUAAAAAUAUUGGGUGUGG	3826
TAGAAGGCTCCTCACAGAAC	733	TGG	-	UAGAAGGCUCCUCACAGAAC	3827
GGTGTAACCCAGGACCATCC	734	AGG	+	GGUGUAACCCAGGACCAUCC	3828
TTTCTTGCTGAGGAAAGAGG	735	AGG	-	UUUCUUGCUGAGGAAAGAGG	3829
TCTCTTCTTTGTGATTGGCA	736	TGG	+	UCUCUUCUUUGUGAUUGGCA	3830
AATCGTCTGATTGCTGGAGA	737	GGG	+	AAUCGUCUGAUUGCUGGAGA	3831
CATTGCGGTGTGAAGATGGA	738	AGG	+	CAUUGCGGUGUGAAGAUGGA	3832
TGTCGTGGGGTGGGGGAGCG	739	GGG	+	UGUCGUGGGGUGGGGGAGCG	3833
GCAAGCTAGTGATACTGGAG	740	GGG	+	GCAAGCUAGUGAUACUGGAG	3834
AGTTTTGATAGGCACAGGAT	741	GGG	-	AGUUUUGAUAGGCACAGGAU	3835
TCACCATGGGCATGGTCCAG	742	TGG	+	UCACCAUGGGCAUGGUCCAG	3836
GGGTACTTGAGAATTGAGAG	743	AGG	-	GGGUACUUGAGAAUUGAGAG	3837
TTTCACTATTTTTCCTTTAG	744	AGG	+	UUUCACUAUUUUUCCUUUAG	3838
GGAACAGGGACTGGTCCTTT	745	TGG	-	GGAACAGGGACUGGUCCUUU	3839
TGTGTTGGGTGGGAGTGGGT	746	GGG	-	UGUGUUGGGUGGGAGUGGGU	3840
ACAAGCGGCTAGATGTCGAG	747	AGG	+	ACAAGCGGCUAGAUGUCGAG	3841
GTGAGTAACCAGTGGAACAA	748	GGG	+	GUGAGUAACCAGUGGAACAA	3842
TTCATCCAGATGTTAGAAAT	749	TGG	+	UUCAUCCAGAUGUUAGAAAU	3843
CGTCTGATTGCTGGAGAGGG	750	AGG	+	CGUCUGAUUGCUGGAGAGGG	3844
GAGGGGGGAGACAGGGAGAA	751	TGG	-	GAGGGGGGAGACAGGGAGAA	3845
TCCTGCGCAAAACGCTTTGG	752	CGG	-	UCCUGCGCAAAACGCUUUGG	3846
AGTTTACCAATCTTTGTGCA	753	TGG	+	AGUUUACCAAUCUUUGUGCA	3847
GCATCCCTCTGGCCCAGGGT	754	AGG	-	GCAUCCCUCUGGCCCAGGGU	3848
GTGTTGGGTGGGAGTGGGTG	755	GGG	-	GUGUUGGGUGGGAGUGGGUG	3849
GAGGCCCCACCCTCTCTCCC	756	AGG	-	GAGGCCCCACCCUCUCUCCC	3850
TCTATCGTGCGCTCTTCCGC	757	CGG	-	UCUAUCGUGCGCUCUUCCGC	3851
TCATTAATTGTTGCAACTTA	758	TGG	-	UCAUUAAUUGUUGCAACUUA	3852
CCAATCCAAGAGACTAAACT	759	AGG	+	CCAAUCCAAGAGACUAAACU	3853
GAACAGGGACTGGTCCTTTT	760	GGG	-	GAACAGGGACUGGUCCUUUU	3854
GAGACAAGTCTCTCTTTAGC	761	AGG	+	GAGACAAGUCUCUCUUUAGC	3855
GGTGAGTAACCAGTGGAACA	762	AGG	+	GGUGAGUAACCAGUGGAACA	3856
TGACCCTTTCTCTCCTGAAA	763	TGG	-	UGACCCUUUCUCUCCUGAAA	3857
TGAGAAAGTATGGCATATTC	764	TGG	+	UGAGAAAGUAUGGCAUAUUC	3858
ATTTGTCTGTAGACAGCCTG	765	GGG	-	AUUUGUCUGUAGACAGCCUG	3859
AAGGGGGAGCCCAGACAGGC	766	TGG	-	AAGGGGGAGCCCAGACAGGC	3860
CTGAGGCTGGGGCAACTGGG	767	AGG	-	CUGAGGCUGGGGCAACUGGG	3861
GACGGAGGCAAGAGACAGTC	768	TGG	+	GACGGAGGCAAGAGACAGUC	3862
AAGGGCTGTTTTTGTTTCTA	769	TGG	+	AAGGGCUGUUUUUGUUUCUA	3863
GAACAACAGTTGTCTTTGTG	770	TGG	+	GAACAACAGUUGUCUUUGUG	3864
AATCCAACAGAAAACAGGAT	771	GGG	+	AAUCCAACAGAAAACAGGAU	3865
CTGACTCAATTATAAGACAT	772	AGG	-	CUGACUCAAUUAUAAGACAU	3866
CCATGGGAAGGAACGCTCTA	773	TGG	+	CCAUGGGAAGGAACGCUCUA	3867
CGCAGGAGAGTGGCGGCATC	774	AGG	+	CGCAGGAGAGUGGCGGCAUC	3868
GGCAAGCTAGTGATACTGGA	775	GGG	+	GGCAAGCUAGUGAUACUGGA	3869
TTTAGTTTGCCGTCTGTAGG	776	CGG	-	UUUAGUUUGCCGUCUGUAGG	3870
ATGGAGTTTTGGTGGTGTAG	777	GGG	+	AUGGAGUUUUGGUGGUGUAG	3871
TGTAGCAAAGTGGACAGGGC	778	AGG	-	UGUAGCAAAGUGGACAGGGC	3872
AAATAATGATTATCTTTTTT	779	TGG	+	AAAUAAUGAUUAUCUUUUUU	3873
GACCCTTTCTCTCCTGAAAT	780	GGG	-	GACCCUUUCUCUCCUGAAAU	3874
GTGAAAGAAACGGAAGTCTG	781	AGG	-	GUGAAAGAAACGGAAGUCUG	3875
GGGTGGGGGTGTGAAAGAAA	782	CGG	-	GGGUGGGGGUGUGAAAGAAA	3876
GCATCATAGGGGTGTGGAAA	783	TGG	+	GCAUCAUAGGGGUGUGGAAA	3877
ATCTGTGTAAAAGAGACTCC	784	AGG	-	AUCUGUGUAAAAGAGACUCC	3878

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGCTATGCAGGGGCTGCAGT	785	GGG	-	GGCUAUGCAGGGGCUGCAGU	3879
AAGTCTGGGAAATGGAGTTT	786	TGG	+	AAGUCUGGGAAAUGGAGUUU	3880
GGAATTCGATGGCCCATTTC	787	AGG	+	GGAAUUCGAUGGCCCAUUUC	3881
TTATCTTACTTGCTTAACGC	788	AGG	+	UUAUCUUACUUGCUUAACGC	3882
GCTGTGAGGCAGTTTCTGAA	789	GGG	-	GCUGUGAGGCAGUUUCUGAA	3883
CGGATGTCTCATGTAACATG	790	TGG	+	CGGAUGUCUCAUGUAACAUG	3884
GGGCACCATCCAGCCTGTCT	791	GGG	+	GGGCACCAUCCAGCCUGUCU	3885
TTATATGCATCATAGGGGTG	792	TGG	+	UUAUAUGCAUCAUAGGGGUG	3886
CTACCATCAAATATGGCACC	793	TGG	-	CUACCAUCAAAUAUGGCACC	3887
TTATTGTATTTCTTGTAGAG	794	AGG	+	UUAUUGUAUUUCUUGUAGAG	3888
TGCCCCCTAGCAGATAGTCA	795	GGG	-	UGCCCCCUAGCAGAUAGUCA	3889
TTTCTGCGGGAGGGGAGTCT	796	GGG	+	UUUCUGCGGGAGGGGAGUCU	3890
TGGCGGGTCCCTGTTGCCAG	797	GGG	-	UGGCGGGUCCCUGUUGCCAG	3891
TTTGTTCAAAATGTCTGGGA	798	AGG	+	UUUGUUCAAAAUGUCUGGGA	3892
TGGCTGGGGGCTGCTAGTCC	799	AGG	-	UGGCUGGGGGCUGCUAGUCC	3893
CCAGCTCGATTTCAACACTG	800	GGG	+	CCAGCUCGAUUUCAACACUG	3894
CAAACTCCACCTCGTTCCGC	801	CGG	-	CAAACUCCACCUCGUUCCGC	3895
TCTGTGTAAAAGAGACTCCA	802	GGG	-	UCUGUGUAAAAGAGACUCCA	3896
GTGTAACCCAGGACCATCCA	803	GGG	+	GUGUAACCCAGGACCAUCCA	3897
TAAATTAAAAAGCAGGGAGA	804	AGG	+	UAAAUUAAAAAGCAGGGAGA	3898
GCTCTTAAAAGAAGGGACCA	805	AGG	-	GCUCUUAAAAGAAGGGACCA	3899
TGTGCCCTACCCTGGGCCAG	806	AGG	+	UGUGCCCUACCCUGGGCCAG	3900
ATTATAAGACATAGGAAGCC	807	AGG	-	AUUAUAAGACAUAGGAAGCC	3901
AATGAATTTGAGACGCCTAG	808	GGG	-	AAUGAAUUUGAGACGCCUAG	3902
AAACACTTGGAGACAGAGCG	809	AGG	+	AAACACUUGGAGACAGAGCG	3903
GTCTGCCCAATGTGCTATTC	810	AGG	-	GUCUGCCCAAUGUGCUAUUC	3904
TCATCCCTGACTATCTGCTA	811	GGG	+	UCAUCCCUGACUAUCUGCUA	3905
AGATAAGAAATAGAGAAAAG	812	AGG	+	AGAUAAGAAAUAGAGAAAAG	3906
AAGAAGTTGGCTGGGTGCAG	813	TGG	+	AAGAAGUUGGCUGGGUGCAG	3907
GCAAACATTGTGTATGACGG	814	CGG	+	GCAAACAUUGUGUAUGACGG	3908
GGCCCACGTTGCAGGGCACC	815	TGG	-	GGCCCACGUUGCAGGGCACC	3909
ACCTAGAGTCCAAGGAAGAG	816	AGG	-	ACCUAGAGUCCAAGGAAGAG	3910
GTCTAGAACTACATGCCACA	817	GGG	-	GUCUAGAACUACAUGCCACA	3911
TTTTCTCCTGGAGTCGGGCT	818	TGG	-	UUUUCUCCUGGAGUCGGGCU	3912
ATTCCTTCCCTCCGGGTATA	819	GGG	+	AUUCCUUCCCUCCGGGUAUA	3913
CAAACATTGTGTATGACGGC	820	GGG	+	CAAACAUUGUGUAUGACGGC	3914
CTTTCATAACCAAATCCACT	821	TGG	-	CUUUCAUAACCAAAUCCACU	3915
TGTCACAGCAGGAAATTTCA	822	AGG	-	UGUCACAGCAGGAAAUUUCA	3916
CCTCAGCTCACCATTTGCTC	823	TGG	-	CCUCAGCUCACCAUUUGCUC	3917
GGTGGGAGGATCATGAGAGT	824	TGG	-	GGUGGGAGGAUCAUGAGAGU	3918
GGACTCAGCCCCTGGCAACA	825	GGG	+	GGACUCAGCCCCUGGCAACA	3919
TGGGGCCTCAAAGCAGGCAG	826	TGG	+	UGGGGCCUCAAAGCAGGCAG	3920
CGTGGGGTGGGGGAGCGGGG	827	AGG	+	CGUGGGGUGGGGGAGCGGGG	3921
TTCTATGGCCAGCTTCAGGA	828	GGG	+	UUCUAUGGCCAGCUUCAGGA	3922
AGTAAGATATTTTAATACGA	829	TGG	-	AGUAAGAUAUUUUAAUACGA	3923
ACTGAAAAGGCCAGAGCAAA	830	TGG	+	ACUGAAAAGGCCAGAGCAAA	3924
CTGGTGGCCATAGGATGGTA	831	GGG	+	CUGGUGGCCAUAGGAUGGUA	3925
GAGAAAAGAGGCCAGGTGCA	832	GGG	+	GAGAAAAGAGGCCAGGUGCA	3926
TCTTTTAGTTTGCCGTCTGT	833	AGG	-	UCUUUUAGUUUGCCGUCUGU	3927
TGGTACTTATACTTTGATTG	834	TGG	+	UGGUACUUAUACUUUGAUUG	3928
CTTTCTGCGGGAGGGGAGTC	835	TGG	+	CUUUCUGCGGGAGGGGAGUC	3929
TAATGAATTTGAGACGCCTA	836	GGG	-	UAAUGAAUUUGAGACGCCUA	3930
TCACTGCCTCAGCAGCGTGG	837	AGG	+	UCACUGCCUCAGCAGCGUGG	3931
AAAAAAAGTGGTTGCAGAAC	838	AGG	+	AAAAAAAGUGGUUGCAGAAC	3932
TCTGGGAAGGCCGGGTGTGG	839	TGG	+	UCUGGGAAGGCCGGGUGUGG	3933
CCAGCCAAAAATAGCCAGTG	840	TGG	+	CCAGCCAAAAAUAGCCAGUG	3934
CCACACTGGCTATTTTTGGC	841	TGG	-	CCACACUGGCUAUUUUUGGC	3935
CAATTAGGGTCTAGTTGGCT	842	GGG	-	CAAUUAGGGUCUAGUUGGCU	3936

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TGCCAGCTCGATTTCAACAC	843	TGG	+	UGCCAGCUCGAUUUCAACAC	3937
GAGCTAGCAAGAGACATCTA	844	CGG	+	GAGCUAGCAAGAGACAUCUA	3938
ACTTTTTTTGTGTCCACTGT	845	AGG	-	ACUUUUUUUGUGUCCACUGU	3939
TTAAATAGCAAAGTGGTTAA	846	AGG	+	UUAAAUAGCAAAGUGGUUAA	3940
TGAGGAAAGAGGAGGGGGCA	847	AGG	-	UGAGGAAAGAGGAGGGGGCA	3941
GGGGCCTCAAAGCAGGCAGT	848	GGG	+	GGGGCCUCAAAGCAGGCAGU	3942
ATAGCAAAGTGGTTAAAGGA	849	GGG	+	AUAGCAAAGUGGUUAAAGGA	3943
ATTGTATTTCTTGTAGAGAG	850	GGG	+	AUUGUAUUUCUUGUAGAGAG	3944
AGGGCTTCCCTAAGCCCAAA	851	AGG	+	AGGGCUUCCCUAAGCCCAAA	3945
CAGGCTGTCGGAGAAGAGTG	852	GGG	+	CAGGCUGUCGGAGAAGAGUG	3946
TTCAAAATGTCTGGGAAGGC	853	CGG	+	UUCAAAAUGUCUGGGAAGGC	3947
TGATGTTGACCTAACTGACA	854	GGG	+	UGAUGUUGACCUAACUGACA	3948
AATCTAAGATTGGTGACTGG	855	CGG	+	AAUCUAAGAUUGGUGACUGG	3949
CCATTTGCGGGGCGGGCGGA	856	AGG	-	CCAUUUGCGGGGCGGGCGGA	3950
AGTACTTCGATGGGCTATGC	857	AGG	-	AGUACUUCGAUGGGCUAUGC	3951
GGTTGTTAGACTCAGCACTG	858	TGG	-	GGUUGUUAGACUCAGCACUG	3952
CCTCTCGTGAAAGAGAAAGA	859	AGG	-	CCUCUCGUGAAAGAGAAAGA	3953
AGGTAGAGTACCTGGGAGAG	860	AGG	+	AGGUAGAGUACCUGGGAGAG	3954
AGCTAGCAAGAGACATCTAC	861	GGG	+	AGCUAGCAAGAGACAUCUAC	3955
GACAGCAGTGTGGTGGCTGC	862	AGG	+	GACAGCAGUGUGGUGGCUGC	3956
TGAGACAGGCCGATCACTTG	863	AGG	-	UGAGACAGGCCGAUCACUUG	3957
TGACTACTTTAAGATGCTTT	864	GGG	+	UGACUACUUUAAGAUGCUUU	3958
TTGCCCCCTAGCAGATAGTC	865	AGG	-	UUGCCCCCUAGCAGAUAGUC	3959
AACGTCCTCTTTATAGGGAA	866	TGG	+	AACGUCCUCUUUAUAGGGAA	3960
CTCAGTTTCCTTGTGTGTCC	867	AGG	+	CUCAGUUUCCUUGUGUGUCC	3961
TTCCTTTCAAGTGCTGTTAA	868	AGG	-	UUCCUUUCAAGUGCUGUUAA	3962
CAAAACTCCAAGACCCTAGC	869	AGG	+	CAAAACUCCAAGACCCUAGC	3963
CCAGGCTGTCGGAGAAGAGT	870	GGG	+	CCAGGCUGUCGGAGAAGAGU	3964
GGGTGGCCCTGAATCTTCCT	871	AGG	+	GGGUGGCCCUGAAUCUUCCU	3965
AGCCTCACTCTCTGCTCACA	872	AGG	-	AGCCUCACUCUCUGCUCACA	3966
TATTTCCATTTTTTACACTC	873	AGG	+	UAUUUCCAUUUUUUACACUC	3967
TTTTTGTTATTACTTCTCTT	874	TGG	+	UUUUUGUUAUUACUUCUCUU	3968
GATGGCTTTCCAGTTCTGTG	875	AGG	+	GAUGGCUUUCCAGUUCUGUG	3969
TTCTTCGCATCGCGGGGGTG	876	GGG	-	UUCUUCGCAUCGCGGGGGUG	3970
ACGATGTTCTCTGCTCTTCG	877	TGG	-	ACGAUGUUCUCUGCUCUUCG	3971
CTTCATCTGTTTCCATGGGA	878	AGG	+	CUUCAUCUGUUUCCAUGGGA	3972
TCAACACTGGGGTGTCCCCT	879	AGG	+	UCAACACUGGGGUGUCCCCU	3973
GGGTCTATGCTATGTTACCC	880	AGG	-	GGGUCUAUGCUAUGUUACCC	3974
AGAGTACCTGGGAGAGAGGG	881	TGG	+	AGAGUACCUGGGAGAGAGGG	3975
AGGAGCCTTCTAAAGTTCAA	882	AGG	+	AGGAGCCUUCUAAAGUUCAA	3976
GCTTCAATTAGGAAAAAATA	883	AGG	+	GCUUCAAUUAGGAAAAAAUA	3977
ATAAATCTAAGATTGGTGAC	884	TGG	+	AUAAAUCUAAGAUUGGUGAC	3978
ACTCAAGGGGGAGCCCAGAC	885	AGG	-	ACUCAAGGGGGAGCCCAGAC	3979
GTGGAACTTGAGGATAGACG	886	AGG	-	GUGGAACUUGAGGAUAGACG	3980
GTAGCAAAGTGGACAGGGCA	887	GGG	-	GUAGCAAAGUGGACAGGGCA	3981
AGGAGAAATTCTGAGCAGAT	888	GGG	-	AGGAGAAAUUCUGAGCAGAU	3982
AATAATAAAGTGTCCTTCAA	889	GGG	-	AAUAAUAAAGUGUCCUUCAA	3983
TTGGCGGGTCCCTGTTGCCA	890	GGG	-	UUGGCGGGUCCCUGUUGCCA	3984
AACAGTTGTCTTTGTGTGGA	891	TGG	+	AACAGUUGUCUUUGUGUGGA	3985
CTACAGACAAATGCTTTCTG	892	CGG	+	CUACAGACAAAUGCUUUCUG	3986
TGTAGGCTTGGCGCTATACT	893	AGG	-	UGUAGGCUUGGCGCUAUACU	3987
TTGTGGCACAGTGAGGGAAC	894	TGG	+	UUGUGGCACAGUGAGGGAAC	3988
CTTAAAGTAGTCAGAGTTAC	895	TGG	-	CUUAAAGUAGUCAGAGUUAC	3989
CTTGTAGTCCCAGCTACCAT	896	GGG	+	CUUGUAGUCCCAGCUACCAU	3990
GAGCGAGGAACATCACACAC	897	TGG	+	GAGCGAGGAACAUCACACAC	3991
CTCCAGGGAGCTAGTGAAAC	898	AGG	-	CUCCAGGGAGCUAGUGAAAC	3992
CTCCTGTTTCTCAAGCAGAT	899	TGG	+	CUCCUGUUUCUCAAGCAGAU	3993
GGCGCGCTTTAGATACACAC	900	AGG	-	GGCGCGCUUUAGAUACACAC	3994

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CATAGGCTAGTGTGTGTGTT	901	GGG	-	CAUAGGCUAGUGUGUGUGUU	3995
TAGAACTACATGCCACAGGG	902	TGG	-	UAGAACUACAUGCCACAGGG	3996
CATCTCAGGGCATGCAAGGG	903	AGG	-	CAUCUCAGGGCAUGCAAGGG	3997
CGGAATGCTATTGACGGTGG	904	AGG	+	CGGAAUGCUAUUGACGGUGG	3998
TTCGCATCGCGGGGGTGGGG	905	AGG	-	UUCGCAUCGCGGGGGUGGGG	3999
ATCAGCAATTAGGGTCTAGT	906	TGG	-	AUCAGCAAUUAGGGUCUAGU	4000
ATTTGCGGGGCGGGCGGAAG	907	GGG	-	AUUUGCGGGGCGGGCGGAAG	4001
GGTGGAAGCTAAAGGAAGAT	908	GGG	+	GGUGGAAGCUAAAGGAAGAU	4002
ACTTCTTCGCATCGCGGGGG	909	TGG	-	ACUUCUUCGCAUCGCGGGGG	4003
CTCCTCATTACAGTGAAATA	910	AGG	+	CUCCUCAUUACAGUGAAAUA	4004
GGAGAAAAGCATCTCCCTTC	911	TGG	+	GGAGAAAAGCAUCUCCCUUC	4005
TGGTGATTAAATCATTGTGT	912	GGG	+	UGGUGAUUAAAUCAUUGUGU	4006
TTTTATAATTGTGATGATAA	913	AGG	+	UUUUAUAAUUGUGAUGAUAA	4007
ATTTAAATTCCAATACCACA	914	TGG	+	AUUUAAAUUCCAAUACCACA	4008
TGATTGTGTCCTCTGTCCCC	915	TGG	-	UGAUUGUGUCCUCUGUCCCC	4009
ATAATGAATTTGAGACGCCT	916	AGG	-	AUAAUGAAUUUGAGACGCCU	4010
TGAGAAACATAAATCCCGAG	917	AGG	+	UGAGAAACAUAAAUCCCGAG	4011
GTGCCCTACCCTGGGCCAGA	918	GGG	+	GUGCCCUACCCUGGGCCAGA	4012
TGAAATGAACTGTCCAGAAC	919	TGG	-	UGAAAUGAACUGUCCAGAAC	4013
CCATAGAGCGTTCCTTCCCA	920	TGG	-	CCAUAGAGCGUUCCUUCCCA	4014
ATGTCTCATGTAACATGTGG	921	TGG	+	AUGUCUCAUGUAACAUGUGG	4015
TATTATTATATGCATCATAG	922	GGG	+	UAUUAUUAUAUGCAUCAUAG	4016
AGAGAAAAGAGGCCAGGTGC	923	AGG	+	AGAGAAAAGAGGCCAGGUGC	4017
AGCAATGTTTACTGGGTGGG	924	TGG	+	AGCAAUGUUUACUGGGUGGG	4018
CAGTGTTGGGAAGAGTCCAT	925	TGG	+	CAGUGUUGGGAAGAGUCCAU	4019
TGGGACGACAGGTGTAACCC	926	AGG	+	UGGGACGACAGGUGUAACCC	4020
TTCAAAGATTGAGAGGGAAT	927	TGG	-	UUCAAAGAUUGAGAGGGAAU	4021
GGGCCAGAGGGATGCTCCAG	928	GGG	+	GGGCCAGAGGGAUGCUCCAG	4022
CATTTGCGGGGCGGGCGGAA	929	GGG	-	CAUUUGCGGGGCGGGCGGAA	4023
CACTGCTAGGGTTAAATGAA	930	AGG	+	CACUGCUAGGGUUAAAUGAA	4024
AATACACATAGAAAAATGAC	931	AGG	+	AAUACACAUAGAAAAAUGAC	4025
AGACCCATCCTGTTTTCTGT	932	TGG	-	AGACCCAUCCUGUUUUCUGU	4026
CTATGCTATGTTACCCAGGC	933	TGG	-	CUAUGCUAUGUUACCCAGGC	4027
TTGCTGGTGGGGGTGCAAGG	934	TGG	-	UUGCUGGUGGGGGUGCAAGG	4028
CACTGGTTACTCACCCGCTA	935	TGG	-	CACUGGUUACUCACCCGCUA	4029
CACCACACTGCTGTCTCTCC	936	TGG	-	CACCACACUGCUGUCUCUCC	4030
GGGACTAGTCATGACTACAT	937	TGG	+	GGGACUAGUCAUGACUACAU	4031
TTATTAGATTCCAGTGGTAC	938	TGG	-	UUAUUAGAUUCCAGUGGUAC	4032
CTACTTAGAAATATACCTTG	939	GGG	-	CUACUUAGAAAUAUACCUUG	4033
TCGAACAATAGACTTTCAAT	940	GGG	-	UCGAACAAUAGACUUUCAAU	4034
GACAGGGAGAATGGGGAGCG	941	AGG	-	GACAGGGAGAAUGGGGAGCG	4035
CCAACATCACCTTCTCAGTG	942	AGG	+	CCAACAUCACCUUCUCAGUG	4036
CCTCAAAGCAGGCAGTGGGA	943	TGG	+	CCUCAAAGCAGGCAGUGGGA	4037
ATCGGGGTATGTTTACACAA	944	TGG	-	AUCGGGGUAUGUUUACACAA	4038
TTTCGTACAATACTGGAGGC	945	TGG	+	UUUCGUACAAUACUGGAGGC	4039
TTCGCCCCTAGACACTGCCG	946	TGG	+	UUCGCCCCUAGACACUGCCG	4040
TGCCCTGAGATGGGGACAAG	947	GGG	+	UGCCCUGAGAUGGGGACAAG	4041
GGGAGCTAGTGAAACAGGAA	948	AGG	-	GGGAGCUAGUGAAACAGGAA	4042
AATCTTAAATCATCTAATTG	949	TGG	+	AAUCUUAAAUCAUCUAAUUG	4043
AAATCCAACAGAAAACAGGA	950	TGG	+	AAAUCCAACAGAAAACAGGA	4044
ATGTAAACCAGTTGTCTGTT	951	AGG	+	AUGUAAACCAGUUGUCUGUU	4045
ACCAAATCCACTTGGCTCAA	952	AGG	-	ACCAAAUCCACUUGGCUCAA	4046
ATCGAACAATAGACTTTCAA	953	TGG	-	AUCGAACAAUAGACUUUCAA	4047
AGGCTAGTGTGTGTGTTGGG	954	TGG	-	AGGCUAGUGUGUGUGUUGGG	4048
GGGACTCAGCCCCTGGCAAC	955	AGG	+	GGGACUCAGCCCCUGGCAAC	4049
TTTGGCGGGTCCCTGTTGCC	956	AGG	-	UUUGGCGGGUCCCUGUUGCC	4050
TTAGCTGAGCGTGGCAGCGC	957	AGG	+	UUAGCUGAGCGUGGCAGCGC	4051
AGTCTAGAACTACATGCCAC	958	AGG	-	AGUCUAGAACUACAUGCCAC	4052

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TGTCGAGAGGAGCACATTGG	959	CGG	+	UGUCGAGAGGAGCACAUUGG	4053
TGTGGTAGTAGTAAGATGTG	960	GGG	-	UGUGGUAGUAGUAAGAUGUG	4054
ACTGCTAGGGTTAAATGAAA	961	GGG	+	ACUGCUAGGGUUAAAUGAAA	4055
AAAAAATCCTAACAGACAAC	962	TGG	-	AAAAAAUCCUAACAGACAAC	4056
TGTGGCACAGTGAGGGAACT	963	GGG	+	UGUGGCACAGUGAGGGAACU	4057
TAATTAAAATTGGCCGGGCG	964	GGG	-	UAAUUAAAAUUGGCCGGGCG	4058
CCAAACCGGAATGCTATTGA	965	CGG	+	CCAAACCGGAAUGCUAUUGA	4059
TCTATTTCTTATCTCCTGAG	966	TGG	-	UCUAUUUCUUAUCUCCUGAG	4060
TCTGTCCCTGTGAGAAGGCA	967	GGG	+	UCUGUCCCUGUGAGAAGGCA	4061
CTCGGCTTCATCTGTTTCCA	968	TGG	+	CUCGGCUUCAUCUGUUUCCA	4062
GAGAGTTGGAGGCTGCAGTG	969	AGG	-	GAGAGUUGGAGGCUGCAGUG	4063
TAAAAGAGAAACCTTGGGCC	970	GGG	+	UAAAAGAGAAACCUUGGGCC	4064
TATTCCTTCCCTCCGGGTAT	971	AGG	+	UAUUCCUUCCCUCCGGGUAU	4065
CCAAGGTATATTTCTAAGTA	972	GGG	+	CCAAGGUAUAUUUCUAAGUA	4066
CAAAAATAGCCAGTGTGGCT	973	TGG	+	CAAAAAUAGCCAGUGUGGCU	4067
ACAGGGAGAATGGGGAGCGA	974	GGG	-	ACAGGGAGAAUGGGGAGCGA	4068
AATATTGGGTGTGGTGGCAC	975	GGG	+	AAUAUUGGGUGUGGUGGCAC	4069
TTCCTGTTTCACTAGCTCCC	976	TGG	+	UUCCUGUUUCACUAGCUCCC	4070
AAGTACCATATACATAATGT	977	GGG	-	AAGUACCAUAUACAUAAUGU	4071
AAATTATGAATGATTAAAAA	978	TGG	-	AAAUUAUGAAUGAUUAAAAA	4072
TTCGTACAATACTGGAGGCT	979	GGG	+	UUCGUACAAUACUGGAGGCU	4073
ATAAAAGAGAAACCTTGGGC	980	CGG	+	AUAAAAGAGAAACCUUGGGC	4074
TGTTTTTTATTAGATTCCAG	981	TGG	-	UGUUUUUUAUUAGAUUCCAG	4075
CCTACTTAGAAATATACCTT	982	GGG	-	CCUACUUAGAAAUAUACCUU	4076
GCAGAGAACATCGTCTCTCC	983	AGG	+	GCAGAGAACAUCGUCUCUCC	4077
TGTCCCCTGGAGCATCCCTC	984	TGG	-	UGUCCCCUGGAGCAUCCCUC	4078
CTCTAAAGATTTGCCAGTTC	985	TGG	+	CUCUAAAGAUUUGCCAGUUC	4079
TTCTCAAGCAGATTGGAAGC	986	TGG	+	UUCUCAAGCAGAUUGGAAGC	4080
GGTAGAGTACCTGGGAGAGA	987	GGG	+	GGUAGAGUACCUGGGAGAGA	4081
CCAGGGATGAAGCAGGAAGC	988	AGG	-	CCAGGGAUGAAGCAGGAAGC	4082
CCAAACCCACGGCAGTGTCT	989	AGG	-	CCAAACCCACGGCAGUGUCU	4083
CAGGAGAGACAGCAGTGTGG	990	TGG	+	CAGGAGAGACAGCAGUGUGG	4084
GCCTCACTCTCTGCTCACAA	991	GGG	-	GCCUCACUCUCUGCUCACAA	4085
GAAAAATGACAGGAAATGAC	992	GGG	+	GAAAAAUGACAGGAAAUGAC	4086
CAAGCCACACTGGCTATTTT	993	TGG	-	CAAGCCACACUGGCUAUUUU	4087
TCGGATGACTGCACTCACCT	994	CGG	+	UCGGAUGACUGCACUCACCU	4088
AGTAGGTTGCCAAGCCACAC	995	TGG	-	AGUAGGUUGCCAAGCCACAC	4089
ACTCTCTTCAGTGACATACA	996	TGG	+	ACUCUCUUCAGUGACAUACA	4090
AAAAGGGCCACTCTGCTGTG	997	AGG	-	AAAAGGGCCACUCUGCUGUG	4091
ATGTTGGAGCAAAGATTCGA	998	AGG	-	AUGUUGGAGCAAAGAUUCGA	4092
CAACATCACCTTCTCAGTGA	999	GGG	+	CAACAUCACCUUCUCAGUGA	4093
TAATAATAAAGTGTCCTTCA	1000	AGG	-	UAAUAAUAAAGUGUCCUUCA	4094
AAAAAAGTGGTTGCAGAACA	1001	GGG	+	AAAAAAGUGGUUGCAGAACA	4095
AATGGAGTTTTGGTGGTGTA	1002	GGG	+	AAUGGAGUUUUGGUGGUGUA	4096
TTAAAGTAGTCAGAGTTACT	1003	GGG	-	UUAAAGUAGUCAGAGUUACU	4097
GGTCTAATTTTAAAATGCTT	1004	TGG	-	GGUCUAAUUUUAAAAUGCUU	4098
TAAATGAGAAAAGCATAACA	1005	AGG	+	UAAAUGAGAAAAGCAUAACA	4099
AATTAGGGTCTAGTTGGCTG	1006	GGG	-	AAUUAGGGUCUAGUUGGCUG	4100
GGGAGCGAGGGCTTAATGTT	1007	TGG	-	GGGAGCGAGGGCUUAAUGUU	4101
TGTCTTTCGCTTAGCATATG	1008	TGG	+	UGUCUUUCGCUUAGCAUAUG	4102
TTGGAGCAAAGATTCGAAGG	1009	AGG	-	UUGGAGCAAAGAUUCGAAGG	4103
TTAACACAATTAGCTGAGCG	1010	TGG	+	UUAACACAAUUAGCUGAGCG	4104
TTGTACGAAATAAATTTCTG	1011	TGG	-	UUGUACGAAAUAAAUUUCUG	4105
GAAAGCAGGCCTCTCTTCCT	1012	TGG	+	GAAAGCAGGCCUCUCUUCCU	4106
CATGAGATGCATCCACCCTG	1013	TGG	+	CAUGAGAUGCAUCCACCCUG	4107
TCAGCACTGTGGAAGAAACA	1014	AGG	-	UCAGCACUGUGGAAGAAACA	4108
CTTCTTCGCATCGCGGGGGT	1015	GGG	-	CUUCUUCGCAUCGCGGGGGU	4109
AACCAGTGGAACAAGGGCCC	1016	AGG	+	AACCAGUGGAACAAGGGCCC	4110

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CTTCCCTCCGGGTATAGGGC	1017	AGG	+	CUUCCCUCCGGGUAUAGGGC	4111
ATCCCTGACTATCTGCTAGG	1018	GGG	+	AUCCCUGACUAUCUGCUAGG	4112
TCGGCTTCATCTGTTTCCAT	1019	GGG	+	UCGGCUUCAUCUGUUUCCAU	4113
AGGCAAAGAAATTCTATGGA	1020	CGG	+	AGGCAAAGAAAUUCUAUGGA	4114
CCTGGTGGCCATAGGATGGT	1021	AGG	+	CCUGGUGGCCAUAGGAUGGU	4115
CAGCGCGTGGTCCCTGCCTC	1022	GGG	+	CAGCGCGUGGUCCCUGCCUC	4116
TGGGGGTGGAGCCCGAGGCA	1023	GGG	-	UGGGGGUGGAGCCCGAGGCA	4117
GAGAAACCTTGGGCCGGGCA	1024	TGG	+	GAGAAACCUUGGGCCGGGCA	4118
CTGACTACTTTAAGATGCTT	1025	TGG	+	CUGACUACUUUAAGAUGCUU	4119
GATGGATGGCGAAGCCACTC	1026	AGG	+	GAUGGAUGGCGAAGCCACUC	4120
GCTTTGGTGGTAAAAAATAC	1027	AGG	+	GCUUUGGUGGUAAAAAAUAC	4121
CCCAAGGTATATTTCTAAGT	1028	AGG	+	CCCAAGGUAUAUUUCUAAGU	4122
GGCTAGTGTGTGTGTTGGGT	1029	GGG	-	GGCUAGUGUGUGUGUUGGGU	4123
CCTCAGCCTCCCATGGTAGC	1030	TGG	-	CCUCAGCCUCCCAUGGUAGC	4124
GCAGCGCGTGGTCCCTGCCT	1031	CGG	+	GCAGCGCGUGGUCCCUGCCU	4125
TATTAGATTCCAGTGGTACT	1032	GGG	-	UAUUAGAUUCCAGUGGUACU	4126
GAAAACCTAGTTTAGTCTCT	1033	TGG	-	GAAAACCUAGUUUAGUCUCU	4127
CTGGAGTCGGGCTTGGTAGG	1034	TGG	-	CUGGAGUCGGGCUUGGUAGG	4128
ATTAATTAAAATTGGCCGGG	1035	CGG	-	AUUAAUUAAAAUUGGCCGGG	4129
AGATTCATTCATTCATTCCT	1036	TGG	+	AGAUUCAUUCAUUCAUUCCU	4130
TTCACTTAGGCGTTGGATGA	1037	AGG	+	UUCACUUAGGCGUUGGAUGA	4131
TTTGAGGCAAAGAAATTCTA	1038	TGG	+	UUUGAGGCAAAGAAAUUCUA	4132
GAGACAGTCTGGCACATTTG	1039	TGG	+	GAGACAGUCUGGCACAUUUG	4133
CTCAGCCTCCCATGGTAGCT	1040	GGG	-	CUCAGCCUCCCAUGGUAGCU	4134
CTCCCTTGCATGCCCTGAGA	1041	TGG	+	CUCCCUUGCAUGCCCUGAGA	4135
TTAATTAAAATTGGCCGGGC	1042	GGG	-	UUAAUUAAAAUUGGCCGGGC	4136
TAGTCATGACTAGTCCCATG	1043	TGG	-	UAGUCAUGACUAGUCCCAUG	4137
TAGTCACATTTCTCAGTATT	1044	TGG	+	UAGUCACAUUUCUCAGUAUU	4138
TGAGGAGACACTGCCACTGA	1045	AGG	-	UGAGGAGACACUGCCACUGA	4139
CTGAGCAAACCTAGAGTCCA	1046	AGG	-	CUGAGCAAACCUAGAGUCCA	4140
AGCTTCAGGAGGGAGGGGGC	1047	AGG	+	AGCUUCAGGAGGGAGGGGGC	4141
AACGTTTCTATCATCGCAGA	1048	AGG	-	AACGUUUCUAUCAUCGCAGA	4142
TGTATGACGGCGGGGCGCGG	1049	TGG	+	UGUAUGACGGCGGGGCGCGG	4143
TGGGCCAGAGGGATGCTCCA	1050	GGG	+	UGGGCCAGAGGGAUGCUCCA	4144
ATTAGGGTCTAGTTGGCTGG	1051	GGG	-	AUUAGGGUCUAGUUGGCUGG	4145
AGAAAAATGACAGGAAATGA	1052	CGG	+	AGAAAAAUGACAGGAAAUGA	4146
GTGTGATATTCCTTCCCTCC	1053	GGG	+	GUGUGAUAUUCCUUCCCUCC	4147
TTGTGTATGACGGCGGGGCG	1054	CGG	+	UUGUGUAUGACGGCGGGGCG	4148
CCAATCTTTGTGCATGGCTG	1055	GGG	+	CCAAUCUUUGUGCAUGGCUG	4149
TGCAAAGGAAATCAGCAATT	1056	AGG	-	UGCAAAGGAAAUCAGCAAUU	4150
AGAAAGCCCCTGGTGGCCAT	1057	AGG	+	AGAAAGCCCCUGGUGGCCAU	4151
GCTATTAAAAAGGATGAGGT	1058	CGG	+	GCUAUUAAAAAGGAUGAGGU	4152
TATGGCCAGCTTCAGGAGGG	1059	AGG	+	UAUGGCCAGCUUCAGGAGGG	4153
TTATCAGTCATTTTTTGTAT	1060	GGG	+	UUAUCAGUCAUUUUUUGUAU	4154
CCATAGGATGGTAGGGATTT	1061	AGG	+	CCAUAGGAUGGUAGGGAUUU	4155
CCACTGAAGGACAGAAGCAG	1062	TGG	-	CCACUGAAGGACAGAAGCAG	4156
AGGGTTTCCTCACAGCAGAG	1063	TGG	+	AGGGUUUCCUCACAGCAGAG	4157
GCCAGCTCGATTTCAACACT	1064	GGG	+	GCCAGCUCGAUUUCAACACU	4158
AAGTTCCACAAAGCCTCACA	1065	TGG	+	AAGUUCCACAAAGCCUCACA	4159
ATTATCAGTCATTTTTTGTA	1066	TGG	+	AUUAUCAGUCAUUUUUUGUA	4160
CTATCTCCATGACCCCTTGA	1067	AGG	+	CUAUCUCCAUGACCCCUUGA	4161
GACTACTTTAAGATGCTTTG	1068	GGG	+	GACUACUUUAAGAUGCUUUG	4162
GACTGGTCCTTTTGGGCTTA	1069	GGG	-	GACUGGUCCUUUUGGGCUUA	4163
ATGTGGTAGTAGTAAGATGT	1070	GGG	-	AUGUGGUAGUAGUAAGAUGU	4164
TACATGGCCTTTGAGCCAAG	1071	TGG	+	UACAUGGCCUUUGAGCCAAG	4165
CATCAACCGGCGGAACGAGG	1072	TGG	+	CAUCAACCGGCGGAACGAGG	4166
GGACTGGTCCTTTTGGGCTT	1073	AGG	-	GGACUGGUCCUUUUGGGCUU	4167
TGGTGTATCTGCTTCAGCAT	1074	TGG	+	UGGUGUAUCUGCUUCAGCAU	4168

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AAATATTGGGTGTGGTGGCA	1075	CGG	+	AAAUAUUGGGUGUGGUGGCA	4169
GAAACATAAATCCCGAGAGG	1076	TGG	+	GAAACAUAAAUCCCGAGAGG	4170
CTACCTCCTGCCCACCTCTC	1077	GGG	-	CUACCUCCUGCCCACCUCUC	4171
AGATGTCGAGAGGAGCACAT	1078	TGG	+	AGAUGUCGAGAGGAGCACAU	4172
CTCTGTAACACACGCCTGCT	1079	GGG	+	CUCUGUAACACACGCCUGCU	4173
CTGGGCCAGAGGGATGCTCC	1080	AGG	+	CUGGGCCAGAGGGAUGCUCC	4174
AAAATAGGTTTAAAAATATT	1081	GGG	+	AAAAUAGGUUUAAAAAUAUU	4175
AAACCTTGGGCCGGGCATGG	1082	TGG	+	AAACCUUGGGCCGGGCAUGG	4176
CAGAGCAAATGGTGAGCTGA	1083	GGG	+	CAGAGCAAAUGGUGAGCUGA	4177
ACCTTCATTGCTACTTGAGA	1084	AGG	+	ACCUUCAUUGCUACUUGAGA	4178
CTAGTTTAGTCTCTTGGATT	1085	GGG	-	CUAGUUUAGUCUCUUGGAUU	4179
AGTCTGGCACATTTGTGGCT	1086	TGG	+	AGUCUGGCACAUUUGUGGCU	4180
ACATATGAGCATTTCCAGTT	1087	TGG	+	ACAUAUGAGCAUUUCCAGUU	4181
CCCTACTTAGAAATATACCT	1088	TGG	-	CCCUACUUAGAAAUAUACCU	4182
ATATGGTACCTCAGAACCCC	1089	AGG	+	AUAUGGUACCUCAGAACCCC	4183
GAAACCCTTGTTGTCACAGC	1090	AGG	-	GAAACCCUUGUUGUCACAGC	4184
TGGTGGAAGCTAAAGGAAGA	1091	TGG	+	UGGUGGAAGCUAAAGGAAGA	4185
CATATGAGCATTTCCAGTTT	1092	GGG	+	CAUAUGAGCAUUUCCAGUUU	4186
TCGCCCCTAGACACTGCCGT	1093	GGG	+	UCGCCCCUAGACACUGCCGU	4187
CATCCCTGACTATCTGCTAG	1094	GGG	+	CAUCCCUGACUAUCUGCUAG	4188
GCAAAGGAAATCAGCAATTA	1095	GGG	-	GCAAAGGAAAUCAGCAAUUA	4189
CCAGAGCAAATGGTGAGCTG	1096	AGG	+	CCAGAGCAAAUGGUGAGCUG	4190
TTTGTTTTGTTCAAAATGTC	1097	TGG	+	UUUGUUUUGUUCAAAAUGUC	4191
GTGTCCTGCCCTATACCCGG	1098	AGG	-	GUGUCCUGCCCUAUACCCGG	4192
GTAGACAGCCTGGGGTTCTG	1099	AGG	-	GUAGACAGCCUGGGGUUCUG	4193
TAATTTTATGCTTTTCTTCT	1100	CGG	+	UAAUUUUAUGCUUUUCUUCU	4194
CCCCAGTGTTGAAATCGAGC	1101	TGG	-	CCCCAGUGUUGAAAUCGAGC	4195
TGGGGTTTGCAGAATGACCT	1102	TGG	-	UGGGGUUUGCAGAAUGACCU	4196
CCTAAATCCCTACCATCCTA	1103	TGG	-	CCUAAAUCCCUACCAUCCUA	4197
CAAACCCACGGCAGTGTCTA	1104	GGG	-	CAAACCCACGGCAGUGUCUA	4198
CAGTGGAAGTGTTAAGTGGC	1105	TGG	-	CAGUGGAAGUGUUAAGUGGC	4199
GTGATGATAAAGGCTTTGTG	1106	TGG	+	GUGAUGAUAAAGGCUUUGUG	4200
TATGGGTTTCTATAATCTGT	1107	GGG	+	UAUGGGUUUCUAUAAUCUGU	4201
ACTGCTGTGGAGAAAACACA	1108	AGG	-	ACUGCUGUGGAGAAAACACA	4202
CCCCATCTCAGGGCATGCAA	1109	GGG	-	CCCCAUCUCAGGGCAUGCAA	4203
AATGTGGTAGTAGTAAGATG	1110	TGG	-	AAUGUGGUAGUAGUAAGAUG	4204
TATTATTTCAAAGATTGAGA	1111	GGG	-	UAUUAUUUCAAAGAUUGAGA	4205
ATATTATTTCAAAGATTGAG	1112	AGG	-	AUAUUAUUUCAAAGAUUGAG	4206
AAACCCACGGCAGTGTCTAG	1113	GGG	-	AAACCCACGGCAGUGUCUAG	4207
AGGGATGCTCCAGGGGACAG	1114	AGG	+	AGGGAUGCUCCAGGGGACAG	4208
CAGAGGTGGGACTCAGCCCC	1115	TGG	+	CAGAGGUGGGACUCAGCCCC	4209
AGCAACATATGACACAGTTC	1116	AGG	+	AGCAACAUAUGACACAGUUC	4210
AGTGAGGCTTCCATTTCAGA	1117	AGG	+	AGUGAGGCUUCCAUUUCAGA	4211
TTCAGGGAAGAAGAGGTGAA	1118	AGG	+	UUCAGGGAAGAAGAGGUGAA	4212
TTTCAGGAGAGAAAGGGTCA	1119	AGG	+	UUUCAGGAGAGAAAGGGUCA	4213
TCCCCATCTCAGGGCATGCA	1120	AGG	-	UCCCCAUCUCAGGGCAUGCA	4214
AGCCCCTGGTGGCCATAGGA	1121	TGG	+	AGCCCCUGGUGGCCAUAGGA	4215
GAATCGTCTGATTGCTGGAG	1122	AGG	+	GAAUCGUCUGAUUGCUGGAG	4216
AAGACATAGGAAGCCAGGTG	1123	CGG	-	AAGACAUAGGAAGCCAGGUG	4217
GCCTCTCTTCCTTGGACTCT	1124	AGG	+	GCCUCUCUUCCUUGGACUCU	4218
GGAGCGAGGGCTTAATGTTT	1125	GGG	-	GGAGCGAGGGCUUAAUGUUU	4219
ACATTGCATAAATCTAAGAT	1126	TGG	+	ACAUUGCAUAAAUCUAAGAU	4220
GGGAGCCCAGACAGGCTGGA	1127	TGG	-	GGGAGCCCAGACAGGCUGGA	4221
AAACATAAATCCCGAGAGGT	1128	GGG	+	AAACAUAAAUCCCGAGAGGU	4222
TCTACCTCCTGCCCACCTCT	1129	CGG	-	UCUACCUCCUGCCCACCUCU	4223
GGCCATCAACCGGCGGAACG	1130	AGG	+	GGCCAUCAACCGGCGGAACG	4224
AGGCGTTGAGAGGAGTGCGC	1131	CGG	+	AGGCGUUGAGAGGAGUGCGC	4225
TGTCCTGCCCTATACCCGGA	1132	GGG	-	UGUCCUGCCCUAUACCCGGA	4226

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ATGCTTTGGTAAACATTTCT	1133	AGG	-	AUGCUUUGGUAAACAUUUCU	4227
GTGGGCCATGTGAGGCTTTG	1134	TGG	-	GUGGGCCAUGUGAGGCUUUG	4228
GGCGGGATAATCACTTGAGC	1135	TGG	-	GGCGGGAUAAUCACUUGAGC	4229
CTGGTGACAATATAATTCCA	1136	AGG	+	CUGGUGACAAUAUAAUUCCA	4230
GGCCAGCTTCAGGAGGGAGG	1137	GGG	+	GGCCAGCUUCAGGAGGGAGG	4231
TAGGACTGGGCCTTCTGAAA	1138	TGG	-	UAGGACUGGGCCUUCUGAAA	4232
TCCCTTGCATGCCCTGAGAT	1139	GGG	+	UCCCUUGCAUGCCCUGAGAU	4233
GTCCCCTTGTCCCCATCTCA	1140	GGG	-	GUCCCCUUGUCCCCAUCUCA	4234
TGTGTGATATTCCTTCCCTC	1141	CGG	+	UGUGUGAUAUUCCUUCCCUC	4235
CTGCCCTATACCCGGAGGGA	1142	AGG	-	CUGCCCUAUACCCGGAGGGA	4236
CTTAAAATCCAACAGAAAAC	1143	AGG	+	CUUAAAAUCCAACAGAAAAC	4237
ACATAGGAAGCCAGGTGCGG	1144	TGG	-	ACAUAGGAAGCCAGGUGCGG	4238
GTGGCAGTGGAAGTGTTAAG	1145	TGG	-	GUGGCAGUGGAAGUGUUAAG	4239
CCCTTGCATGCCCTGAGATG	1146	GGG	+	CCCUUGCAUGCCCUGAGAUG	4240
GGTCCCCTTGTCCCCATCTC	1147	AGG	-	GGUCCCCUUGUCCCCAUCUC	4241
ATAAATCCCGAGAGGTGGGC	1148	AGG	+	AUAAAUCCCGAGAGGUGGGC	4242
TATGAGCATTTCCAGTTTGG	1149	GGG	+	UAUGAGCAUUUCCAGUUUGG	4243
TATTTGTAATACCCCCAAAC	1150	TGG	-	UAUUUGUAAUACCCCCAAAC	4244
GCCCAGGAGAGACAGCAGTG	1151	TGG	+	GCCCAGGAGAGACAGCAGUG	4245
TCACTTTTTAAATAGCAAAG	1152	TGG	+	UCACUUUUUAAAUAGCAAAG	4246
TCTGTGCTCTGCAAATGACC	1153	TGG	+	UCUGUGCUCUGCAAAUGACC	4247
AGGTCATTCTGCAAACCCCA	1154	AGG	+	AGGUCAUUCUGCAAACCCCA	4248
TAGGTTTGTCTATAATGTAC	1155	AGG	-	UAGGUUUGUCUAUAAUGUAC	4249
ACCTTCTCAAGTAGCAATGA	1156	AGG	-	ACCUUCUCAAGUAGCAAUGA	4250
GTATGGGTTTCTATAATCTG	1157	TGG	+	GUAUGGGUUUCUAUAAUCUG	4251
TACCAATCTTTGTGCATGGC	1158	TGG	+	UACCAAUCUUUGUGCAUGGC	4252
GACGTGCAGAAGGCGTTGAG	1159	AGG	+	GACGUGCAGAAGGCGUUGAG	4253
CTCACTTATCTCAAGGTTCA	1160	TGG	+	CUCACUUAUCUCAAGGUUCA	4254
TTCAGGAGAGAAAGGGTCAA	1161	GGG	+	UUCAGGAGAGAAAGGGUCAA	4255
TAGGACTTCTTCGCATCGCG	1162	GGG	-	UAGGACUUCUUCGCAUCGCG	4256
CTGCTCTTCGTGGACATTTC	1163	TGG	-	CUGCUCUUCGUGGACAUUUC	4257
AGAAATAGAGAAAAGAGGCC	1164	AGG	+	AGAAAUAGAGAAAAGAGGCC	4258
ACTGGTTACTCACCCGCTAT	1165	GGG	-	ACUGGUUACUCACCCGCUAU	4259
AGGACTTCTTCGCATCGCGG	1166	GGG	-	AGGACUUCUUCGCAUCGCGG	4260
CAACACGAATCTGAACAAAC	1167	AGG	-	CAACACGAAUCUGAACAAAC	4261
AGAAGTGATATGCTTCAATT	1168	AGG	+	AGAAGUGAUAUGCUUCAAUU	4262
GATAATCACTTGAGCTGGGG	1169	AGG	-	GAUAAUCACUUGAGCUGGGG	4263
ATATGAGCATTTCCAGTTTG	1170	GGG	+	AUAUGAGCAUUUCCAGUUUG	4264
CGGGATAATCACTTGAGCTG	1171	GGG	-	CGGGAUAAUCACUUGAGCUG	4265
CGGGAGAATCGTCTGATTGC	1172	TGG	+	CGGGAGAAUCGUCUGAUUGC	4266
TCACCAAAGGAGAAGCAACA	1173	TGG	-	UCACCAAAGGAGAAGCAACA	4267
GGGGAGGAGGATGATCTGTT	1174	TGG	-	GGGGAGGAGGAUGAUCUGUU	4268
CGTTGGATGAAGGTTCATTT	1175	CGG	+	CGUUGGAUGAAGGUUCAUUU	4269
AGGTCAATTCTGCCCATAGC	1176	GGG	+	AGGUCAAUUCUGCCCAUAGC	4270
ATGGCCAGCTTCAGGAGGGA	1177	GGG	+	AUGGCCAGCUUCAGGAGGGA	4271
AAATTTATTTCGTACAATAC	1178	TGG	+	AAAUUUAUUUCGUACAAUAC	4272
GAAGAGCGCACGATAGACGC	1179	CGG	+	GAAGAGCGCACGAUAGACGC	4273
CCACTGCTTCTGTCCTTCAG	1180	TGG	+	CCACUGCUUCUGUCCUUCAG	4274
CAAAGAAATTCTATGGACGG	1181	AGG	+	CAAAGAAAUUCUAUGGACGG	4275
AAGGATGAGGTCGGGTGCAG	1182	TGG	+	AAGGAUGAGGUCGGGUGCAG	4276
GATCAAAGGGTATGATCTAA	1183	TGG	+	GAUCAAAGGGUAUGAUCUAA	4277
TTTTGTGTCCACTGTAGGCT	1184	TGG	-	UUUUGUGUCCACUGUAGGCU	4278
AGGGAAGGAATATCACACAG	1185	GGG	-	AGGGAAGGAAUAUCACACAG	4279
CTAGGACTTCTTCGCATCGC	1186	GGG	-	CUAGGACUUCUUCGCAUCGC	4280
ACCAATCTTTGTGCATGGCT	1187	GGG	+	ACCAAUCUUUGUGCAUGGCU	4281
TTGGCGACAACATATTGTAA	1188	TGG	-	UUGGCGACAACAUAUUGUAA	4282
TGGCCAGCTTCAGGAGGGAG	1189	GGG	+	UGGCCAGCUUCAGGAGGGAG	4283
GGGAGGAGGATGATCTGTTT	1190	GGG	-	GGGAGGAGGAUGAUCUGUUU	4284

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TAGGGAAGCCCTCACTGAGA	1191	AGG	-	UAGGGAAGCCCUCACUGAGA	4285
AAGGTCAATTCTGCCCATAG	1192	CGG	+	AAGGUCAAUUCUGCCCAUAG	4286
ATCTAATTGTGGCACAGTGA	1193	GGG	+	AUCUAAUUGUGGCACAGUGA	4287
TACTGGAGGGGGGCAGCGCG	1194	TGG	+	UACUGGAGGGGGGCAGCGCG	4288
CATCTAATTGTGGCACAGTG	1195	AGG	+	CAUCUAAUUGUGGCACAGUG	4289
GCTCTGAAGCACTTGAAATG	1196	TGG	+	GCUCUGAAGCACUUGAAAUG	4290
CTATGTCTTATAATTGAGTC	1197	AGG	+	CUAUGUCUUAUAAUUGAGUC	4291
TTTATTTCGTACAATACTGG	1198	AGG	+	UUUAUUUCGUACAAUACUGG	4292
AATCCCGAGAGGTGGGCAGG	1199	AGG	+	AAUCCCGAGAGGUGGGCAGG	4293
GTATAGCGCCAAGCCTACAG	1200	TGG	+	GUAUAGCGCCAAGCCUACAG	4294
AACCGGAATGCTATTGACGG	1201	TGG	+	AACCGGAAUGCUAUUGACGG	4295
TTGTTTTGTTCAAAATGTCT	1202	GGG	+	UUGUUUUGUUCAAAAUGUCU	4296
TCTAGGACTTCTTCGCATCG	1203	**CGG**	-	**UCUAGGACUUCUUCGCAUCG**	4297
CCTAGTTTAGTCTCTTGGAT	1204	TGG	-	CCUAGUUUAGUCUCUUGGAU	4298
TTTAAATTCCAATACCACAT	1205	GGG	+	UUUAAAUUCCAAUACCACAU	4299
AGCATCCTTTGAACTTTAGA	1206	AGG	-	AGCAUCCUUUGAACUUUAGA	4300
GCGGGATAATCACTTGAGCT	1207	GGG	-	GCGGGAUAAUCACUUGAGCU	4301
ACTCTGTAACACACGCCTGC	1208	TGG	+	ACUCUGUAACACACGCCUGC	4302
AAGCGTTTTGCGCAGGAGAG	1209	TGG	+	AAGCGUUUUGCGCAGGAGAG	4303
AGAGCTTTGGCCGAGGACGA	1210		+	**AGAGCUUUGGCCGAGGACGA**	4304
AGAGACAGAAAGTCGATCCG	1211	TGG	-	AGAGACAGAAAGUCGAUCCG	4305
CTATTAAAAAGGATGAGGTC	1212	GGG	+	CUAUUAAAAAGGAUGAGGUC	4306
TGAAGGACAGAAGCAGTGGA	1213	GGG	-	UGAAGGACAGAAGCAGUGGA	4307
CTGAAGGACAGAAGCAGTGG	1214	AGG	-	CUGAAGGACAGAAGCAGUGG	4308
TACCTTATTTCACTGTAATG	1215	AGG	-	UACCUUAUUUCACUGUAAUG	4309
AATTAGACCTGCCCAGTTAG	1216	TGG	+	AAUUAGACCUGCCCAGUUAG	4310
CTGATATCCACTAACTGGGC	1217	AGG	-	CUGAUAUCCACUAACUGGGC	4311
ATTGCTGATATCCACTAACT	1218	GGG	-	AUUGCUGAUAUCCACUAACU	4312
CAGGCTGTCAGGAGGCATGA	1219	AGG	+	CAGGCUGUCAGGAGGCAUGA	4313
AAATATCTTACTTCATTACT	1220	TGG	+	AAAUAUCUUACUUCAUUACU	4314
TTTTCTGGTATGATACTATT	1221	AGG	+	UUUUCUGGUAUGAUACUAUU	4315
CTGGTATGATACTATTAGGT	1222	TGG	+	CUGGUAUGAUACUAUUAGGU	4316
ATCTCTTGATGAACAGCTAC	1223	AGG	+	AUCUCUUGAUGAACAGCUAC	4317
GATGAACAGCTACAGGCCTG	1224	AGG	+	GAUGAACAGCUACAGGCCUG	4318
ACAGCTACAGGCCTGAGGTT	1225	AGG	+	ACAGCUACAGGCCUGAGGUU	4319
CTACATGAACTCCTAACCTC	1226	AGG	-	CUACAUGAACUCCUAACCUC	4320
GTCCTTCAGCCTCAACTTAT	1227	AGG	+	GUCCUUCAGCCUCAACUUAU	4321
CACCTATAAGTTGAGGCTGA	1228	AGG	-	CACCUAUAAGUUGAGGCUGA	4322
ACACAGTCACCTATAAGTTG	1229	AGG	-	ACACAGUCACCUAUAAGUUG	4323
TCGTTAACTCCGCAGAGCAC	1230	AGG	+	UCGUUAACUCCGCAGAGCAC	4324
CGTTAACTCCGCAGAGCACA	1231	GGG	+	CGUUAACUCCGCAGAGCACA	4325
GGATGGTGCCCTGTGCTCTG	1232	CGG	-	GGAUGGUGCCCUGUGCUCUG	4326
CTATCCCCAGCACCTAAAGC	1233	TGG	+	CUAUCCCCAGCACCUAAAGC	4327
TATCCCCAGCACCTAAAGCT	1234	GGG	+	UAUCCCCAGCACCUAAAGCU	4328
GGTAGGGATTTAGGAAAAAG	1235	AGG	+	GGUAGGGAUUUAGGAAAAAG	4329
AGGGATTTAGGAAAAAGAGG	1236	AGG	+	AGGGAUUUAGGAAAAAGAGG	4330
TTTAGGAAAAAGAGGAGGCT	1237	TGG	+	UUUAGGAAAAAGAGGAGGCU	4331
AGGAAAAAGAGGAGGCTTGG	1238	AGG	+	AGGAAAAAGAGGAGGCUUGG	4332
GATCATCTAGCACATGTTTT	1239	TGG	+	GAUCAUCUAGCACAUGUUUU	4333
CTAGCACATGTTTTTGGTTT	1240	TGG	+	CUAGCACAUGUUUUUGGUUU	4334
TATCTGGTGGGTAGCTACTT	1241	AGG	+	UAUCUGGUGGGUAGCUACUU	4335
GGTGGGTAGCTACTTAGGAG	1242	TGG	+	GGUGGGUAGCUACUUAGGAG	4336
CCTGCTTCCTGCTTCATCCC	1243	TGG	+	CCUGCUUCCUGCUUCAUCCC	4337
GAAGGTGCCAGGGATGAAGC	1244	AGG	-	GAAGGUGCCAGGGAUGAAGC	4338
TTGCCCCTTCCGCCATGGGA	1245	GGG	-	UUGCCCCUUCCGCCAUGGGA	4339
CTTGCCCCTTCCGCCATGGG	1246	AGG	-	CUUGCCCCUUCCGCCAUGGG	4340
TAGCTTGCCCCTTCCGCCAT	1247	GGG	-	UAGCUUGCCCCUUCCGCCAU	4341
CTAGCTTGCCCCTTCCGCCA	1248	TGG	-	CUAGCUUGCCCCUUCCGCCA	4342

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ACTAACATTCACATCATAGC	1249	AGG	+	ACUAACAUUCACAUCAUAGC	4343
CTAACATTCACATCATAGCA	1250	GGG	+	CUAACAUUCACAUCAUAGCA	4344
TCACATCATAGCAGGGACTC	1251	TGG	+	UCACAUCAUAGCAGGGACUC	4345
CATCATAGCAGGGACTCTGG	1252	TGG	+	CAUCAUAGCAGGGACUCUGG	4346
GGGAGCGCCATCTTTGTAGC	1253	AGG	-	GGGAGCGCCAUCUUUGUAGC	4347
TCAAGTAGCAATGAAGGTTA	1254	GGG	-	UCAAGUAGCAAUGAAGGUUA	4348
CTCAAGTAGCAATGAAGGTT	1255	AGG	-	CUCAAGUAGCAAUGAAGGUU	4349
GGAAGACCTGTGCATTTTTG	1256	TGG	+	GGAAGACCUGUGCAUUUUUG	4350
AACTGTCCACAAAAATGCAC	1257	AGG	-	AACUGUCCACAAAAAUGCAC	4351
ATTTATTTTTAGCAGAGACA	1258	GGG	+	AUUUAUUUUUAGCAGAGACA	4352
GGTTGGTGCAAGAGTAACTG	1259	CGG	+	GGUUGGUGCAAGAGUAACUG	4353
ATATCGGCTCACTGCAACCC	1260	GGG	+	AUAUCGGCUCACUGCAACCC	4354
AGCCAGCATCTTCTCTCCCT	1261	CGG	+	AGCCAGCAUCUUCUCUCCCU	4355
CACCTGTGATCCGAGCTACT	1262	TGG	-	CACCUGUGAUCCGAGCUACU	4356
TACTCGGGGGAGGCTGAAGC	1263	GGG	+	UACUCGGGGGAGGCUGAAGC	4357
GAACGAGGTGGAGTTTGGCC	1264	AGG	+	GAACGAGGUGGAGUUUGGCC	4358
GCATCACTGCACCTCAGTCT	1265	GGG	-	GCAUCACUGCACCUCAGUCU	4359
TGGGTTTTGCCATATTGCTC	1266	AGG	-	UGGGUUUUGCCAUAUUGCUC	4360
CTACTTAGGAGTGGAATTGC	1267	TGG	+	CUACUUAGGAGUGGAAUUGC	4361
TTCAGCCTCCCCCGAGTAGC	1268	TGG	-	UUCAGCCUCCCCCGAGUAGC	4362
CACTTCCACTGCCACCACCC	1269	TGG	+	CACUUCCACUGCCACCACCC	4363
TGCATCACTGCACCTCAGTC	1270	TGG	-	UGCAUCACUGCACCUCAGUC	4364
TCCACAGCCTTGTCAACACT	1271	AGG	+	UCCACAGCCUUGUCAACACU	4365
CTCTGGGGGAGCATACAGAC	1272	GGG	-	CUCUGGGGGAGCAUACAGAC	4366
TCCCCCAGAGGTTTGAGCAG	1273	CGG	+	UCCCCCAGAGGUUUGAGCAG	4367
AGGGAGAGAAGATGCTGGCT	1274	TGG	-	AGGGAGAGAAGAUGCUGGCU	4368
TCTTAGCCTAGGAAGATTCA	1275	GGG	-	UCUUAGCCUAGGAAGAUUCA	4369
GTATCTCAGGGTTCTTGCCT	1276	TGG	-	GUAUCUCAGGGUUCUUGCCU	4370
CTCAAGTGGTTTGCCCGCCT	1277	CGG	+	CUCAAGUGGUUUGCCCGCCU	4371
CAAAACCAGCCTGAGCAATA	1278	TGG	+	CAAAACCAGCCUGAGCAAUA	4372
TGAGGCGGGTCTATCACCTG	1279	AGG	+	UGAGGCGGGUCUAUCACCUG	4373
TGCCCAGGGGCTGGGGGAGG	1280	GGG	-	UGCCCAGGGGCUGGGGGAGG	4374
CAGCCATGAGCCACTGCGTC	1281	CGG	+	CAGCCAUGAGCCACUGCGUC	4375
TAAGATGTGGGGCCTTTGGG	1282	AGG	-	UAAGAUGUGGGGCCUUUGGG	4376
CAAAAAGTATCTGGGCATGA	1283	TGG	+	CAAAAAGUAUCUGGGCAUGA	4377
TTAAATTAATTAAAATTGGC	1284	CGG	-	UUAAAUUAAUUAAAAUUGGC	4378
TGGAAGTGAGCAGAGAGAGA	1285	AGG	-	UGGAAGUGAGCAGAGAGAGA	4379
CCGTCTGTATGCTCCCCCAG	1286	AGG	+	CCGUCUGUAUGCUCCCCCAG	4380
CAGCATTTGGGCACGAAGGC	1287	AGG	-	CAGCAUUUGGGCACGAAGGC	4381
CATGCCCTGAGATGGGGACA	1288	AGG	+	CAUGCCCUGAGAUGGGGACA	4382
ACTTCCACTGCCACCACCCT	1289	GGG	+	ACUUCCACUGCCACCACCCU	4383
TTTACAGCTGAGGCCCCAGC	1290	AGG	-	UUUACAGCUGAGGCCCCAGC	4384
ATGCCCTGAGATGGGGACAA	1291	GGG	+	AUGCCCUGAGAUGGGGACAA	4385
TTGAATGAATGAATGCTTTG	1292	AGG	+	UUGAAUGAAUGAAUGCUUUG	4386
AATGCAGCATTTGGGCACGA	1293	AGG	-	AAUGCAGCAUUUGGGCACGA	4387
TAAAATTACAAAAATGATCC	1294	GGG	-	UAAAAUUACAAAAAUGAUCC	4388
GGAAGGAGCTCTCAGCAGAT	1295	AGG	-	GGAAGGAGCUCUCAGCAGAU	4389
AAGTATCTGGGCATGATGGC	1296	AGG	+	AAGUAUCUGGGCAUGAUGGC	4390
TGCCCCGCTGCTCAAACCTC	1297	TGG	-	UGCCCCGCUGCUCAAACCUC	4391
TGCAGCTATTAAAAAGGATG	1298	AGG	+	UGCAGCUAUUAAAAAGGAUG	4392
ACGCCACTGTACTCCCAGCC	1299	TGG	-	ACGCCACUGUACUCCCAGCC	4393
TATTGTATTTCTTGTAGAGA	1300	GGG	+	UAUUGUAUUUCUUGUAGAGA	4394
TGAGTCTCGCTCAGTCACCC	1301	AGG	-	UGAGUCUCGCUCAGUCACCC	4395
TTGGCGGAAGAAGACACAAG	1302	TGG	+	UUGGCGGAAGAAGACACAAG	4396
CCGAGCTACTTGGAAGACTG	1303	AGG	-	CCGAGCUACUUGGAAGACUG	4397
AAGGAGCTCTCAGCAGATAG	1304	GGG	-	AAGGAGCUCUCAGCAGAUAG	4398
TGAAGCACTTGAAATGTGGC	1305	TGG	+	UGAAGCACUUGAAAUGUGGC	4399
GAAGGAGCTCTCAGCAGATA	1306	GGG	-	GAAGGAGCUCUCAGCAGAUA	4400

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGAGGGAAGGAATATCACAC	1307	AGG	-	GGAGGGAAGGAAUAUCACAC	4401
GAGGGAAGGAATATCACACA	1308	GGG	-	GAGGGAAGGAAUAUCACACA	4402
TGGCCGGACGCAGTGGCTCA	1309	TGG	-	UGGCCGGACGCAGUGGCUCA	4403
CGGGTCTATCACCTGAGGTC	1310	AGG	+	CGGGUCUAUCACCUGAGGUC	4404
TGGCTGTAATTCTAACACTT	1311	TGG	-	UGGCUGUAAUUCUAACACUU	4405
GGCTGTAATTCTAACACTTT	1312	GGG	-	GGCUGUAAUUCUAACACUUU	4406
GGCTTGAGCCCTGGAGTTCA	1313	AGG	+	GGCUUGAGCCCUGGAGUUCA	4407
CCTCTGGGGGAGCATACAGA	1314	CGG	-	CCUCUGGGGGAGCAUACAGA	4408
GGGGGAGCATACAGACGGGC	1315	AGG	-	GGGGGAGCAUACAGACGGGC	4409
AGGGTCTCGCCATGCTGCCC	1316	AGG	+	AGGGUCUCGCCAUGCUGCCC	4410
ATGAGCCACTGCGTCCGGCC	1317	AGG	+	AUGAGCCACUGCGUCCGGCC	4411
GAGGAGAATCGATTGAACCC	1318	AGG	-	GAGGAGAAUCGAUUGAACCC	4412
CAAGGCAGGAGTATCACCTG	1319	AGG	+	CAAGGCAGGAGUAUCACCUG	4413
ACAAAAGGGGCCGGGCGCGG	1320	TGG	-	ACAAAAGGGGCCGGGCGCGG	4414
AAAAAGGATGAGTTCATGTA	1321	GGG	+	AAAAAGGAUGAGUUCAUGUA	4415
CTGTCGTGGGGTGGGGGAGC	1322	GGG	+	CUGUCGUGGGGUGGGGGAGC	4416
CGCCACTGTACTCCCAGCCT	1323	GGG	-	CGCCACUGUACUCCCAGCCU	4417
TGCCCAGGCTGGGAGTACAG	1324	TGG	+	UGCCCAGGCUGGGAGUACAG	4418
CAAAAATACAAAAAGTATCT	1325	GGG	+	CAAAAAUACAAAAAGUAUCU	4419
ACAAAAATACAAAAAGTATC	1326	TGG	+	ACAAAAAUACAAAAAGUAUC	4420
CGGGCAAACCACTTGAGGTC	1327	AGG	-	CGGGCAAACCACUUGAGGUC	4421
TTGTATCTTTAGTAGAGCCA	1328	GGG	+	UUGUAUCUUUAGUAGAGCCA	4422
AATAAATTAGCCGGATGTGG	1329	TGG	-	AAUAAAUUAGCCGGAUGUGG	4423
GAGACAGGGTTTCAGCATGC	1330	TGG	+	GAGACAGGGUUUCAGCAUGC	4424
CCCCCAGAGGTTTGAGCAGC	1331	GGG	+	CCCCCAGAGGUUUGAGCAGC	4425
AAAAAAGGATGAGTTCATGT	1332	AGG	+	AAAAAAGGAUGAGUUCAUGU	4426
AGCACCACTGCACTCCAGTG	1333	TGG	+	AGCACCACUGCACUCCAGUG	4427
CAGGTGTCCTGCCCTATACC	1334	CGG	-	CAGGUGUCCUGCCCUAUACC	4428
GCTTGTAGTCCCAGCTACCA	1335	TGG	+	GCUUGUAGUCCCAGCUACCA	4429
GATGAGTTCATGTAGGGACA	1336	TGG	+	GAUGAGUUCAUGUAGGGACA	4430
CCATGGGAGGCTGAGGTGAG	1337	AGG	+	CCAUGGGAGGCUGAGGUGAG	4431
TATTTATTTTTAGCAGAGAC	1338	AGG	+	UAUUUAUUUUUAGCAGAGAC	4432
TTTGTATCTTTAGTAGAGCC	1339	AGG	+	UUUGUAUCUUUAGUAGAGCC	4433
AGACGGGGTTTCACCAGGTT	1340	GGG	-	AGACGGGGUUUCACCAGGUU	4434
GGGCTTGCAGAATGAGTGCA	1341	AGG	-	GGGCUUGCAGAAUGAGUGCA	4435
GGCCTGCTTCAGGGAAGAAG	1342	AGG	+	GGCCUGCUUCAGGGAAGAAG	4436
AGGGTTTCAGCATGCTGGCC	1343	AGG	+	AGGGUUUCAGCAUGCUGGCC	4437
TGAGACCAGCCTGCCCAACC	1344	TGG	+	UGAGACCAGCCUGCCCAACC	4438
CAGGCATGAGCTACCCCGCC	1345	CGG	+	CAGGCAUGAGCUACCCCGCC	4439
AAAAAAAAAAAAAACAAGTCT	1346	GGG	+	AAAAAAAAAAAAAACAAGUCU	4440
ATAAAATACCATAAACTAGG	1347	TGG	+	AUAAAAUACCAUAAACUAGG	4441
GCCGGTAATCCCAGTTACTC	1348	AGG	+	GCCGGUAAUCCCAGUUACUC	4442
TGTTAATTTTTTTGTAGAGA	1349	CGG	-	UGUUAAUUUUUUUGUAGAGA	4443
CTCAAACTTCTGAGCTCAAG	1350	GGG	+	CUCAAACUUCUGAGCUCAAG	4444
ATACTATGCAGCTATTAAAA	1351	AGG	+	AUACUAUGCAGCUAUUAAAA	4445
AGAAAAGAGGCCAGGTGCAG	1352	GGG	+	AGAAAAGAGGCCAGGUGCAG	4446
GTTAATTTTTTTGTAGAGAC	1353	GGG	-	GUUAAUUUUUUUGUAGAGAC	4447
CTTCAGGAGGCCAAGGCCAG	1354	AGG	+	CUUCAGGAGGCCAAGGCCAG	4448
CAAAAATGATCCGGGCGTGG	1355	TGG	-	CAAAAAUGAUCCGGGCGUGG	4449
GCACAGGATGGGGGCGTGGT	1356	GGG	-	GCACAGGAUGGGGGCGUGGU	4450
GGTCTGGAACTCTCGACCTC	1357	AGG	-	GGUCUGGAACUCUCGACCUC	4451
TGCCAAATAGTTTTCCAAAG	1358	AGG	+	UGCCAAAUAGUUUUCCAAAG	4452
CCTGTCACATGAGGGTCTTC	1359	AGG	+	CCUGUCACAUGAGGGUCUUC	4453
CCTCTAATCCCAGCTACTCG	1360	GGG	+	CCUCUAAUCCCAGCUACUCG	4454
GGCTGGGAAGTCCACGATCA	1361	AGG	+	GGCUGGGAAGUCCACGAUCA	4455
CCTGAAGACCCTCATGTGAC	1362	AGG	-	CCUGAAGACCCUCAUGUGAC	4456
CGGAAGAAGACACAAGTGGC	1363	TGG	+	CGGAAGAAGACACAAGUGGC	4457
GCAGGACACCTGTCACATGA	1364	GGG	+	GCAGGACACCUGUCACAUGA	4458

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TGTCACATGAGGGTCTTCAG	1365	GGG	+	UGUCACAUGAGGGUCUUCAG	4459
CAGCAGATAGGGGAGCCAGA	1366	AGG	-	CAGCAGAUAGGGGAGCCAGA	4460
GGGTTTTCACCATGTTTCCC	1367	AGG	+	GGGUUUUCACCAUGUUUCCC	4461
CCCCGAGTAGCTGGGATTAG	1368	AGG	-	CCCCGAGUAGCUGGGAUUAG	4462
CACTTGAGCTGGGGAGGCGG	1369	AGG	-	CACUUGAGCUGGGGAGGCGG	4463
ATCAAGGTGCCAGCAGATTT	1370	GGG	+	AUCAAGGUGCCAGCAGAUUU	4464
CTGTCACATGAGGGTCTTCA	1371	GGG	+	CUGUCACAUGAGGGUCUUCA	4465
GTCCGTGGGCACAGGCCTGG	1372	GGG	-	GUCCGUGGGCACAGGCCUGG	4466
GGCAGGACACCTGTCACATG	1373	AGG	+	GGCAGGACACCUGUCACAUG	4467
AGCAGATAGGGGAGCCAGAA	1374	GGG	-	AGCAGAUAGGGGAGCCAGAA	4468
CGCTTGTGTGTCTGCCTGCT	1375	AGG	-	CGCUUGUGUGUCUGCCUGCU	4469
CCGGCATGCGCCACCATGCC	1376	TGG	-	CCGGCAUGCGCCACCAUGCC	4470
CCAGACCAGCCTGGCCAAGG	1377	TGG	-	CCAGACCAGCCUGGCCAAGG	4471
CGTGGGCACAGGCCTGGGGG	1378	TGG	-	CGUGGGCACAGGCCUGGGGG	4472
AGAAAACTGCAGAAGCAGGA	1379	AGG	-	AGAAAACUGCAGAAGCAGGA	4473
CTCTAATCCCAGCTACTCGG	1380	GGG	+	CUCUAAUCCCAGCUACUCGG	4474
GGATGGGGGCGTGGTGGGCC	1381	AGG	-	GGAUGGGGGCGUGGUGGGCC	4475
CTCAGGTGATACTCCTGCCT	1382	TGG	-	CUCAGGUGAUACUCCUGCCU	4476
CCACCTTGGCCAGGCTGGTC	1383	TGG	+	CCACCUUGGCCAGGCUGGUC	4477
GTCTTGGGAAATGCAGCATT	1384	TGG	-	GUCUUGGGAAAUGCAGCAUU	4478
CCAGGCATGGTGGCGCATGC	1385	CGG	+	CCAGGCAUGGUGGCGCAUGC	4479
CGGAAGAATCAGATCACACG	1386	TGG	-	CGGAAGAAUCAGAUCACACG	4480
GGGGTTTCACCAGGTTGGGC	1387	AGG	-	GGGGUUUCACCAGGUUGGGC	4481
TCACTGATGGACGTTTGGGT	1388	TGG	-	UCACUGAUGGACGUUUGGGU	4482
AACGTCCATCAGTGATAGAC	1389	TGG	+	AACGUCCAUCAGUGAUAGAC	4483
TCTATCACTGATGGACGTTT	1390	GGG	-	UCUAUCACUGAUGGACGUUU	4484
GATGGGGGCGTGGTGGGCCA	1391	GGG	-	GAUGGGGGCGUGGUGGGCCA	4485
GCGAGGAACATCACACACTG	1392	GGG	+	GCGAGGAACAUCACACACUG	4486
AATCACTTGAGCTGGGGAGG	1393	CGG	-	AAUCACUUGAGCUGGGGAGG	4487
CAGGAGTATCACCTGAGGTC	1394	AGG	+	CAGGAGUAUCACCUGAGGUC	4488
GTGGGGTGGGGGAGCGGGGA	1395	GGG	+	GUGGGGUGGGGGAGCGGGGA	4489
TTTCACCAGGTTGGGCAGGC	1396	TGG	-	UUUCACCAGGUUGGGCAGGC	4490
TCTTGGGAAATGCAGCATTT	1397	GGG	-	UCUUGGGAAAUGCAGCAUUU	4491
GTTCCAGACCAGCCTGGCCA	1398	AGG	-	GUUCCAGACCAGCCUGGCCA	4492
TCTCGCCATGCTGCCCAGGC	1399	TGG	+	UCUCGCCAUGCUGCCCAGGC	4493
TTTCAGCATGCTGGCCAGGC	1400	TGG	+	UUUCAGCAUGCUGGCCAGGC	4494
GGGGGCGTGGTGGGCCAGGG	1401	TGG	-	GGGGGCGUGGUGGGCCAGGG	4495
GTCTATCACTGATGGACGTT	1402	TGG	-	GUCUAUCACUGAUGGACGUU	4496
TAACACAAGCCGCCTACAGA	1403	CGG	+	UAACACAAGCCGCCUACAGA	4497
AATAGCTGCATAGTATTCCA	1404	TGG	-	AAUAGCUGCAUAGUAUUCCA	4498
TCCTGCCTCAGGCTCCCGAG	1405	TGG	+	UCCUGCCUCAGGCUCCCGAG	4499
CTAGCAGGCAGACACACAAG	1406	CGG	+	CUAGCAGGCAGACACACAAG	4500
GCCACTCGGGAGCCTGAGGC	1407	AGG	-	GCCACUCGGGAGCCUGAGGC	4501
AGGTCCGTGGGCACAGGCCT	1408	GGG	-	AGGUCCGUGGGCACAGGCCU	4502
GCCTCAGGCTCCCGAGTGGC	1409	TGG	+	GCCUCAGGCUCCCGAGUGGC	4503
CCCAGCCACTCGGGAGCCTG	1410	AGG	-	CCCAGCCACUCGGGAGCCUG	4504
TGTAGAGACGGGGTTTCACC	1411	AGG	-	UGUAGAGACGGGGUUUCACC	4505
ACTGCCACTGTACTCCAGCC	1412	TGG	+	ACUGCCACUGUACUCCAGCC	4506
TCTCAAACTTCTGAGCTCAA	1413	GGG	+	UCUCAAACUUCUGAGCUCAA	4507
GTCTCAAACTTCTGAGCTCA	1414	AGG	+	GUCUCAAACUUCUGAGCUCA	4508
CCTCAGGCTCCCGAGTGGCT	1415	GGG	+	CCUCAGGCUCCCGAGUGGCU	4509
AGGCCTGTGCCCACGGACCT	1416	AGG	+	AGGCCUGUGCCCACGGACCU	4510
ACCTGTAATCCCAGCCACCT	1417	GGG	-	ACCUGUAAUCCCAGCCACCU	4511
AGAGTCTCGCTGTGTTGCTC	1418	AGG	+	AGAGUCUCGCUGUGUUGCUC	4512
TAGGTCCGTGGGCACAGGCC	1419	TGG	-	UAGGUCCGUGGGCACAGGCC	4513
CACCCCCAGGCCTGTGCCCA	1420	CGG	+	CACCCCCAGGCCUGUGCCCA	4514
TCAGCCTCCCCCGAGTAGCT	1421	GGG	-	UCAGCCUCCCCCGAGUAGCU	4515
GGCACAGGATGGGGGCGTGG	1422	TGG	-	GGCACAGGAUGGGGGCGUGG	4516

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GCTTGTGTGTCTGCCTGCTA	1423	GGG	-	GCUUGUGUGUCUGCCUGCUA	4517
CCTCTCACCTCAGCCTCCCA	1424	TGG	-	CCUCUCACCUCAGCCUCCCA	4518
ATAATAAAATACCATAAACT	1425	AGG	+	AUAAUAAAAUACCAUAAACU	4519
CACCTGTAATCCCAGCCACC	1426	TGG	-	CACCUGUAAUCCCAGCCACC	4520
CGTCTGTAATCCCAGCCACT	1427	CGG	-	CGUCUGUAAUCCCAGCCACU	4521
AGGAGAATCACTTGAAACCC	1428	GGG	-	AGGAGAAUCACUUGAAACCC	4522
TAATCCCAGCTACTCGGGGG	1429	AGG	+	UAAUCCCAGCUACUCGGGGG	4523
GGGATTACAGCCACCATGCC	1430	TGG	-	GGGAUUACAGCCACCAUGCC	4524
CTGGAACTCCTGACCTCAAG	1431	TGG	+	CUGGAACUCCUGACCUCAAG	4525
TACTTAGGAGTGGAATTGCT	1432	GGG	+	UACUUAGGAGUGGAAUUGCU	4526
GGTCCGTGGGCACAGGCCTG	1433	GGG	-	GGUCCGUGGGCACAGGCCUG	4527
GAGCCACCATGCCCGGCCCA	1434	AGG	-	GAGCCACCAUGCCCGGCCCA	4528
CTTACTCTTGTCGCCCAGGC	1435	TGG	-	CUUACUCUUGUCGCCCAGGC	4529
CCTGTCGTGGGGTGGGGGAG	1436	CGG	+	CCUGUCGUGGGGUGGGGGAG	4530
AGGAGGTCGAGGCTGCAGTG	1437	AGG	+	AGGAGGUCGAGGCUGCAGUG	4531
GGAAGAATCAGATCACACGT	1438	GGG	-	GGAAGAAUCAGAUCACACGU	4532
ATACAGACGGGCAGGCTGTG	1439	GGG	-	AUACAGACGGGCAGGCUGUG	4533
GAGTGGAATTGCTGGGTCAT	1440	AGG	+	GAGUGGAAUUGCUGGGUCAU	4534
GCCTGTCATCCCAGCTACTA	1441	GGG	-	GCCUGUCAUCCCAGCUACUA	4535
TTTATAAGCCACCTAGTTTA	1442	TGG	-	UUUAUAAGCCACCUAGUUUA	4536
GCATACAGACGGGCAGGCTG	1443	TGG	-	GCAUACAGACGGGCAGGCUG	4537
TGGTGGGCCAGGGTGGTCTT	1444	GGG	-	UGGUGGGCCAGGGUGGUCUU	4538
TCACTCTGTCACCCAGACTG	1445	AGG	+	UCACUCUGUCACCCAGACUG	4539
CATACAGACGGGCAGGCTGT	1446	GGG	-	CAUACAGACGGGCAGGCUGU	4540
AACTGAACAATGAAAACACT	1447	TGG	+	AACUGAACAAUGAAAACACU	4541
CTTACTCTGTTGCCCAGGCT	1448	GGG	+	CUUACUCUGUUGCCCAGGCU	4542
GGTTTTGAACTCCTGACCTT	1449	AGG	-	GGUUUUGAACUCCUGACCUU	4543
GTGGTGGGCCAGGGTGGTCT	1450	TGG	-	GUGGUGGGCCAGGGUGGUCU	4544
CAGGTGTGCACCACCATGGC	1451	CGG	+	CAGGUGUGCACCACCAUGGC	4545
TCCCTAGTAGCTGGGATGAC	1452	AGG	+	UCCCUAGUAGCUGGGAUGAC	4546
TTTTGCCATATTGCTCAGGC	1453	TGG	-	UUUUGCCAUAUUGCUCAGGC	4547
ACTTGGAAGACTGAGGTGGG	1454	AGG	-	ACUUGGAAGACUGAGGUGGG	4548
CTCAGCACTTTGGGAGGGCG	1455	AGG	+	CUCAGCACUUUGGGAGGGCG	4549
GTGTCTGCCTGCTAGGGTCT	1456	TGG	-	GUGUCUGCCUGCUAGGGUCU	4550
TGTCATCCCAGCTACTAGGG	1457	AGG	-	UGUCAUCCCAGCUACUAGGG	4551
TGTGCCCACGGACCTAGGTG	1458	AGG	+	UGUGCCCACGGACCUAGGUG	4552
GCTACTTGGAAGACTGAGGT	1459	GGG	-	GCUACUUGGAAGACUGAGGU	4553
GCCCCGCTGCTCAAACCTCT	1460	GGG	-	GCCCCGCUGCUCAAACCUCU	4554
TCTAACACTTTGGGAGGCTG	1461	AGG	-	UCUAACACUUUGGGAGGCUG	4555
TCACCTAGGTCCGTGGGCAC	1462	AGG	-	UCACCUAGGUCCGUGGGCAC	4556
TCCCAGGTGGCTGGGATTAC	1463	AGG	+	UCCCAGGUGGCUGGGAUUAC	4557
AGCTACTTGGAAGACTGAGG	1464	TGG	-	AGCUACUUGGAAGACUGAGG	4558
CAGGCCGATCACTTGAGGTC	1465	AGG	-	CAGGCCGAUCACUUGAGGUC	4559
CAGACGTGTGCCACCACGCC	1466	CGG	+	CAGACGUGUGCCACCACGCC	4560
GTCTCAAATTCCTGGGCTCA	1467	AGG	-	GUCUCAAAUUCCUGGGCUCA	4561
ACTACAGGTGTGCACCACCA	1468	TGG	+	ACUACAGGUGUGCACCACCA	4562
GAGACAGGGTTTCACCACCT	1469	TGG	+	GAGACAGGGUUUCACCACCU	4563
TGTAATCCCAGCCACCTGGG	1470	AGG	-	UGUAAUCCCAGCCACCUGGG	4564
GACTGTAATCCCAGCACTTC	1471	AGG	+	GACUGUAAUCCCAGCACUUC	4565
CTCAAGCAATCCTCTGGCCT	1472	TGG	-	CUCAAGCAAUCCUCUGGCCU	4566
TCTCAAATTCCTGGGCTCAA	1473	GGG	-	UCUCAAAUUCCUGGGCUCAA	4567
AGGGTTTCACCACCTTGGCC	1474	AGG	+	AGGGUUUCACCACCUUGGCC	4568
CAGGCGTGAGCCCCTGCACC	1475	TGG	-	CAGGCGUGAGCCCCUGCACC	4569
TTGCTCAGGGTGGAGTGCAG	1476	TGG	+	UUGCUCAGGGUGGAGUGCAG	4570
ATAGGCACAGGATGGGGGCG	1477	TGG	-	AUAGGCACAGGAUGGGGGCG	4571
CACTGCAGCCTTGAACTCCA	1478	GGG	-	CACUGCAGCCUUGAACUCCA	4572
GCTACTAGGGAGGCTGAGGG	1479	AGG	-	GCUACUAGGGAGGCUGAGGG	4573
CAGGCACGCACCACCACATC	1480	CGG	+	CAGGCACGCACCACCACAUC	4574

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GCCACCTGGGAGGCTGAGGC	1481	AGG	-	GCCACCUGGGAGGCUGAGGC	4575
GAAGACTGCTTGAGCCCAGG	1482	AGG	+	GAAGACUGCUUGAGCCCAGG	4576
TCCTGCCTCAGCCTCCCAGG	1483	TGG	+	UCCUGCCUCAGCCUCCCAGG	4577
TAAATAAATAAAAATTAGCC	1484	AGG	-	UAAAUAAAUAAAAAUUAGCC	4578
TTAATTTTTTTGTAGAGACG	1485	GGG	-	UUAAUUUUUUUGUAGAGACG	4579
GCCTCAGCCTCCCAGGTGGC	1486	TGG	+	GCCUCAGCCUCCCAGGUGGC	4580
CCCTCAGCCTCCCTAGTAGC	1487	TGG	+	CCCUCAGCCUCCCUAGUAGC	4581
AAAAAAAAAAAAAAAAGACAT	1488	AGG	-	AAAAAAAAAAAAAAAAGACAU	4582
AAAAAAAAAGCCAGGCACAG	1489	TGG	+	AAAAAAAAAGCCAGGCACAG	4583
CCTGTCCTCACCTAGGTCCG	1490	TGG	-	CCUGUCCUCACCUAGGUCCG	4584
AACCCGGGAGATGGAGGTTG	1491	TGG	-	AACCCGGGAGAUGGAGGUUG	4585
GTCTGTAATCCCAGCCACTC	1492	GGG	-	GUCUGUAAUCCCAGCCACUC	4586
GGGTTTCAACATATGAATTT	1493	TGG	+	GGGUUUCAACAUAUGAAUUU	4587
CTGTCCTCACCTAGGTCCGT	1494	GGG	-	CUGUCCUCACCUAGGUCCGU	4588
CCACGGACCTAGGTGAGGAC	1495	AGG	+	CCACGGACCUAGGUGAGGAC	4589
TCTTGCTCTGTCACCCACAC	1496	TGG	-	UCUUGCUCUGUCACCCACAC	4590
TCTTAAGTAGCTGGGACTAC	1497	AGG	-	UCUUAAGUAGCUGGGACUAC	4591
TCACCACAACCTCCATCTCC	1498	CGG	+	UCACCACAACCUCCAUCUCC	4592
CCAGCTACTAGGGAGGCTGA	1499	GGG	-	CCAGCUACUAGGGAGGCUGA	4593
AGGAGTGCCTGTCCTCACCT	1500	AGG	-	AGGAGUGCCUGUCCUCACCU	4594
CACCACAACCTCCATCTCCC	1501	GGG	+	CACCACAACCUCCAUCUCCC	4595
GTTACTCAGGAAGCTGAGGC	1502	AGG	+	GUUACUCAGGAAGCUGAGGC	4596
TGTAGTCCCAGCTACTTAAG	1503	AGG	+	UGUAGUCCCAGCUACUUAAG	4597
CCAGAGGATTGCTTGAGCCC	1504	AGG	+	CCAGAGGAUUGCUUGAGCCC	4598
GGCTTGAGCCTGGGAGGTTG	1505	AGG	-	GGCUUGAGCCUGGGAGGUUG	4599
GATCAAGGTGCCAGCAGATT	1506	TGG	+	GAUCAAGGUGCCAGCAGAUU	4600
CCTCAGCCTCCCAGGTGGCT	1507	GGG	+	CCUCAGCCUCCCAGGUGGCU	4601
GGGAGGCTGAGGTGAGAGGA	1508	TGG	+	GGGAGGCUGAGGUGAGAGGA	4602
CCCAGCCACCTGGGAGGCTG	1509	AGG	-	CCCAGCCACCUGGGAGGCUG	4603
ACAGGTGCAGCACACCAACA	1510	TGG	+	ACAGGUGCAGCACACCAACA	4604
GCACCACTGCACTCCAGTGT	1511	GGG	+	GCACCACUGCACUCCAGUGU	4605
GCCTCAGCTTCCTGAGTAAC	1512	TGG	-	GCCUCAGCUUCCUGAGUAAC	4606
TGAGAGGATGGCTTGAGCCC	1513	TGG	+	UGAGAGGAUGGCUUGAGCCC	4607
TGTAATTCTAACACTTTGGG	1514	AGG	-	UGUAAUUCUAACACUUUGGG	4608
CCTGGGCTCAAGCAATCCTC	1515	TGG	-	CCUGGGCUCAAGCAAUCCUC	4609
GAGCCAGGGTTTCACCATGT	1516	TGG	+	GAGCCAGGGUUUCACCAUGU	4610
GAGAATCGATTGAACCCAGG	1517	CGG	-	GAGAAUCGAUUGAACCCAGG	4611
GGTTTCAACATATGAATTTT	1518	GGG	+	GGUUUCAACAUAUGAAUUUU	4612
GGAGTGCAGTGGTGCAATAT	1519	CGG	+	GGAGUGCAGUGGUGCAAUAU	4613
ATACAAAAATTAGCCGGCCA	1520	TGG	-	AUACAAAAAUUAGCCGGCCA	4614
CCCAGTTACTCAGGAAGCTG	1521	AGG	+	CCCAGUUACUCAGGAAGCUG	4615
CAAAAATTAGCCGGCCATGG	1522	TGG	-	CAAAAAUUAGCCGGCCAUGG	4616
AAATAAAAATTAGCCAGGTG	1523	TGG	-	AAAUAAAAAUUAGCCAGGUG	4617
CAGGTGCATACCACCACACC	1524	TGG	-	CAGGUGCAUACCACCACACC	4618
GCACTTTGGGAAGCTGAGAC	1525	AGG	-	GCACUUUGGGAAGCUGAGAC	4619
CCTCAGCTTCCTGAGTAACT	1526	GGG	-	CCUCAGCUUCCUGAGUAACU	4620
CTTTGGGAGGGCGAGGTGGG	1527	AGG	+	CUUUGGGAGGGCGAGGUGGG	4621
GCTACTTAAGAGGCTGAGGC	1528	AGG	+	GCUACUUAAGAGGCUGAGGC	4622
TGGGAAGACTGCTTGAGCCC	1529	AGG	+	UGGGAAGACUGCUUGAGCCC	4623
GTTTCAACATATGAATTTTG	1530	GGG	+	GUUUCAACAUAUGAAUUUUG	4624
GCCTCAGCCTCTTAAGTAGC	1531	TGG	-	GCCUCAGCCUCUUAAGUAGC	4625
CAAGGCAGGTGGATCACCTA	1532	AGG	+	CAAGGCAGGUGGAUCACCUA	4626
TTCAACATATGAATTTTGGG	1533	GGG	+	UUCAACAUAUGAAUUUUGGG	4627
CTGCCACTGTACTCCAGCCT	1534	GGG	+	CUGCCACUGUACUCCAGCCU	4628
TTGTGTTTTTTGTAGAGACA	1535	GGG	+	UUGUGUUUUUUGUAGAGACA	4629
TTTCACCACCTTGGCCAGGC	1536	TGG	+	UUUCACCACCUUGGCCAGGC	4630
TCTCGCTCAGTCACCCAGGC	1537	TGG	-	UCUCGCUCAGUCACCCAGGC	4631
TGCATTTCCCAAGACCACCC	1538	TGG	+	UGCAUUUCCCAAGACCACCC	4632

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TCTTTCTTTCTTTTTTGAGA	1539	TGG	-	UCUUUCUUUCUUUUUUGAGA	4633
CCTCAGTCTTCCAAGTAGCT	1540	CGG	+	CCUCAGUCUUCCAAGUAGCU	4634
CTTTGGGAGGACAAGGCAGG	1541	TGG	+	CUUUGGGAGGACAAGGCAGG	4635
GAGTGCAAGGTTTTATTGAG	1542	TGG	-	GAGUGCAAGGUUUUAUUGAG	4636
GTAATCTCAGCACTTTGGGA	1543	GGG	+	GUAAUCUCAGCACUUUGGGA	4637
CAAACATTAGCCAGGCATGG	1544	TGG	+	CAAACAUUAGCCAGGCAUGG	4638
TGAGACCAGCCTGGGCAGCA	1545	TGG	-	UGAGACCAGCCUGGGCAGCA	4639
ATACAAACATTAGCCAGGCA	1546	TGG	+	AUACAAACAUUAGCCAGGCA	4640
TATTATTATTATTTTGAGAC	1547	AGG	-	UAUUAUUAUUAUUUUGAGAC	4641
CCCAGCTACTTAAGAGGCTG	1548	AGG	+	CCCAGCUACUUAAGAGGCUG	4642
TCACCCACACTGGAGTGCAG	1549	TGG	-	UCACCCACACUGGAGUGCAG	4643
GCACTTTGGGAGGGCGAGGT	1550	GGG	+	GCACUUUGGGAGGGCGAGGU	4644
GAGACAGGGTTTTGCTATGT	1551	TGG	-	GAGACAGGGUUUUGCUAUGU	4645
CCTCAGCCTCTTAAGTAGCT	1552	GGG	-	CCUCAGCCUCUUAAGUAGCU	4646
AGGGTTTTGCTATGTTGGCC	1553	AGG	-	AGGGUUUUGCUAUGUUGGCC	4647
TTTGTGTTTTTTGTAGAGAC	1554	AGG	+	UUUGUGUUUUUUGUAGAGAC	4648
TTTCAACATATGAATTTTGG	1555	GGG	+	UUUCAACAUAUGAAUUUUGG	4649
GGGGCCTTTGGGAGGTGATT	1556	AGG	-	GGGGCCUUUGGGAGGUGAUU	4650
AGTGGAATTGCTGGGTCATA	1557	GGG	+	AGUGGAAUUGCUGGGUCAUA	4651
CACTGTAGCCTCAACCTCCC	1558	AGG	+	CACUGUAGCCUCAACCUCCC	4652
CAAAAAACACAAAAATTAGC	1559	TGG	-	CAAAAAACACAAAAAUUAGC	4653
GGAGTACAATGGCGCGATCT	1560	CGG	-	GGAGUACAAUGGCGCGAUCU	4654
TTTTTTTTTTCTTTTGAGAC	1561	TGG	-	UUUUUUUUUUCUUUUGAGAC	4655
TGGCCAACATGGTGAAACCC	1562	TGG	-	UGGCCAACAUGGUGAAACCC	4656
CGCTTGAACCCGGAAGGCAG	1563	AGG	+	CGCUUGAACCCGGAAGGCAG	4657
GAGACGGGGTTTCACCAGGT	1564	TGG	-	GAGACGGGGUUUCACCAGGU	4658
TGGGGCCTGTCGTGGGGTGG	1565	GGG	+	UGGGGCCUGUCGUGGGGUGG	4659
TCTCAGCTTCCCAAAGTGCT	1566	AGG	+	UCUCAGCUUCCCAAAGUGCU	4660
CTGGGGCCTGTCGTGGGGTG	1567	GGG	+	CUGGGGCCUGUCGUGGGGUG	4661
CGTTTGAACCTGGGAGGCGG	1568	AGG	-	CGUUUGAACCUGGGAGGCGG	4662
CACTGGGGCCTGTCGTGGGG	1569	TGG	+	CACUGGGGCCUGUCGUGGGG	4663
AACTCCTGACCTCAAGTGAT	1570	CGG	+	AACUCCUGACCUCAAGUGAU	4664
CAGGCTGGTCTTGAGCTCCT	1571	GGG	-	CAGGCUGGUCUUGAGCUCCU	4665
CGCGCCATTGTACTCCAGCC	1572	TGG	+	CGCGCCAUUGUACUCCAGCC	4666
CGCTTGAACCCGGGAGATGG	1573	AGG	-	CGCUUGAACCCGGGAGAUGG	4667
AGAGTCTTACTCTGTCGCCC	1574	AGG	+	AGAGUCUUACUCUGUCGCCC	4668
AAGAGGCTGAGGCAGGAGAA	1575	TGG	+	AAGAGGCUGAGGCAGGAGAA	4669
AAAAAACACAAAAATTAGCT	1576	GGG	-	AAAAAACACAAAAAUUAGCU	4670
ACTGGGGCCTGTCGTGGGGT	1577	GGG	+	ACUGGGGCCUGUCGUGGGGU	4671
GCACTTTGGGAGGACAAGGC	1578	AGG	+	GCACUUUGGGAGGACAAGGC	4672
GCCTTGGCCTCCTGAAGTGC	1579	TGG	-	GCCUUGGCCUCCUGAAGUGC	4673
GCCTTGTCCTCCCAAAGTGC	1580	TGG	-	GCCUUGUCCUCCCAAAGUGC	4674
AGCACTTTGGGAGGGCGAGG	1581	TGG	+	AGCACUUUGGGAGGGCGAGG	4675
ACACACTGGGGCCTGTCGTG	1582	GGG	+	ACACACUGGGGCCUGUCGUG	4676
TGGCTGTAATCCCAGCTACT	1583	CGG	+	UGGCUGUAAUCCCAGCUACU	4677
CAGGAGAATCACTTGAAACC	1584	CGG	-	CAGGAGAAUCACUUGAAACC	4678
TGCCTGTCATCCCAGCTACT	1585	AGG	-	UGCCUGUCAUCCCAGCUACU	4679
CACACACTGGGGCCTGTCGT	1586	GGG	+	CACACACUGGGGCCUGUCGU	4680
CAGGTGGATCACCTAAGGTC	1587	AGG	+	CAGGUGGAUCACCUAAGGUC	4681
TGTTGTTGTTGTTTTTGAGA	1588	TGG	+	UGUUGUUGUUGUUUUUGAGA	4682
TGCCTCTAATCCCAGCTACT	1589	CGG	+	UGCCUCUAAUCCCAGCUACU	4683
GGAGTGCAGTGGAGCGATCT	1590	TGG	+	GGAGUGCAGUGGAGCGAUCU	4684
CCCAGCACTTCAGGAGGCCA	1591	AGG	+	CCCAGCACUUCAGGAGGCCA	4685
GGCTGTAATCCCAGCTACTC	1592	GGG	+	GGCUGUAAUCCCAGCUACUC	4686
TGCTTGAGCCCAGGAGGTCG	1593	AGG	+	UGCUUGAGCCCAGGAGGUCG	4687
TTTGTATTTTTGTAGAGACG	1594	GGG	-	UUUGUAUUUUUGUAGAGACG	4688
GGAGTCTTACTCTGTTGCCC	1595	AGG	+	GGAGUCUUACUCUGUUGCCC	4689
CCTTGGCCTCCTGAAGTGCT	1596	GGG	-	CCUUGGCCUCCUGAAGUGCU	4690

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CAGGAGCTCAAGACCAGCCT	1597	GGG	+	CAGGAGCUCAAGACCAGCCU	4691
CCAGGCTGGTCTTGAGCTCC	1598	TGG	-	CCAGGCUGGUCUUGAGCUCC	4692
CCAGGAGCTCAAGACCAGCC	1599	TGG	+	CCAGGAGCUCAAGACCAGCC	4693
GAATTTCACTCTTGTTGCCC	1600	AGG	+	GAAUUUCACUCUUGUUGCCC	4694
CGATAGTTTGCTCAGAATGA	1601	TGG	-	CGAUAGUUUGCUCAGAAUGA	4695
CCCAGCACTTTGGGAGGACA	1602	AGG	+	CCCAGCACUUUGGGAGGACA	4696
ATTCTGAGCAAACTATCGCA	1603	AGG	+	AUUCUGAGCAAACUAUCGCA	4697
CTAAAGATACAAAAATTAGC	1604	CGG	-	CUAAAGAUACAAAAAUUAGC	4698
CCTTGTCCTCCCAAAGTGCT	1605	GGG	-	CCUUGUCCUCCCAAAGUGCU	4699
CGCTCCACTGCACTCCAGCC	1606	TGG	-	CGCUCCACUGCACUCCAGCC	4700
GCGCCATTGTACTCCAGCCT	1607	GGG	+	GCGCCAUUGUACUCCAGCCU	4701
ATCTCAAAAAAAAAAAAAAA	1608	AGG	+	AUCUCAAAAAAAAAAAAAAA	4702
GAGCGGGGAGGGATAGCATT	1609	AGG	+	GAGCGGGGAGGGAUAGCAUU	4703
TCAAGCGATTCTCCTGCCTC	1610	AGG	+	UCAAGCGAUUCUCCUGCCUC	4704
TTAATCCAGTCTATCACTGA	1611	TGG	-	UUAAUCCAGUCUAUCACUGA	4705
GAGAATCGCTTGAACCCGGA	1612	AGG	+	GAGAAUCGCUUGAACCCGGA	4706
GCCTCTAATCCCAGCTACTC	1613	GGG	+	GCCUCUAAUCCCAGCUACUC	4707
TTGTAATTTTAGTAGAGACG	1614	GGG	+	UUGUAAUUUUAGUAGAGACG	4708
AATCGCTTGAACCCGGGAGA	1615	TGG	-	AAUCGCUUGAACCCGGGAGA	4709
TTTTGCTATGTTGGCCAGGC	1616	TGG	-	UUUUGCUAUGUUGGCCAGGC	4710
TAAAAATACAAACATTAGCC	1617	AGG	+	UAAAAAUACAAACAUUAGCC	4711
TCACACACTGGGGCCTGTCG	1618	TGG	+	UCACACACUGGGGCCUGUCG	4712
CCATGTTGGTCAGGCTGGTC	1619	TGG	-	CCAUGUUGGUCAGGCUGGUC	4713
CCAGACCAGCCTGACCAACA	1620	TGG	+	CCAGACCAGCCUGACCAACA	4714
TGACTGTAATCCCAGCACTT	1621	TGG	+	UGACUGUAAUCCCAGCACUU	4715
CAGGCTGGTCTCAAATTCCT	1622	GGG	-	CAGGCUGGUCUCAAAUUCCU	4716
TGGGAGGATCCCTTGAGCCC	1623	AGG	+	UGGGAGGAUCCCUUGAGCCC	4717
TGCACCACTGCACTCCAGTC	1624	TGG	-	UGCACCACUGCACUCCAGUC	4718
TCTTACTCTGTCGCCCAGGC	1625	TGG	+	UCUUACUCUGUCGCCCAGGC	4719
TCTCACTCTGTTGCCCAGAC	1626	TGG	+	UCUCACUCUGUUGCCCAGAC	4720
AACTGCTTGAACCCAGGAGG	1627	CGG	+	AACUGCUUGAACCCAGGAGG	4721
ACACAAAAATTAGCTGGGCA	1628	TGG	-	ACACAAAAAUUAGCUGGGCA	4722
GGATTACAGGCGTGAGCCAC	1629	GGG	+	GGAUUACAGGCGUGAGCCAC	4723
GCCGAGGTGGGCGGATCATG	1630	AGG	+	GCCGAGGUGGGCGGAUCAUG	4724
CAGAAGTTTGAGACCAGCCT	1631	GGG	-	CAGAAGUUUGAGACCAGCCU	4725
GAGGTCTCACTATGTTGCCC	1632	AGG	-	GAGGUCUCACUAUGUUGCCC	4726
TTTTGTATTTTTGTAGAGAC	1633	GGG	-	UUUUGUAUUUUUGUAGAGAC	4727
TCGGGAGTTCGAGACCAGCC	1634	TGG	-	UCGGGAGUUCGAGACCAGCC	4728
TGCCTGTAACCCCAGCACTT	1635	TGG	+	UGCCUGUAACCCCAGCACUU	4729
TTTGTAATTTTAGTAGAGAC	1636	GGG	+	UUUGUAAUUUUAGUAGAGAC	4730
GCTCCACTGCACTCCAGCCT	1637	GGG	-	GCUCCACUGCACUCCAGCCU	4731
GCAGGCAGATCACCTGAGGT	1638	CGG	-	GCAGGCAGAUCACCUGAGGU	4732
TCCTGAGTAACTGGGATTAC	1639	CGG	-	UCCUGAGUAACUGGGAUUAC	4733
CTTGGCCTCCCAAAGTGCTG	1640	GGG	-	CUUGGCCUCCCAAAGUGCUG	4734
CAGGTGTGTGCCACCACACC	1641	TGG	+	CAGGUGUGUGCCACCACACC	4735
CCAGGCTGGTCTCAAATTCC	1642	TGG	-	CCAGGCUGGUCUCAAAUUCC	4736
CCAGGAATTTGAGACCAGCC	1643	TGG	+	CCAGGAAUUUGAGACCAGCC	4737
TTTTTGTATTTTTGTAGAGA	1644	CGG	-	UUUUUGUAUUUUUGUAGAGA	4738
TGTAATCCCAGCACTTCAGG	1645	AGG	+	UGUAAUCCCAGCACUUCAGG	4739
GAGAACTGCTTGAACCCAGG	1646	AGG	+	GAGAACUGCUUGAACCCAGG	4740
CAGGAATTTGAGACCAGCCT	1647	GGG	+	CAGGAAUUUGAGACCAGCCU	4741
TCAGAAGTTTGAGACCAGCC	1648	TGG	-	UCAGAAGUUUGAGACCAGCC	4742
GACTGTAATCCCAGCACTTT	1649	GGG	+	GACUGUAAUCCCAGCACUUU	4743
GCACCACTGCACTCCAGTCT	1650	GGG	-	GCACCACUGCACUCCAGUCU	4744
TAAAAATTAGCCAGGTGTGG	1651	TGG	-	UAAAAAUUAGCCAGGUGUGG	4745
TTTTGTAATTTTAGTAGAGA	1652	CGG	+	UUUUGUAAUUUUAGUAGAGA	4746
TAAAAATACAAAAATCAGCT	1653	GGG	-	UAAAAAUACAAAAAUCAGCU	4747
GCCTCGGTCTCCCAAAGTGC	1654	TGG	+	GCCUCGGUCUCCCAAAGUGC	4748

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GCACTTTGGGAGACCGAGGC	1655	GGG	-	GCACUUUGGGAGACCGAGGC	4749
AGCACTTTGGGAGACCGAGG	1656	CGG	-	AGCACUUUGGGAGACCGAGG	4750
ACCTCATGATCCGCCCACCT	1657	CGG	-	ACCUCAUGAUCCGCCCACCU	4751
CACTGCAACCTCTGCCTTCC	1658	GGG	-	CACUGCAACCUCUGCCUUCC	4752
TCTTACTCTGTTGCCCAGGC	1659	TGG	+	UCUUACUCUGUUGCCCAGGC	4753
CACTGCAGCCTCGACCTCCT	1660	GGG	-	CACUGCAGCCUCGACCUCCU	4754
AAAAAAAAAAAAAAAAAAGCC	1661	AGG	+	AAAAAAAAAAAAAAAAAAGCC	4755
GGGGTTTCTCCATGTTGGCC	1662	AGG	+	GGGGUUUCUCCAUGUUGGCC	4756
CAGGAGAACTGCTTGAACCC	1663	AGG	+	CAGGAGAACUGCUUGAACCC	4757
GGTGGGCGGATCATGAGGTC	1664	AGG	+	GGUGGGCGGAUCAUGAGGUC	4758
TTGCCCAGGCTGGAGTACAA	1665	TGG	-	UUGCCCAGGCUGGAGUACAA	4759
TCACTGCAGCCTCGACCTCC	1666	TGG	-	UCACUGCAGCCUCGACCUCC	4760
TCACTGCAACCTCTGCCTTC	1667	CGG	-	UCACUGCAACCUCUGCCUUC	4761
GGGATTACAGGCGTGAGCCA	1668	CGG	+	GGGAUUACAGGCGUGAGCCA	4762
GCCTGTAGTCCCAGCTACTA	1669	GGG	-	GCCUGUAGUCCCAGCUACUA	4763
CTAAAAATACAAAAATCAGC	1670	TGG	-	CUAAAAAUACAAAAAUCAGC	4764
CAGGCGCCCGCCACCACACC	1671	CGG	-	CAGGCGCCCGCCACCACACC	4765
CCCAGCACTTTGGGAGACCG	1672	AGG	-	CCCAGCACUUUGGGAGACCG	4766
TTGCCCAGACTGGAGTGCAG	1673	TGG	+	UUGCCCAGACUGGAGUGCAG	4767
CAAAAAAAAAAAAAAAAAAA	1674	AGG	+	CAAAAAAAAAAAAAAAAAAA	4768
ACACTTTGGGAGGCTGAGGT	1675	GGG	-	ACACUUUGGGAGGCUGAGGU	4769
CCTCGGTCTCCCAAAGTGCT	1676	GGG	+	CCUCGGUCUCCCAAAGUGCU	4770
TCACTGCAGCCTTGAACTCC	1677	AGG	-	UCACUGCAGCCUUGAACUCC	4771
AAAAAAAAAAAAAAAAAAGA	1678	GGG	-	AAAAAAAAAAAAAAAAAAGA	4772
GCCTGTAACCCCAGCACTTT	1679	GGG	+	GCCUGUAACCCCAGCACUUU	4773
ATTATTATACTTTAAGTTCT	1680	AGG	-	AUUAUUAUACUUUAAGUUCU	4774
TTGTATTTTTAGTAGAGACT	1681	GGG	-	UUGUAUUUUUAGUAGAGACU	4775
TCGCCCAGGCTGGAGTACAG	1682	TGG	-	UCGCCCAGGCUGGAGUACAG	4776
CGAGGCAGGCAGATCACCTG	1683	AGG	-	CGAGGCAGGCAGAUCACCUG	4777
CGAGGCAGGCAGATCACCTG	1684	AGG	-	CGAGGCAGGCAGAUCACCUG	4778
TCCCTAGTAGCTGGGACTAC	1685	AGG	+	UCCCUAGUAGCUGGGACUAC	4779
TTTCTCCATGTTGGCCAGGC	1686	TGG	+	UUUCUCCAUGUUGGCCAGGC	4780
AATACAAAATTAGCCAGGCA	1687	TGG	+	AAUACAAAAUUAGCCAGGCA	4781
TGTAGTCCCAGCTACTAGGG	1688	AGG	-	UGUAGUCCCAGCUACUAGGG	4782
GGAGTGCAATGGCGCAATCT	1689	CGG	-	GGAGUGCAAUGGCGCAAUCU	4783
ACAAAATTAGCCAGGCATGG	1690	TGG	+	ACAAAAUUAGCCAGGCAUGG	4784
AATGGTGTGAACCCGGGAGG	1691	CGG	+	AAUGGUGUGAACCCGGGAGG	4785
TTCTCCTGCCTCAGCCTCCC	1692	AGG	+	UUCUCCUGCCUCAGCCUCCC	4786
TCAGGAGTTCCAGACCAGCC	1693	TGG	-	UCAGGAGUUCCAGACCAGCC	4787
TCCCAAAGTGCTGGGGTTAC	1694	AGG	-	UCCCAAAGUGCUGGGGUUAC	4788
TGTAACCCCAGCACTTTGGG	1695	AGG	+	UGUAACCCCAGCACUUUGGG	4789
AAAAAAAAAAAAAAAAAAAG	1696	AGG	-	AAAAAAAAAAAAAAAAAAAG	4790
TACAAAAAATTAGCCGGGTG	1697	TGG	+	UACAAAAAAUUAGCCGGGUG	4791
CAGGTGTGAGCCACCACACC	1698	CGG	-	CAGGUGUGAGCCACCACACC	4792
AACACTTTGGGAGGCTGAGG	1699	TGG	-	AACACUUUGGGAGGCUGAGG	4793
GGTCTCGAACTCCCGACCTC	1700	AGG	+	GGUCUCGAACUCCCGACCUC	4794
TACAAAAAATTAGCCAGGTG	1701	TGG	+	UACAAAAAAUUAGCCAGGUG	4795
CAGGCATGAGCCACCGCACC	1702	TGG	+	CAGGCAUGAGCCACCGCACC	4796
TGCGCCATTGCACTCCAGCC	1703	TGG	+	UGCGCCAUUGCACUCCAGCC	4797
TTATTATACTTTAAGTTCTA	1704	GGG	-	UUAUUAUACUUUAAGUUCUA	4798
GAGAATGGTGTGAACCCGGG	1705	AGG	+	GAGAAUGGUGUGAACCCGGG	4799
AATTAGCCGGGTGTGGTGGC	1706	GGG	+	AAUUAGCCGGGUGUGGUGGC	4800
CACCGCATGTTCTCACTCAT	1707	AGG	+	CACCGCAUGUUCUCACUCAU	4801
CAAGGCAGGTGGATCACCTG	1708	AGG	+	CAAGGCAGGUGGAUCACCUG	4802
CGCATGTTCTCACTCATAGG	1709	TGG	+	CGCAUGUUCUCACUCAUAGG	4803
AAATTAGCCGGGTGTGGTGG	1710	CGG	+	AAAUUAGCCGGGUGUGGUGG	4804
CGCCTGTAATCTCAGCACTT	1711	TGG	+	CGCCUGUAAUCUCAGCACUU	4805
CTCAGGTGATCTGCCTGCCT	1712	CGG	+	CUCAGGUGAUCUGCCUGCCU	4806

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CTCAGGTGATCTGCCTGCCT	1713	CGG	+	CUCAGGUGAUCUGCCUGCCU	4807
GCTACTAGGGAGGCTGAGGC	1714	AGG	-	GCUACUAGGGAGGCUGAGGC	4808
TGCTTGAACCCAGGAGGCGG	1715	AGG	+	UGCUUGAACCCAGGAGGCGG	4809
GCCTCAGCCTCCCTAGTAGC	1716	TGG	+	GCCUCAGCCUCCCUAGUAGC	4810
GAGACGGGGTTTCTCCATGT	1717	TGG	+	GAGACGGGGUUUCUCCAUGU	4811
GAGTGAGAACATGCGGTGTT	1718	TGG	-	GAGUGAGAACAUGCGGUGUU	4812
GGTACATGTGCACAACGTGC	1719	AGG	-	GGUACAUGUGCACAACGUGC	4813
AAAAAATTAGCCGGGTGTGG	1720	TGG	+	AAAAAAUUAGCCGGGUGUGG	4814
CCTAGCACTTTGGGAGGCCG	1721	AGG	+	CCUAGCACUUUGGGAGGCCG	4815
CAGGCGTGAGCCACTGTGCC	1722	TGG	-	CAGGCGUGAGCCACUGUGCC	4816
CCCAGCTACTAGGGAGGCTG	1723	AGG	-	CCCAGCUACUAGGGAGGCUG	4817
CCCAGCTACTAGGGAGGCTG	1724	AGG	-	CCCAGCUACUAGGGAGGCUG	4818
CGCCTGTAATCCTAGCACTT	1725	TGG	+	CGCCUGUAAUCCUAGCACUU	4819
TGTAGGGACATGGATGAAGC	1726	TGG	+	UGUAGGGACAUGGAUGAAGC	4820
CCTCAGCCTCCCTAGTAGCT	1727	GGG	+	CCUCAGCCUCCCUAGUAGCU	4821
CCTCAGCCTCCCTAGTAGCT	1728	GGG	+	CCUCAGCCUCCCUAGUAGCU	4822
TGCCTGTAATCCTAGCACTT	1729	TGG	-	UGCCUGUAAUCCUAGCACUU	4823
CAGGCAGATCACCTGAGGTC	1730	AGG	-	CAGGCAGAUCACCUGAGGUC	4824
CAGGCAGATCACCTGAGGTC	1731	GGG	-	CAGGCAGAUCACCUGAGGUC	4825
GCATGTTCTCACTCATAGGT	1732	GGG	+	GCAUGUUCUCACUCAUAGGU	4826
CACCTATGAGTGAGAACATG	1733	CGG	-	CACCUAUGAGUGAGAACAUG	4827
GCAGGAGAATGGTGTGAACC	1734	CGG	+	GCAGGAGAAUGGUGUGAACC	4828
CAGGCATGAGCCACCATGCC	1735	CGG	-	CAGGCAUGAGCCACCAUGCC	4829
CAGGAGAATGGTGTGAACCC	1736	GGG	+	CAGGAGAAUGGUGUGAACCC	4830
CATGCCATTGCACTCCAGCC	1737	TGG	+	CAUGCCAUUGCACUCCAGCC	4831
TCTCACTATGTTGCCCAGGC	1738	TGG	-	UCUCACUAUGUUGCCCAGGC	4832
ATGCCATTGCACTCCAGCCT	1739	GGG	+	AUGCCAUUGCACUCCAGCCU	4833
AAAAAATTAGCCAGGTGTGG	1740	TGG	+	AAAAAAUUAGCCAGGUGUGG	4834
TATGTATACATGTGCCATGT	1741	TGG	-	UAUGUAUACAUGUGCCAUGU	4835
GGGGTTTCACTGTGTTAGCC	1742	AGG	-	GGGGUUUCACUGUGUUAGCC	4836
GAGTTTCACTCTTGTTGCCC	1743	AGG	-	GAGUUUCACUCUUGUUGCCC	4837
CTCAGGTGATCCACCTGCCT	1744	TGG	-	CUCAGGUGAUCCACCUGCCU	4838
TTCACTCTTGTTGCCCAGGC	1745	TGG	+	UUCACUCUUGUUGCCCAGGC	4839
TTCACTCTTGTTGCCCAGGC	1746	TGG	-	UUCACUCUUGUUGCCCAGGC	4840
GCCTGTAATCTCAGCACTTT	1747	GGG	+	GCCUGUAAUCUCAGCACUUU	4841
GCGCCATTGCACTCCAGCCT	1748	GGG	+	GCGCCAUUGCACUCCAGCCU	4842
GGGGTTTCACCATGTTGGTC	1749	AGG	-	GGGGUUUCACCAUGUUGGUC	4843
ACCTCAGCCTCCTGAGTAGC	1750	TGG	-	ACCUCAGCCUCCUGAGUAGC	4844
AGCTACTCAGGAGGCTGAGG	1751	CGG	-	AGCUACUCAGGAGGCUGAGG	4845
AGCTACTCAGGAGGCTGAGG	1752	TGG	+	AGCUACUCAGGAGGCUGAGG	4846
GCTACTCAGGAGGCTGAGGT	1753	GGG	+	GCUACUCAGGAGGCUGAGGU	4847
TTTTTTTTTTTTTTTGAGAC	1754	AGG	+	UUUUUUUUUUUUUUUGAGAC	4848
TTTTTTTTTTTTTTTGAGAC	1755	AGG	-	UUUUUUUUUUUUUUUGAGAC	4849
TTTTTTTTTTTTTTGAGACA	1756	GGG	+	UUUUUUUUUUUUUUGAGACA	4850
TTTTTTTTTTTTTTGAGACA	1757	GGG	-	UUUUUUUUUUUUUUGAGACA	4851
TTTCACTGTGTTAGCCAGGA	1758	TGG	-	UUUCACUGUGUUAGCCAGGA	4852
TCACCCAGGCTGGAGTGCAA	1759	TGG	-	UCACCCAGGCUGGAGUGCAA	4853
ACCTGTAATCCCAGCTACTT	1760	GGG	+	ACCUGUAAUCCCAGCUACUU	4854
CTAAAAATACAAAATTAGCC	1761	AGG	+	CUAAAAAUACAAAAUUAGCC	4855
GCCTGTAATCCTAGCACTTT	1762	GGG	-	GCCUGUAAUCCUAGCACUUU	4856
GCCTGTAATCCTAGCACTTT	1763	GGG	+	GCCUGUAAUCCUAGCACUUU	4857
GGAGTGCAGTGGCGTGATCT	1764	TGG	+	GGAGUGCAGUGGCGUGAUCU	4858
GGAGTGCAGTGGCGTGATCT	1765	CGG	+	GGAGUGCAGUGGCGUGAUCU	4859
TCCCAAAGTGCTGAGATTAC	1766	AGG	-	UCCCAAAGUGCUGAGAUUAC	4860
TGTAATCTCAGCACTTTGGG	1767	AGG	+	UGUAAUCUCAGCACUUUGGG	4861
TAGACTGGATTAAGAAAATG	1768	TGG	+	UAGACUGGAUUAAGAAAAUG	4862
TTTCACCATGTTGGTCAGGC	1769	TGG	-	UUUCACCAUGUUGGUCAGGC	4863
CACTGCAACCTCCGCCTCCT	1770	GGG	-	CACUGCAACCUCCGCCUCCU	4864

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGAGTCTCGCTCTGTCGCCC	1771	AGG	-	GGAGUCUCGCUCUGUCGCCC	4865
AATGTGGCACATATACACCA	1772	TGG	+	AAUGUGGCACAUAUACACCA	4866
ACGCCACTGCACTCCAGCCT	1773	GGG	-	ACGCCACUGCACUCCAGCCU	4867
TCCCAAAGTGCTAGGATTAC	1774	AGG	+	UCCCAAAGUGCUAGGAUUAC	4868
TCCCAAAGTGCTAGGATTAC	1775	AGG	-	UCCCAAAGUGCUAGGAUUAC	4869
GGAGTGCAGTGGCGCGATCT	1776	TGG	-	GGAGUGCAGUGGCGCGAUCU	4870
CACGCCACTGCACTCCAGCC	1777	TGG	-	CACGCCACUGCACUCCAGCC	4871
TGTAATCCTAGCACTTTGGG	1778	AGG	+	UGUAAUCCUAGCACUUUGGG	4872
CAAAAATTAGCTGGGCATGG	1779	TGG	-	CAAAAAUUAGCUGGGCAUGG	4873
CTTTGGGAGGCTGAGGTGGG	1780	AGG	-	CUUUGGGAGGCUGAGGUGGG	4874
GGTCTCAAACTCCTGACCTC	1781	AGG	-	GGUCUCAAACUCCUGACCUC	4875
GAGAATCGCTTGAACCCAGG	1782	AGG	+	GAGAAUCGCUUGAACCCAGG	4876
CAGGCGTGAGCCACCGCGCC	1783	CGG	+	CAGGCGUGAGCCACCGCGCC	4877
CTTTGGGAGGCCAAGGCAGG	1784	TGG	+	CUUUGGGAGGCCAAGGCAGG	4878
GGTCTTGAACTCCTGACCTC	1785	AGG	+	GGUCUUGAACUCCUGACCUC	4879
TCTCGCTCTGTCGCCCAGGC	1786	TGG	-	UCUCGCUCUGUCGCCCAGGC	4880
CTTTGGGAGGCCGAGGTGGG	1787	CGG	+	CUUUGGGAGGCCGAGGUGGG	4881
CACCTGTAATCCCAGCTACT	1788	TGG	+	CACCUGUAAUCCCAGCUACU	4882
CGCGCCACTGCACTCCAGCC	1789	TGG	+	CGCGCCACUGCACUCCAGCC	4883
ACCTGTAGTCCCAGCTACTC	1790	AGG	-	ACCUGUAGUCCCAGCUACUC	4884
AGGGTTTCACCATGTTGGCC	1791	AGG	+	AGGGUUUCACCAUGUUGGCC	4885
CACTGCAAGCTCCGCCTCCC	1792	GGG	-	CACUGCAAGCUCCGCCUCCC	4886
TGCCTGTAGTCCCAGCTACT	1793	AGG	-	UGCCUGUAGUCCCAGCUACU	4887
GGTCTCGAACTCCTGACCTC	1794	AGG	-	GGUCUCGAACUCCUGACCUC	4888
TCACTGCAAGCTCCGCCTCC	1795	CGG	-	UCACUGCAAGCUCCGCCUCC	4889
CACTGCAACCTCCGCCTCCC	1796	AGG	+	CACUGCAACCUCCGCCUCCC	4890
GCACTTTGGGAGGCCGAGGT	1797	GGG	+	GCACUUUGGGAGGCCGAGGU	4891
TCACTGCAACCTCCGCCTCC	1798	TGG	-	UCACUGCAACCUCCGCCUCC	4892
CAAGACCAGCCTGGCCAACA	1799	TGG	-	CAAGACCAGCCUGGCCAACA	4893
GAGATGGGGTTTCACCATGT	1800	TGG	-	GAGAUGGGGUUUCACCAUGU	4894
TGTAGTCCCAGCTACTCAGG	1801	AGG	+	UGUAGUCCCAGCUACUCAGG	4895
TGTAGTCCCAGCTACTCAGG	1802	AGG	-	UGUAGUCCCAGCUACUCAGG	4896
TCCTGAGTAGCTGGGACTAC	1803	AGG	+	UCCUGAGUAGCUGGGACUAC	4897
TCCTGAGTAGCTGGGACTAC	1804	AGG	-	UCCUGAGUAGCUGGGACUAC	4898
CAGGAGAATCGCTTGAACCC	1805	AGG	+	CAGGAGAAUCGCUUGAACCC	4899
CAGGAGAATCGCTTGAACCC	1806	GGG	-	CAGGAGAAUCGCUUGAACCC	4900
CGAGACCAGCCTGGCCAACA	1807	TGG	-	CGAGACCAGCCUGGCCAACA	4901
GCAGGAGAATCGCTTGAACC	1808	CGG	+	GCAGGAGAAUCGCUUGAACC	4902
GCAGGAGAATCGCTTGAACC	1809	CGG	-	GCAGGAGAAUCGCUUGAACC	4903
TGTAATCCCAGCTACTCGGG	1810	AGG	+	UGUAAUCCCAGCUACUCGGG	4904
TAAAAATACAAAAAATTAGC	1811	CGG	+	UAAAAAUACAAAAAAUUAGC	4905
GCGCCACTGCACTCCAGCCT	1812	GGG	+	GCGCCACUGCACUCCAGCCU	4906
TCCCAAGTAGCTGGGATTAC	1813	AGG	-	UCCCAAGUAGCUGGGAUUAC	4907
TGTAATCCCAGCTACTTGGG	1814	AGG	+	UGUAAUCCCAGCUACUUGGG	4908
TCAGGAGTTCAAGACCAGCC	1815	TGG	-	UCAGGAGUUCAAGACCAGCC	4909
TCAGGAGTTCGAGACCAGCC	1816	TGG	+	UCAGGAGUUCGAGACCAGCC	4910
TCAGGAGTTCGAGACCAGCC	1817	TGG	-	UCAGGAGUUCGAGACCAGCC	4911
AAAAATACAAAAAATTAGCC	1818	AGG	+	AAAAAUACAAAAAAUUAGCC	4912
AAAAATACAAAAAATTAGCC	1819	GGG	+	AAAAAUACAAAAAAUUAGCC	4913
TCAGGAGATCGAGACCATCC	1820	TGG	+	UCAGGAGAUCGAGACCAUCC	4914
GCCTTGGCCTCCCAAAGTGC	1821	TGG	-	GCCUUGGCCUCCCAAAGUGC	4915
GCCTTGGCCTCCCAAAGTGC	1822	TGG	-	GCCUUGGCCUCCCAAAGUGC	4916
GCACTTTGGGAGGCCAAGGC	1823	AGG	+	GCACUUUGGGAGGCCAAGGC	4917
GCACTTTGGGAGGCCAAGGC	1824	AGG	+	GCACUUUGGGAGGCCAAGGC	4918
GCCTCAGCCTCCCAAAGTGC	1825	TGG	-	GCCUCAGCCUCCCAAAGUGC	4919
GCACTTTGGGAGGCTGAGGC	1826	GGG	+	GCACUUUGGGAGGCUGAGGC	4920
AGCACTTTGGGAGGCTGAGG	1827	CGG	+	AGCACUUUGGGAGGCUGAGG	4921
TCGCCCAGGCTGGAGTGCAG	1828	TGG	-	UCGCCCAGGCUGGAGUGCAG	4922

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TCGCCCAGGCTGGAGTGCAG	1829	TGG	+	UCGCCCAGGCUGGAGUGCAG	4923
TTGTATTTTTAGTAGAGACA	1830	GGG	+	UUGUAUUUUUAGUAGAGACA	4924
TTGTATTTTTAGTAGAGACA	1831	GGG	-	UUGUAUUUUUAGUAGAGACA	4925
TTGCCCAGGCTGGAGTGCAG	1832	TGG	+	UUGCCCAGGCUGGAGUGCAG	4926
CACCTGTAATCCCAGCACTT	1833	TGG	+	CACCUGUAAUCCCAGCACUU	4927
GCCTGTAGTCCCAGCTACTC	1834	AGG	+	GCCUGUAGUCCCAGCUACUC	4928
ACCTGTAATCCCAGCACTTT	1835	GGG	+	ACCUGUAAUCCCAGCACUUU	4929
GCTACTTGGGAGGCTGAGGC	1836	AGG	+	GCUACUUGGGAGGCUGAGGC	4930
GCTACTCAGGAGGCTGAGGC	1837	GGG	-	GCUACUCAGGAGGCUGAGGC	4931
CCCAGCACTTTGGGAGGCCA	1838	AGG	+	CCCAGCACUUUGGGAGGCCA	4932
CCCAGCACTTTGGGAGGCCA	1839	AGG	+	CCCAGCACUUUGGGAGGCCA	4933
GCCTCAGCCTCCCAAGTAGC	1840	TGG	-	GCCUCAGCCUCCCAAGUAGC	4934
GCCTCGGCCTCCCAAAGTGC	1841	TGG	+	GCCUCGGCCUCCCAAAGUGC	4935
GCCTCGGCCTCCCAAAGTGC	1842	TGG	+	GCCUCGGCCUCCCAAAGUGC	4936
TTGTATTTTTAGTAGAGATG	1843	GGG	-	UUGUAUUUUUAGUAGAGAUG	4937
TTTTTTTTTTTTTTTTGAGA	1844	TGG	-	UUUUUUUUUUUUUUUUGAGA	4938
TTTTTTTTTTTTTTTTGAGA	1845	CGG	-	UUUUUUUUUUUUUUUUGAGA	4939
CCTTGGCCTCCCAAAGTGCT	1846	GGG	-	CCUUGGCCUCCCAAAGUGCU	4940
CCTTGGCCTCCCAAAGTGCT	1847	GGG	-	CCUUGGCCUCCCAAAGUGCU	4941
CCCAGCACTTTGGGAGGCTG	1848	AGG	+	CCCAGCACUUUGGGAGGCUG	4942
GCACTTTGGGAGGCCGAGGC	1849	AGG	-	GCACUUUGGGAGGCCGAGGC	4943
GCACTTTGGGAGGCCGAGGC	1850	AGG	-	GCACUUUGGGAGGCCGAGGC	4944
GCCTCAGCCTCCTGAGTAGC	1851	TGG	+	GCCUCAGCCUCCUGAGUAGC	4945
TTTGTATTTTTAGTAGAGAT	1852	GGG	-	UUUGUAUUUUUAGUAGAGAU	4946
TTTCACCATGTTGGCCAGGC	1853	TGG	+	UUUCACCAUGUUGGCCAGGC	4947
GCTACTCGGGAGGCTGAGGC	1854	AGG	+	GCUACUCGGGAGGCUGAGGC	4948
AGCACTTTGGGAGGCCGAGG	1855	TGG	+	AGCACUUUGGGAGGCCGAGG	4949
CCCAGCTACTTGGGAGGCTG	1856	AGG	+	CCCAGCUACUUGGGAGGCUG	4950
TGCCTGTAATCCCAGCACTT	1857	TGG	-	UGCCUGUAAUCCCAGCACUU	4951
CCTCAGCCTCCCAAAGTGCT	1858	GGG	-	CCUCAGCCUCCCAAAGUGCU	4952
CCTCAGCCTCCCAAGTAGCT	1859	GGG	-	CCUCAGCCUCCCAAGUAGCU	4953
CCCAGCTACTCAGGAGGCTG	1860	AGG	-	CCCAGCUACUCAGGAGGCUG	4954
CCCAGCTACTCAGGAGGCTG	1861	AGG	+	CCCAGCUACUCAGGAGGCUG	4955
GCCTCAGCCTCCCGAGTAGC	1862	TGG	-	GCCUCAGCCUCCCGAGUAGC	4956
CCTCAGCCTCCTGAGTAGCT	1863	GGG	-	CCUCAGCCUCCUGAGUAGCU	4957
CCTCAGCCTCCTGAGTAGCT	1864	GGG	+	CCUCAGCCUCCUGAGUAGCU	4958
CCCAGCTACTCGGGAGGCTG	1865	AGG	+	CCCAGCUACUCGGGAGGCUG	4959
TTGTATTTTTAGTAGAGACG	1866	GGG	-	UUGUAUUUUUAGUAGAGACG	4960
CCTCAGCCTCCCGAGTAGCT	1867	GGG	-	CCUCAGCCUCCCGAGUAGCU	4961
CCCAGCACTTTGGGAGGCCG	1868	AGG	-	CCCAGCACUUUGGGAGGCCG	4962
CCCAGCACTTTGGGAGGCCG	1869	AGG	-	CCCAGCACUUUGGGAGGCCG	4963
CCTCGGCCTCCCAAAGTGCT	1870	AGG	-	CCUCGGCCUCCCAAAGUGCU	4964
CCTCGGCCTCCCAAAGTGCT	1871	GGG	+	CCUCGGCCUCCCAAAGUGCU	4965
CCTCGGCCTCCCAAAGTGCT	1872	GGG	+	CCUCGGCCUCCCAAAGUGCU	4966
CGCCTGTAATCCCAGCACTT	1873	TGG	+	CGCCUGUAAUCCCAGCACUU	4967
CGCCTGTAATCCCAGCACTT	1874	TGG	-	CGCCUGUAAUCCCAGCACUU	4968
CGCCTGTAATCCCAGCACTT	1875	TGG	-	CGCCUGUAAUCCCAGCACUU	4969
TTTGTATTTTTAGTAGAGAC	1876	AGG	+	UUUGUAUUUUUAGUAGAGAC	4970
TTTGTATTTTTAGTAGAGAC	1877	AGG	-	UUUGUAUUUUUAGUAGAGAC	4971
TTTGTATTTTTAGTAGAGAC	1878	GGG	-	UUUGUAUUUUUAGUAGAGAC	4972
TTTGTATTTTTAGTAGAGAC	1879	TGG	-	UUUGUAUUUUUAGUAGAGAC	4973
TTTTGTATTTTTAGTAGAGA	1880	TGG	-	UUUUGUAUUUUUAGUAGAGA	4974
TTTTGTATTTTTAGTAGAGA	1881	CGG	-	UUUUGUAUUUUUAGUAGAGA	4975
GCCTGTAATCCCAGCACTTT	1882	GGG	+	GCCUGUAAUCCCAGCACUUU	4976
GCCTGTAATCCCAGCACTTT	1883	GGG	-	GCCUGUAAUCCCAGCACUUU	4977
GCCTGTAATCCCAGCACTTT	1884	GGG	-	GCCUGUAAUCCCAGCACUUU	4978
GCCTGTAATCCCAGCACTTT	1885	GGG	-	GCCUGUAAUCCCAGCACUUU	4979
TCCCAAAGTGCTGGGATTAC	1886	AGG	-	UCCCAAAGUGCUGGGAUUAC	4980

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TCCCAAAGTGCTGGGATTAC	1887	AGG	-	UCCCAAAGUGCUGGGAUUAC	4981
TCCCAAAGTGCTGGGATTAC	1888	AGG	+	UCCCAAAGUGCUGGGAUUAC	4982
TCCCAAAGTGCTGGGATTAC	1889	AGG	+	UCCCAAAGUGCUGGGAUUAC	4983
TCCCAAAGTGCTGGGATTAC	1890	AGG	+	UCCCAAAGUGCUGGGAUUAC	4984
TGTAATCCCAGCACTTTGGG	1891	AGG	+	UGUAAUCCCAGCACUUUGGG	4985
TGTAATCCCAGCACTTTGGG	1892	AGG	+	UGUAAUCCCAGCACUUUGGG	4986
TGTAATCCCAGCACTTTGGG	1893	AGG	+	UGUAAUCCCAGCACUUUGGG	4987
TGTAATCCCAGCACTTTGGG	1894	AGG	-	UGUAAUCCCAGCACUUUGGG	4988
TGTAATCCCAGCACTTTGGG	1895	AGG	-	UGUAAUCCCAGCACUUUGGG	4989

[00307] Table 3. Mouse *Rs1* Intron 1 Guide RNA Target Sequences and Guide Sequences.

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ACCTGAGTAATAAAAATTGT	1896	TGG	+	ACCUGAGUAAUAAAAAUUGU	4990
TCCAACAATTTTTATTACTC	1897	AGG	-	UCCAACAAUUUUUAUUACUC	4991
CAACATGCCAATTAGTTCAC	1898	AGG	+	CAACAUGCCAAUUAGUUCAC	4992
TTAATAACCTGTGAACTAAT	1899	TGG	-	UUAAUAACCUGUGAACUAAU	4993
TCACAGGTTATTAATTTAAA	1900	AGG	+	UCACAGGUUAUUAAUUUAAA	4994
TTTAAAAGGTCACTGTTGTG	1901	TGG	+	UUUAAAAGGUCACUGUUGUG	4995
CTTTGTCACTGTCTTGCTCC	1902	TGG	+	CUUUGUCACUGUCUUGCUCC	4996
CTGTCTTGCTCCTGGCTTCC	1903	TGG	+	CUGUCUUGCUCCUGGCUUCC	4997
CCTGGCTTCCTGGTTTCATG	1904	AGG	+	CCUGGCUUCCUGGUUUCAUG	4998
CCTCATGAAACCAGGAAGCC	1905	AGG	-	CCUCAUGAAACCAGGAAGCC	4999
AGAAGGTTCCTCATGAAACC	1906	AGG	-	AGAAGGUUCCUCAUGAAACC	5000
AATATCATTTGAACTTTAGA	1907	AGG	-	AAUAUCAUUUGAACUUUAGA	5001
ATTGAAACTCAACAGAAAGA	1908	AGG	+	AUUGAAACUCAACAGAAAGA	5002
AAACTCAACAGAAAGAAGGA	1909	AGG	+	AAACUCAACAGAAAGAAGGA	5003
AACTCAACAGAAAGAAGGAA	1910	GGG	+	AACUCAACAGAAAGAAGGAA	5004
TTGTTTTTATAGAAACTCTG	1911	AGG	-	UUGUUUUUAUAGAAACUCUG	5005
TAATGAGAAGTCATGTTTCT	1912	TGG	+	UAAUGAGAAGUCAUGUUUCU	5006
TCATGTTTCTTGGAAATTTT	1913	AGG	+	UCAUGUUUCUUGGAAAUUUU	5007
TGTTTCTTGGAAATTTTAGG	1914	AGG	+	UGUUUCUUGGAAAUUUUAGG	5008
TAGAATTTAATATTGTAAGC	1915	CGG	+	UAGAAUUUAAUAUUGUAAGC	5009
TGTAAGCCGGACTATTACTG	1916	AGG	+	UGUAAGCCGGACUAUUACUG	5010
GTAAGCCGGACTATTACTGA	1917	GGG	+	GUAAGCCGGACUAUUACUGA	5011
CTTAACCCTCAGTAATAGTC	1918	CGG	-	CUUAACCCUCAGUAAUAGUC	5012
CGGACTATTACTGAGGGTTA	1919	AGG	+	CGGACUAUUACUGAGGGUUA	5013
GAAGCTATTAATAGCTCTGC	1920	TGG	+	GAAGCUAUUAAUAGCUCUGC	5014
AAGCTATTAATAGCTCTGCT	1921	GGG	+	AAGCUAUUAAUAGCUCUGCU	5015
TGCTGGGACAAGTGTAAATG	1922	AGG	+	UGCUGGGACAAGUGUAAAUG	5016
GTGTAAATGAGGACTGTCCC	1923	AGG	+	GUGUAAAUGAGGACUGUCCC	5017
AGGACTGTCCCAGGCAAATC	1924	AGG	+	AGGACUGUCCCAGGCAAAUC	5018
TCCCAGGCAAATCAGGACAA	1925	AGG	+	UCCCAGGCAAAUCAGGACAA	5019
CCCAGGCAAATCAGGACAAA	1926	GGG	+	CCCAGGCAAAUCAGGACAAA	5020
CCCTTTGTCCTGATTTGCCT	1927	GGG	-	CCCUUUGUCCUGAUUUGCCU	5021
ACCCTTTGTCCTGATTTGCC	1928	TGG	-	ACCCUUUGUCCUGAUUUGCC	5022
GGGTCACCCTAGTTTAAATA	1929	TGG	+	GGGUCACCCUAGUUUAAAUA	5023
GCTAATCCATATTTAAACTA	1930	GGG	-	GCUAAUCCAUAUUUAAACUA	5024
TGCTAATCCATATTTAAACT	1931	AGG	-	UGCUAAUCCAUAUUUAAACU	5025
TTTAAATATGGATTAGCATA	1932	AGG	+	UUUAAAUAUGGAUUAGCAUA	5026
TATTAGACTGCAGTGGTGCT	1933	TGG	-	UAUUAGACUGCAGUGGUGCU	5027
AGACTTCTATTAGACTGCAG	1934	TGG	-	AGACUUCUAUUAGACUGCAG	5028
TAGGGTTTATTGACTTTCTT	1935	TGG	-	UAGGGUUUAUUGACUUUCUU	5029
TGGAATATATTGAGTCTTTA	1936	GGG	-	UGGAAUAUAUUGAGUCUUUA	5030
ATGGAATATATTGAGTCTTT	1937	AGG	-	AUGGAAUAUAUUGAGUCUUU	5031

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ATTTAGAAGTTTAAAAGTGA	1938	TGG	-	AUUUAGAAGUUUAAAAGUGA	5032
CACTTTTAAACTTCTAAATG	1939	TGG	+	CACUUUUAAACUUCUAAAUG	5033
TAAACTTCTAAATGTGGCCC	1940	TGG	+	UAAACUUCUAAAUGUGGCCC	5034
CTTCTAAATGTGGCCCTGGC	1941	AGG	+	CUUCUAAAUGUGGCCCUGGC	5035
TAAGCATATTATGCCTGCCA	1942	GGG	-	UAAGCAUAUUAUGCCUGCCA	5036
TTAAGCATATTATGCCTGCC	1943	AGG	-	UUAAGCAUAUUAUGCCUGCC	5037
AGGCATAATATGCTTAAATA	1944	AGG	+	AGGCAUAAUAUGCUUAAAUA	5038
AGAGAGTATTATGTCTAAGA	1945	TGG	-	AGAGAGUAUUAUGUCUAAGA	5039
GGGCACTAGAGAGGTAAGAG	1946	AGG	-	GGGCACUAGAGAGGUAAGAG	5040
TGAATTGATGGGCACTAGAG	1947	AGG	-	UGAAUUGAUGGGCACUAGAG	5041
AAAAGGAATTCTGAATTGAT	1948	GGG	-	AAAAGGAAUUCUGAAUUGAU	5042
TAAAAGGAATTCTGAATTGA	1949	TGG	-	UAAAAGGAAUUCUGAAUUGA	5043
TTGAAAGTATAAACTTTAAA	1950	AGG	-	UUGAAAGUAUAAACUUUAAA	5044
TACTTTCAAATATTAAAAAT	1951	TGG	+	UACUUUCAAAUAUUAAAAAU	5045
AAATATTAAAAATTGGTTGA	1952	AGG	+	AAAUAUUAAAAAUUGGUUGA	5046
AATATTAAAAATTGGTTGAA	1953	GGG	+	AAUAUUAAAAAUUGGUUGAA	5047
TTAAAAATTGGTTGAAGGGT	1954	TGG	+	UUAAAAAUUGGUUGAAGGGU	5048
TAAAAATTGGTTGAAGGGTT	1955	GGG	+	UAAAAAUUGGUUGAAGGGUU	5049
AAAAATTGGTTGAAGGGTTG	1956	GGG	+	AAAAAUUGGUUGAAGGGUUG	5050
TGGTTGAAGGGTTGGGGATG	1957	TGG	+	UGGUUGAAGGGUUGGGGAUG	5051
GGGTTGGGGATGTGGCTCAG	1958	TGG	+	GGGUUGGGGAUGUGGCUCAG	5052
GACTCATGAAGGCTGGGCAG	1959	GGG	-	GACUCAUGAAGGCUGGGCAG	5053
GGACTCATGAAGGCTGGGCA	1960	GGG	-	GGACUCAUGAAGGCUGGGCA	5054
GGGACTCATGAAGGCTGGGC	1961	AGG	-	GGGACUCAUGAAGGCUGGGC	5055
CTCAGGGACTCATGAAGGCT	1962	GGG	-	CUCAGGGACUCAUGAAGGCU	5056
ACTCAGGGACTCATGAAGGC	1963	TGG	-	ACUCAGGGACUCAUGAAGGC	5057
TTTAACTCAGGGACTCATGA	1964	AGG	-	UUUAACUCAGGGACUCAUGA	5058
GCTTCTGGGAATTTAACTCA	1965	GGG	-	GCUUCUGGGAAUUUAACUCA	5059
TGCTTCTGGGAATTTAACTC	1966	AGG	-	UGCUUCUGGGAAUUUAACUC	5060
GTTTGTCTTATACTGCTTCT	1967	GGG	-	GUUUGUCUUAUACUGCUUCU	5061
TGTTTGTCTTATACTGCTTC	1968	TGG	-	UGUUUGUCUUAUACUGCUUC	5062
ACCTTACTTCATCCATTTTC	1969	TGG	+	ACCUUACUUCAUCCAUUUUC	5063
GCCAGAAAATGGATGAAGTA	1970	AGG	-	GCCAGAAAAUGGAUGAAGUA	5064
TGTGTAGCTATGCCAGAAAA	1971	TGG	-	UGUGUAGCUAUGCCAGAAAA	5065
CATACCTGCAATCCTAGTTC	1972	TGG	+	CAUACCUGCAAUCCUAGUUC	5066
TTGTCCAGAACTAGGATTGC	1973	AGG	-	UUGUCCAGAACUAGGAUUGC	5067
GCTTGAATTTGTCCAGAACT	1974	AGG	-	GCUUGAAUUUGUCCAGAACU	5068
AGTTCTGGACAAATTCAAGC	1975	AGG	+	AGUUCUGGACAAAUUCAAGC	5069
AATGCCAACCTCAGCTACTT	1976	AGG	+	AAUGCCAACCUCAGCUACUU	5070
CAAACCTAAGTAGCTGAGGT	1977	TGG	-	CAAACCUAAGUAGCUGAGGU	5071
CAACCTCAGCTACTTAGGTT	1978	TGG	+	CAACCUCAGCUACUUAGGUU	5072
AACCTCAGCTACTTAGGTTT	1979	GGG	+	AACCUCAGCUACUUAGGUUU	5073
AGCCCAAACCTAAGTAGCTG	1980	AGG	-	AGCCCAAACCUAAGUAGCUG	5074
CTGAGACCCTGTCTTAACAT	1981	TGG	+	CUGAGACCCUGUCUUAACAU	5075
TGAGACCCTGTCTTAACATT	1982	GGG	+	UGAGACCCUGUCUUAACAUU	5076
GAGACCCTGTCTTAACATTG	1983	GGG	+	GAGACCCUGUCUUAACAUUG	5077
AGACCCTGTCTTAACATTGG	1984	GGG	+	AGACCCUGUCUUAACAUUGG	5078
TCTCCCCCAATGTTAAGACA	1985	GGG	-	UCUCCCCCAAUGUUAAGACA	5079
CTCTCCCCCAATGTTAAGAC	1986	AGG	-	CUCUCCCCCAAUGUUAAGAC	5080
TTAACATTGGGGGAGAGCCT	1987	AGG	+	UUAACAUUGGGGGAGAGCCU	5081
ATTGGGGGAGAGCCTAGGCT	1988	AGG	+	AUUGGGGGAGAGCCUAGGCU	5082
TGTATAGCTCAGCCTAGCCT	1989	AGG	-	UGUAUAGCUCAGCCUAGCCU	5083
CTATACAGTAAGCCTCTGTC	1990	TGG	+	CUAUACAGUAAGCCUCUGUC	5084
TATACAGTAAGCCTCTGTCT	1991	GGG	+	UAUACAGUAAGCCUCUGUCU	5085
CAGTAAGCCTCTGTCTGGGA	1992	AGG	+	CAGUAAGCCUCUGUCUGGGA	5086
TAAGCCTCTGTCTGGGAAGG	1993	AGG	+	UAAGCCUCUGUCUGGGAAGG	5087
AAGCCTCTGTCTGGGAAGGA	1994	GGG	+	AAGCCUCUGUCUGGGAAGGA	5088
TTTCCCTCCTTCCCAGACAG	1995	AGG	-	UUUCCCUCCUUCCCAGACAG	5089

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TGTCTGGGAAGGAGGGAAAA	1996	AGG	+	UGUCUGGGAAGGAGGGAAAA	5090
TGGGAAGGAGGGAAAAAGGA	1997	AGG	+	UGGGAAGGAGGGAAAAAGGA	5091
GAAAAAGGAAGGAAAGACAT	1998	TGG	+	GAAAAAGGAAGGAAAGACAU	5092
GACATTGGTTGAATGACTTG	1999	TGG	+	GACAUUGGUUGAAUGACUUG	5093
ACATTGGTTGAATGACTTGT	2000	GGG	+	ACAUUGGUUGAAUGACUUGU	5094
ACTGTATGTTTAGAGTGTTC	2001	AGG	+	ACUGUAUGUUUAGAGUGUUC	5095
CTGTATGTTTAGAGTGTTCA	2002	GGG	+	CUGUAUGUUUAGAGUGUUCA	5096
GTTTAGAGTGTTCAGGGAAA	2003	AGG	+	GUUUAGAGUGUUCAGGGAAA	5097
TAGAGTGTTCAGGGAAAAGG	2004	AGG	+	UAGAGUGUUCAGGGAAAAGG	5098
AGGTCATTTGCAGAGCAGAG	2005	AGG	-	AGGUCAUUUGCAGAGCAGAG	5099
ATTCGTCTTATCTGAGTGGC	2006	AGG	-	AUUCGUCUUAUCUGAGUGGC	5100
GTGTATTCGTCTTATCTGAG	2007	TGG	-	GUGUAUUCGUCUUAUCUGAG	5101
CATGAATATAGAAGAAACAG	2008	AGG	-	CAUGAAUAUAGAAGAAACAG	5102
AATAAAAGACACAAGACTGC	2009	TGG	-	AAUAAAAGACACAAGACUGC	5103
CTAATAATATTTCTAGTGTC	2010	TGG	+	CUAAUAAUAUUUCUAGUGUC	5104
CTTGAGATTTTATTTTCACA	2011	AGG	-	CUUGAGAUUUUAUUUUCACA	5105
CTTGTGAAAATAAAATCTCA	2012	AGG	+	CUUGUGAAAAUAAAAUCUCA	5106
AATAAAATCTCAAGGTCATA	2013	CGG	+	AAUAAAAUCUCAAGGUCAUA	5107
TACGGCAAACACAAAGAAAC	2014	AGG	+	UACGGCAAACACAAAGAAAC	5108
GTCTTCAGACACTCCAGAAG	2015	AGG	+	GUCUUCAGACACUCCAGAAG	5109
TCTTCAGACACTCCAGAAGA	2016	GGG	+	UCUUCAGACACUCCAGAAGA	5110
CGAGATCTGATGCCCTCTTC	2017	TGG	-	CGAGAUCUGAUGCCCUCUUC	5111
GAGGGCATCAGATCTCGTTA	2018	CGG	+	GAGGGCAUCAGAUCUCGUUA	5112
GCATCAGATCTCGTTACGGA	2019	TGG	+	GCAUCAGAUCUCGUUACGGA	5113
GATGGTTGTGAGCCACCATG	2020	TGG	+	GAUGGUUGUGAGCCACCAUG	5114
GTGAGCCACCATGTGGTTGC	2021	TGG	+	GUGAGCCACCAUGUGGUUGC	5115
TGAGCCACCATGTGGTTGCT	2022	GGG	+	UGAGCCACCAUGUGGUUGCU	5116
AAATCCCAGCAACCACATGG	2023	TGG	-	AAAUCCCAGCAACCACAUGG	5117
TTCAAATCCCAGCAACCACA	2024	TGG	-	UUCAAAUCCCAGCAACCACA	5118
GGATTTGAACTTCAGACCTT	2025	CGG	+	GGAUUUGAACUUCAGACCUU	5119
CAGACCTTCGGAAGAGCAGT	2026	CGG	+	CAGACCUUCGGAAGAGCAGU	5120
AGACCTTCGGAAGAGCAGTC	2027	GGG	+	AGACCUUCGGAAGAGCAGUC	5121
GCACCCGACTGCTCTTCCGA	2028	AGG	-	GCACCCGACUGCUCUUCCGA	5122
GGCTGGTGAGATGGCTCAGT	2029	GGG	-	GGCUGGUGAGAUGGCUCAGU	5123
GGGCTGGTGAGATGGCTCAG	2030	TGG	-	GGGCUGGUGAGAUGGCUCAG	5124
TGAGCCATCTCACCAGCCCC	2031	AGG	+	UGAGCCAUCUCACCAGCCCC	5125
AAAACCTGGGGCTGGTGAGA	2032	TGG	-	AAAACCUGGGGCUGGUGAGA	5126
AGAAAAAAAAAACCTGGGGC	2033	TGG	-	AGAAAAAAAAAACCUGGGGC	5127
ACTAAGAAAAAAAAAACCTG	2034	GGG	-	ACUAAGAAAAAAAAAACCUG	5128
AACTAAGAAAAAAAAAACCT	2035	GGG	-	AACUAAGAAAAAAAAAACCU	5129
CAACTAAGAAAAAAAAAACC	2036	TGG	-	CAACUAAGAAAAAAAAAACC	5130
TATTGTTATTCTTTTGAGAC	2037	AGG	+	UAUUGUUAUUCUUUUGAGAC	5131
AGGATCCCACACTGAAACTC	2038	AGG	+	AGGAUCCCACACUGAAACUC	5132
GCTAGCCTGAGTTTCAGTGT	2039	GGG	-	GCUAGCCUGAGUUUCAGUGU	5133
GGCTAGCCTGAGTTTCAGTG	2040	TGG	-	GGCUAGCCUGAGUUUCAGUG	5134
GGCTAGCCTTCAATTTCCTA	2041	TGG	+	GGCUAGCCUUCAAUUUCCUA	5135
TCAAATCCATAGGAAATTGA	2042	AGG	-	UCAAAUCCAUAGGAAAUUGA	5136
TGCTATGAGGTCAAATCCAT	2043	AGG	-	UGCUAUGAGGUCAAAUCCAU	5137
GAAACTGGCTGACTGCTATG	2044	AGG	-	GAAACUGGCUGACUGCUAUG	5138
AGCACTCAGAAGGTGGAAAC	2045	TGG	-	AGCACUCAGAAGGUGGAAAC	5139
AAATCTTAGCACTCAGAAGG	2046	TGG	-	AAAUCUUAGCACUCAGAAGG	5140
TTGAAATCTTAGCACTCAGA	2047	AGG	-	UUGAAAUCUUAGCACUCAGA	5141
GGAATTGGGTGTGGTGGCTC	2048	AGG	-	GGAAUUGGGUGUGGUGGCUC	5142
ATCTTAGGAATTGGGTGTGG	2049	TGG	-	AUCUUAGGAAUUGGGUGUGG	5143
CATATCTTAGGAATTGGGTG	2050	TGG	-	CAUAUCUUAGGAAUUGGGUG	5144
AAAAACATATCTTAGGAATT	2051	GGG	-	AAAAACAUAUCUUAGGAAUU	5145
TAAAAACATATCTTAGGAAT	2052	TGG	-	UAAAAACAUAUCUUAGGAAU	5146
CTTGCTTAAAAACATATCTT	2053	AGG	-	CUUGCUUAAAAACAUAUCUU	5147

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CTAAGATATGTTTTTAAGCA	2054	AGG	+	CUAAGAUAUGUUUUUAAGCA	5148
ATGTTTTTAAGCAAGGTTAT	2055	TGG	+	AUGUUUUUAAGCAAGGUUAU	5149
CACGTCATTGCAAAGGGTTA	2056	AGG	-	CACGUCAUUGCAAAGGGUUA	5150
ACCCTTTGCAATGACGTGTC	2057	AGG	+	ACCCUUUGCAAUGACGUGUC	5151
TCCTGACACGTCATTGCAAA	2058	GGG	-	UCCUGACACGUCAUUGCAAA	5152
TTCCTGACACGTCATTGCAA	2059	AGG	-	UUCCUGACACGUCAUUGCAA	5153
GACAACTGGAAAAAAAAAAA	2060	AGG	-	GACAACUGGAAAAAAAAAAA	5154
TTTTTTTTTTTCCAGTTGTC	2061	AGG	+	UUUUUUUUUUUCCAGUUGUC	5155
TTTTTTCCAGTTGTCAGGCC	2062	TGG	+	UUUUUUCCAGUUGUCAGGCC	5156
CCAGTTGTCAGGCCTGGCAA	2063	TGG	+	CCAGUUGUCAGGCCUGGCAA	5157
CCATTGCCAGGCCTGACAAC	2064	TGG	-	CCAUUGCCAGGCCUGACAAC	5158
CAGGCCTGGCAATGGAGCCC	2065	AGG	+	CAGGCCUGGCAAUGGAGCCC	5159
AGGCCTGGCAATGGAGCCCA	2066	GGG	+	AGGCCUGGCAAUGGAGCCCA	5160
GGGCCCTGGGCTCCATTGCC	2067	AGG	-	GGGCCCUGGGCUCCAUUGCC	5161
TTTAGCAAGCACAGGGCCCT	2068	GGG	-	UUUAGCAAGCACAGGGCCCU	5162
GTTTAGCAAGCACAGGGCCC	2069	TGG	-	GUUUAGCAAGCACAGGGCCC	5163
GCCCTGTGCTTGCTAAACGA	2070	TGG	+	GCCCUGUGCUUGCUAAACGA	5164
GCCATCGTTTAGCAAGCACA	2071	GGG	-	GCCAUCGUUUAGCAAGCACA	5165
AGCCATCGTTTAGCAAGCAC	2072	AGG	-	AGCCAUCGUUUAGCAAGCAC	5166
GCTGGGTATATATAACTTAG	2073	GGG	-	GCUGGGUAUAUAUAACUUAG	5167
AGCTGGGTATATATAACTTA	2074	GGG	-	AGCUGGGUAUAUAUAACUUA	5168
AAGCTGGGTATATATAACTT	2075	AGG	-	AAGCUGGGUAUAUAUAACUU	5169
ATATATACCCAGCTTATCCC	2076	AGG	+	AUAUAUACCCAGCUUAUCCC	5170
GAAAGATCCTGGGATAAGCT	2077	GGG	-	GAAAGAUCCUGGGAUAAGCU	5171
AGAAAGATCCTGGGATAAGC	2078	TGG	-	AGAAAGAUCCUGGGAUAAGC	5172
GAAAGTCTGAGAAAGATCCT	2079	GGG	-	GAAAGUCUGAGAAAGAUCCU	5173
AGAAAGTCTGAGAAAGATCC	2080	TGG	-	AGAAAGUCUGAGAAAGAUCC	5174
CTATCGATGTTGCTTCTCTT	2081	TGG	+	CUAUCGAUGUUGCUUCUCUU	5175
CTTTTTGTGAGAAAAGAGCA	2082	AGG	-	CUUUUUGUGAGAAAAGAGCA	5176
TCTAAGCAAGAACCCTTGTC	2083	TGG	+	UCUAAGCAAGAACCCUUGUC	5177
ATCTCTCTGACTCCAGACAA	2084	GGG	-	AUCUCUCUGACUCCAGACAA	5178
TATCTCTCTGACTCCAGACA	2085	AGG	-	UAUCUCUCUGACUCCAGACA	5179
GTCAGAGAGATAGCTTAGTC	2086	TGG	+	GUCAGAGAGAUAGCUUAGUC	5180
TTTAAACAAGAAAAGTTCTG	2087	GGG	-	UUUAAACAAGAAAAGUUCUG	5181
TTTTAAACAAGAAAAGTTCT	2088	GGG	-	UUUUAAACAAGAAAAGUUCU	5182
TTTTTAAACAAGAAAAGTTC	2089	TGG	-	UUUUUAAACAAGAAAAGUUC	5183
CAAACAAACAAACAAAAACT	2090	AGG	+	CAAACAAACAAACAAAAACU	5184
CAAAAACTAGGCATGACAGC	2091	TGG	+	CAAAAACUAGGCAUGACAGC	5185
ACTAGGCATGACAGCTGGAG	2092	AGG	+	ACUAGGCAUGACAGCUGGAG	5186
AGGCATGACAGCTGGAGAGG	2093	TGG	+	AGGCAUGACAGCUGGAGAGG	5187
ATTAACAGCACTTGTTAAAG	2094	AGG	+	AUUAACAGCACUUGUUAAAG	5188
TGTTAAAGAGGACCTCAGCT	2095	TGG	+	UGUUAAAGAGGACCUCAGCU	5189
AGGACCTCAGCTTGGTTCCC	2096	AGG	+	AGGACCUCAGCUUGGUUCCC	5190
GGACCTCAGCTTGGTTCCCA	2097	GGG	+	GGACCUCAGCUUGGUUCCCA	5191
GAGCCCTGGGAACCAAGCTG	2098	AGG	-	GAGCCCUGGGAACCAAGCUG	5192
AACTGGAGTTATAGAGCCCT	2099	GGG	-	AACUGGAGUUAUAGAGCCCU	5193
GAACTGGAGTTATAGAGCCC	2100	TGG	-	GAACUGGAGUUAUAGAGCCC	5194
GGCTCTATAACTCCAGTTCC	2101	AGG	+	GGCUCUAUAACUCCAGUUCC	5195
GCTCTATAACTCCAGTTCCA	2102	GGG	+	GCUCUAUAACUCCAGUUCCA	5196
GCTGTCTGAGGCCCTGGAAC	2103	TGG	-	GCUGUCUGAGGCCCUGGAAC	5197
CAGGAGGCTGTCTGAGGCCC	2104	TGG	-	CAGGAGGCUGUCUGAGGCCC	5198
AGGCTACAGGAGGCTGTCTG	2105	AGG	-	AGGCUACAGGAGGCUGUCUG	5199
ATGCATGAAGAGGCTACAGG	2106	AGG	-	AUGCAUGAAGAGGCUACAGG	5200
CTGATGCATGAAGAGGCTAC	2107	AGG	-	CUGAUGCAUGAAGAGGCUAC	5201
GCCTCTTCATGCATCAGACA	2108	TGG	+	GCCUCUUCAUGCAUCAGACA	5202
ACCATGTCTGATGCATGAAG	2109	AGG	-	ACCAUGUCUGAUGCAUGAAG	5203
GACATGGTCCATATGCATGC	2110	AGG	+	GACAUGGUCCAUAUGCAUGC	5204
GTGTTTGACCTGCATGCATA	2111	TGG	-	GUGUUUGACCUGCAUGCAUA	5205

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AAAAATAAAAGTCTAAAAAA	2112	AGG	+	AAAAAUAAAAGUCUAAAAAA	5206
CATGCTATAATCCCAGTGCT	2113	AGG	+	CAUGCUAUAAUCCCAGUGCU	5207
ATGCTATAATCCCAGTGCTA	2114	GGG	+	AUGCUAUAAUCCCAGUGCUA	5208
CTATAATCCCAGTGCTAGGG	2115	AGG	+	CUAUAAUCCCAGUGCUAGGG	5209
ACATTTGCCTCCCTAGCACT	2116	GGG	-	ACAUUUGCCUCCCUAGCACU	5210
GACATTTGCCTCCCTAGCAC	2117	TGG	-	GACAUUUGCCUCCCUAGCAC	5211
GTGCTAGGGAGGCAAATGTC	2118	TGG	+	GUGCUAGGGAGGCAAAUGUC	5212
GGCAAATGTCTGGACCTTGT	2119	CGG	+	GGCAAAUGUCUGGACCUUGU	5213
AATGTCTGGACCTTGTCGGT	2120	CGG	+	AAUGUCUGGACCUUGUCGGU	5214
GACTGACTGGCCGACCGACA	2121	AGG	-	GACUGACUGGCCGACCGACA	5215
TGACTGGCTGGCTGACTGAC	2122	TGG	-	UGACUGGCUGGCUGACUGAC	5216
GCTAGGCTAGACTGACTGGC	2123	TGG	-	GCUAGGCUAGACUGACUGGC	5217
CTATGCTAGGCTAGACTGAC	2124	TGG	-	CUAUGCUAGGCUAGACUGAC	5218
CTAGCCTAGCATAGCCTACT	2125	AGG	+	CUAGCCUAGCAUAGCCUACU	5219
CACACCTAGTAGGCTATGCT	2126	AGG	-	CACACCUAGUAGGCUAUGCU	5220
TGGGCTGGAGCACACCTAGT	2127	AGG	-	UGGGCUGGAGCACACCUAGU	5221
GACAAGGTCTCTCATTGGGC	2128	TGG	-	GACAAGGUCUCUCAUUGGGC	5222
TAGAGACAAGGTCTCTCATT	2129	GGG	-	UAGAGACAAGGUCUCUCAUU	5223
TTAGAGACAAGGTCTCTCAT	2130	TGG	-	UUAGAGACAAGGUCUCUCAU	5224
AGACCTTGTCTCTAAAAAAA	2131	AGG	+	AGACCUUGUCUCUAAAAAAA	5225
GACCTTGTCTCTAAAAAAAA	2132	GGG	+	GACCUUGUCUCUAAAAAAAA	5226
TACCCTTTTTTTTAGAGACA	2133	AGG	-	UACCCUUUUUUUUAGAGACA	5227
GGTAGAAAGAAAAGACAAAA	2134	TGG	+	GGUAGAAAGAAAAGACAAAA	5228
GACAAAATGGATGTCTCCTG	2135	AGG	+	GACAAAAUGGAUGUCUCCUG	5229
CTCCTGAGGAATGATACCCA	2136	CGG	+	CUCCUGAGGAAUGAUACCCA	5230
ATCCGTGGGTATCATTCCTC	2137	AGG	-	AUCCGUGGGUAUCAUUCCUC	5231
GGACAATTGTGGGTATCCGT	2138	GGG	-	GGACAAUUGUGGGUAUCCGU	5232
AGGACAATTGTGGGTATCCG	2139	TGG	-	AGGACAAUUGUGGGUAUCCG	5233
GGATACCCACAATTGTCCTC	2140	TGG	+	GGAUACCCACAAUUGUCCUC	5234
GGAGACCAGAGGACAATTGT	2141	GGG	-	GGAGACCAGAGGACAAUUGU	5235
CGGAGACCAGAGGACAATTG	2142	TGG	-	CGGAGACCAGAGGACAAUUG	5236
GTATTGCACACGGAGACCAG	2143	AGG	-	GUAUUGCACACGGAGACCAG	5237
ACATGGGTTTGTATTGCACA	2144	CGG	-	ACAUGGGUUUGUAUUGCACA	5238
TGTTGCAAATATTCAGACAT	2145	GGG	-	UGUUGCAAAUAUUCAGACAU	5239
CTGTTGCAAATATTCAGACA	2146	TGG	-	CUGUUGCAAAUAUUCAGACA	5240
CATGTCTGAATATTTGCAAC	2147	AGG	+	CAUGUCUGAAUAUUUGCAAC	5241
ATGTCTGAATATTTGCAACA	2148	GGG	+	AUGUCUGAAUAUUUGCAACA	5242
AGCACAGCTTTACTAGATGA	2149	GGG	-	AGCACAGCUUUACUAGAUGA	5243
AAGCACAGCTTTACTAGATG	2150	AGG	-	AAGCACAGCUUUACUAGAUG	5244
CATCTAGTAAAGCTGTGCTT	2151	TGG	+	CAUCUAGUAAAGCUGUGCUU	5245
GAAAAATAAGACAGATGGGA	2152	GGG	-	GAAAAAUAAGACAGAUGGGA	5246
AGAAAAATAAGACAGATGGG	2153	AGG	-	AGAAAAAUAAGACAGAUGGG	5247
AGTAGAAAAATAAGACAGAT	2154	GGG	-	AGUAGAAAAAUAAGACAGAU	5248
CAGTAGAAAAATAAGACAGA	2155	TGG	-	CAGUAGAAAAAUAAGACAGA	5249
CATCTGTCTTATTTTTCTAC	2156	TGG	+	CAUCUGUCUUAUUUUUCUAC	5250
ATTTTTCTACTGGTGTGCTA	2157	AGG	+	AUUUUUCUACUGGUGUGCUA	5251
GTGCTAAGGCACCATGACCA	2158	AGG	+	GUGCUAAGGCACCAUGACCA	5252
TCTCTAAGTTGCCTTGGTCA	2159	TGG	-	UCUCUAAGUUGCCUUGGUCA	5253
TCTTCTTCTCTAAGTTGCCT	2160	TGG	-	UCUUCUUCUCUAAGUUGCCU	5254
GAAGAAGAAACAGTTTATTG	2161	AGG	+	GAAGAAGAAACAGUUUAUUG	5255
AAGAAGAAACAGTTTATTGA	2162	GGG	+	AAGAAGAAACAGUUUAUUGA	5256
TTGAGGGTTTATAGTTATAG	2163	AGG	+	UUGAGGGUUUAUAGUUAUAG	5257
TGAGGGTTTATAGTTATAGA	2164	GGG	+	UGAGGGUUUAUAGUUAUAGA	5258
GTTTATAGTTATAGAGGGTA	2165	AGG	+	GUUUAUAGUUAUAGAGGGUA	5259
TAGTTATAGAGGGTAAGGCA	2166	AGG	+	UAGUUAUAGAGGGUAAGGCA	5260
TAGAGGGTAAGGCAAGGACT	2167	TGG	+	UAGAGGGUAAGGCAAGGACU	5261
TAAGGCAAGGACTTGGTAGC	2168	AGG	+	UAAGGCAAGGACUUGGUAGC	5262
GCAAGGACTTGGTAGCAGGC	2169	AGG	+	GCAAGGACUUGGUAGCAGGC	5263

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GGACTTGGTAGCAGGCAGGC	2170	AGG	+	GGACUUGGUAGCAGGCAGGC	5264
TTGGTAGCAGGCAGGCAGGC	2171	AGG	+	UUGGUAGCAGGCAGGCAGGC	5265
AGCAGGCAGGCAGGCAGGCA	2172	TGG	+	AGCAGGCAGGCAGGCAGGCA	5266
CAGGCAGGCAGGCATGGTAC	2173	TGG	+	CAGGCAGGCAGGCAUGGUAC	5267
CATGGTACTGGTGTAGTAGC	2174	TGG	+	CAUGGUACUGGUGUAGUAGC	5268
ATGGTACTGGTGTAGTAGCT	2175	GGG	+	AUGGUACUGGUGUAGUAGCU	5269
GGAGCTTGTATCTTGAGACA	2176	CGG	+	GGAGCUUGUAUCUUGAGACA	5270
AAAGCTGAGAGATAGCTAAG	2177	TGG	+	AAAGCUGAGAGAUAGCUAAG	5271
AAGCTGAGAGATAGCTAAGT	2178	GGG	+	AAGCUGAGAGAUAGCUAAGU	5272
GAGAGATAGCTAAGTGGGCC	2179	TGG	+	GAGAGAUAGCUAAGUGGGCC	5273
AGGTTTCAAAAGCTCACACC	2180	AGG	-	AGGUUUCAAAAGCUCACACC	5274
TTTCACTGGGGACAGCTTTG	2181	AGG	-	UUUCACUGGGGACAGCUUUG	5275
TTGATGGAGGCATTTCACTG	2182	GGG	-	UUGAUGGAGGCAUUUCACUG	5276
GTTGATGGAGGCATTTCACT	2183	GGG	-	GUUGAUGGAGGCAUUUCACU	5277
TGTTGATGGAGGCATTTCAC	2184	TGG	-	UGUUGAUGGAGGCAUUUCAC	5278
GTGAAATGCCTCCATCAACA	2185	AGG	+	GUGAAAUGCCUCCAUCAACA	5279
AGGTGTGGCCTTGTTGATGG	2186	AGG	-	AGGUGUGGCCUUGUUGAUGG	5280
GTTAGGTGTGGCCTTGTTGA	2187	TGG	-	GUUAGGUGUGGCCUUGUUGA	5281
TGTTTGGGAAGGGTTAGGTG	2188	TGG	-	UGUUUGGGAAGGGUUAGGUG	5282
AGAACTGTTTGGGAAGGGTT	2189	AGG	-	AGAACUGUUUGGGAAGGGUU	5283
TTGGTAGAACTGTTTGGGAA	2190	GGG	-	UUGGUAGAACUGUUUGGGAA	5284
GTTGGTAGAACTGTTTGGGA	2191	AGG	-	GUUGGUAGAACUGUUUGGGA	5285
TCCCAAACAGTTCTACCAAC	2192	TGG	+	UCCCAAACAGUUCUACCAAC	5286
CCCAAACAGTTCTACCAACT	2193	GGG	+	CCCAAACAGUUCUACCAACU	5287
CCCAGTTGGTAGAACTGTTT	2194	GGG	-	CCCAGUUGGUAGAACUGUUU	5288
TCCCAGTTGGTAGAACTGTT	2195	TGG	-	UCCCAGUUGGUAGAACUGUU	5289
ATGAATGCTTGGTTCCCAGT	2196	TGG	-	AUGAAUGCUUGGUUCCCAGU	5290
CTGAGGCTCATATGAATGCT	2197	TGG	-	CUGAGGCUCAUAUGAAUGCU	5291
CAAGCATTCATATGAGCCTC	2198	AGG	+	CAAGCAUUCAUAUGAGCCUC	5292
AAGCATTCATATGAGCCTCA	2199	GGG	+	AAGCAUUCAUAUGAGCCUCA	5293
AGCATTCATATGAGCCTCAG	2200	GGG	+	AGCAUUCAUAUGAGCCUCAG	5294
TGAATGAGACTGCTCCCCTG	2201	AGG	-	UGAAUGAGACUGCUCCCCUG	5295
GTACTCAGTACACTGCCCCA	2202	TGG	+	GUACUCAGUACACUGCCCCA	5296
TACTCAGTACACTGCCCCAT	2203	GGG	+	UACUCAGUACACUGCCCCAU	5297
TTAGAGGACTTCTGCCCATG	2204	GGG	-	UUAGAGGACUUCUGCCCAUG	5298
TTTAGAGGACTTCTGCCCAT	2205	GGG	-	UUUAGAGGACUUCUGCCCAU	5299
GTTTAGAGGACTTCTGCCCA	2206	TGG	-	GUUUAGAGGACUUCUGCCCA	5300
ATGGGCAGAAGTCCTCTAAA	2207	CGG	+	AUGGGCAGAAGUCCUCUAAA	5301
ACACATTAGTAACCGTTTAG	2208	AGG	-	ACACAUUAGUAACCGUUUAG	5302
TATCCCTCACCATACTTAGA	2209	AGG	+	UAUCCCUCACCAUACUUAGA	5303
TTTCCTTCTAAGTATGGTGA	2210	GGG	-	UUUCCUUCUAAGUAUGGUGA	5304
GTTTCCTTCTAAGTATGGTG	2211	AGG	-	GUUUCCUUCUAAGUAUGGUG	5305
GGCTTGTTTCCTTCTAAGTA	2212	TGG	-	GGCUUGUUUCCUUCUAAGUA	5306
GGAAACAAGCCATCCCAAAA	2213	TGG	+	GGAAACAAGCCAUCCCAAAA	5307
TCATGCTATCCATTTTGGGA	2214	TGG	-	UCAUGCUAUCCAUUUUGGGA	5308
TGAGTCATGCTATCCATTTT	2215	GGG	-	UGAGUCAUGCUAUCCAUUUU	5309
CTGAGTCATGCTATCCATTT	2216	TGG	-	CUGAGUCAUGCUAUCCAUUU	5310
TTGCTCTGGGTGAAGTACAC	2217	AGG	-	UUGCUCUGGGUGAAGUACAC	5311
TACTTCACCCAGAGCAAATG	2218	TGG	+	UACUUCACCCAGAGCAAAUG	5312
ACCCAGAGCAAATGTGGATA	2219	CGG	+	ACCCAGAGCAAAUGUGGAUA	5313
ACCGTATCCACATTTGCTCT	2220	GGG	-	ACCGUAUCCACAUUUGCUCU	5314
CACCGTATCCACATTTGCTC	2221	TGG	-	CACCGUAUCCACAUUUGCUC	5315
CAGAGCAAATGTGGATACGG	2222	TGG	+	CAGAGCAAAUGUGGAUACGG	5316
AGAGCAAATGTGGATACGGT	2223	GGG	+	AGAGCAAAUGUGGAUACGGU	5317
GTGGATACGGTGGGAAACAG	2224	AGG	+	GUGGAUACGGUGGGAAACAG	5318
GGGAAACAGAGGACAGTGAT	2225	AGG	+	GGGAAACAGAGGACAGUGAU	5319
AGGACAGTGATAGGACACAA	2226	AGG	+	AGGACAGUGAUAGGACACAA	5320
GTCTCAGTTTCTAGTTCAGA	2227	TGG	+	GUCUCAGUUUCUAGUUCAGA	5321

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AGACATAAACCAACACACAT	2228	AGG	+	AGACAUAAACCAACACACAU	5322
TGTATCTTTCCTATGTGTGT	2229	TGG	-	UGUAUCUUUCCUAUGUGUGU	5323
ATAGGAAAGATACACTCCAC	2230	TGG	+	AUAGGAAAGAUACACUCCAC	5324
AGATACACTCCACTGGACAG	2231	TGG	+	AGAUACACUCCACUGGACAG	5325
GATACACTCCACTGGACAGT	2232	GGG	+	GAUACACUCCACUGGACAGU	5326
CATGGGCTCCCACTGTCCAG	2233	TGG	-	CAUGGGCUCCCACUGUCCAG	5327
CAGTGGGAGCCCATGCACAA	2234	AGG	+	CAGUGGGAGCCCAUGCACAA	5328
GCCCATGCACAAAGGTTCTT	2235	TGG	+	GCCCAUGCACAAAGGUUCUU	5329
GCCAAAGAACCTTTGTGCAT	2236	GGG	-	GCCAAAGAACCUUUGUGCAU	5330
AGCCAAAGAACCTTTGTGCA	2237	TGG	-	AGCCAAAGAACCUUUGUGCA	5331
TGACAGCAGTTTCTTATTAT	2238	TGG	+	UGACAGCAGUUUCUUAUUAU	5332
GACAGCAGTTTCTTATTATT	2239	GGG	+	GACAGCAGUUUCUUAUUAUU	5333
GATGAGCTTTGTTTCTGCCC	2240	TGG	+	GAUGAGCUUUGUUUCUGCCC	5334
ATGAGCTTTGTTTCTGCCCT	2241	GGG	+	AUGAGCUUUGUUUCUGCCCU	5335
CCCTGGGTGAGAGTTTATTT	2242	AGG	+	CCCUGGGUGAGAGUUUAUUU	5336
CCTAAATAAACTCTCACCCA	2243	GGG	-	CCUAAAUAAACUCUCACCCA	5337
TCCTAAATAAACTCTCACCC	2244	AGG	-	UCCUAAAUAAACUCUCACCC	5338
CACACTAAGACAACCACTGA	2245	TGG	+	CACACUAAGACAACCACUGA	5339
ACTTTGTTCTAGGCCATCAG	2246	TGG	-	ACUUUGUUCUAGGCCAUCAG	5340
CAGTGCTCTGACTTTGTTCT	2247	AGG	-	CAGUGCUCUGACUUUGUUCU	5341
AGTCAGAGCACTGTGCGTTG	2248	TGG	+	AGUCAGAGCACUGUGCGUUG	5342
CACTGTGCGTTGTGGATCTT	2249	AGG	+	CACUGUGCGUUGUGGAUCUU	5343
GATGCTTTAATCATCATGAA	2250	TGG	-	GAUGCUUUAAUCAUCAUGAA	5344
ACTAACACATTTTAATAGGA	2251	TGG	-	ACUAACACAUUUUAAUAGGA	5345
ATGGACTAACACATTTTAAT	2252	AGG	-	AUGGACUAACACAUUUUAAU	5346
AAAATGTGTTAGTCCATTTT	2253	TGG	+	AAAAUGUGUUAGUCCAUUUU	5347
AAATGTGTTAGTCCATTTTT	2254	GGG	+	AAAUGUGUUAGUCCAUUUUU	5348
AATGTGTTAGTCCATTTTTG	2255	GGG	+	AAUGUGUUAGUCCAUUUUUG	5349
AGTAAACATAACCCCAAAAA	2256	TGG	-	AGUAAACAUAACCCCAAAAA	5350
GGGTTATGTTTACTATACAA	2257	AGG	+	GGGUUAUGUUUACUAUACAA	5351
TTATGTTTACTATACAAAGG	2258	TGG	+	UUAUGUUUACUAUACAAAGG	5352
GTTTACTATACAAAGGTGGA	2259	AGG	+	GUUUACUAUACAAAGGUGGA	5353
TTTACTATACAAAGGTGGAA	2260	GGG	+	UUUACUAUACAAAGGUGGAA	5354
TTACTATACAAAGGTGGAAG	2261	GGG	+	UUACUAUACAAAGGUGGAAG	5355
CAAAGGTGGAAGGGGTTATC	2262	TGG	+	CAAAGGUGGAAGGGGUUAUC	5356
GTTATCTGGCCCCATCTTAC	2263	AGG	+	GUUAUCUGGCCCCAUCUUAC	5357
ACACAGTTACCTGTAAGATG	2264	GGG	-	ACACAGUUACCUGUAAGAUG	5358
GACACAGTTACCTGTAAGAT	2265	GGG	-	GACACAGUUACCUGUAAGAU	5359
GGACACAGTTACCTGTAAGA	2266	TGG	-	GGACACAGUUACCUGUAAGA	5360
AGGTAACTGTGTCCCTTTTG	2267	TGG	+	AGGUAACUGUGUCCCUUUUG	5361
TGGTTTTTATATCCACAAAA	2268	GGG	-	UGGUUUUUAUAUCCACAAAA	5362
ATGGTTTTTATATCCACAAA	2269	AGG	-	AUGGUUUUUAUAUCCACAAA	5363
AATAAAATTGCAGTATCTGA	2270	TGG	-	AAUAAAAUUGCAGUAUCUGA	5364
TATTTTTGATTATGTGTCTG	2271	AGG	+	UAUUUUUGAUUAUGUGUCUG	5365
ATTTTTGATTATGTGTCTGA	2272	GGG	+	AUUUUUGAUUAUGUGUCUGA	5366
CAGTATGCACATCTGCATGC	2273	AGG	+	CAGUAUGCACAUCUGCAUGC	5367
CATCTGCATGCAGGTGCCTG	2274	TGG	+	CAUCUGCAUGCAGGUGCCUG	5368
CAGGTGCCTGTGGAAGCCTG	2275	TGG	+	CAGGUGCCUGUGGAAGCCUG	5369
ATGTCTCCACAGGCTTCCAC	2276	AGG	-	AUGUCUCCACAGGCUUCCAC	5370
AGGTGATCTGATGTCTCCAC	2277	AGG	-	AGGUGAUCUGAUGUCUCCAC	5371
GACATCAGATCACCTGAAGC	2278	TGG	+	GACAUCAGAUCACCUGAAGC	5372
TCACCTGAAGCTGGAGTTTC	2279	AGG	+	UCACCUGAAGCUGGAGUUUC	5373
CTGCCTGAAACTCCAGCTTC	2280	AGG	-	CUGCCUGAAACUCCAGCUUC	5374
AGTTTTATGCCACCAGACAC	2281	TGG	+	AGUUUUAUGCCACCAGACAC	5375
TTCTCAGCACCAGTGTCTGG	2282	TGG	-	UUCUCAGCACCAGUGUCUGG	5376
CTATTCTCAGCACCAGTGTC	2283	TGG	-	CUAUUCUCAGCACCAGUGUC	5377
ATAAAATATACTGCTTTTGC	2284	AGG	-	AUAAAAUAUACUGCUUUUGC	5378
GTTGCTTTTGGCTGGAGAGA	2285	TGG	-	GUUGCUUUUGGCUGGAGAGA	5379

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TTTTAAAGGTTGCTTTTGGC	2286	TGG	-	UUUUAAAGGUUGCUUUUGGC	5380
CAGCTTTTAAAGGTTGCTTT	2287	TGG	-	CAGCUUUUAAAGGUUGCUUU	5381
AAAGCAACCTTTAAAAGCTG	2288	TGG	+	AAAGCAACCUUUAAAAGCUG	5382
ACATAAACCACAGCTTTTAA	2289	AGG	-	ACAUAAACCACAGCUUUUAA	5383
CTCTAAGTGGATGAATTAAA	2290	TGG	-	CUCUAAGUGGAUGAAUUAAA	5384
CATTTAATTCATCCACTTAG	2291	AGG	+	CAUUUAAUUCAUCCACUUAG	5385
ATTTAATTCATCCACTTAGA	2292	GGG	+	AUUUAAUUCAUCCACUUAGA	5386
ATTGAAGTGTACCCTCTAAG	2293	TGG	-	AUUGAAGUGUACCCUCUAAG	5387
AAAATATGCTTACAGATCTG	2294	TGG	+	AAAAUAUGCUUACAGAUCUG	5388
AAATATGCTTACAGATCTGT	2295	GGG	+	AAAUAUGCUUACAGAUCUGU	5389
TGCTAAAAGGGTTCTAAAAT	2296	TGG	-	UGCUAAAAGGGUUCUAAAAU	5390
TAATATCTGTAGTGCTAAAA	2297	GGG	-	UAAUAUCUGUAGUGCUAAAA	5391
TTAATATCTGTAGTGCTAAA	2298	AGG	-	UUAAUAUCUGUAGUGCUAAA	5392
GCTGGGGGCTGGGGAGAATA	2299	GGG	-	GCUGGGGGCUGGGGAGAAUA	5393
AGCTGGGGGCTGGGGAGAAT	2300	AGG	-	AGCUGGGGGCUGGGGAGAAU	5394
GGGACTGGAGCTGGGGGCTG	2301	GGG	-	GGGACUGGAGCUGGGGGCUG	5395
AGGGACTGGAGCTGGGGGCT	2302	GGG	-	AGGGACUGGAGCUGGGGGCU	5396
CAGGGACTGGAGCTGGGGGC	2303	TGG	-	CAGGGACUGGAGCUGGGGGC	5397
CAGCCCCCAGCTCCAGTCCC	2304	TGG	+	CAGCCCCCAGCUCCAGUCCC	5398
ATGCCAGGGACTGGAGCTGG	2305	GGG	-	AUGCCAGGGACUGGAGCUGG	5399
GATGCCAGGGACTGGAGCTG	2306	GGG	-	GAUGCCAGGGACUGGAGCUG	5400
GGATGCCAGGGACTGGAGCT	2307	GGG	-	GGAUGCCAGGGACUGGAGCU	5401
TGGATGCCAGGGACTGGAGC	2308	TGG	-	UGGAUGCCAGGGACUGGAGC	5402
GATTTGTGGATGCCAGGGAC	2309	TGG	-	GAUUUGUGGAUGCCAGGGAC	5403
AAGGGGATTTGTGGATGCCA	2310	GGG	-	AAGGGGAUUUGUGGAUGCCA	5404
GAAGGGGATTTGTGGATGCC	2311	AGG	-	GAAGGGGAUUUGUGGAUGCC	5405
GATGGCAGGAAGGGGATTTG	2312	TGG	-	GAUGGCAGGAAGGGGAUUUG	5406
AATCTATAGATGGCAGGAAG	2313	GGG	-	AAUCUAUAGAUGGCAGGAAG	5407
GAATCTATAGATGGCAGGAA	2314	GGG	-	GAAUCUAUAGAUGGCAGGAA	5408
TGAATCTATAGATGGCAGGA	2315	AGG	-	UGAAUCUAUAGAUGGCAGGA	5409
GAGGTGAATCTATAGATGGC	2316	AGG	-	GAGGUGAAUCUAUAGAUGGC	5410
AGAAGAGGTGAATCTATAGA	2317	TGG	-	AGAAGAGGUGAAUCUAUAGA	5411
TTCAGTGTAAAGTCTAGAAG	2318	AGG	-	UUCAGUGUAAAGUCUAGAAG	5412
TTCTAGACTTTACACTGAAA	2319	TGG	+	UUCUAGACUUUACACUGAAA	5413
AAATGGTATTACACAACATG	2320	TGG	+	AAAUGGUAUUACACAACAUG	5414
TTGCTTGCCGTGTGTCTGTG	2321	AGG	+	UUGCUUGCCGUGUGUCUGUG	5415
AGATGAACCTCACAGACACA	2322	CGG	-	AGAUGAACCUCACAGACACA	5416
TCATCTGTGTTGAGCATGAA	2323	AGG	+	UCAUCUGUGUUGAGCAUGAA	5417
TAGGCGACAGGCATATTCAT	2324	GGG	-	UAGGCGACAGGCAUAUUCAU	5418
TTAGGCGACAGGCATATTCA	2325	TGG	-	UUAGGCGACAGGCAUAUUCA	5419
GTGTTGCTATTTTAGGCGAC	2326	AGG	-	GUGUUGCUAUUUUAGGCGAC	5420
TGTGAAAGTGTTGCTATTTT	2327	AGG	-	UGUGAAAGUGUUGCUAUUUU	5421
CCTTCTTTCTTCTTCCCAAG	2328	AGG	+	CCUUCUUUCUUCUUCCCAAG	5422
CCTCTTGGGAAGAAGAAAGA	2329	AGG	-	CCUCUUGGGAAGAAGAAAGA	5423
CTTCTTTCTTCTTCCCAAGA	2330	GGG	+	CUUCUUUCUUCUUCCCAAGA	5424
GCTGCAATAATGTCCCTCTT	2331	GGG	-	GCUGCAAUAAUGUCCCUCUU	5425
GGCTGCAATAATGTCCCTCT	2332	TGG	-	GGCUGCAAUAAUGUCCCUCU	5426
AGACACAGGAGAAAACTAGC	2333	AGG	-	AGACACAGGAGAAAACUAGC	5427
AGAAACACGAAAAAAGACAC	2334	AGG	-	AGAAACACGAAAAAAGACAC	5428
CGTGTTTCTCAAGTAGATGA	2335	AGG	+	CGUGUUUCUCAAGUAGAUGA	5429
GTGTTTCTCAAGTAGATGAA	2336	GGG	+	GUGUUUCUCAAGUAGAUGAA	5430
TCTCAAGTAGATGAAGGGAC	2337	TGG	+	UCUCAAGUAGAUGAAGGGAC	5431
TTAGGAAACTGACAAAGCAG	2338	GGG	-	UUAGGAAACUGACAAAGCAG	5432
TTTAGGAAACTGACAAAGCA	2339	GGG	-	UUUAGGAAACUGACAAAGCA	5433
ATTTAGGAAACTGACAAAGC	2340	AGG	-	AUUUAGGAAACUGACAAAGC	5434
AATACAGTATTCACTCATTT	2341	AGG	-	AAUACAGUAUUCACUCAUUU	5435
AAATGAGTGAATACTGTATT	2342	TGG	+	AAAUGAGUGAAUACUGUAUU	5436
TATTTTGGTCTTTCTGATTG	2343	GGG	-	UAUUUUGGUCUUUCUGAUUG	5437

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TTATTTTGGTCTTTCTGATT	2344	GGG	-	UUAUUUUGGUCUUUCUGAUU	5438
ATTATTTTGGTCTTTCTGAT	2345	TGG	-	AUUAUUUUGGUCUUUCUGAU	5439
GAGATTATTTTAAATTATTT	2346	TGG	-	GAGAUUAUUUUAAAUUAUUU	5440
ATCTCGTCTCTATATCACTA	2347	AGG	+	AUCUCGUCUCUAUAUCACUA	5441
CTAAGGTATCCTTTTCCACT	2348	TGG	+	CUAAGGUAUCCUUUUCCACU	5442
TAAGGTATCCTTTTCCACTT	2349	GGG	+	UAAGGUAUCCUUUUCCACUU	5443
CTGCATGGCCCAAGTGGAAA	2350	AGG	-	CUGCAUGGCCCAAGUGGAAA	5444
ATTTCTCTGCATGGCCCAAG	2351	TGG	-	AUUUCUCUGCAUGGCCCAAG	5445
GGCATGCTTATTTCTCTGCA	2352	TGG	-	GGCAUGCUUAUUUCUCUGCA	5446
GGGTGGGGTGGGGTCAATCA	2353	AGG	-	GGGUGGGGUGGGGUCAAUCA	5447
CTGGGGGTTGGGGTGGGGTG	2354	GGG	-	CUGGGGGUUGGGGUGGGGUG	5448
ACTGGGGGTTGGGGTGGGGT	2355	GGG	-	ACUGGGGGUUGGGGUGGGGU	5449
CACTGGGGGTTGGGGTGGGG	2356	TGG	-	CACUGGGGGUUGGGGUGGGG	5450
CACCCCACCCCAACCCCCAG	2357	TGG	+	CACCCCACCCCAACCCCCAG	5451
CACCACTGGGGGTTGGGGTG	2358	GGG	-	CACCACUGGGGGUUGGGGUG	5452
TCACCACTGGGGGTTGGGGT	2359	GGG	-	UCACCACUGGGGGUUGGGGU	5453
GTCACCACTGGGGGTTGGGG	2360	TGG	-	GUCACCACUGGGGGUUGGGG	5454
CTTGTCACCACTGGGGGTTG	2361	GGG	-	CUUGUCACCACUGGGGGUUG	5455
CCCAACCCCCAGTGGTGACA	2362	AGG	+	CCCAACCCCCAGUGGUGACA	5456
CCTTGTCACCACTGGGGGTT	2363	GGG	-	CCUUGUCACCACUGGGGGUU	5457
TCCTTGTCACCACTGGGGGT	2364	TGG	-	UCCUUGUCACCACUGGGGGU	5458
GAGGTCCTTGTCACCACTGG	2365	GGG	-	GAGGUCCUUGUCACCACUGG	5459
GGAGGTCCTTGTCACCACTG	2366	GGG	-	GGAGGUCCUUGUCACCACUG	5460
GGGAGGTCCTTGTCACCACT	2367	GGG	-	GGGAGGUCCUUGUCACCACU	5461
GGGGAGGTCCTTGTCACCAC	2368	TGG	-	GGGGAGGUCCUUGUCACCAC	5462
GGTGACAAGGACCTCCCCAG	2369	CGG	+	GGUGACAAGGACCUCCCCAG	5463
AAATGGTTGAGCCGCTGGGG	2370	AGG	-	AAAUGGUUGAGCCGCUGGGG	5464
TTCAAATGGTTGAGCCGCTG	2371	GGG	-	UUCAAAUGGUUGAGCCGCUG	5465
ATTCAAATGGTTGAGCCGCT	2372	GGG	-	AUUCAAAUGGUUGAGCCGCU	5466
TATTCAAATGGTTGAGCCGC	2373	TGG	-	UAUUCAAAUGGUUGAGCCGC	5467
GCTCAACCATTTGAATAAAA	2374	AGG	+	GCUCAACCAUUUGAAUAAAA	5468
AAGTGTCCTTTTTATTCAAA	2375	TGG	-	AAGUGUCCUUUUUAUUCAAA	5469
ATCTTAGATTTGTGCAAAAT	2376	GGG	-	AUCUUAGAUUUGUGCAAAAU	5470
CATCTTAGATTTGTGCAAAA	2377	TGG	-	CAUCUUAGAUUUGUGCAAAA	5471
ACAAATCTAAGATGAGCAAG	2378	TGG	+	ACAAAUCUAAGAUGAGCAAG	5472
GTCACCTGTAAACATGTGCC	2379	TGG	+	GUCACCUGUAAACAUGUGCC	5473
TGCTCCAGGCACATGTTTAC	2380	AGG	-	UGCUCCAGGCACAUGUUUAC	5474
GTAAACATGTGCCTGGAGCA	2381	CGG	+	GUAAACAUGUGCCUGGAGCA	5475
CTCAGGTCGTTCCGTGCTCC	2382	AGG	-	CUCAGGUCGUUCCGUGCUCC	5476
GAGCACGGAACGACCTGAGT	2383	**AGG**	**+**	**GAGCACGGAACGACCUGAGU**	5477
CGACCTGAGTAGGCAGAAAA	2384	AGG	+	CGACCUGAGUAGGCAGAAAA	5478
CCTGAGTAGGCAGAAAAAGG	2385	TGG	+	CCUGAGUAGGCAGAAAAAGG	5479
CCACCTTTTTCTGCCTACTC	2386	AGG	-	CCACCUUUUUCUGCCUACUC	5480
ATTTATGATTTTTCTTTTAA	2387	AGG	+	AUUUAUGAUUUUUCUUUUAA	5481
AGTGAGAAAGGTGGGGGAAA	2388	TGG	-	AGUGAGAAAGGUGGGGGAAA	5482
TTGGAAAGTGAGAAAGGTGG	2389	GGG	-	UUGGAAAGUGAGAAAGGUGG	5483
GTTGGAAAGTGAGAAAGGTG	2390	GGG	-	GUUGGAAAGUGAGAAAGGUG	5484
TGTTGGAAAGTGAGAAAGGT	2391	GGG	-	UGUUGGAAAGUGAGAAAGGU	5485
TTGTTGGAAAGTGAGAAAGG	2392	TGG	-	UUGUUGGAAAGUGAGAAAGG	5486
ATATTGTTGGAAAGTGAGAA	2393	AGG	-	AUAUUGUUGGAAAGUGAGAA	5487
TTTCCAACAATATTATATTC	2394	TGG	+	UUUCCAACAAUAUUAUAUUC	5488
ACACCAGAATATAATATTGT	2395	TGG	-	ACACCAGAAUAUAAUAUUGU	5489
CTTTTATTCCCCAGTTGTTT	2396	TGG	+	CUUUUAUUCCCCAGUUGUUU	5490
TTTAATCACCAAAACAACTG	2397	GGG	-	UUUAAUCACCAAAACAACUG	5491
ATTTAATCACCAAAACAACT	2398	GGG	-	AUUUAAUCACCAAAACAACU	5492
GATTTAATCACCAAAACAAC	2399	TGG	-	GAUUUAAUCACCAAAACAAC	5493
TTTTGGTGATTAAATCATTT	2400	TGG	+	UUUUGGUGAUUAAAUCAUUU	5494
TTTGGTGATTAAATCATTTT	2401	GGG	+	UUUGGUGAUUAAAUCAUUUU	5495

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TTGGTGATTAAATCATTTTG	2402	GGG	+	UUGGUGAUUAAAUCAUUUUG	5496
TGGTGATTAAATCATTTTGG	2403	GGG	+	UGGUGAUUAAAUCAUUUUGG	5497
ACTCTCATTCACTGCTCCCT	2404	TGG	+	ACUCUCAUUCACUGCUCCCU	5498
AAAAAAAACACTGTCACCAA	2405	GGG	-	AAAAAAAACACUGUCACCAA	5499
GAAAAAAAACACTGTCACCA	2406	AGG	-	GAAAAAAAACACUGUCACCA	5500
GCAGAGACAGAGGGAACAGC	2407	AGG	-	GCAGAGACAGAGGGAACAGC	5501
TGAAAATGAGCAGAGACAGA	2408	GGG	-	UGAAAAUGAGCAGAGACAGA	5502
ATGAAAATGAGCAGAGACAG	2409	AGG	-	AUGAAAAUGAGCAGAGACAG	5503
CTGTCTCTGCTCATTTTCAT	2410	AGG	+	CUGUCUCUGCUCAUUUUCAU	5504
TGCTCATTTTCATAGGAATC	2411	TGG	+	UGCUCAUUUUCAUAGGAAUC	5505
ATCTGGCACCTTAGAATCCC	2412	AGG	+	AUCUGGCACCUUAGAAUCCC	5506
GCAGGCAGCCTGGGATTCTA	2413	AGG	-	GCAGGCAGCCUGGGAUUCUA	5507
CATTTGACTGCAGGCAGCCT	2414	GGG	-	CAUUUGACUGCAGGCAGCCU	5508
GCATTTGACTGCAGGCAGCC	2415	TGG	-	GCAUUUGACUGCAGGCAGCC	5509
CCTGCAGTCAAATGCTTGCT	2416	AGG	+	CCUGCAGUCAAAUGCUUGCU	5510
CCTAGCAAGCATTTGACTGC	2417	AGG	-	CCUAGCAAGCAUUUGACUGC	5511
CTGCAGTCAAATGCTTGCTA	2418	GGG	+	CUGCAGUCAAAUGCUUGCUA	5512
CAGTCAAATGCTTGCTAGGG	2419	AGG	+	CAGUCAAAUGCUUGCUAGGG	5513
AGTCAAATGCTTGCTAGGGA	2420	GGG	+	AGUCAAAUGCUUGCUAGGGA	5514
GTCAAATGCTTGCTAGGGAG	2421	GGG	+	GUCAAAUGCUUGCUAGGGAG	5515
TTGCTAGGGAGGGGAGCCAG	2422	TGG	+	UUGCUAGGGAGGGGAGCCAG	5516
TGCTAGGGAGGGGAGCCAGT	2423	GGG	+	UGCUAGGGAGGGGAGCCAGU	5517
TAGGGAGGGGAGCCAGTGGG	2424	TGG	+	UAGGGAGGGGAGCCAGUGGG	5518
AGGGAGGGGAGCCAGTGGGT	2425	GGG	+	AGGGAGGGGAGCCAGUGGGU	5519
GGGAGGGGAGCCAGTGGGTG	2426	GGG	+	GGGAGGGGAGCCAGUGGGUG	5520
GGAGGGGAGCCAGTGGGTGG	2427	GGG	+	GGAGGGGAGCCAGUGGGUGG	5521
TTGCAGGTTCCCCCACCCAC	2428	TGG	-	UUGCAGGUUCCCCCACCCAC	5522
ATTTGGAGTTGATTTCTTGC	2429	AGG	-	AUUUGGAGUUGAUUUCUUGC	5523
AAATCAACTCCAAATTTAAT	2430	AGG	+	AAAUCAACUCCAAAUUUAAU	5524
GAGCTTAGTCCTATTAAATT	2431	TGG	-	GAGCUUAGUCCUAUUAAAUU	5525
CTGCTAATCAGGCATGGTGT	2432	AGG	-	CUGCUAAUCAGGCAUGGUGU	5526
ACCATGCCTGATTAGCAGCT	2433	AGG	+	ACCAUGCCUGAUUAGCAGCU	5527
ACCTAGCTGCTAATCAGGCA	2434	TGG	-	ACCUAGCUGCUAAUCAGGCA	5528
ATGAGACCTAGCTGCTAATC	2435	AGG	-	AUGAGACCUAGCUGCUAAUC	5529
GGTCTCATCCTTACCCCTCC	2436	AGG	+	GGUCUCAUCCUUACCCCUCC	5530
TCCTTACCCCTCCAGGAATA	2437	AGG	+	UCCUUACCCCUCCAGGAAUA	5531
TCCTTATTCCTGGAGGGGTA	2438	AGG	-	UCCUUAUUCCUGGAGGGGUA	5532
TACCCCTCCAGGAATAAGGA	2439	AGG	+	UACCCCUCCAGGAAUAAGGA	5533
AACCTTCCTTATTCCTGGAG	2440	GGG	-	AACCUUCCUUAUUCCUGGAG	5534
CAACCTTCCTTATTCCTGGA	2441	GGG	-	CAACCUUCCUUAUUCCUGGA	5535
GCAACCTTCCTTATTCCTGG	2442	AGG	-	GCAACCUUCCUUAUUCCUGG	5536
TCTGCAACCTTCCTTATTCC	2443	TGG	-	UCUGCAACCUUCCUUAUUCC	5537
AGGTTGCAGAAGTCCCTGAA	2444	AGG	+	AGGUUGCAGAAGUCCCUGAA	5538
GGCTCAGCAAATACCTTTCA	2445	GGG	-	GGCUCAGCAAAUACCUUUCA	5539
AGGCTCAGCAAATACCTTTC	2446	AGG	-	AGGCUCAGCAAAUACCUUUC	5540
GCTGAGCCTCTTGTGAACCG	2447	TGG	+	GCUGAGCCUCUUGUGAACCG	5541
CTGAGCCTCTTGTGAACCGT	2448	GGG	+	CUGAGCCUCUUGUGAACCGU	5542
GCCTCTTGTGAACCGTGGGT	2449	TGG	+	GCCUCUUGUGAACCGUGGGU	5543
GCCAACCCACGGTTCACAAG	2450	AGG	-	GCCAACCCACGGUUCACAAG	5544
AGTTCAGGTGAGCCAACCCA	2451	CGG	-	AGUUCAGGUGAGCCAACCCA	5545
AAGAATATCTACTAAAGTTC	2452	AGG	-	AAGAAUAUCUACUAAAGUUC	5546
ATATTCTTTAGCGACATATT	2453	TGG	+	AUAUUCUUUAGCGACAUAUU	5547
TATTCTTTAGCGACATATTT	2454	GGG	+	UAUUCUUUAGCGACAUAUUU	5548
TTAGCGACATATTTGGGCTT	2455	TGG	+	UUAGCGACAUAUUUGGGCUU	5549
TGTGGAGAGATAAAAGAGAG	2456	AGG	-	UGUGGAGAGAUAAAAGAGAG	5550
TTATCTCTCCACAAAAGTCT	2457	TGG	+	UUAUCUCUCCACAAAAGUCU	5551
TCAGTTTGCCAAGACTTTTG	2458	TGG	-	UCAGUUUGCCAAGACUUUUG	5552
TGATGTTTATGCAACCAGTA	2459	AGG	+	UGAUGUUUAUGCAACCAGUA	5553

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TATGCAACCAGTAAGGCCTC	2460	TGG	+	UAUGCAACCAGUAAGGCCUC	5554
ATGCAACCAGTAAGGCCTCT	2461	GGG	+	AUGCAACCAGUAAGGCCUCU	5555
TGCAACCAGTAAGGCCTCTG	2462	GGG	+	UGCAACCAGUAAGGCCUCUG	5556
CTCAGCCCCAGAGGCCTTAC	2463	TGG	-	CUCAGCCCCAGAGGCCUUAC	5557
CAGTAAGGCCTCTGGGGCTG	2464	AGG	+	CAGUAAGGCCUCUGGGGCUG	5558
AGTAAGGCCTCTGGGGCTGA	2465	GGG	+	AGUAAGGCCUCUGGGGCUGA	5559
GTAAGGCCTCTGGGGCTGAG	2466	GGG	+	GUAAGGCCUCUGGGGCUGAG	5560
TAAGGCCTCTGGGGCTGAGG	2467	GGG	+	UAAGGCCUCUGGGGCUGAGG	5561
GGCCTCTGGGGCTGAGGGGG	2468	TGG	+	GGCCUCUGGGGCUGAGGGGG	5562
GCCTCTGGGGCTGAGGGGGT	2469	GGG	+	GCCUCUGGGGCUGAGGGGGU	5563
TCCCACCCCCTCAGCCCCAG	2470	AGG	-	UCCCACCCCCUCAGCCCCAG	5564
CTGAGGGGGTGGGAAGCCAG	2471	TGG	+	CUGAGGGGGUGGGAAGCCAG	5565
TGAGGGGGTGGGAAGCCAGT	2472	GGG	+	UGAGGGGGUGGGAAGCCAGU	5566
GGGGGTGGGAAGCCAGTGGG	2473	TGG	+	GGGGGUGGGAAGCCAGUGGG	5567
GGTGGGAAGCCAGTGGGTGG	2474	CGG	+	GGUGGGAAGCCAGUGGGUGG	5568
TTGCAGGTTCCGCCACCCAC	2475	TGG	-	UUGCAGGUUCCGCCACCCAC	5569
ATTTGGGGCGGATTTCTTGC	2476	AGG	-	AUUUGGGGCGGAUUUCUUGC	5570
AGAAATCCGCCCCAAATTTA	2477	AGG	+	AGAAAUCCGCCCCAAAUUUA	5571
ATGCTTCCTTAAATTTGGGG	2478	CGG	-	AUGCUUCCUUAAAUUUGGGG	5572
CCCCAAATTTAAGGAAGCAT	2479	AGG	+	CCCCAAAUUUAAGGAAGCAU	5573
CCTATGCTTCCTTAAATTTG	2480	GGG	-	CCUAUGCUUCCUUAAAUUUG	5574
TCCTATGCTTCCTTAAATTT	2481	GGG	-	UCCUAUGCUUCCUUAAAUUU	5575
TTCCTATGCTTCCTTAAATT	2482	TGG	-	UUCCUAUGCUUCCUUAAAUU	5576
CCTACCCTATGTCTGCCTTG	2483	TGG	+	CCUACCCUAUGUCUGCCUUG	5577
CCACAAGGCAGACATAGGGT	2484	AGG	-	CCACAAGGCAGACAUAGGGU	5578
CTAGCCACAAGGCAGACATA	2485	GGG	-	CUAGCCACAAGGCAGACAUA	5579
CCTATGTCTGCCTTGTGGCT	2486	AGG	+	CCUAUGUCUGCCUUGUGGCU	5580
CCTAGCCACAAGGCAGACAT	2487	AGG	-	CCUAGCCACAAGGCAGACAU	5581
GAACTAAGGACCTAGCCACA	2488	AGG	-	GAACUAAGGACCUAGCCACA	5582
TCCTTAGTTCATTGTCTCTT	2489	AGG	+	UCCUUAGUUCAUUGUCUCUU	5583
ACCTAAGAGACAATGAACTA	2490	AGG	-	ACCUAAGAGACAAUGAACUA	5584
TTCTACTCTCAAGAGAATCT	2491	GGG	-	UUCUACUCUCAAGAGAAUCU	5585
CTTCTACTCTCAAGAGAATC	2492	TGG	-	CUUCUACUCUCAAGAGAAUC	5586
CAGATTCTCTTGAGAGTAGA	2493	AGG	+	CAGAUUCUCUUGAGAGUAGA	5587
ATTCTCTTGAGAGTAGAAGG	2494	TGG	+	AUUCUCUUGAGAGUAGAAGG	5588
TCTCGGATTTGTTAATCAGG	2495	AGG	-	UCUCGGAUUUGUUAAUCAGG	5589
TCCTGATTAACAAATCCGAG	2496	AGG	+	UCCUGAUUAACAAAUCCGAG	5590
ACCTCTCGGATTTGTTAATC	2497	AGG	-	ACCUCUCGGAUUUGUUAAUC	5591
TTCATGAGATTAGCACCTCT	2498	CGG	-	UUCAUGAGAUUAGCACCUCU	5592
GCTAATCTCATGAATTCTCC	2499	CGG	+	GCUAAUCUCAUGAAUUCUCC	5593
TCATGAATTCTCCCGGACTA	2500	TGG	+	UCAUGAAUUCUCCCGGACUA	5594
GCTATATATAACCATAGTCC	2501	GGG	-	GCUAUAUAUAACCAUAGUCC	5595
AGCTATATATAACCATAGTC	2502	CGG	-	AGCUAUAUAUAACCAUAGUC	5596
TCAGTGATCTCATGTAGCTT	2503	AGG	+	UCAGUGAUCUCAUGUAGCUU	5597
TGATCTCATGTAGCTTAGGC	2504	TGG	+	UGAUCUCAUGUAGCUUAGGC	5598
CTCATGTAGCTTAGGCTGGC	2505	TGG	+	CUCAUGUAGCUUAGGCUGGC	5599
TTGAGCTCACTATGAAGCAA	2506	AGG	+	UUGAGCUCACUAUGAAGCAA	5600
AAGGATGACCCTGAACTTCT	2507	TGG	+	AAGGAUGACCCUGAACUUCU	5601
CAGGAAAACCAAGAAGTTCA	2508	GGG	-	CAGGAAAACCAAGAAGUUCA	5602
GCAGGAAAACCAAGAAGTTC	2509	AGG	-	GCAGGAAAACCAAGAAGUUC	5603
AGCACTTGGAAGATGAAAGC	2510	AGG	-	AGCACUUGGAAGAUGAAAGC	5604
ATTCTTGTAATCGCAGCACT	2511	TGG	-	AUUCUUGUAAUCGCAGCACU	5605
GCTGCCATGTCTAGTTCATG	2512	TGG	+	GCUGCCAUGUCUAGUUCAUG	5606
AGCACCACATGAACTAGACA	2513	TGG	-	AGCACCACAUGAACUAGACA	5607
TGTCTAGTTCATGTGGTGCT	2514	AGG	+	UGUCUAGUUCAUGUGGUGCU	5608
GTCTAGTTCATGTGGTGCTA	2515	GGG	+	GUCUAGUUCAUGUGGUGCUA	5609
GGTGCTAGGGATCAACCCTG	2516	AGG	+	GGUGCUAGGGAUCAACCCUG	5610
TTAACATGCACAAAGCCTCA	2517	GGG	-	UUAACAUGCACAAAGCCUCA	5611

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CTTAACATGCACAAAGCCTC	2518	AGG	-	CUUAACAUGCACAAAGCCUC	5612
ATTCTTTTGAGTCAGGGTCA	2519	GGG	-	AUUCUUUUGAGUCAGGGUCA	5613
TATTCTTTTGAGTCAGGGTC	2520	AGG	-	UAUUCUUUUGAGUCAGGGUC	5614
TGACCCTGACTCAAAAGAAT	2521	AGG	+	UGACCCUGACUCAAAAGAAU	5615
GACCCTGACTCAAAAGAATA	2522	GGG	+	GACCCUGACUCAAAAGAAUA	5616
ACCCTGACTCAAAAGAATAG	2523	GGG	+	ACCCUGACUCAAAAGAAUAG	5617
CCCTGACTCAAAAGAATAGG	2524	GGG	+	CCCUGACUCAAAAGAAUAGG	5618
CCCCCTATTCTTTTGAGTCA	2525	GGG	-	CCCCCUAUUCUUUUGAGUCA	5619
CCTGACTCAAAAGAATAGGG	2526	GGG	+	CCUGACUCAAAAGAAUAGGG	5620
CCCCCCTATTCTTTTGAGTC	2527	AGG	-	CCCCCCUAUUCUUUUGAGUC	5621
CTGACTCAAAAGAATAGGGG	2528	GGG	+	CUGACUCAAAAGAAUAGGGG	5622
ACTCAAAAGAATAGGGGGGG	2529	AGG	+	ACUCAAAAGAAUAGGGGGGG	5623
AAAAGAATAGGGGGGGAGGA	2530	AGG	+	AAAAGAAUAGGGGGGGAGGA	5624
GAATAGGGGGGGAGGAAGGA	2531	AGG	+	GAAUAGGGGGGGAGGAAGGA	5625
AGGGGGGGAGGAAGGAAGGA	2532	AGG	+	AGGGGGGGAGGAAGGAAGGA	5626
GGGGAGGAAGGAAGGAAGGA	2533	AGG	+	GGGGAGGAAGGAAGGAAGGA	5627
AGGAAGGAAGGAAGGAAGGA	2534	AGG	+	AGGAAGGAAGGAAGGAAGGA	5628
AGGAAGGAAGGAAGGAAGGA	2535	AGG	+	AGGAAGGAAGGAAGGAAGGA	5629
GGAAGGAAGGAAGGAAGGAA	2536	GGG	+	GGAAGGAAGGAAGGAAGGAA	5630
AGGAAGGAAGGAAGGAAGGG	2537	AGG	+	AGGAAGGAAGGAAGGAAGGG	5631
GGAAGGAAGGAAGGAAGGGA	2538	GGG	+	GGAAGGAAGGAAGGAAGGGA	5632
AGGAAGGAAGGAAGGGAGGG	2539	AGG	+	AGGAAGGAAGGAAGGGAGGG	5633
GGAAGGAAGGAAGGGAGGGA	2540	GGG	+	GGAAGGAAGGAAGGGAGGGA	5634
AGGAAGGAAGGGAGGGAGGG	2541	AGG	+	AGGAAGGAAGGGAGGGAGGG	5635
GGAAGGAAGGGAGGGAGGGA	2542	GGG	+	GGAAGGAAGGGAGGGAGGGA	5636
AGGAAGGGAGGGAGGGAGGG	2543	AGG	+	AGGAAGGGAGGGAGGGAGGG	5637
GGAAGGGAGGGAGGGAGGGA	2544	GGG	+	GGAAGGGAGGGAGGGAGGGA	5638
AGGGAGGGAGGGAGGGAGGG	2545	AGG	+	AGGGAGGGAGGGAGGGAGGG	5639
GGGAGGGAGGGAGGGAGGGA	2546	GGG	+	GGGAGGGAGGGAGGGAGGGA	5640
AGGGAGGGAGGGAGGGAGGG	2547	AGG	+	AGGGAGGGAGGGAGGGAGGG	5641
GGGAGGGAGGGAGGGAGGGA	2548	GGG	+	GGGAGGGAGGGAGGGAGGGA	5642
AGGGAGGGAGGGAGGGAGGG	2549	AGG	+	AGGGAGGGAGGGAGGGAGGG	5643
GGGAGGGAGGGAGGGAGGGA	2550	GGG	+	GGGAGGGAGGGAGGGAGGGA	5644
AGGGAGGGAGGGAGGGAGGG	2551	AGG	+	AGGGAGGGAGGGAGGGAGGG	5645
GGGAGGGAGGGAGGGAGGGA	2552	GGG	+	GGGAGGGAGGGAGGGAGGGA	5646
AGGGAGGGAGGGAGGGAGGG	2553	AGG	+	AGGGAGGGAGGGAGGGAGGG	5647
GGGAGGGAGGGAGGGAGGGA	2554	GGG	+	GGGAGGGAGGGAGGGAGGGA	5648
AGGGAGGGAGGGAGGGAGGG	2555	AGG	+	AGGGAGGGAGGGAGGGAGGG	5649
AGGGAGGGAGGGAGGGAGGA	2556	AGG	+	AGGGAGGGAGGGAGGGAGGA	5650
GATGTTGAGAACAAAAGCCA	2557	AGG	+	GAUGUUGAGAACAAAAGCCA	5651
GAACAAAAGCCAAGGCCAAC	2558	AGG	+	GAACAAAAGCCAAGGCCAAC	5652
GCCAAGGCCAACAGGTGAGT	2559	TGG	+	GCCAAGGCCAACAGGUGAGU	5653
CCAAGGCCAACAGGTGAGTT	2560	GGG	+	CCAAGGCCAACAGGUGAGUU	5654
CCCAACTCACCTGTTGGCCT	2561	TGG	-	CCCAACUCACCUGUUGGCCU	5655
CCAACAGGTGAGTTGGGATT	2562	AGG	+	CCAACAGGUGAGUUGGGAUU	5656
CCTAATCCCAACTCACCTGT	2563	TGG	-	CCUAAUCCCAACUCACCUGU	5657
AAAAGTCCTTTTCATAGCAA	2564	AGG	+	AAAAGUCCUUUUCAUAGCAA	5658
AAAGTCCTTTTCATAGCAAA	2565	GGG	+	AAAGUCCUUUUCAUAGCAAA	5659
AAGTCCTTTTCATAGCAAAG	2566	GGG	+	AAGUCCUUUUCAUAGCAAAG	5660
ACATCCCCTTTGCTATGAAA	2567	AGG	-	ACAUCCCCUUUGCUAUGAAA	5661
TTTTCATAGCAAAGGGGATG	2568	TGG	+	UUUUCAUAGCAAAGGGGAUG	5662
GTGGAAGAAGAGAAGTTGAC	2569	AGG	+	GUGGAAGAAGAGAAGUUGAC	5663
AGAGAAGTTGACAGGTAAGT	2570	AGG	+	AGAGAAGUUGACAGGUAAGU	5664
GAGAAGTTGACAGGTAAGTA	2571	GGG	+	GAGAAGUUGACAGGUAAGUA	5665
AGAAAAAAGAAAAAAATCTG	2572	TGG	+	AGAAAAAAGAAAAAAAUCUG	5666
AAGAAAAAAATCTGTGGACC	2573	AGG	+	AAGAAAAAAAUCUGUGGACC	5667
AGAAAAAAATCTGTGGACCA	2574	GGG	+	AGAAAAAAAUCUGUGGACCA	5668
AAAATCTGTGGACCAGGGCA	2575	TGG	+	AAAAUCUGUGGACCAGGGCA	5669

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CTGCGTTTCTTGCCATGCCC	2576	TGG	-	CUGCGUUUCUUGCCAUGCCC	5670
GAGCAAATGTTCATAAAGAG	2577	AGG	+	GAGCAAAUGUUCAUAAAGAG	5671
CAAATGTTCATAAAGAGAGG	2578	TGG	+	CAAAUGUUCAUAAAGAGAGG	5672
AAATGTTCATAAAGAGAGGT	2579	GGG	+	AAAUGUUCAUAAAGAGAGGU	5673
GTTCATAAAGAGAGGTGGGC	2580	AGG	+	GUUCAUAAAGAGAGGUGGGC	5674
CATAAAGAGAGGTGGGCAGG	2581	AGG	+	CAUAAAGAGAGGUGGGCAGG	5675
GGTGGGCAGGAGGCAGACCA	2582	TGG	+	GGUGGGCAGGAGGCAGACCA	5676
TCTGATGAGTCTTGAGACCA	2583	TGG	-	UCUGAUGAGUCUUGAGACCA	5677
CAAGACTCATCAGAGAGATA	2584	AGG	+	CAAGACUCAUCAGAGAGAUA	5678
AGAGATAAGGCAATATCCTG	2585	AGG	+	AGAGAUAAGGCAAUAUCCUG	5679
TCCTGAGGAGCACCCATCTG	2586	AGG	+	UCCUGAGGAGCACCCAUCUG	5680
CCTGAGGAGCACCCATCTGA	2587	GGG	+	CCUGAGGAGCACCCAUCUGA	5681
CCCTCAGATGGGTGCTCCTC	2588	AGG	-	CCCUCAGAUGGGUGCUCCUC	5682
CTGAGGAGCACCCATCTGAG	2589	GGG	+	CUGAGGAGCACCCAUCUGAG	5683
TTGCTCAACGCCCCTCAGAT	2590	GGG	-	UUGCUCAACGCCCCUCAGAU	5684
CTTGCTCAACGCCCCTCAGA	2591	TGG	-	CUUGCUCAACGCCCCUCAGA	5685
TCTGAGGGGCGTTGAGCAAG	2592	TGG	+	UCUGAGGGGCGUUGAGCAAG	5686
CTGAGGGGCGTTGAGCAAGT	2593	GGG	+	CUGAGGGGCGUUGAGCAAGU	5687
CATTTCTCTGCATTCACAGA	2594	TGG	+	CAUUUCUCUGCAUUCACAGA	5688
TTCTCTGCATTCACAGATGG	2595	AGG	+	UUCUCUGCAUUCACAGAUGG	5689
TGCAACTATCTTTAGCAGTG	2596	AGG	+	UGCAACUAUCUUUAGCAGUG	5690
TCTTTAGCAGTGAGGCATGC	2597	TGG	+	UCUUUAGCAGUGAGGCAUGC	5691
CTTTAGCAGTGAGGCATGCT	2598	GGG	+	CUUUAGCAGUGAGGCAUGCU	5692
ATGCTGGGTAGTGCTAAGAG	2599	CGG	+	AUGCUGGGUAGUGCUAAGAG	5693
TGCTGGGTAGTGCTAAGAGC	2600	GGG	+	UGCUGGGUAGUGCUAAGAGC	5694
TGGGTAGTGCTAAGAGCGGG	2601	CGG	+	UGGGUAGUGCUAAGAGCGGG	5695
GGGTAGTGCTAAGAGCGGGC	2602	GGG	+	GGGUAGUGCUAAGAGCGGGC	5696
GGTAGTGCTAAGAGCGGGCG	2603	GGG	+	GGUAGUGCUAAGAGCGGGCG	5697
CTAAGAGCGGGCGGGGTCTG	2604	TGG	+	CUAAGAGCGGGCGGGGUCUG	5698
AGCGGGCGGGGTCTGTGGAA	2605	TGG	+	AGCGGGCGGGGUCUGUGGAA	5699
GCGGGGTCTGTGGAATGGAG	2606	AGG	+	GCGGGGUCUGUGGAAUGGAG	5700
AATGACGTGGTAATAAGGAG	2607	TGG	-	AAUGACGUGGUAAUAAGGAG	5701
AGTAAAATGACGTGGTAATA	2608	AGG	-	AGUAAAAUGACGUGGUAAUA	5702
TAATCAAAAGTAAAATGACG	2609	TGG	-	UAAUCAAAAGUAAAAUGACG	5703
CTATGTATCTTGCCTCTTGA	2610	CGG	+	CUAUGUAUCUUGCCUCUUGA	5704
GTACTTGTGTGACCGTCAAG	2611	AGG	-	GUACUUGUGUGACCGUCAAG	5705
AGTACTTCCAGTTGAAGAGT	2612	AGG	+	AGUACUUCCAGUUGAAGAGU	5706
TGTAATGCCTACTCTTCAAC	2613	TGG	-	UGUAAUGCCUACUCUUCAAC	5707
GTTGAAGAGTAGGCATTACA	2614	AGG	+	GUUGAAGAGUAGGCAUUACA	5708
TCTGCTTCCATGAATATTCT	2615	AGG	+	UCUGCUUCCAUGAAUAUUCU	5709
CTGCTTCCATGAATATTCTA	2616	GGG	+	CUGCUUCCAUGAAUAUUCUA	5710
AATGTGCCCTAGAATATTCA	2617	TGG	-	AAUGUGCCCUAGAAUAUUCA	5711
CTAGGGCACATTTTTATTCC	2618	AGG	+	CUAGGGCACAUUUUUAUUCC	5712
TGCTTTCATATATTTTTTCC	2619	TGG	-	UGCUUUCAUAUAUUUUUUCC	5713
AAAGCATTTCTAATTATGAA	2620	TGG	+	AAAGCAUUUCUAAUUAUGAA	5714
TTTCTAATTATGAATGGAAT	2621	TGG	+	UUUCUAAUUAUGAAUGGAAU	5715
CTAATTATGAATGGAATTGG	2622	TGG	+	CUAAUUAUGAAUGGAAUUGG	5716
TGAATGGAATTGGTGGTTTA	2623	TGG	+	UGAAUGGAAUUGGUGGUUUA	5717
GAATGGAATTGGTGGTTTAT	2624	GGG	+	GAAUGGAAUUGGUGGUUUAU	5718
AATGGAATTGGTGGTTTATG	2625	GGG	+	AAUGGAAUUGGUGGUUUAUG	5719
GGAACACATATGCTCAGCCT	2626	TGG	+	GGAACACAUAUGCUCAGCCU	5720
GGTTTTGGTAATTTATTCCA	2627	AGG	-	GGUUUUGGUAAUUUAUUCCA	5721
ATAAATTACCAAAACCAAAG	2628	AGG	+	AUAAAUUACCAAAACCAAAG	5722
GAGTCTAGCCTCTTTGGTTT	2629	TGG	-	GAGUCUAGCCUCUUUGGUUU	5723
AGAAGGGAGTCTAGCCTCTT	2630	TGG	-	AGAAGGGAGUCUAGCCUCUU	5724
GCTTCTGTTGATCGGTAGAA	2631	GGG	-	GCUUCUGUUGAUCGGUAGAA	5725
TGCTTCTGTTGATCGGTAGA	2632	AGG	-	UGCUUCUGUUGAUCGGUAGA	5726
TCTTATATGCTTCTGTTGAT	2633	CGG	-	UCUUAUAUGCUUCUGUUGAU	5727

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
CAACAGAAGCATATAAGAGT	2634	TGG	+	CAACAGAAGCAUAUAAGAGU	5728
TTCTCTACTCTACCAACTTG	2635	TGG	+	UUCUCUACUCUACCAACUUG	5729
CTACTCTACCAACTTGTGGT	2636	TGG	+	CUACUCUACCAACUUGUGGU	5730
TACTCTACCAACTTGTGGTT	2637	GGG	+	UACUCUACCAACUUGUGGUU	5731
AACAGGACCCAACCACAAGT	2638	TGG	-	AACAGGACCCAACCACAAGU	5732
GTCCTGTTTAGAATAATAAG	2639	AGG	+	GUCCUGUUUAGAAUAAUAAG	5733
TGCCTCTTATTATTCTAAAC	2640	AGG	-	UGCCUCUUAUUAUUCUAAAC	5734
GCATCTACATGTAGAGCTCT	2641	AGG	+	GCAUCUACAUGUAGAGCUCU	5735
TCTAGGAAGCCATTCAATGC	2642	AGG	+	UCUAGGAAGCCAUUCAAUGC	5736
GGTTTAGATCCTGCATTGAA	2643	TGG	-	GGUUUAGAUCCUGCAUUGAA	5737
TCAATGCAGGATCTAAACCT	2644	TGG	+	UCAAUGCAGGAUCUAAACCU	5738
CACAGACATTGCTAAAGCCA	2645	AGG	-	CACAGACAUUGCUAAAGCCA	5739
TGGCTTTAGCAATGTCTGTG	2646	CGG	+	UGGCUUUAGCAAUGUCUGUG	5740
AGCAATGTCTGTGCGGTACT	2647	TGG	+	AGCAAUGUCUGUGCGGUACU	5741
CAAAGAAAACCAAGATTTAT	2648	CGG	+	CAAAGAAAACCAAGAUUUAU	5742
AAAACCAAGATTTATCGGAA	2649	TGG	+	AAAACCAAGAUUUAUCGGAA	5743
ACCAAGATTTATCGGAATGG	2650	TGG	+	ACCAAGAUUUAUCGGAAUGG	5744
TCCACCATTCCGATAAATCT	2651	TGG	-	UCCACCAUUCCGAUAAAUCU	5745
GGTGGACACTGAAAGTAGAC	2652	AGG	+	GGUGGACACUGAAAGUAGAC	5746
ACACTGAAAGTAGACAGGAA	2653	AGG	+	ACACUGAAAGUAGACAGGAA	5747
CCTAGTGTGCAGTGTGCAGA	2654	TGG	+	CCUAGUGUGCAGUGUGCAGA	5748
CCATCTGCACACTGCACACT	2655	AGG	-	CCAUCUGCACACUGCACACU	5749
GTGTGCAGTGTGCAGATGGA	2656	AGG	+	GUGUGCAGUGUGCAGAUGGA	5750
TGTGCAGTGTGCAGATGGAA	2657	GGG	+	UGUGCAGUGUGCAGAUGGAA	5751
TGCAGATGGAAGGGCAGTGC	2658	TGG	+	UGCAGAUGGAAGGGCAGUGC	5752
GCAGATGGAAGGGCAGTGCT	2659	GGG	+	GCAGAUGGAAGGGCAGUGCU	5753
CAGATGGAAGGGCAGTGCTG	2660	GGG	+	CAGAUGGAAGGGCAGUGCUG	5754
TGGGGAGAATCCATTGCATT	2661	TGG	+	UGGGGAGAAUCCAUUGCAUU	5755
CTTCTTCTTTCCAAATGCAA	2662	TGG	-	CUUCUUCUUUCCAAAUGCAA	5756
TTAGCAAAAGAAATCGCAGA	2663	GGG	-	UUAGCAAAAGAAAUCGCAGA	5757
ATTAGCAAAAGAAATCGCAG	2664	AGG	-	AUUAGCAAAAGAAAUCGCAG	5758
GCGATTTCTTTTGCTAATGC	2665	AGG	+	GCGAUUUCUUUUGCUAAUGC	5759
AAGCAAGTACTACAGTGAAA	2666	AGG	-	AAGCAAGUACUACAGUGAAA	5760
TTCACTGTAGTACTTGCTTG	2667	TGG	+	UUCACUGUAGUACUUGCUUG	5761
ACTGTAGTACTTGCTTGTGG	2668	TGG	+	ACUGUAGUACUUGCUUGUGG	5762
TGTGGTGGTTTGAATGAAAA	2669	TGG	+	UGUGGUGGUUUGAAUGAAAA	5763
TGAATGAAAATGGTCCCCAC	2670	AGG	+	UGAAUGAAAAUGGUCCCCAC	5764
GTTTTCAAAAATAGCCTGTG	2671	GGG	-	GUUUUCAAAAAUAGCCUGUG	5765
AGTTTTCAAAAATAGCCTGT	2672	GGG	-	AGUUUUCAAAAAUAGCCUGU	5766
AAGTTTTCAAAAATAGCCTG	2673	TGG	-	AAGUUUUCAAAAAUAGCCUG	5767
TTGAAAACTTGATCCCCAGT	2674	TGG	+	UUGAAAACUUGAUCCCCAGU	5768
CCCCAGTTGGTGAAACTGTT	2675	TGG	+	CCCCAGUUGGUGAAACUGUU	5769
CCAAACAGTTTCACCAACTG	2676	GGG	-	CCAAACAGUUUCACCAACUG	5770
TCCAAACAGTTTCACCAACT	2677	GGG	-	UCCAAACAGUUUCACCAACU	5771
TTCCAAACAGTTTCACCAAC	2678	TGG	-	UUCCAAACAGUUUCACCAAC	5772
GTTGGTGAAACTGTTTGGAA	2679	AGG	+	GUUGGUGAAACUGUUUGGAA	5773
GAAACTGTTTGGAAAGGATT	2680	AGG	+	GAAACUGUUUGGAAAGGAUU	5774
TTGGAAAGGATTAGGAAGTG	2681	TGG	+	UUGGAAAGGAUUAGGAAGUG	5775
ATTAGGAAGTGTGGCCTTGC	2682	TGG	+	AUUAGGAAGUGUGGCCUUGC	5776
AAGTGTGGCCTTGCTGGATG	2683	AGG	+	AAGUGUGGCCUUGCUGGAUG	5777
TTGACAGACCTCATCCAGCA	2684	AGG	-	UUGACAGACCUCAUCCAGCA	5778
TGCTGGATGAGGTCTGTCAA	2685	TGG	+	UGCUGGAUGAGGUCUGUCAA	5779
TGGATGAGGTCTGTCAATGG	2686	CGG	+	UGGAUGAGGUCUGUCAAUGG	5780
TGAGGTCTGTCAATGGCGGA	2687	AGG	+	UGAGGUCUGUCAAUGGCGGA	5781
GAGACAGAGAGAGGCACACT	2688	GGG	-	GAGACAGAGAGAGGCACACU	5782
GGAGACAGAGAGAGGCACAC	2689	TGG	-	GGAGACAGAGAGAGGCACAC	5783
AATAAGTAGGAGACAGAGAG	2690	AGG	-	AAUAAGUAGGAGACAGAGAG	5784
TCATTTTTCAATCAATAAGT	2691	AGG	-	UCAUUUUUCAAUCAAUAAGU	5785

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AATTCTGGATGGCAGAGCAA	2692	AGG	-	AAUUCUGGAUGGCAGAGCAA	5786
TGGGTGATTAGAATTCTGGA	2693	TGG	-	UGGGUGAUUAGAAUUCUGGA	5787
GTTTTGGGTGATTAGAATTC	2694	TGG	-	GUUUUGGGUGAUUAGAAUUC	5788
TAATTAGGCTATATGGTTTT	2695	GGG	-	UAAUUAGGCUAUAUGGUUUU	5789
TTAATTAGGCTATATGGTTT	2696	TGG	-	UUAAUUAGGCUAUAUGGUUU	5790
CAGTATTTAATTAGGCTATA	2697	TGG	-	CAGUAUUUAAUUAGGCUAUA	5791
TATAAAAACAGTATTTAATT	2698	AGG	-	UAUAAAAACAGUAUUUAAUU	5792
ACTGTTTTTATAAGTTACCT	2699	TGG	+	ACUGUUUUUAUAAGUUACCU	5793
TTTATAAGTTACCTTGGTCA	2700	TGG	+	UUUAUAAGUUACCUUGGUCA	5794
TACCTTGGTCATGGTACTTT	2701	AGG	+	UACCUUGGUCAUGGUACUUU	5795
ATCCTAAAGTACCATGACCA	2702	AGG	-	AUCCUAAAGUACCAUGACCA	5796
TAGTTAAGAAACTAAGACAT	2703	TGG	+	UAGUUAAGAAACUAAGACAU	5797
CTTAACCAAAACACCACAGA	2704	CGG	+	CUUAACCAAAACACCACAGA	5798
TTAACCAAAACACCACAGAC	2705	GGG	+	UUAACCAAAACACCACAGAC	5799
CAAGCCCGTCTGTGGTGTTT	2706	TGG	-	CAAGCCCGUCUGUGGUGUUU	5800
GTCATATGCAAGCCCGTCTG	2707	TGG	-	GUCAUAUGCAAGCCCGUCUG	5801
GCATATGACAACAGTTCTAG	2708	AGG	+	GCAUAUGACAACAGUUCUAG	5802
CCCTGATCAAATGCCCTAGC	2709	AGG	+	CCCUGAUCAAAUGCCCUAGC	5803
CCTGCTAGGGCATTTGATCA	2710	GGG	-	CCUGCUAGGGCAUUUGAUCA	5804
ACCTGCTAGGGCATTTGATC	2711	AGG	-	ACCUGCUAGGGCAUUUGAUC	5805
ATCAGACACAGAACCTGCTA	2712	GGG	-	AUCAGACACAGAACCUGCUA	5806
CATCAGACACAGAACCTGCT	2713	AGG	-	CAUCAGACACAGAACCUGCU	5807
AGCAGGTTCTGTGTCTGATG	2714	AGG	+	AGCAGGUUCUGUGUCUGAUG	5808
GCAGGTTCTGTGTCTGATGA	2715	GGG	+	GCAGGUUCUGUGUCUGAUGA	5809
CTGTGTCTGATGAGGGCTGC	2716	TGG	+	CUGUGUCUGAUGAGGGCUGC	5810
TGATGAGGGCTGCTGGTTCA	2717	TGG	+	UGAUGAGGGCUGCUGGUUCA	5811
GATGAGGGCTGCTGGTTCAT	2718	GGG	+	GAUGAGGGCUGCUGGUUCAU	5812
GAGGGCTGCTGGTTCATGGG	2719	TGG	+	GAGGGCUGCUGGUUCAUGGG	5813
CTTTTTCTCAGTGTCCTTAC	2720	AGG	+	CUUUUUCUCAGUGUCCUUAC	5814
TTTCTCAGTGTCCTTACAGG	2721	TGG	+	UUUCUCAGUGUCCUUACAGG	5815
TCAGTGTCCTTACAGGTGGA	2722	AGG	+	UCAGUGUCCUUACAGGUGGA	5816
CTGGATACCTTCCACCTGTA	2723	AGG	-	CUGGAUACCUUCCACCUGUA	5817
TGGAAGGTATCCAGCTTTCT	2724	TGG	+	UGGAAGGUAUCCAGCUUUCU	5818
AATATAAGGTCCAAGAAAGC	2725	TGG	-	AAUAUAAGGUCCAAGAAAGC	5819
GGAATGCGTGCTTTAATATA	2726	AGG	-	GGAAUGCGUGCUUUAAUAUA	5820
AAGCTATCTACTCACATCAA	2727	TGG	-	AAGCUAUCUACUCACAUCAA	5821
GAGGTGCTTAAATCACTTTG	2728	GGG	-	GAGGUGCUUAAAUCACUUUG	5822
GGAGGTGCTTAAATCACTTT	2729	GGG	-	GGAGGUGCUUAAAUCACUUU	5823
TGGAGGTGCTTAAATCACTT	2730	TGG	-	UGGAGGUGCUUAAAUCACUU	5824
CCTCCAAAACCCTCCGTCTC	2731	CGG	+	CCUCCAAAACCCUCCGUCUC	5825
CCGGAGACGGAGGGTTTTGG	2732	AGG	-	CCGGAGACGGAGGGUUUUGG	5826
CATCCGGAGACGGAGGGTTT	2733	TGG	-	CAUCCGGAGACGGAGGGUUU	5827
GGGGAGCATCCGGAGACGGA	2734	GGG	-	GGGGAGCAUCCGGAGACGGA	5828
GGGGGAGCATCCGGAGACGG	2735	AGG	-	GGGGGAGCAUCCGGAGACGG	5829
AATGGGGGAGCATCCGGAGA	2736	CGG	-	AAUGGGGGAGCAUCCGGAGA	5830
GTCTCCGGATGCTCCCCCAT	2737	TGG	+	GUCUCCGGAUGCUCCCCCAU	5831
TTCACCAATGGGGGAGCATC	2738	CGG	-	UUCACCAAUGGGGGAGCAUC	5832
TGAAATCTGTTCACCAATGG	2739	GGG	-	UGAAAUCUGUUCACCAAUGG	5833
TTGAAATCTGTTCACCAATG	2740	GGG	-	UUGAAAUCUGUUCACCAAUG	5834
GTTGAAATCTGTTCACCAAT	2741	GGG	-	GUUGAAAUCUGUUCACCAAU	5835
TGTTGAAATCTGTTCACCAA	2742	TGG	-	UGUUGAAAUCUGUUCACCAA	5836
TTTCAACATAGCAATTGAGC	2743	AGG	+	UUUCAACAUAGCAAUUGAGC	5837
CAACATAGCAATTGAGCAGG	2744	AGG	+	CAACAUAGCAAUUGAGCAGG	5838
TAGCAATTGAGCAGGAGGAA	2745	AGG	+	UAGCAAUUGAGCAGGAGGAA	5839
CTGGCTGTGATCAAATAATT	2746	TGG	-	CUGGCUGUGAUCAAAUAAUU	5840
TTTGATCACAGCCAGTACTT	2747	TGG	+	UUUGAUCACAGCCAGUACUU	5841
TTGGGTGTTCACCAAAGTAC	2748	TGG	-	UUGGGUGUUCACCAAAGUAC	5842
GTGAACACCCAAACCCAGCA	2749	TGG	+	GUGAACACCCAAACCCAGCA	5843

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
TTCAGCTCCATGCTGGGTTT	2750	GGG	-	UUCAGCUCCAUGCUGGGUUU	5844
ATTCAGCTCCATGCTGGGTT	2751	TGG	-	AUUCAGCUCCAUGCUGGGUU	5845
CAGATATTCAGCTCCATGCT	2752	GGG	-	CAGAUAUUCAGCUCCAUGCU	5846
ACAGATATTCAGCTCCATGC	2753	TGG	-	ACAGAUAUUCAGCUCCAUGC	5847
CTGTAATCTCAGTATCCAGC	2754	AGG	+	CUGUAAUCUCAGUAUCCAGC	5848
TAATCTCAGTATCCAGCAGG	2755	TGG	+	UAAUCUCAGUAUCCAGCAGG	5849
AGTATCCAGCAGGTGGATAT	2756	TGG	+	AGUAUCCAGCAGGUGGAUAU	5850
GTATCCAGCAGGTGGATATT	2757	GGG	+	GUAUCCAGCAGGUGGAUAUU	5851
TATCCAGCAGGTGGATATTG	2758	GGG	+	UAUCCAGCAGGUGGAUAUUG	5852
ATTCCCCAATATCCACCTGC	2759	TGG	-	AUUCCCCAAUAUCCACCUGC	5853
ATTGGGGAATTAAGACTTCA	2760	AGG	+	AUUGGGGAAUUAAGACUUCA	5854
TAAGACTTCAAGGCCAGACT	2761	TGG	+	UAAGACUUCAAGGCCAGACU	5855
ACTTACTATGTAGCCAAGTC	2762	TGG	-	ACUUACUAUGUAGCCAAGUC	5856
TAGTAAGTTTGATGCCATCC	2763	TGG	+	UAGUAAGUUUGAUGCCAUCC	5857
AGTAAGTTTGATGCCATCCT	2764	GGG	+	AGUAAGUUUGAUGCCAUCCU	5858
GGATCTCATATAGCCCAGGA	2765	TGG	-	GGAUCUCAUAUAGCCCAGGA	5859
AACAGGATCTCATATAGCCC	2766	AGG	-	AACAGGAUCUCAUAUAGCCC	5860
TATTTGGGGGTTTTGGAAAC	2767	AGG	-	UAUUUGGGGGUUUUGGAAAC	5861
TTGTTTCTATTTGGGGGTTT	2768	TGG	-	UUGUUUCUAUUUGGGGGUUU	5862
TTTTTATTGTTTCTATTTGG	2769	GGG	-	UUUUUAUUGUUUCUAUUUGG	5863
TTTTTTATTGTTTCTATTTG	2770	GGG	-	UUUUUUAUUGUUUCUAUUUG	5864
TTTTTTTATTGTTTCTATTT	2771	GGG	-	UUUUUUUAUUGUUUCUAUUU	5865
ATTTTTTTATTGTTTCTATT	2772	TGG	-	AUUUUUUUAUUGUUUCUAUU	5866
AAATAGAAACAATAAAAAAA	2773	TGG	+	AAAUAGAAACAAUAAAAAAA	5867
TAGGGTAGCAGTGAACATGA	2774	AGG	-	UAGGGUAGCAGUGAACAUGA	5868
ATGTTCACTGCTACCCTAGA	2775	AGG	+	AUGUUCACUGCUACCCUAGA	5869
CAAGCAAAACTGACCTTCTA	2776	GGG	-	CAAGCAAAACUGACCUUCUA	5870
ACAAGCAAAACTGACCTTCT	2777	AGG	-	ACAAGCAAAACUGACCUUCU	5871
TTGTAACAGATGACTAACAG	2778	AGG	+	UUGUAACAGAUGACUAACAG	5872
AGATGACTAACAGAGGAACA	2779	AGG	+	AGAUGACUAACAGAGGAACA	5873
CTAACAGAGGAACAAGGTCC	2780	CGG	+	CUAACAGAGGAACAAGGUCC	5874
AGCATACTGGTAGCTCTTCC	2781	GGG	-	AGCAUACUGGUAGCUCUUCC	5875
CAGCATACTGGTAGCTCTTC	2782	CGG	-	CAGCAUACUGGUAGCUCUUC	5876
CGGAAGAGCTACCAGTATGC	2783	TGG	+	CGGAAGAGCUACCAGUAUGC	5877
TTCATTGGCAGCCAGCATAC	2784	TGG	-	UUCAUUGGCAGCCAGCAUAC	5878
TAAGACTTCAGCAGCTTCAT	2785	TGG	-	UAAGACUUCAGCAGCUUCAU	5879
GCTGAAGTCTTAGTAGATTT	2786	AGG	+	GCUGAAGUCUUAGUAGAUUU	5880
CTTAGTAGATTTAGGAGCTA	2787	TGG	+	CUUAGUAGAUUUAGGAGCUA	5881
CTGGGGCTGTGATGGAAGTC	2788	AGG	-	CUGGGGCUGUGAUGGAAGUC	5882
TGGTGGTGCTGGGGCTGTGA	2789	TGG	-	UGGUGGUGCUGGGGCUGUGA	5883
TCACAGCCCCAGCACCACCA	2790	TGG	+	UCACAGCCCCAGCACCACCA	5884
CACAGCCCCAGCACCACCAT	2791	GGG	+	CACAGCCCCAGCACCACCAU	5885
CAGTGCCCATGGTGGTGCTG	2792	GGG	-	CAGUGCCCAUGGUGGUGCUG	5886
GCAGTGCCCATGGTGGTGCT	2793	GGG	-	GCAGUGCCCAUGGUGGUGCU	5887
GGCAGTGCCCATGGTGGTGC	2794	TGG	-	GGCAGUGCCCAUGGUGGUGC	5888
CTGCTGGGCAGTGCCCATGG	2795	TGG	-	CUGCUGGGCAGUGCCCAUGG	5889
AGACTGCTGGGCAGTGCCCA	2796	TGG	-	AGACUGCUGGGCAGUGCCCA	5890
CTAAAAGAAGATAGACTGCT	2797	GGG	-	CUAAAAGAAGAUAGACUGCU	5891
CCAGCAGTCTATCTTCTTTT	2798	AGG	+	CCAGCAGUCUAUCUUCUUUU	5892
CCTAAAAGAAGATAGACTGC	2799	TGG	-	CCUAAAAGAAGAUAGACUGC	5893
CTATCTTCTTTTAGGTCTTC	2800	AGG	+	CUAUCUUCUUUUAGGUCUUC	5894
TTTAGGTCTTCAGGTGTCCC	2801	TGG	+	UUUAGGUCUUCAGGUGUCCC	5895
TTAGGTCTTCAGGTGTCCCT	2802	GGG	+	UUAGGUCUUCAGGUGUCCCU	5896
GTGTCCCTGGGTAGAGAACA	2803	AGG	+	GUGUCCCUGGGUAGAGAACA	5897
GAAGCCTTGTTCTCTACCCA	2804	GGG	-	GAAGCCUUGUUCUCUACCCA	5898
AGAAGCCTTGTTCTCTACCC	2805	AGG	-	AGAAGCCUUGUUCUCUACCC	5899
AACAAGGCTTCTGCTTGCAT	2806	AGG	+	AACAAGGCUUCUGCUUGCAU	5900
ACAAGGCTTCTGCTTGCATA	2807	GGG	+	ACAAGGCUUCUGCUUGCAUA	5901

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
GTGAGACTGTCACTGTACTG	2808	GGG	-	GUGAGACUGUCACUGUACUG	5902
TGTGAGACTGTCACTGTACT	2809	GGG	-	UGUGAGACUGUCACUGUACU	5903
TTGTGAGACTGTCACTGTAC	2810	TGG	-	UUGUGAGACUGUCACUGUAC	5904
TCACAATGAGTGCAGCAATG	2811	TGG	+	UCACAAUGAGUGCAGCAAUG	5905
GCAGCAATGTGGCCACAGAA	2812	CGG	+	GCAGCAAUGUGGCCACAGAA	5906
CAGCAATGTGGCCACAGAAC	2813	GGG	+	CAGCAAUGUGGCCACAGAAC	5907
AGCAATGTGGCCACAGAACG	2814	GGG	+	AGCAAUGUGGCCACAGAACG	5908
ACACACATTTCCCCGTTCTG	2815	TGG	-	ACACACAUUUCCCCGUUCUG	5909
TCAAGCTGAAAGCTTTTCAC	2816	AGG	+	UCAAGCUGAAAGCUUUUCAC	5910
TCTCTTGCTAAATGACAAAG	2817	TGG	+	UCUCUUGCUAAAUGACAAAG	5911
CTAAATGACAAAGTGGTTAG	2818	AGG	+	CUAAAUGACAAAGUGGUUAG	5912
AATGACAAAGTGGTTAGAGG	2819	AGG	+	AAUGACAAAGUGGUUAGAGG	5913
ATGACAAAGTGGTTAGAGGA	2820	GGG	+	AUGACAAAGUGGUUAGAGGA	5914
GTGTAGACAATGAAAGCTGT	2821	AGG	-	GUGUAGACAAUGAAAGCUGU	5915
AGCTTTCATTGTCTACACTC	2822	AGG	+	AGCUUUCAUUGUCUACACUC	5916
TCTTTTGAGAATTTTACAGT	2823	AGG	+	UCUUUUGAGAAUUUUACAGU	5917
CTAGTCAACACAGAAATAAA	2824	AGG	+	CUAGUCAACACAGAAAUAAA	5918
TTTGTTTTGTTGAAAAGAAG	2825	TGG	+	UUUGUUUUGUUGAAAAGAAG	5919
TTGTTTTGTTGAAAAGAAGT	2826	GGG	+	UUGUUUUGUUGAAAAGAAGU	5920
TGTTTTGTTGAAAAGAAGTG	2827	GGG	+	UGUUUUGUUGAAAAGAAGUG	5921
GTTTTGTTGAAAAGAAGTGG	2828	GGG	+	GUUUUGUUGAAAAGAAGUGG	5922
TTGAAAAGAAGTGGGGGTTT	2829	TGG	+	UUGAAAAGAAGUGGGGGUUU	5923
TGGGGGTTTTGGTACTGATG	2830	AGG	+	UGGGGGUUUUGGUACUGAUG	5924
GGGGGTTTTGGTACTGATGA	2831	GGG	+	GGGGGUUUUGGUACUGAUGA	5925
GGGGTTTTGGTACTGATGAG	2832	GGG	+	GGGGUUUUGGUACUGAUGAG	5926
TATGTTACTTATTCATTCTC	2833	AGG	+	UAUGUUACUUAUUCAUUCUC	5927
CTTATTCATTCTCAGGTTCA	2834	TGG	+	CUUAUUCAUUCUCAGGUUCA	5928
TTAATCTATCATTTGTCATA	2835	AGG	-	UUAAUCUAUCAUUUGUCAUA	5929
TAATAAAAGCATGCAAATGC	2836	TGG	+	UAAUAAAAGCAUGCAAAUGC	5930
TGCAAATGCTGGAGCTCTGC	2837	TGG	+	UGCAAAUGCUGGAGCUCUGC	5931
AAATGCTGGAGCTCTGCTGG	2838	CGG	+	AAAUGCUGGAGCUCUGCUGG	5932
GGCGGAGTACCTGATATGCA	2839	AGG	+	GGCGGAGUACCUGAUAUGCA	5933
GATCTTCATCCTTGCATATC	2840	AGG	-	GAUCUUCAUCCUUGCAUAUC	5934
GCTTTTTTATTTGATTTCTG	2841	GGG	-	GCUUUUUUAUUUGAUUUCUG	5935
AGCTTTTTTATTTGATTTCT	2842	GGG	-	AGCUUUUUUAUUUGAUUUCU	5936
CAGCTTTTTTATTTGATTTC	2843	TGG	-	CAGCUUUUUUAUUUGAUUUC	5937
CAGAAATCAAATAAAAAAGC	2844	TGG	+	CAGAAAUCAAAUAAAAAAGC	5938
AGAAATCAAATAAAAAAGCT	2845	GGG	+	AGAAAUCAAAUAAAAAAGCU	5939
TAAAAAAGCTGGGCATAGTG	2846	AGG	+	UAAAAAAGCUGGGCAUAGUG	5940
AGCTGGGCATAGTGAGGAAG	2847	AGG	+	AGCUGGGCAUAGUGAGGAAG	5941
GCATAGTGAGGAAGAGGAGA	2848	TGG	+	GCAUAGUGAGGAAGAGGAGA	5942
CATAGTGAGGAAGAGGAGAT	2849	GGG	+	CAUAGUGAGGAAGAGGAGAU	5943
AGTGAGGAAGAGGAGATGGG	2850	AGG	+	AGUGAGGAAGAGGAGAUGGG	5944
AGGAGATGGGAGGATTCCTG	2851	TGG	+	AGGAGAUGGGAGGAUUCCUG	5945
GATGGGAGGATTCCTGTGGC	2852	TGG	+	GAUGGGAGGAUUCCUGUGGC	5946
GGAGGATTCCTGTGGCTGGC	2853	TGG	+	GGAGGAUUCCUGUGGCUGGC	5947
TTAGCTGTCCAGCCAGCCAC	2854	AGG	-	UUAGCUGUCCAGCCAGCCAC	5948
TGGAAATAGATGAATAGGCT	2855	AGG	-	UGGAAAUAGAUGAAUAGGCU	5949
TAGCCTATTCATCTATTTCC	2856	AGG	+	UAGCCUAUUCAUCUAUUUCC	5950
TGGCCTGGAAATAGATGAAT	2857	AGG	-	UGGCCUGGAAAUAGAUGAAU	5951
CATCTATTTCCAGGCCACTA	2858	AGG	+	CAUCUAUUUCCAGGCCACUA	5952
ATCTATTTCCAGGCCACTAA	2859	GGG	+	AUCUAUUUCCAGGCCACUAA	5953
ATTTCCAGGCCACTAAGGGA	2860	TGG	+	AUUUCCAGGCCACUAAGGGA	5954
GGTACCATCCCTTAGTGGCC	2861	TGG	-	GGUACCAUCCCUUAGUGGCC	5955
TTTAAGGTACCATCCCTTAG	2862	TGG	-	UUUAAGGUACCAUCCCUUAG	5956
ATTATTATTGTTATTTTTTA	2863	AGG	-	AUUAUUAUUGUUAUUUUUUA	5957
ACAATAATAATAAATAAAAA	2864	AGG	+	ACAAUAAUAAUAAAUAAAAA	5958
TAATAAATAAAAAAGGTAGC	2865	TGG	+	UAAUAAAUAAAAAAGGUAGC	5959

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AAAAAGGTAGCTGGTTCCTG	2866	AGG	+	AAAAAGGUAGCUGGUUCCUG	5960
TGGTTCCTGAGGAGCATCTA	2867	AGG	+	UGGUUCCUGAGGAGCAUCUA	5961
GATATCCTTAGATGCTCCTC	2868	AGG	-	GAUAUCCUUAGAUGCUCCUC	5962
GTATGTGCACGTGGAGTTAA	2869	AGG	-	GUAUGUGCACGUGGAGUUAA	5963
GTATACAGTGTATGTGCACG	2870	TGG	-	GUAUACAGUGUAUGUGCACG	5964
TGCACATACACTGTATACAC	2871	AGG	+	UGCACAUACACUGUAUACAC	5965
TTTTTTTGGTAAAACTTGAT	2872	AGG	-	UUUUUUUGGUAAAACUUGAU	5966
TTTTTTTTTTTTTTTTTTTT	2873	TGG	-	UUUUUUUUUUUUUUUUUUUU	5967
AAGAACACTTTATTCACTGT	2874	TGG	+	AAGAACACUUUAUUCACUGU	5968
AACACTTTATTCACTGTTGG	2875	TGG	+	AACACUUUAUUCACUGUUGG	5969
ACACTTTATTCACTGTTGGT	2876	GGG	+	ACACUUUAUUCACUGUUGGU	5970
CTGTTGGTGGGTATGTAAAC	2877	TGG	+	CUGUUGGUGGGUAUGUAAAC	5971
CTCTGTGCTGATTTTCATAG	2878	TGG	-	CUCUGUGCUGAUUUUCAUAG	5972
CACTATGAAAATCAGCACAG	2879	AGG	+	CACUAUGAAAAUCAGCACAG	5973
TTGTTTTGTTGGAGTGGGAA	2880	GGG	-	UUGUUUUGUUGGAGUGGGAA	5974
TTTGTTTTGTTGGAGTGGGA	2881	AGG	-	UUUGUUUUGUUGGAGUGGGA	5975
TTGTTTTGTTTTGTTGGAGT	2882	GGG	-	UUGUUUUGUUUUGUUGGAGU	5976
TTTGTTTTGTTTTGTTGGAG	2883	TGG	-	UUUGUUUUGUUUUGUUGGAG	5977
TTTGTTTTGTTTTGTTTTGT	2884	TGG	-	UUUGUUUUGUUUUGUUUUGU	5978
GTTCTAGTTCTAGTTCTAGA	2885	GGG	-	GUUCUAGUUCUAGUUCUAGA	5979
AGTTCTAGTTCTAGTTCTAG	2886	AGG	-	AGUUCUAGUUCUAGUUCUAG	5980
CTAGAACTACTGCACGATCC	2887	AGG	+	CUAGAACUACUGCACGAUCC	5981
GATCCAGGTTTACCACTCCT	2888	AGG	+	GAUCCAGGUUUACCACUCCU	5982
ATACCTAGGAGTGGTAAACC	2889	TGG	-	AUACCUAGGAGUGGUAAACC	5983
TTTGAGTAAATACCTAGGAG	2890	TGG	-	UUUGAGUAAAUACCUAGGAG	5984
ACTCCTAGGTATTTACTCAA	2891	AGG	+	ACUCCUAGGUAUUUACUCAA	5985
AGTCCTTTGAGTAAATACCT	2892	AGG	-	AGUCCUUUGAGUAAAUACCU	5986
TATACGTCAACCTGTGACAG	2893	AGG	+	UAUACGUCAACCUGUGACAG	5987
GTATATGATGCCTCTGTCAC	2894	AGG	-	GUAUAUGAUGCCUCUGUCAC	5988
CAGAGGCATCATATACAAGC	2895	AGG	+	CAGAGGCAUCAUAUACAAGC	5989
AGAGGCATCATATACAAGCA	2896	GGG	+	AGAGGCAUCAUAUACAAGCA	5990
CATCATATACAAGCAGGGAG	2897	TGG	+	CAUCAUAUACAAGCAGGGAG	5991
CATATACAAGCAGGGAGTGG	2898	TGG	+	CAUAUACAAGCAGGGAGUGG	5992
TGAGGGGTGGGATTAAAGGC	2899	AGG	-	UGAGGGGUGGGAUUAAAGGC	5993
TGCCTTTAATCCCACCCCTC	2900	AGG	+	UGCCUUUAAUCCCACCCCUC	5994
CTCCTGAGGGGTGGGATTAA	2901	AGG	-	CUCCUGAGGGGUGGGAUUAA	5995
CTTTAATCCCACCCCTCAGG	2902	AGG	+	CUUUAAUCCCACCCCUCAGG	5996
GACTCTGCCTCCTGAGGGGT	2903	GGG	-	GACUCUGCCUCCUGAGGGGU	5997
TGACTCTGCCTCCTGAGGGG	2904	TGG	-	UGACUCUGCCUCCUGAGGGG	5998
ACCCCTCAGGAGGCAGAGTC	2905	AGG	+	ACCCCUCAGGAGGCAGAGUC	5999
ACCTGACTCTGCCTCCTGAG	2906	GGG	-	ACCUGACUCUGCCUCCUGAG	6000
TACCTGACTCTGCCTCCTGA	2907	GGG	-	UACCUGACUCUGCCUCCUGA	6001
CTACCTGACTCTGCCTCCTG	2908	AGG	-	CUACCUGACUCUGCCUCCUG	6002
GGTAGATCTCTGTGAGTTCG	2909	AGG	+	GGUAGAUCUCUGUGAGUUCG	6003
CTGTGAGTTCGAGGCCAGTT	2910	TGG	+	CUGUGAGUUCGAGGCCAGUU	6004
ACTCACTATGTAGACCAAAC	2911	TGG	-	ACUCACUAUGUAGACCAAAC	6005
CATATGGCTATATGGTCTTA	2912	TGG	-	CAUAUGGCUAUAUGGUCUUA	6006
CATAAGACCATATAGCCATA	2913	TGG	+	CAUAAGACCAUAUAGCCAUA	6007
AAGACCATATAGCCATATGG	2914	AGG	+	AAGACCAUAUAGCCAUAUGG	6008
AGACCATATAGCCATATGGA	2915	GGG	+	AGACCAUAUAGCCAUAUGGA	6009
GACCATATAGCCATATGGAG	2916	GGG	+	GACCAUAUAGCCAUAUGGAG	6010
ACCATATAGCCATATGGAGG	2917	GGG	+	ACCAUAUAGCCAUAUGGAGG	6011
CCATATAGCCATATGGAGGG	2918	GGG	+	CCAUAUAGCCAUAUGGAGGG	6012
CCCCCCTCCATATGGCTATA	2919	TGG	-	CCCCCCUCCAUAUGGCUAUA	6013
CTCACAATCCCCCCTCCATA	2920	TGG	-	CUCACAAUCCCCCCUCCAUA	6014
GAGAGAATTATTAAATGAAT	2921	TGG	+	GAGAGAAUUAUUAAAUGAAU	6015
TATACACTGTCTTCTTTCAT	2922	AGG	+	UAUACACUGUCUUCUUUCAU	6016
CATAGGAAAAATGAACAGAA	2923	AGG	+	CAUAGGAAAAAUGAACAGAA	6017

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ATAGGAAAAATGAACAGAAA	2924	GGG	+	AUAGGAAAAAUGAACAGAAA	6018
AACAGAAAGGGAGAGATCTT	2925	TGG	+	AACAGAAAGGGAGAGAUCUU	6019
ACAGAAAGGGAGAGATCTTT	2926	GGG	+	ACAGAAAGGGAGAGAUCUUU	6020
GAAAGGGAGAGATCTTTGGG	2927	TGG	+	GAAAGGGAGAGAUCUUUGGG	6021
AAAGGGAGAGATCTTTGGGT	2928	GGG	+	AAAGGGAGAGAUCUUUGGGU	6022
AAGGGAGAGATCTTTGGGTG	2929	GGG	+	AAGGGAGAGAUCUUUGGGUG	6023
ATACATGACATGAAAGCAGA	2930	AGG	+	AUACAUGACAUGAAAGCAGA	6024
TACATGACATGAAAGCAGAA	2931	GGG	+	UACAUGACAUGAAAGCAGAA	6025
ACATGACATGAAAGCAGAAG	2932	GGG	+	ACAUGACAUGAAAGCAGAAG	6026
AGCAGAAGGGGATTAGTTAT	2933	TGG	+	AGCAGAAGGGGAUUAGUUAU	6027
GAAGGGGATTAGTTATTGGA	2934	AGG	+	GAAGGGGAUUAGUUAUUGGA	6028
GGGATTAGTTATTGGAAGGC	2935	AGG	+	GGGAUUAGUUAUUGGAAGGC	6029
GGATTAGTTATTGGAAGGCA	2936	GGG	+	GGAUUAGUUAUUGGAAGGCA	6030
TTAGTTATTGGAAGGCAGGG	2937	AGG	+	UUAGUUAUUGGAAGGCAGGG	6031
TAGTTATTGGAAGGCAGGGA	2938	GGG	+	UAGUUAUUGGAAGGCAGGGA	6032
TGGAAGGCAGGGAGGGACCC	2939	AGG	+	UGGAAGGCAGGGAGGGACCC	6033
AAGGCAGGGAGGGACCCAGG	2940	AGG	+	AAGGCAGGGAGGGACCCAGG	6034
GGAGGGACCCAGGAGGATGA	2941	AGG	+	GGAGGGACCCAGGAGGAUGA	6035
GAGGGACCCAGGAGGATGAA	2942	GGG	+	GAGGGACCCAGGAGGAUGAA	6036
TCTCTTCCCTTCATCCTCCT	2943	GGG	-	UCUCUUCCCUUCAUCCUCCU	6037
TTCTCTTCCCTTCATCCTCC	2944	TGG	-	UUCUCUUCCCUUCAUCCUCC	6038
GATGAAGGGAAGAGAATGCA	2945	AGG	+	GAUGAAGGGAAGAGAAUGCA	6039
ATGAAGGGAAGAGAATGCAA	2946	GGG	+	AUGAAGGGAAGAGAAUGCAA	6040
GGGAAGAGAATGCAAGGGAA	2947	AGG	+	GGGAAGAGAAUGCAAGGGAA	6041
AATGCAAGGGAAAGGAGTAA	2948	AGG	+	AAUGCAAGGGAAAGGAGUAA	6042
GCAAGGGAAAGGAGTAAAGG	2949	AGG	+	GCAAGGGAAAGGAGUAAAGG	6043
AGGGAAAGGAGTAAAGGAGG	2950	AGG	+	AGGGAAAGGAGUAAAGGAGG	6044
GAAAGGAGTAAAGGAGGAGG	2951	AGG	+	GAAAGGAGUAAAGGAGGAGG	6045
AGTAAAGGAGGAGGAGGTAG	2952	AGG	+	AGUAAAGGAGGAGGAGGUAG	6046
TATTAATTCCAGCACTTGTG	2953	AGG	+	UAUUAAUUCCAGCACUUGUG	6047
TAATTCCAGCACTTGTGAGG	2954	AGG	+	UAAUUCCAGCACUUGUGAGG	6048
AATTCCAGCACTTGTGAGGA	2955	GGG	+	AAUUCCAGCACUUGUGAGGA	6049
ATTCCAGCACTTGTGAGGAG	2956	GGG	+	AUUCCAGCACUUGUGAGGAG	6050
TGTCCCCTCCTCACAAGTGC	2957	TGG	-	UGUCCCCUCCUCACAAGUGC	6051
GACAGAGCTCTGTGAGTTCA	2958	AGG	+	GACAGAGCUCUGUGAGUUCA	6052
TGGAACTGACTATGCAGAGG	2959	GGG	-	UGGAACUGACUAUGCAGAGG	6053
CTGGAACTGACTATGCAGAG	2960	GGG	-	CUGGAACUGACUAUGCAGAG	6054
CCCTCTGCATAGTCAGTTCC	2961	AGG	+	CCCUCUGCAUAGUCAGUUCC	6055
CCTGGAACTGACTATGCAGA	2962	GGG	-	CCUGGAACUGACUAUGCAGA	6056
TCCTGGAACTGACTATGCAG	2963	AGG	-	UCCUGGAACUGACUAUGCAG	6057
GTCAGTTCCAGGATAGCCAA	2964	TGG	+	GUCAGUUCCAGGAUAGCCAA	6058
TATGTAGCCATTGGCTATCC	2965	TGG	-	UAUGUAGCCAUUGGCUAUCC	6059
GGGTCTCATTATGTAGCCAT	2966	TGG	-	GGGUCUCAUUAUGUAGCCAU	6060
ATGAGACCCTGTGTCAGAGA	2967	AGG	+	AUGAGACCCUGUGUCAGAGA	6061
AGACCCTGTGTCAGAGAAGG	2968	TGG	+	AGACCCUGUGUCAGAGAAGG	6062
CGGCCACCTTCTCTGACACA	2969	GGG	-	CGGCCACCUUCUCUGACACA	6063
CCTGTGTCAGAGAAGGTGGC	2970	CGG	+	CCUGUGUCAGAGAAGGUGGC	6064
CCGGCCACCTTCTCTGACAC	2971	AGG	-	CCGGCCACCUUCUCUGACAC	6065
GTCAGAGAAGGTGGCCGGCG	2972	AGG	+	GUCAGAGAAGGUGGCCGGCG	6066
TCAGAGAAGGTGGCCGGCGA	2973	GGG	+	UCAGAGAAGGUGGCCGGCGA	6067
TAAGCATCAATATCCCTCGC	2974	CGG	-	UAAGCAUCAAUAUCCCUCGC	6068
TAACTCATTCAGCTGTGTGG	2975	TGG	-	UAACUCAUUCAGCUGUGUGG	6069
GTTTAACTCATTCAGCTGTG	2976	TGG	-	GUUUAACUCAUUCAGCUGUG	6070
AGTGCAAAAGTAGCAGGAAC	2977	AGG	-	AGUGCAAAAGUAGCAGGAAC	6071
ATTGAAAGTGCAAAAGTAGC	2978	AGG	-	AUUGAAAGUGCAAAAGUAGC	6072
ATGCTCCCAGATTACTTACA	2979	TGG	+	AUGCUCCCAGAUUACUUACA	6073
AAATTCCATGTAAGTAATCT	2980	GGG	-	AAAUUCCAUGUAAGUAAUCU	6074
TAAATTCCATGTAAGTAATC	2981	TGG	-	UAAAUUCCAUGUAAGUAAUC	6075

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
ATGGAATTTATATTTTATTG	2982	TGG	+	AUGGAAUUUAUAUUUUAUUG	6076
GAGGTTTGTCTATGATGTAC	2983	AGG	-	GAGGUUUGUCUAUGAUGUAC	6077
TTGGTTGAAAATTTTTCTTG	2984	AGG	-	UUGGUUGAAAAUUUUUCUUG	6078
AAATTTTCAACCAATAGTCC	2985	AGG	+	AAAUUUUCAACCAAUAGUCC	6079
GTTATTTTTGCCTGGACTAT	2986	TGG	-	GUUAUUUUUGCCUGGACUAU	6080
AAAAAGAGGTTATTTTTGCC	2987	TGG	-	AAAAAGAGGUUAUUUUUGCC	6081
AGAAAGAAAGAAAGAAAAAG	2988	AGG	-	AGAAAGAAAGAAAGAAAAAG	6082
TCTTTTTTTTTTTTTTTTTT	2989	TGG	+	UCUUUUUUUUUUUUUUUUUU	6083
TTTTTTTGGTTTTTTGAAAC	2990	AGG	+	UUUUUUUGGUUUUUUGAAAC	6084
TTTTTTGGTTTTTTGAAACA	2991	GGG	+	UUUUUUGGUUUUUUGAAACA	6085
AGGGTTTCTCTGTATAGCCC	2992	TGG	+	AGGGUUUCUCUGUAUAGCCC	6086
CTGTATAGCCCTGGCTGTCC	2993	TGG	+	CUGUAUAGCCCUGGCUGUCC	6087
AGTGAGTTCCAGGACAGCCA	2994	GGG	-	AGUGAGUUCCAGGACAGCCA	6088
AAGTGAGTTCCAGGACAGCC	2995	AGG	-	AAGUGAGUUCCAGGACAGCC	6089
TGGTGTACAAAGTGAGTTCC	2996	AGG	-	UGGUGUACAAAGUGAGUUCC	6090
TGGAACTCACTTTGTACACC	2997	AGG	+	UGGAACUCACUUUGUACACC	6091
ACTCACTTTGTACACCAGGC	2998	TGG	+	ACUCACUUUGUACACCAGGC	6092
TTCTGAGTTCGAGGCCAGCC	2999	TGG	-	UUCUGAGUUCGAGGCCAGCC	6093
CAGGTGGATTTCTGAGTTCG	3000	AGG	-	CAGGUGGAUUUCUGAGUUCG	6094
ACTCGGGAGGCAGAGGCAGG	3001	TGG	-	ACUCGGGAGGCAGAGGCAGG	6095
AGCACTCGGGAGGCAGAGGC	3002	AGG	-	AGCACUCGGGAGGCAGAGGC	6096
GCCTCTGCCTCCCGAGTGCT	3003	CGG	+	GCCUCUGCCUCCCGAGUGCU	6097
TCCGAGCACTCGGGAGGCAG	3004	AGG	-	UCCGAGCACUCGGGAGGCAG	6098
CTTTAATCCGAGCACTCGGG	3005	AGG	-	CUUUAAUCCGAGCACUCGGG	6099
CTCCCGAGTGCTCGGATTAA	3006	AGG	+	CUCCCGAGUGCUCGGAUUAA	6100
CGCCTTTAATCCGAGCACTC	3007	GGG	-	CGCCUUUAAUCCGAGCACUC	6101
ACGCCTTTAATCCGAGCACT	3008	CGG	-	ACGCCUUUAAUCCGAGCACU	6102
TATTTTTTGCGGGACGGTGG	3009	TGG	-	UAUUUUUUGCGGGACGGUGG	6103
GGTTATTTTTTGCGGGACGG	3010	TGG	-	GGUUAUUUUUUGCGGGACGG	6104
ACAGGTTATTTTTTGCGGGA	3011	CGG	-	ACAGGUUAUUUUUUGCGGGA	6105
AAAAACAGGTTATTTTTTGC	3012	GGG	-	AAAAACAGGUUAUUUUUUGC	6106
CAAAAACAGGTTATTTTTTG	3013	CGG	-	CAAAAACAGGUUAUUUUUUG	6107
ATAATTAAAACAACAAAAAC	3014	AGG	-	AUAAUUAAAACAACAAAAAC	6108
TTTTTAAGATTTTATGTGTA	3015	TGG	+	UUUUUAAGAUUUUAUGUGUA	6109
TTTTAAGATTTTATGTGTAT	3016	GGG	+	UUUUAAGAUUUUAUGUGUAU	6110
CTGTGTACCACTTGTATGCT	3017	TGG	+	CUGUGUACCACUUGUAUGCU	6111
TTGGGCACCAAGCATACAAG	3018	TGG	-	UUGGGCACCAAGCAUACAAG	6112
TGTATGCTTGGTGCCCAAGA	3019	AGG	+	UGUAUGCUUGGUGCCCAAGA	6113
TGATCTGTTCTGTCCTTCTT	3020	GGG	-	UGAUCUGUUCUGUCCUUCUU	6114
ATGATCTGTTCTGTCCTTCT	3021	TGG	-	AUGAUCUGUUCUGUCCUUCU	6115
AGAAGGACAGAACAGATCAT	3022	AGG	+	AGAAGGACAGAACAGAUCAU	6116
GAAGGACAGAACAGATCATA	3023	GGG	+	GAAGGACAGAACAGAUCAUA	6117
GAACAGATCATAGGGTTTCC	3024	TGG	+	GAACAGAUCAUAGGGUUUCC	6118
AACAGATCATAGGGTTTCCT	3025	GGG	+	AACAGAUCAUAGGGUUUCCU	6119
TTTCCTGGGATAAGAGTTAT	3026	AGG	+	UUUCCUGGGAUAAGAGUUAU	6120
ACTCCTATAACTCTTATCCC	3027	AGG	-	ACUCCUAUAACUCUUAUCCC	6121
GATGACTGTAAGCCACCAAG	3028	TGG	+	GAUGACUGUAAGCCACCAAG	6122
GTAAGCCACCAAGTGGACGC	3029	TGG	+	GUAAGCCACCAAGUGGACGC	6123
TAAGCCACCAAGTGGACGCT	3030	GGG	+	UAAGCCACCAAGUGGACGCU	6124
AATTCCCAGCGTCCACTTGG	3031	TGG	-	AAUUCCCAGCGUCCACUUGG	6125
TTCAATTCCCAGCGTCCACT	3032	TGG	-	UUCAAUUCCCAGCGUCCACU	6126
GGACGCTGGGAATTGAACCT	3033	TGG	+	GGACGCUGGGAAUUGAACCU	6127
GGAATTGAACCTTGGTTAGC	3034	TGG	+	GGAAUUGAACCUUGGUUAGC	6128
GACACTCTTCCAGCTAACCA	3035	AGG	-	GACACUCUUCCAGCUAACCA	6129
GGGACTGGAATTAAAAATAC	3036	TGG	-	GGGACUGGAAUUAAAAAUAC	6130
CATAGACAAACAACGGGGAC	3037	TGG	-	CAUAGACAAACAACGGGGAC	6131
ATCTTCATAGACAAACAACG	3038	GGG	-	AUCUUCAUAGACAAACAACG	6132
AATCTTCATAGACAAACAAC	3039	GGG	-	AAUCUUCAUAGACAAACAAC	6133

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AAATCTTCATAGACAAACAA	3040	CGG	-	AAAUCUUCAUAGACAAACAA	6134
TTGTCTATGAAGATTTTGCT	3041	TGG	+	UUGUCUAUGAAGAUUUUGCU	6135
TTTTGCTTGGTAGTCCAGTC	3042	TGG	+	UUUUGCUUGGUAGUCCAGUC	6136
CCAGTCTGGCTTTAAGAAAC	3043	AGG	+	CCAGUCUGGCUUUAAGAAAC	6137
CCTGTTTCTTAAAGCCAGAC	3044	TGG	-	CCUGUUUCUUAAAGCCAGAC	6138
CAGTCTGGCTTTAAGAAACA	3045	GGG	+	CAGUCUGGCUUUAAGAAACA	6139
AACAGGGTCTCATGCATCCC	3046	TGG	+	AACAGGGUCUCAUGCAUCCC	6140
ATGGAAGTCTGAGCAAGCCA	3047	GGG	-	AUGGAAGUCUGAGCAAGCCA	6141
CATGGAAGTCTGAGCAAGCC	3048	AGG	-	CAUGGAAGUCUGAGCAAGCC	6142
CTCAGACTTCCATGCAGCTG	3049	AGG	+	CUCAGACUUCCAUGCAGCUG	6143
GACTTCCATGCAGCTGAGGA	3050	TGG	+	GACUUCCAUGCAGCUGAGGA	6144
TAAAGCCATCCTCAGCTGCA	3051	TGG	-	UAAAGCCAUCCUCAGCUGCA	6145
TCTACGAGGAAAAGGCAGGA	3052	GGG	-	UCUACGAGGAAAAGGCAGGA	6146
CTCTACGAGGAAAAGGCAGG	3053	AGG	-	CUCUACGAGGAAAAGGCAGG	6147
GCACTCTACGAGGAAAAGGC	3054	AGG	-	GCACUCUACGAGGAAAAGGC	6148
CTCAGCACTCTACGAGGAAA	3055	AGG	-	CUCAGCACUCUACGAGGAAA	6149
TGTAATCTCAGCACTCTACG	3056	AGG	-	UGUAAUCUCAGCACUCUACG	6150
GAGAAGTCTGCTGGTGGCAC	3057	AGG	-	GAGAAGUCUGCUGGUGGCAC	6151
TGTTAGGAGAAGTCTGCTGG	3058	TGG	-	UGUUAGGAGAAGUCUGCUGG	6152
TCTTGTTAGGAGAAGTCTGC	3059	TGG	-	UCUUGUUAGGAGAAGUCUGC	6153
GACTTCTCCTAACAAGAATG	3060	TGG	+	GACUUCUCCUAACAAGAAUG	6154
TCCTAACAAGAATGTGGCAC	3061	AGG	+	UCCUAACAAGAAUGUGGCAC	6155
CCTAACAAGAATGTGGCACA	3062	GGG	+	CCUAACAAGAAUGUGGCACA	6156
CCCTGTGCCACATTCTTGTT	3063	AGG	-	CCCUGUGCCACAUUCUUGUU	6157
AACAAGAATGTGGCACAGGG	3064	AGG	+	AACAAGAAUGUGGCACAGGG	6158
ATGTGGCACAGGGAGGCAAC	3065	TGG	+	AUGUGGCACAGGGAGGCAAC	6159
TGTGGCACAGGGAGGCAACT	3066	GGG	+	UGUGGCACAGGGAGGCAACU	6160
GAGGCAACTGGGAATCAAAC	3067	AGG	+	GAGGCAACUGGGAAUCAAAC	6161
CAACTGGGAATCAAACAGGA	3068	AGG	+	CAACUGGGAAUCAAACAGGA	6162
CTGGGAATCAAACAGGAAGG	3069	AGG	+	CUGGGAAUCAAACAGGAAGG	6163
AAACAGGAAGGAGGAGTAAA	3070	AGG	+	AAACAGGAAGGAGGAGUAAA	6164
AGGAAGGAGGAGTAAAAGGA	3071	AGG	+	AGGAAGGAGGAGUAAAAGGA	6165
GGAAGGAGGAGTAAAAGGAA	3072	GGG	+	GGAAGGAGGAGUAAAAGGAA	6166
GAAGGAGGAGTAAAAGGAAG	3073	GGG	+	GAAGGAGGAGUAAAAGGAAG	6167
GGAGGAGTAAAAGGAAGGGG	3074	AGG	+	GGAGGAGUAAAAGGAAGGGG	6168
GAGTAAAAGGAAGGGGAGGA	3075	AGG	+	GAGUAAAAGGAAGGGGAGGA	6169
AAAGGAAGGGGAGGAAGGAG	3076	AGG	+	AAAGGAAGGGGAGGAAGGAG	6170
GGGAGGAAGGAGAGGAGAAG	3077	TGG	+	GGGAGGAAGGAGAGGAGAAG	6171
GGAAGGAGAGGAGAAGTGGC	3078	AGG	+	GGAAGGAGAGGAGAAGUGGC	6172
AGGAGAGGAGAAGTGGCAGG	3079	AGG	+	AGGAGAGGAGAAGUGGCAGG	6173
GTGGCAGGAGGAGAAGAAAA	3080	AGG	+	GUGGCAGGAGGAGAAGAAAA	6174
GCAGGAGGAGAAGAAAAAGG	3081	AGG	+	GCAGGAGGAGAAGAAAAAGG	6175
GGAGGAGAAGAAAAAGGAGG	3082	CGG	+	GGAGGAGAAGAAAAAGGAGG	6176
AGAAAAAGGAGGCGGAGAAA	3083	AGG	+	AGAAAAAGGAGGCGGAGAAA	6177
AAAAGGAGGCGGAGAAAAGG	3084	TGG	+	AAAAGGAGGCGGAGAAAAGG	6178
AGGCGGAGAAAAGGTGGTAG	3085	TGG	+	AGGCGGAGAAAAGGUGGUAG	6179
CGGAGAAAAGGTGGTAGTGG	3086	TGG	+	CGGAGAAAAGGUGGUAGUGG	6180
AGAAAAGGTGGTAGTGGTGG	3087	AGG	+	AGAAAAGGUGGUAGUGGUGG	6181
GGTGGTAGTGGTGGAGGAAG	3088	AGG	+	GGUGGUAGUGGUGGAGGAAG	6182
GGTAGTGGTGGAGGAAGAGG	3089	AGG	+	GGUAGUGGUGGAGGAAGAGG	6183
AGTGGTGGAGGAAGAGGAGG	3090	AGG	+	AGUGGUGGAGGAAGAGGAGG	6184
GGTGGAGGAAGAGGAGGAGG	3091	AGG	+	GGUGGAGGAAGAGGAGGAGG	6185
GGAGGAAGAGGAGGAGGAGG	3092	AGG	+	GGAGGAAGAGGAGGAGGAGG	6186
GGAAGAGGAGGAGGAGGAGG	3093	AGG	+	GGAAGAGGAGGAGGAGGAGG	6187
AGAGGAGGAGGAGGAGGAGG	3094	AGG	+	AGAGGAGGAGGAGGAGGAGG	6188
GGAGGAGGAGGAGGAGGAGG	3095	AGG	+	GGAGGAGGAGGAGGAGGAGG	6189
GGAGGAGGAGGAGGAGGAGG	3096	AGG	+	GGAGGAGGAGGAGGAGGAGG	6190
GAGGAGGAGGAGGAGGAGGA	3097	GGG	+	GAGGAGGAGGAGGAGGAGGA	6191

Target	SEQ ID NO.	PAM	Direction	Guide	SEQ ID NO.
AGGAGGAGGAGGAGGAGGAG	3098	GGG	+	AGGAGGAGGAGGAGGAGGAG	6192
GGAGGAGGAGGAGGAGGAGG	3099	GGG	+	GGAGGAGGAGGAGGAGGAGG	6193
GAGGGGGACTTAATCAGTAA	3100	AGG	+	GAGGGGGACUUAAUCAGUAA	6194
ACTTAATCAGTAAAGGAAAA	3101	AGG	+	ACUUAAUCAGUAAAGGAAAA	6195
AAAAGGCTGAGCTCAGTCCT	3102	TGG	+	AAAAGGCUGAGCUCAGUCCU	6196
CTCAGTCCTTGGAACCTACA	3103	TGG	+	CUCAGUCCUUGGAACCUACA	6197
GTCCTTGGAACCTACATGGT	3104	AGG	+	GUCCUUGGAACCUACAUGGU	6198
CTCCTACCATGTAGGTTCCA	3105	AGG	-	CUCCUACCAUGUAGGUUCCA	6199
CTCATTCTCTCCTACCATGT	3106	AGG	-	CUCAUUCUCUCCUACCAUGU	6200
GTTTCCTCTGATCCCCACAC	3107	TGG	+	GUUUCCUCUGAUCCCCACAC	6201
TGCTCCAGTGTGGGGATCAG	3108	AGG	-	UGCUCCAGUGUGGGGAUCAG	6202
TGATCCCCACACTGGAGCAA	3109	TGG	+	UGAUCCCCACACUGGAGCAA	6203
GGCACCATTGCTCCAGTGTG	3110	GGG	-	GGCACCAUUGCUCCAGUGUG	6204
TGGCACCATTGCTCCAGTGT	3111	GGG	-	UGGCACCAUUGCUCCAGUGU	6205
ATGGCACCATTGCTCCAGTG	3112	TGG	-	AUGGCACCAUUGCUCCAGUG	6206
GAAGTGGGAGAGTGATGTGA	3113	TGG	-	GAAGUGGGAGAGUGAUGUGA	6207
TGTGAGGAGTTGGGTGAAGT	3114	GGG	-	UGUGAGGAGUUGGGUGAAGU	6208
TTGTGAGGAGTTGGGTGAAG	3115	TGG	-	UUGUGAGGAGUUGGGUGAAG	6209
TTTTATTTTTGTGAGGAGTT	3116	GGG	-	UUUUAUUUUUGUGAGGAGUU	6210
TTTTTATTTTTGTGAGGAGT	3117	TGG	-	UUUUUAUUUUUGUGAGGAGU	6211
TTTTGTTTTTTATTTTTGTG	3118	AGG	-	UUUUGUUUUUUAUUUUUGUG	6212
TTAAAAAAGAAAGCTGTAGC	3119	CGG	+	UUAAAAAAGAAAGCUGUAGC	6213
TAAAAAAGAAAGCTGTAGCC	3120	GGG	+	UAAAAAAGAAAGCUGUAGCC	6214
AAGAAAGCTGTAGCCGGGCG	3121	TGG	+	AAGAAAGCUGUAGCCGGGCG	6215
AAAGCTGTAGCCGGGCGTGG	3122	TGG	+	AAAGCUGUAGCCGGGCGUGG	6216
AAAGTGTGTGCCACCACGCC	3123	CGG	-	AAAGUGUGUGCCACCACGCC	6217
GTGGCACACACTTTTAATCC	3124	CGG	+	GUGGCACACACUUUUAAUCC	6218
ACACTTTTAATCCCGGCACT	3125	TGG	+	ACACUUUUAAUCCCGGCACU	6219
CACTTTTAATCCCGGCACTT	3126	GGG	+	CACUUUUAAUCCCGGCACUU	6220
TTTTAATCCCGGCACTTGGG	3127	AGG	+	UUUUAAUCCCGGCACUUGGG	6221
TCCCGGCACTTGGGAGGCAG	3128	AGG	+	UCCCGGCACUUGGGAGGCAG	6222
GCCTCTGCCTCCCAAGTGCC	3129	GGG	-	GCCUCUGCCUCCCAAGUGCC	6223
TGCCTCTGCCTCCCAAGTGC	3130	CGG	-	UGCCUCUGCCUCCCAAGUGC	6224
ACTTGGGAGGCAGAGGCAAG	3131	CGG	+	ACUUGGGAGGCAGAGGCAAG	6225
CAAGCGGATTTCTGAGTTCG	3132	AGG	+	CAAGCGGAUUUCUGAGUUCG	6226
TTCTGAGTTCGAGGCCAGCT	3133	TGG	+	UUCUGAGUUCGAGGCCAGCU	6227
ACTCACTTTGTAGACCAAGC	3134	TGG	-	ACUCACUUUGUAGACCAAGC	6228
TGGTCTACAAAGTGAGTTCC	3135	AGG	+	UGGUCUACAAAGUGAGUUCC	6229
AAGTGAGTTCCAGGACAGCC	3136	AGG	+	AAGUGAGUUCCAGGACAGCC	6230
AGTGAGTTCCAGGACAGCCA	3137	GGG	+	AGUGAGUUCCAGGACAGCCA	6231
CTGTGTAGCCCTGGCTGTCC	3138	TGG	-	CUGUGUAGCCCUGGCUGUCC	6232
AGGGTTTCTCTGTGTAGCCC	3139	TGG	-	AGGGUUUCUCUGUGUAGCCC	6233
TCTTTTGGTTTTTCAAGACA	3140	GGG	-	UCUUUUGGUUUUUCAAGACA	6234
TTCTTTTGGTTTTTCAAGAC	3141	AGG	-	UUCUUUUGGUUUUUCAAGAC	6235
ACAGCTTTCTTTTTTTCTTT	3142	TGG	-	ACAGCUUUCUUUUUUUCUUU	6236
TATATTAATATAGCAAAGCA	3143	GGG	-	UAUAUUAAUAUAGCAAAGCA	6237
ATATATTAATATAGCAAAGC	3144	AGG	-	AUAUAUUAAUAUAGCAAAGC	6238
TGTTTTTTCTTATAGAATTA	3145	TGG	-	UGUUUUUUCUUAUAGAAUUA	6239
TTCTGGAGGGAGGAAAAGGA	3146	TGG	-	UUCUGGAGGGAGGAAAAGGA	6240
CTCATTCTGGAGGGAGGAAA	3147	AGG	-	CUCAUUCUGGAGGGAGGAAA	6241

B. Other Nuclease Agents and Target Sequences for Nuclease Agents

[00308] Any nuclease agent that induces a nick or double-strand break at a desired target sequence can be used in the methods and compositions disclosed herein. A naturally occurring or

native nuclease agent can be employed so long as the nuclease agent induces a nick or double-strand break at a desired target sequence. Alternatively, a modified or engineered nuclease agent can be employed. An "engineered nuclease agent" includes a nuclease that is engineered (modified or derived) from its native form to specifically recognize and induce a nick or double-strand break in the desired target sequence. Thus, an engineered nuclease agent can be derived from a native, naturally occurring nuclease agent or it can be artificially created or synthesized. The engineered nuclease can induce a nick or double-strand break in a target sequence, for example, wherein the target sequence is not a sequence that would have been recognized by a native (non-engineered or non-modified) nuclease agent. The modification of the nuclease agent can be as little as one amino acid in a protein cleavage agent or one nucleotide in a nucleic acid cleavage agent. Producing a nick or double-strand break at a target sequence or other DNA can be referred to herein as "cutting" or "cleaving" the target sequence or other DNA.

[00309] Active variants and fragments of the exemplified target sequences are also provided. Such active variants can comprise at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the given target sequence, wherein the active variants retain biological activity and hence are capable of being recognized and cleaved by a nuclease agent in a sequence-specific manner. Assays to measure the double-strand break of a target sequence by a nuclease agent are well-known. *See, e.g.*, Frendewey et al. (2010) *Methods in Enzymology* 476:295-307, which is herein incorporated by reference herein in its entirety for all purposes.

[00310] The target sequence of the nuclease agent can be positioned anywhere in or near the target locus. The target sequence can be located within a coding region of a gene, or within regulatory regions that influence the expression of the gene. A target sequence of the nuclease agent can be located in an intron, an exon, a promoter, an enhancer, a regulatory region, or any non-protein coding region.

[00311] One type of nuclease agent is a Transcription Activator-Like Effector Nuclease (TALEN). TAL effector nucleases are a class of sequence-specific nucleases that can be used to make double-strand breaks at specific target sequences in the genome of a prokaryotic or eukaryotic organism. TAL effector nucleases are created by fusing a native or engineered transcription activator-like (TAL) effector, or functional part thereof, to the catalytic domain of an endonuclease, such as, for example, FokI. The unique, modular TAL effector DNA binding

domain allows for the design of proteins with potentially any given DNA recognition specificity. Thus, the DNA binding domains of the TAL effector nucleases can be engineered to recognize specific DNA target sites and thus, used to make double-strand breaks at desired target sequences. *See* WO 2010/079430; Morbitzer et al. (2010) *Proc. Natl. Acad. Sci. U.S.A.* 107(50):21617-21622; Scholze & Boch (2010) *Virulence* 1:428-432; Christian et al. *Genetics* (2010) 186:757-761; Li et al. (2010) *Nucleic Acids Res.* (2010) doi:10.1093/nar/gkq704; and Miller et al. (2011) *Nat. Biotechnol.* 29:143–148, each of which is herein incorporated by reference in its entirety for all purposes.

[00312] Examples of suitable TAL nucleases, and methods for preparing suitable TAL nucleases, are disclosed, e.g., in US 2011/0239315 A1, US 2011/0269234 A1, US 2011/0145940 A1, US 2003/0232410 A1, US 2005/0208489 A1, US 2005/0026157 A1, US 2005/0064474 A1, US 2006/0188987 A1, and US 2006/0063231 A1, each of which is herein incorporated by reference in its entirety for all purposes. In various embodiments, TAL effector nucleases are engineered that cut in or near a target nucleic acid sequence in, e.g., a locus of interest or a genomic locus of interest, wherein the target nucleic acid sequence is at or near a sequence to be modified by a targeting vector. The TAL nucleases suitable for use with the various methods and compositions provided herein include those that are specifically designed to bind at or near target nucleic acid sequences to be modified by targeting vectors as described herein.

[00313] In some TALENs, each monomer of the TALEN comprises 33-35 TAL repeats that recognize a single base pair via two hypervariable residues. In some TALENs, the nuclease agent is a chimeric protein comprising a TAL-repeat-based DNA binding domain operably linked to an independent nuclease such as a FokI endonuclease. For example, the nuclease agent can comprise a first TAL-repeat-based DNA binding domain and a second TAL-repeat-based DNA binding domain, wherein each of the first and the second TAL-repeat-based DNA binding domains is operably linked to a FokI nuclease, wherein the first and the second TAL-repeat-based DNA binding domain recognize two contiguous target DNA sequences in each strand of the target DNA sequence separated by a spacer sequence of varying length (12-20 bp), and wherein the FokI nuclease subunits dimerize to create an active nuclease that makes a double strand break at a target sequence.

[00314] The nuclease agent employed in the various methods and compositions disclosed herein can further comprise a zinc-finger nuclease (ZFN). In some ZFNs, each monomer of the

ZFN comprises 3 or more zinc finger-based DNA binding domains, wherein each zinc finger-based DNA binding domain binds to a 3 bp subsite. In other ZFNs, the ZFN is a chimeric protein comprising a zinc finger-based DNA binding domain operably linked to an independent nuclease such as a FokI endonuclease. For example, the nuclease agent can comprise a first ZFN and a second ZFN, wherein each of the first ZFN and the second ZFN is operably linked to a FokI nuclease subunit, wherein the first and the second ZFN recognize two contiguous target DNA sequences in each strand of the target DNA sequence separated by about 5-7 bp spacer, and wherein the FokI nuclease subunits dimerize to create an active nuclease that makes a double strand break. *See, e.g.*, US20060246567; US20080182332; US20020081614; US20030021776; WO/2002/057308A2; US20130123484; US20100291048; WO/2011/017293A2; and Gaj et al. (2013) *Trends Biotechnol.*, 31(7):397-405, each of which is herein incorporated by reference in its entirety for all purposes.

[00315] Active variants and fragments of nuclease agents (i.e., an engineered nuclease agent) are also provided. Such active variants can comprise at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the native nuclease agent, wherein the active variants retain the ability to cut at a desired target sequence and hence retain nick or double-strand-break-inducing activity. For example, any of the nuclease agents described herein can be modified from a native endonuclease sequence and designed to recognize and induce a nick or double-strand break at a target sequence that was not recognized by the native nuclease agent. Thus, some engineered nucleases have a specificity to induce a nick or double-strand break at a target sequence that is different from the corresponding native nuclease agent target sequence. Assays for nick or double-strand-break-inducing activity are known and generally measure the overall activity and specificity of the endonuclease on DNA substrates containing the target sequence.

[00316] The nuclease agent may be introduced into the cell or animal by any known means. A polypeptide encoding the nuclease agent may be directly introduced into the cell or animal. Alternatively, a polynucleotide encoding the nuclease agent can be introduced into the cell or animal. When a polynucleotide encoding the nuclease agent is introduced, the nuclease agent can be transiently, conditionally, or constitutively expressed within the cell. The polynucleotide encoding the nuclease agent can be contained in an expression cassette and be operably linked to a conditional promoter, an inducible promoter, a constitutive promoter, or a tissue-specific

promoter. Examples of promoters are discussed in further detail elsewhere herein. Alternatively, the nuclease agent can be introduced into the cell as an mRNA encoding the nuclease agent.

[00317] A polynucleotide encoding a nuclease agent can be stably integrated in the genome of a cell and operably linked to a promoter active in the cell. Alternatively, a polynucleotide encoding a nuclease agent can be in an expression vector or a targeting vector.

[00318] When the nuclease agent is provided to the cell through the introduction of a polynucleotide encoding the nuclease agent, such a polynucleotide encoding a nuclease agent can be modified to substitute codons having a higher frequency of usage in the cell of interest, as compared to the naturally occurring polynucleotide sequence encoding the nuclease agent. For example, the polynucleotide encoding the nuclease agent can be modified to substitute codons having a higher frequency of usage in a given eukaryotic cell of interest, including a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell or any other host cell of interest, as compared to the naturally occurring polynucleotide sequence.

[00319] The term "target sequence for a nuclease agent" includes a DNA sequence at which a nick or double-strand break is induced by a nuclease agent. The target sequence for a nuclease agent can be endogenous (or native) to the cell or the target sequence can be exogenous to the cell. A target sequence that is exogenous to the cell is not naturally occurring in the genome of the cell. The target sequence can also exogenous to the polynucleotides of interest that one desires to be positioned at the target locus. In some cases, the target sequence is present only once in the genome of the host cell.

[00320] The length of the target sequence can vary, and includes, for example, target sequences that are about 30-36 bp for a zinc finger nuclease (ZFN) pair (i.e., about 15-18 bp for each ZFN), about 36 bp for a Transcription Activator-Like Effector Nuclease (TALEN), or about 20 bp for a CRISPR/Cas9 guide RNA.

VI. Cells or Animals or Genomes Comprising Nucleic Acid Constructs and/or Nuclease Agents or Nucleic Acids Encoding Nuclease Agents

[00321] Genomes, cells, and animals produced by the methods disclosed herein are also provided. Likewise, genomes, cells, and animals comprising the nucleic acid constructs comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus, vectors, lipid

nanoparticles, or compositions as described herein are also provided. Likewise, genomes, cells, and animals comprising the described nuclease agents or nucleic acids encoding the nuclease agents (e.g., targeting an endogenous *RS1* locus) or vectors, lipid nanoparticles, or compositions as described herein are also provided. The genomes, cells, or animals can comprise the nucleic acid construct genomically integrated at a target genomic locus (e.g., at an *RS1* locus) and can express the retinoschisin protein or fragment or variant thereof. The retinoschisin coding sequence can be operably linked to an endogenous promoter at the target genomic locus upon integration into the target genomic locus, or it can be operably linked to an exogenous promoter present in the nucleic acid construct. If the nucleic acid construct is a bidirectional nucleic acid construct disclosed herein, the genome, cell, or animal can express the first retinoschisin protein or fragment or variant thereof or can express the second retinoschisin protein or fragment or variant thereof. In some genomes, cells, or animals, the target genomic locus is an *RS1* locus. For example, the nucleic acid construct can be genomically integrated in intron 1 of the endogenous *RS1* locus. Endogenous *RS1* exon 1 can then splice into the coding sequence for the retinoschisin protein or fragment or variant thereof in the nucleic acid construct. In a specific example, the modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4. In a specific example, the modified *RS1* locus comprising the genomically integrated nucleic acid construct comprises an *RS1* coding sequence comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 6, 11, or 12.

[00322] In some genomes, cells, or animals, integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site. For example, the integration of the nucleic acid construct into the endogenous *RS1* locus can reduce or eliminate expression of the endogenous retinoschisin protein and replace it with expression of the retinoschisin protein or fragment or variant thereof encoded by the nucleic acid construct. In one example, integration of the nucleic acid construct into the

endogenous *RS1* locus reduces expression of the endogenous retinoschisin protein. In another example, integration of the nucleic acid construct into the endogenous *RS1* locus eliminates expression of the endogenous retinoschisin protein. In a specific example, the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis, and expression of the genomically integrated nucleic acid construct reduces or eliminates expression of the mutated *RS1* gene.

[00323] The target genomic locus at which the nucleic acid construct is stably integrated can be heterozygous for the retinoschisin coding sequence from the nucleic acid construct or homozygous for the retinoschisin coding sequence from the nucleic acid construct. A diploid organism has two alleles at each genetic locus. Each pair of alleles represents the genotype of a specific genetic locus. Genotypes are described as homozygous if there are two identical alleles at a particular locus and as heterozygous if the two alleles differ. An animal comprising a genomically integrated nucleic acid construct as described herein can comprise the nucleic acid construct in a target genomic locus in its germline.

[00324] The genomes, cells, or animals provided herein can be, for example, eukaryotic, including, for example, animal, mammalian, non-human mammalian, and human. The term "animal" includes mammals, fishes, and birds. A mammal can be, for example, a non-human mammal, a human, a rodent, a rat, a mouse, or a hamster. Other non-human mammals include, for example, non-human primates, monkeys, apes, cats, dogs, rabbits, horses, bulls, deer, bison, livestock (e.g., bovine species such as cows, steer, and so forth; ovine species such as sheep, goats, and so forth; and porcine species such as pigs and boars). Birds include, for example, chickens, turkeys, ostrich, geese, ducks, and so forth. Domesticated animals and agricultural animals are also included. The term "non-human" excludes humans.

[00325] The cells can be isolated cells (e.g., *in vitro*) or can be *in vivo* within an animal. Cells can also be any type of undifferentiated or differentiated state. For example, a cell can be a totipotent cell, a pluripotent cell (e.g., a human pluripotent cell or a non-human pluripotent cell such as a mouse embryonic stem (ES) cell or a rat ES cell), or a non-pluripotent cell. Totipotent cells include undifferentiated cells that can give rise to any cell type, and pluripotent cells include undifferentiated cells that possess the ability to develop into more than one differentiated cell types.

[00326] The cells provided herein can also be germ cells (e.g., sperm or oocytes). The cells

can be mitotically competent cells or mitotically-inactive cells, meiotically competent cells or meiotically-inactive cells. Similarly, the cells can also be primary somatic cells or cells that are not a primary somatic cell. Somatic cells include any cell that is not a gamete, germ cell, gametocyte, or undifferentiated stem cell. For example, the cells can be liver cells, kidney cells, hematopoietic cells, endothelial cells, epithelial cells, fibroblasts, mesenchymal cells, keratinocytes, blood cells, melanocytes, monocytes, mononuclear cells, monocytic precursors, B cells, erythroid-megakaryocytic cells, eosinophils, macrophages, T cells, islet beta cells, exocrine cells, pancreatic progenitors, endocrine progenitors, adipocytes, preadipocytes, neurons, glial cells, neural stem cells, neurons, hepatoblasts, hepatocytes, cardiomyocytes, skeletal myoblasts, smooth muscle cells, ductal cells, acinar cells, alpha cells, beta cells, delta cells, PP cells, cholangiocytes, white or brown adipocytes, or ocular cells (e.g., trabecular meshwork cells, retinal pigment epithelial cells, retinal microvascular endothelial cells, retinal pericyte cells, conjunctival epithelial cells, conjunctival fibroblasts, iris pigment epithelial cells, keratocytes, lens epithelial cells, non-pigment ciliary epithelial cells, ocular choroid fibroblasts, photoreceptor cells, ganglion cells, bipolar cells, horizontal cells, or amacrine cells). For example, the cells can be eye cells, such as retinal cells (e.g., photoreceptors).

[00327] The cells provided herein can be normal, healthy cells, or can be diseased or mutant-bearing cells. For example, the cells can comprise one or more mutations associated with or that cause XLRS (e.g., encoding a R141C substitution in the retinoschisin protein).

[00328] The animals provided herein can be humans or they can be non-human animals. Non-human animals comprising a nucleic acid or expression cassette as described herein can be made by the methods described elsewhere herein. The term "animal" includes mammals, fishes, and birds. Mammals include, for example, humans, non-human primates, monkeys, apes, cats, dogs, horses, bulls, deer, bison, sheep, rabbits, rodents (e.g., mice, rats, hamsters, and guinea pigs), and livestock (e.g., bovine species such as cows and steer; ovine species such as sheep and goats; and porcine species such as pigs and boars). Birds include, for example, chickens, turkeys, ostrich, geese, and ducks. Domesticated animals and agricultural animals are also included. The term "non-human animal" excludes humans. Particular examples of non-human animals include rodents, such as mice and rats.

[00329] Non-human animals can be from any genetic background. For example, suitable mice can be from a 129 strain, a C57BL/6 strain, a mix of 129 and C57BL/6, a BALB/c strain, or a

Swiss Webster strain. Examples of 129 strains include 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/Svlm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, and 129T2. *See, e.g.*, Festing et al. (1999) *Mammalian Genome* 10:836, herein incorporated by reference in its entirety for all purposes. Examples of C57BL strains include C57BL/A, C57BL/An, C57BL/GrFa, C57BL/Kal_wN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. Suitable mice can also be from a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain (e.g., 50% 129 and 50% C57BL/6). Likewise, suitable mice can be from a mix of aforementioned 129 strains or a mix of aforementioned BL/6 strains (e.g., the 129S6 (129/SvEvTac) strain).

[00330] Similarly, rats can be from any rat strain, including, for example, an ACI rat strain, a Dark Agouti (DA) rat strain, a Wistar rat strain, a LEA rat strain, a Sprague Dawley (SD) rat strain, or a Fischer rat strain such as Fisher F344 or Fisher F6. Rats can also be obtained from a strain derived from a mix of two or more strains recited above. For example, a suitable rat can be from a DA strain or an ACI strain. The ACI rat strain is characterized as having black agouti, with white belly and feet and an RTI^{av1} haplotype. Such strains are available from a variety of sources including Harlan Laboratories. The Dark Agouti (DA) rat strain is characterized as having an agouti coat and an RTI^{av1} haplotype. Such rats are available from a variety of sources including Charles River and Harlan Laboratories. In some cases, suitable rats can be from an inbred rat strain. *See, e.g.*, US 2014/0235933, herein incorporated by reference in its entirety for all purposes.

VII. Methods for Modifying a Target Genomic Locus, Expressing Retinoschisin in a Cell, or Treating XLRS

[00331] Also provided herein are methods for modifying a target genomic locus or methods for expressing retinoschisin in a cell using the nucleic acid constructs comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus as provided herein. Also provided herein are methods for modifying a target genomic locus or methods for expressing retinoschisin in a cell using the nuclease agents (or nucleic acids encoding) disclosed herein in combination with the nucleic acid constructs comprising a retinoschisin coding sequence (i.e., encoding a

retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus as provided herein. Also provided herein are methods for modifying a target genomic locus using the nuclease agents (or nucleic acids encoding) disclosed herein.

[00332] The methods can be, for example, methods of modifying a target genomic locus (e.g., an endogenous *RS1* locus). Some such methods comprise administering a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus and cleaves the target genomic locus.

[00333] The methods can be, for example, methods of integrating a coding sequence for a retinoschisin protein or fragment thereof into a target genomic locus and expressing the retinoschisin protein or fragment thereof in a cell. Some such methods comprise administering a nucleic acid construct, vector, or lipid nanoparticle as described elsewhere herein to a cell. The coding sequence can then be integrated into the target genomic locus, and the retinoschisin protein or fragment thereof is expressed in the cell. The retinoschisin coding sequence can be operably linked to an endogenous promoter at the target genomic locus upon integration into the target genomic locus, or it can be operably linked to an exogenous promoter present in the nucleic acid construct. Some such methods comprise administering to a cell a nucleic acid construct, vector, or lipid nanoparticle as described elsewhere herein and a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus. The nuclease agent can cleave the target genomic locus, and the coding sequence from the nucleic acid construct can be integrated into the target genomic locus so that the retinoschisin protein or fragment thereof is expressed in the cell. Some such methods comprise administering to a cell a composition comprising a nucleic acid construct, vector, or lipid nanoparticle disclosed herein and a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus. The nuclease agent can cleave the target genomic locus, and the coding sequence from the nucleic acid construct can be integrated into the target genomic locus so that the retinoschisin protein or fragment thereof is expressed in the cell. The nuclease agent or nucleic acid encoding the nuclease agent can be administered, for example, in a vector (e.g., viral vector such as an AAV vector) or in a lipid nanoparticle.

[00334] The cells in the above methods can be isolated cells (e.g., *in vitro*) or can be *in vivo* within an animal. The cells can also be any type of undifferentiated or differentiated state. For

example, a cell can be a totipotent cell, a pluripotent cell (e.g., a human pluripotent cell or a non-human pluripotent cell such as a mouse embryonic stem (ES) cell or a rat ES cell), or a non-pluripotent cell. Totipotent cells include undifferentiated cells that can give rise to any cell type, and pluripotent cells include undifferentiated cells that possess the ability to develop into more than one differentiated cell types.

[00335] The cells can also be germ cells (e.g., sperm or oocytes). The cells can be mitotically competent cells or mitotically-inactive cells, meiotically competent cells or meiotically-inactive cells. Similarly, the cells can also be primary somatic cells or cells that are not a primary somatic cell. Somatic cells include any cell that is not a gamete, germ cell, gametocyte, or undifferentiated stem cell. For example, the cells can be liver cells, kidney cells, hematopoietic cells, endothelial cells, epithelial cells, fibroblasts, mesenchymal cells, keratinocytes, blood cells, melanocytes, monocytes, mononuclear cells, monocytic precursors, B cells, erythroid-megakaryocytic cells, eosinophils, macrophages, T cells, islet beta cells, exocrine cells, pancreatic progenitors, endocrine progenitors, adipocytes, preadipocytes, neurons, glial cells, neural stem cells, neurons, hepatoblasts, hepatocytes, cardiomyocytes, skeletal myoblasts, smooth muscle cells, ductal cells, acinar cells, alpha cells, beta cells, delta cells, PP cells, cholangiocytes, white or brown adipocytes, or ocular cells (e.g., trabecular meshwork cells, retinal pigment epithelial cells, retinal microvascular endothelial cells, retinal pericyte cells, conjunctival epithelial cells, conjunctival fibroblasts, iris pigment epithelial cells, keratocytes, lens epithelial cells, non-pigment ciliary epithelial cells, ocular choroid fibroblasts, photoreceptor cells, ganglion cells, bipolar cells, horizontal cells, or amacrine cells). For example, the cells can be eye cells, such as retinal cells (e.g., photoreceptors).

[00336] The cells provided herein can be normal, healthy cells, or can be diseased or mutant-bearing cells. For example, the cells can comprise one or more mutations associated with or that cause XLRS (e.g., R141C).

[00337] The cells can be, for example, eukaryotic, including, for example, animal, mammalian, non-human mammalian, and human. In a specific example, the cells are human cells. The term "animal" includes mammals, fishes, and birds. A mammal can be, for example, a non-human mammal, a human, a rodent, a rat, a mouse, or a hamster. Other non-human mammals include, for example, non-human primates, monkeys, apes, cats, dogs, rabbits, horses, bulls, deer, bison, livestock (e.g., bovine species such as cows, steer, and so forth; ovine species

such as sheep, goats, and so forth; and porcine species such as pigs and boars). Birds include, for example, chickens, turkeys, ostrich, geese, ducks, and so forth. Domesticated animals and agricultural animals are also included. The term "non-human" excludes humans.

[00338] The cells can be *in vivo* within an animal. The animal can be a human or can be a non-human animal. Non-human animals comprising a nucleic acid or expression cassette as described herein can be made by the methods described elsewhere herein. The term "animal" includes mammals, fishes, and birds. Mammals include, for example, humans, non-human primates, monkeys, apes, cats, dogs, horses, bulls, deer, bison, sheep, rabbits, rodents (e.g., mice, rats, hamsters, and guinea pigs), and livestock (e.g., bovine species such as cows and steer; ovine species such as sheep and goats; and porcine species such as pigs and boars). Birds include, for example, chickens, turkeys, ostrich, geese, and ducks. Domesticated animals and agricultural animals are also included. The term "non-human animal" excludes humans. Particular examples of non-human animals include rodents, such as mice and rats.

[00339] Non-human animals can be from any genetic background. For example, suitable mice can be from a 129 strain, a C57BL/6 strain, a mix of 129 and C57BL/6, a BALB/c strain, or a Swiss Webster strain. Examples of 129 strains include 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/Svlm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, and 129T2. *See, e.g.*, Festing et al. (1999) *Mammalian Genome* 10:836, herein incorporated by reference in its entirety for all purposes. Examples of C57BL strains include C57BL/A, C57BL/An, C57BL/GrFa, C57BL/Kal_wN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. Suitable mice can also be from a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain (e.g., 50% 129 and 50% C57BL/6). Likewise, suitable mice can be from a mix of aforementioned 129 strains or a mix of aforementioned BL/6 strains (e.g., the 129S6 (129/SvEvTac) strain).

[00340] Similarly, rats can be from any rat strain, including, for example, an ACI rat strain, a Dark Agouti (DA) rat strain, a Wistar rat strain, a LEA rat strain, a Sprague Dawley (SD) rat strain, or a Fischer rat strain such as Fisher F344 or Fisher F6. Rats can also be obtained from a strain derived from a mix of two or more strains recited above. For example, a suitable rat can be from a DA strain or an ACI strain. The ACI rat strain is characterized as having black agouti, with white belly and feet and an $RT1^{av1}$ haplotype. Such strains are available from a variety of

sources including Harlan Laboratories. The Dark Agouti (DA) rat strain is characterized as having an agouti coat and an *RT1*^{av1} haplotype. Such rats are available from a variety of sources including Charles River and Harlan Laboratories. In some cases, suitable rats can be from an inbred rat strain. *See, e.g.*, US 2014/0235933, herein incorporated by reference in its entirety for all purposes.

[00341] Also provided are methods of treating a subject with X-linked juvenile retinoschisis (XLRS). Such methods can comprise administering a nucleic acid construct, vector, or lipid nanoparticle as described elsewhere herein to a subject with XLRS, wherein the nucleic acid construct is integrated into and expressed from the target genomic locus in one or more retinal cells (e.g., photoreceptors) in the subject, and a therapeutically effective level of retinoschisin expression is achieved in the subject. The subject can have, for example, an endogenous *RS1* gene comprising at least one mutation associated with or that causes X-linked juvenile retinoschisis (e.g., R141C). The retinoschisin coding sequence can be operably linked to an endogenous promoter at the target genomic locus upon integration into the target genomic locus, or it can be operably linked to an exogenous promoter present in the nucleic acid construct. Such methods can also comprise administering to the subject a nucleic acid construct, vector, or lipid nanoparticle as described elsewhere herein and a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus, the nucleic acid construct is integrated into and expressed from the target genomic locus in one or more retinal cells (e.g., photoreceptors) in the subject, and a therapeutically effective level of retinoschisin expression is achieved in the subject. Such methods can also comprise administering to the subject a composition comprising a nucleic acid construct, vector, or lipid nanoparticle disclosed herein and a nuclease agent or a nucleic acid encoding the nuclease agent, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus, the nucleic acid construct is integrated into and expressed from the target genomic locus in one or more retinal cells (e.g., photoreceptors) in the subject, and a therapeutically effective level of retinoschisin expression is achieved in the subject.

[00342] XLRS is a vitreo-retinal dystrophy characterized by macular pathology and by splitting of the superficial layer of the retina. Macular changes are present in almost all cases. In the fundi, radially oriented intraretinal foveomacular cysts are seen in a spoke-wheel configuration, with the absence of foveal reflex in most cases. In addition, approximately half of

cases have bilateral peripheral retinoschisis in the inferotemporal part of the retina. Aside from the typical fundus appearance, strabismus, nystagmus, axial hyperopia, defective color vision and foveal ectopy can be present. The most important complications are vitreous hemorrhage, retinal detachment, and neovascular glaucoma. Foveal schisis with "spoke-wheel" pattern is the characteristic finding on fundus exam, present in nearly 100% of cases. Schisis may occur peripherally in up to 50% of patients. Peripheral schisis can lead to holes and tears of the inner leaf with potential for hemorrhage from unsupported crossing vessels. Additional peripheral changes include pigmentation resembling RP, retinal fibrosis and white flecks.

[00343] Because XLRS is a recessive disease caused by the loss in retinoschisin function, gene replacement therapy has been considered as a potential treatment for this disease. Furthermore, because retinoschisin functions as an extracellular protein, beneficial treatment is not necessarily restricted to the transfected cell expressing the replacement gene but can encompass a wider area due to the spread of the secreted protein from the site of expression.

[00344] In some methods, integration of the nucleic acid construct results in retinal structural restoration (e.g., at least partial retinal structural restoration). In some methods, integration of the nucleic acid construct results in retinal functional restoration (e.g., at least partial retinal functional restoration).

[00345] The subject with XLRS in the above methods can be any type of animal as disclosed above. In a particular example, the subject is a human.

[00346] In methods targeting a cell *in vivo* in an animal, the nucleic acid construct can be inserted in particular types of cells in the animal. The method and vehicle for introducing the nuclease agent (or nucleic acid encoding the nuclease agent) and the nucleic acid construct into the animal can affect which types of cells in the animal are targeted. In some methods, for example, the nucleic acid construct is inserted into a target genomic locus (e.g., an endogenous *RS1* locus) in retinal cells, such as photoreceptors. Methods and vehicles for introducing the nuclease agent (or nucleic acid encoding the nuclease agent) and the nucleic acid construct into the animal (including methods and vehicles that target the eye or retinal cells (e.g., photoreceptors), such as lipid nanoparticle-mediated delivery and AAV2-mediated delivery, AAV5-mediated delivery, AAV8-mediated delivery, or AAV7m8-mediated delivery and intravitreal or subretinal injection), are disclosed in more detail elsewhere herein.

[00347] Any target genomic locus capable of expressing a gene can be used, such as a safe

harbor locus (safe harbor gene) or an endogenous *RS1* locus. Such loci are described in more detail elsewhere herein. In a specific example, the target genomic locus can be an endogenous *RS1* locus, such as an endogenous *RS1* locus comprising one or more mutations associated with or that cause XLRS (e.g., R141C). For example, the nucleic acid construct can be genomically integrated in intron 1 of the endogenous *RS1* locus. Endogenous *RS1* exon 1 can then splice into the coding sequence for the retinoschisin protein or fragment or variant thereof in the nucleic acid construct. In a specific example, the modified *RS1* locus comprising the genomically integrated nucleic acid construct encodes a protein comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 2 or 4. In a specific example, the modified *RS1* locus comprising the genomically integrated nucleic acid construct comprises an *RS1* coding sequence comprising, consisting essentially of, or consisting of a sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 6, 11, or 12.

[00348] Integration of the nucleic acid construct into the endogenous *RS1* locus can, in some cases, prevent transcription of the endogenous *RS1* gene downstream of the integration site. The integration of the nucleic acid construct into the endogenous *RS1* locus can reduce or eliminate expression of the endogenous retinoschisin protein and replace it with expression of the retinoschisin protein or fragment or variant thereof encoded by the nucleic acid construct. In one example, integration of the nucleic acid construct into the endogenous *RS1* locus reduces expression of the endogenous retinoschisin protein. In another example, integration of the nucleic acid construct into the endogenous *RS1* locus eliminates expression of the endogenous retinoschisin protein. In methods targeting cells *in vivo*, such as methods of treating a subject with XLRS, the integration of the nucleic acid construct into the endogenous *RS1* locus in one or more cells can reduce or eliminate expression of the endogenous retinoschisin protein in those cells and replace it with expression of the retinoschisin protein or fragment or variant thereof encoded by the nucleic acid construct. In this way, integration of the nucleic acid construct can simultaneously knock out the endogenous *RS1* gene (e.g., an endogenous *RS1* gene comprising one or more mutations associated with or that cause XLRS, such as R141C) and knock in a

replacement retinoschisin coding sequence (e.g., a replacement retinoschisin coding sequence that does not comprise mutations associated with or that cause XLRS). In other methods, however, an endogenous *RS1* gene comprising one or more mutations associated with or that cause XLRS can first be inactivated (e.g., using one or more nuclease agents that target and disrupt the endogenous *RS1* gene), and a nucleic acid construct comprising a replacement retinoschisin coding sequence (e.g., a replacement retinoschisin coding sequence that does not comprise mutations associated with or that cause XLRS) can subsequently be integrated into a target genomic locus. In other methods, the nucleic acid construct can be integrated first, and the endogenous *RS1* gene can subsequently be inactivated.

[00349] Targeted insertion of the nucleic acid construct comprising the retinoschisin coding sequence into a target genomic locus, and particularly an endogenous *RS1* locus, offers multiple advantages. Such methods result in stable modification to allow for stable, long-term expression of the retinoschisin coding sequence. With respect to the *RS1* locus, such methods are able to utilize the endogenous *RS1* promoter and regulatory regions to achieve physiologically relevant expression (level of expression, timing of expression, and location of expression). For example, the retinoschisin coding sequence in the nucleic acid construct can comprise a promoterless gene, and the inserted nucleic acid construct can be operably linked to an endogenous promoter in the target genomic locus (e.g., *RS1* locus). Use of an endogenous promoter is advantageous because it obviates the need for inclusion of a promoter in the nucleic acid construct, allowing packaging of larger transgenes that may not normally package efficiently (e.g., in AAV). Alternatively, the retinoschisin coding sequence in the nucleic acid construct can be operably linked to an exogenous promoter in the nucleic acid construct. Examples of types of promoters that can be used are disclosed elsewhere herein.

[00350] Optionally, some or all of the endogenous gene (e.g., endogenous *RS1* gene) at the target genomic locus can be expressed upon insertion of the retinoschisin coding sequence from the nucleic acid construct. Alternatively, in some methods, none of the endogenous gene at the target genomic locus is expressed. As one example, the modified target genomic locus (e.g., modified *RS1* locus) after integration of the nucleic acid construct can encode a chimeric protein comprising an endogenous secretion signal or a fragment thereof and the retinoschisin protein encoded by the nucleic acid construct. In another example, the first intron of an *RS1* locus can be targeted. The secretion signal peptide of *RS1* is encoded by exon 1 and part of exon 2 of the *RS1*

gene. In such a scenario, a promoterless cassette bearing a splice acceptor and the retinoschisin coding sequence will support expression and secretion of the retinoschisin protein. Splicing between endogenous *RS1* exon 1 and the integrated retinoschisin coding sequence creates a chimeric mRNA and protein including the endogenous retinoschisin sequence encoded by exon 1 operably linked to the retinoschisin protein sequence encoded by the integrated nucleic acid construct.

[00351] The retinoschisin coding sequence in the nucleic acid construct can be inserted into the target genomic locus by any means, including homologous recombination (HR) and non-homologous end joining (NHEJ) as described elsewhere herein.

[00352] In a specific example, the nucleic acid construct can be inserted via homology-independent targeted integration (e.g., directional homology-independent targeted integration). For example, the retinoschisin coding sequence in the nucleic acid construct can be flanked on each side by a target site for a nuclease agent (e.g., the same target site as in the target genomic locus, and the same nuclease agent being used to cleave the target site in the target genomic locus). The nuclease agent can then cleave the target sites flanking the retinoschisin coding sequence. In a specific example, the nucleic acid construct is delivered AAV-mediated delivery, and cleavage of the target sites flanking the retinoschisin coding sequence can remove the inverted terminal repeats (ITRs) of the AAV. Removal of the ITRs can make it easier to assess successful targeting, because presence of the ITRs can hamper sequencing efforts due to the repeated sequences. In some methods, the target site in the target genomic locus (e.g., a gRNA target sequence including the flanking protospacer adjacent motif) is no longer present if the retinoschisin coding sequence is inserted into the target genomic locus in the correct orientation but it is reformed if the retinoschisin protein coding sequence is inserted into the target genomic locus in the opposite orientation. This can help ensure that the retinoschisin coding sequence is inserted in the correct orientation for expression.

[00353] In methods in which a nucleic acid construct (or vector or LNP) and a nuclease agent or nucleic acid encoding a nuclease agent are administered together with a nucleic acid construct, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent can be administered simultaneously. Alternatively, the nucleic acid construct and the nuclease agent or the nucleic acid encoding the nuclease agent can be administered sequentially in any order. For example, the nucleic acid construct can be administered after the nuclease agent or the

nucleic acid encoding the nuclease agent, or the nuclease agent or the nucleic acid encoding the nuclease agent can be administered after the nucleic acid construct. For example, the nuclease agent or the nucleic acid encoding the nuclease agent can be administered about 1 hour to about 48 hours, about 1 hour to about 24 hours, about 1 hour to about 12 hours, about 1 hour to about 6 hours, about 1 hour to about 2 hours, about 2 hours to about 48 hours, about 2 hours to about 24 hours, about 2 hours to about 12 hours, about 2 hours to about 6 hours, about 3 hours to about 48 hours, about 6 hours to about 48 hours, about 12 hours to about 48 hours, or about 24 hours to about 48 hours prior to or subsequent to administration of the nucleic acid construct.

[00354] The nucleic acid construct and the nuclease agent or nucleic acid encoding a nuclease agent can be administered in any suitable delivery vehicle. In some methods, the nucleic acid construct and the nuclease agent or nucleic acid encoding a nuclease agent can be administered in the same delivery vehicle (e.g., the same lipid nanoparticle or vector). In some methods in which the nuclease agent comprises a Cas protein or a nucleic acid encoding a Cas protein and a guide RNA or a DNA encoding a guide RNA, both components can be administered in the same delivery vehicle, or alternatively they can be delivered in separate delivery vehicles. In other methods, the nucleic acid construct and the nuclease agent or nucleic acid encoding a nuclease agent can be administered in different delivery vehicles (e.g., administration of the nuclease agent or the nucleic acid encoding the nuclease agent in a first vector or in an LNP, and administration of the nucleic acid construct in a second vector, such as an AAV vector).

[00355] The nuclease agent (or nucleic acid encoding the nuclease agent) and the nucleic acid construct can be introduced in any form (e.g., DNA or RNA for guide RNAs; DNA, RNA, or protein for Cas proteins) via any delivery method (e.g., AAV, LNP, or HDD) and any route of administration (e.g., intravitreal injection or subretinal injection) as disclosed elsewhere herein. As one example, the nucleic acid construct is delivered via adeno-associated virus (AAV)-mediated delivery (e.g., AAV2-mediated delivery, AAV5-mediated delivery, AAV8-mediated delivery, or AAV7m8-mediated delivery). Likewise, the nuclease agent (or nucleic acid encoding the nuclease agent) can be delivered by lipid nanoparticle (LNP)-mediated delivery or AAV-mediated delivery. For example, the nuclease agent can be CRISPR/Cas9, and a Cas9 mRNA and a gRNA targeting an endogenous *RS1* locus (e.g., intron 1 of *RS1*) can be delivered via LNP-mediated delivery or AAV-mediated delivery, and the nucleic acid construct (e.g., a bidirectional construct or a homology-independent targeted integration construct) can be

delivered via AAV2-mediated delivery, AAV5-mediated delivery, AAV8-mediated delivery, or AAV7m8-mediated delivery. In another specific example, both the nucleic acid encoding the nuclease agent and the nucleic acid construct are delivered via AAV-mediated delivery (e.g., in a single AAV or via two separate AAVs). For example, a first AAV can carry a Cas9 expression cassette, and a second AAV can carry a gRNA expression cassette and the nucleic acid construct. Similarly, a first AAV can carry a Cas9 expression cassette, and a second AAV can carry two or more gRNA expression cassettes and the nucleic acid construct. Alternatively, a first AAV can carry a Cas9 expression cassette (e.g., Cas9 coding sequence operably linked to a promoter) and a gRNA expression cassette (e.g., gRNA coding sequence operably linked to a promoter), and the second AAV can carry the nucleic acid construct. Similarly, a first AAV can carry a Cas9 expression cassette (e.g., Cas9 coding sequence operably linked to a promoter) and two gRNA expression cassettes (e.g., gRNA coding sequences operably linked to promoters), and the second AAV can carry the nucleic acid construct. Different promoters can be used to drive expression of the gRNA, such as a U6 promoter or the small tRNA Gln. Likewise, different promoters can be used to drive Cas9 expression. In some methods, small promoters are used so that the Cas9 coding sequence can fit into an AAV construct. In some methods, small Cas9 proteins (e.g., SaCas9 or CjCas9 are used to maximize the AAV packaging capacity).

[00356] The methods disclosed herein comprise administering or introducing into a cell or animal nuclease agents (or nucleic acids encoding nuclease agents) and nucleic acid constructs (i.e., exogenous donor nucleic acids) comprising a retinoschisin coding sequence (i.e., encoding a retinoschisin protein or fragment or variant thereof) for integration into and expression from a target genomic locus. "Introducing" includes presenting to the cell or animal the nucleic acid or protein in such a manner that the nucleic acid or protein gains access to the interior of the cell or to the interior of cells within the animal. The introducing can be accomplished by any means, and two or more of the components (e.g., two of the components, or all of the components) can be introduced into the cell or animal simultaneously or sequentially in any combination. For example, a nuclease agent can be introduced into a cell or animal before introduction of the nucleic acid construct. In addition, two or more of the components can be introduced into the cell or animal by the same delivery method or different delivery methods. Similarly, two or more of the components can be introduced into an animal by the same route of administration or different routes of administration.

[00357] A guide RNA can be introduced into the cell in the form of an RNA (e.g., *in vitro* transcribed RNA) or in the form of a DNA encoding the guide RNA. Likewise, protein components such as Cas9 proteins, ZFNs, or TALENs can be introduced into the cell in the form of DNA, RNA, or protein. For example, a guide RNA and a Cas9 protein can both be introduced in the form of RNA. When introduced in the form of a DNA, the DNA encoding a guide RNA can be operably linked to a promoter active in the cell. For example, a guide RNA may be delivered via AAV and expressed *in vivo* under a U6 promoter. Such DNAs can be in one or more expression constructs. For example, such expression constructs can be components of a single nucleic acid molecule. Alternatively, they can be separated in any combination among two or more nucleic acid molecules (i.e., DNAs encoding one or more CRISPR RNAs and DNAs encoding one or more tracrRNAs can be components of a separate nucleic acid molecules).

[00358] Nucleic acids encoding guide RNAs or nuclease agents can be operably linked to a promoter in an expression construct. Expression constructs include any nucleic acid constructs capable of directing expression of a gene or other nucleic acid sequence of interest and which can transfer such a nucleic acid sequence of interest to a target cell. Suitable promoters that can be used in an expression construct include promoters active, for example, in one or more of a eukaryotic cell, a human cell, a non-human cell, a mammalian cell, a non-human mammalian cell, a rodent cell, a mouse cell, a rat cell, a hamster cell, a rabbit cell, a pluripotent cell, an embryonic stem (ES) cell, an adult stem cell, a developmentally restricted progenitor cell, an induced pluripotent stem (iPS) cell, or a one-cell stage embryo. Such promoters can be, for example, conditional promoters, inducible promoters, constitutive promoters, or tissue-specific promoters. Optionally, the promoter can be a bidirectional promoter driving expression of both a guide RNA in one direction and another component in the other direction. Such bidirectional promoters can consist of (1) a complete, conventional, unidirectional Pol III promoter that contains 3 external control elements: a distal sequence element (DSE), a proximal sequence element (PSE), and a TATA box; and (2) a second basic Pol III promoter that includes a PSE and a TATA box fused to the 5′ terminus of the DSE in reverse orientation. For example, in the H1 promoter, the DSE is adjacent to the PSE and the TATA box, and the promoter can be rendered bidirectional by creating a hybrid promoter in which transcription in the reverse direction is controlled by appending a PSE and TATA box derived from the U6 promoter. *See, e.g.*, US 2016/0074535, herein incorporated by references in its entirety for all purposes. Use of a

bidirectional promoter to express genes encoding a guide RNA and another component simultaneously allows for the generation of compact expression cassettes to facilitate delivery.

[00359] Guide RNAs or nucleic acids encoding guide RNAs (or other components) can be provided in compositions comprising a carrier increasing the stability of the guide RNA (e.g., prolonging the period under given conditions of storage (e.g., -20°C, 4°C, or ambient temperature) for which degradation products remain below a threshold, such below 0.5% by weight of the starting nucleic acid or protein; or increasing the stability in vivo). Non-limiting examples of such carriers include poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules.

[00360] The methods provided herein do not depend on a particular method for introducing a nucleic acid or protein into the cell, only that the nucleic acid or protein gains access to the interior of a least one cell. Methods for introducing nucleic acids and proteins into various cell types are known in the art and include, for example, stable transfection methods, transient transfection methods, and virus-mediated methods.

[00361] Transfection protocols as well as protocols for introducing nucleic acids or proteins into cells may vary. Non-limiting transfection methods include chemical-based transfection methods using liposomes; nanoparticles; calcium phosphate (Graham et al. (1973) *Virology* 52 (2): 456–67, Bacchetti et al. (1977) *Proc. Natl. Acad. Sci. USA* 74 (4): 1590–4, and Kriegler, M (1991). Transfer and Expression: A Laboratory Manual. New York: W. H. Freeman and Company. pp. 96–97); dendrimers; or cationic polymers such as DEAE-dextran or polyethylenimine. Non-chemical methods include electroporation, sonoporation, and optical transfection. Particle-based transfection includes the use of a gene gun, or magnet-assisted transfection (Bertram (2006) *Current Pharmaceutical Biotechnology* 7, 277–28). Viral methods can also be used for transfection.

[00362] Introduction of nucleic acids or proteins into a cell can also be mediated by electroporation, by intracytoplasmic injection, by viral infection, by adenovirus, by lentivirus, by retrovirus, by transfection, by lipid-mediated transfection, or by nucleofection. Introduction of nucleic acids or proteins into a cell can also be mediated by adeno-associated virus. Nucleofection is an improved electroporation technology that enables nucleic acid substrates to be delivered not only to the cytoplasm but also through the nuclear membrane and into the

nucleus. In addition, use of nucleofection in the methods disclosed herein typically requires much fewer cells than regular electroporation (e.g., only about 2 million compared with 7 million by regular electroporation). In one example, nucleofection is performed using the LONZA® NUCLEOFECTOR™ system.

[00363] Introduction of nucleic acids or proteins into a cell (e.g., a one-cell stage embryo) can also be accomplished by microinjection. In one-cell stage embryos, microinjection can be into the maternal and/or paternal pronucleus or into the cytoplasm. If the microinjection is into only one pronucleus, the paternal pronucleus is preferable due to its larger size. Microinjection of an mRNA is preferably into the cytoplasm (e.g., to deliver mRNA directly to the translation machinery), while microinjection of a Cas protein or a nucleic acid encoding a Cas protein or encoding an RNA is preferable into the nucleus/pronucleus. Alternatively, microinjection can be carried out by injection into both the nucleus/pronucleus and the cytoplasm: a needle can first be introduced into the nucleus/pronucleus and a first amount can be injected, and while removing the needle from the one-cell stage embryo a second amount can be injected into the cytoplasm. If a Cas protein is injected into the cytoplasm, the Cas protein preferably comprises a nuclear localization signal to ensure delivery to the nucleus/pronucleus. Methods for carrying out microinjection are well known. *See, e.g.*, Nagy et al. (Nagy A, Gertsenstein M, Vintersten K, Behringer R., 2003, Manipulating the Mouse Embryo. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press); Meyer et al. (2010) *Proc. Natl. Acad. Sci. USA* 107:15022-15026 and Meyer et al. (2012) *Proc. Natl. Acad. Sci. USA* 109:9354-9359. Introduction into one-cell stage embryos can also be accomplished by electroporation.

[00364] Various methods and compositions are provided herein to allow for introduction of a nucleic acid or protein into a cell or animal. Such methods for introducing nucleic acid or proteins into a cell or animal can include, for example, vector delivery, particle-mediated delivery, exosome-mediated delivery, lipid-nanoparticle (LNP)-mediated delivery, cell-penetrating-peptide-mediated delivery, or implantable-device-mediated delivery. As specific examples, a nucleic acid or protein can be introduced into a cell or animal in a carrier such as a poly(lactic acid) (PLA) microsphere, a poly(D,L-lactic-coglycolic-acid) (PLGA) microsphere, a liposome, a micelle, an inverse micelle, a lipid cochleate, or a lipid microtubule. Some specific examples of delivery to an animal include hydrodynamic delivery, virus-mediated delivery (e.g., adeno-associated virus (AAV)-mediated delivery, or by adenovirus, by lentivirus, or by

retrovirus), and lipid-nanoparticle-mediated delivery. In one specific example, both the nuclease agent and nucleic acid construct can be delivered via LNP-mediated delivery. In another specific example, both the nuclease agent and nucleic acid construct can be delivered via AAV-mediated delivery. For example, the nuclease agent and the nucleic acid construct can be delivered via multiple different AAV vectors (e.g., two different AAV vectors). In a specific example in which the nuclease agent is CRISPR/Cas (e.g., CRISPR/Cas9), a first AAV vector can deliver the Cas (e.g., Cas9), and a second AAV vector can deliver the gRNA and the nucleic acid construct. For example, small promoters can be used so that the Cas9 coding sequence can fit into an AAV construct.

[00365] In another specific example, the nuclease agent can be delivered via LNP-mediated delivery and the nucleic acid construct can be delivered via AAV-mediated delivery. In another specific example, the nuclease agent can be delivered via AAV-mediated delivery and the nucleic acid construct can be delivered via LNP-mediated delivery.

[00366] Introduction of nucleic acids can also be accomplished by virus-mediated delivery, such as AAV-mediated delivery or lentivirus-mediated delivery (e.g., an AAV vector or a lentiviral vector). Other exemplary viruses/viral vectors include retroviruses, adenoviruses, vaccinia viruses, poxviruses, and herpes simplex viruses. The viruses can infect dividing cells, non-dividing cells, or both dividing and non-dividing cells. The viruses can integrate into the host genome or alternatively do not integrate into the host genome. Such viruses can also be engineered to have reduced immunity. The viruses can be replication-competent or can be replication-defective (e.g., defective in one or more genes necessary for additional rounds of virion replication and/or packaging). Viruses can cause transient expression, long-lasting expression (e.g., at least 1 week, 2 weeks, 1 month, 2 months, or 3 months), or permanent expression (e.g., of Cas9 and/or gRNA). Exemplary viral titers (e.g., AAV titers) include 10^{12}, 10^{13}, 10^{14}, 10^{15}, and 10^{16} vector genomes/mL. Exemplary viral titers (e.g., AAV titers) include about 10^{12}, about 10^{13}, about 10^{14}, about 10^{15}, and about 10^{16} vector genomes (vg)/mL, or between about 10^{12} to about 10^{16}, between about 10^{12} to about 10^{15}, between about 10^{12} to about 10^{14}, between about 10^{12} to about 10^{13}, between about 10^{13} to about 10^{16}, between about 10^{14} to about 10^{16}, between about 10^{15} to about 10^{16}, or between about 10^{13} to about 10^{15} vg/mL. Other exemplary viral titers (e.g., AAV titers) include about 10^{12}, about 10^{13}, about 10^{14}, about 10^{15}, and about 10^{16} vector genomes (vg)/kg of body weight, or between about 10^{12} to about 10^{16},

between about 10^{12} to about 10^{15}, between about 10^{12} to about 10^{14}, between about 10^{12} to about 10^{13}, between about 10^{13} to about 10^{16}, between about 10^{14} to about 10^{16}, between about 10^{15} to about 10^{16}, or between about 10^{13} to about 10^{15} vg/kg of body weight.

[00367] The ssDNA AAV genome consists of two open reading frames, Rep and Cap, flanked by two inverted terminal repeats that allow for synthesis of the complementary DNA strand. When constructing an AAV transfer plasmid, the transgene is placed between the two ITRs, and Rep and Cap can be supplied *in trans*. In addition to Rep and Cap, AAV can require a helper plasmid containing genes from adenovirus. These genes (E4, E2a, and VA) mediate AAV replication. For example, the transfer plasmid, Rep/Cap, and the helper plasmid can be transfected into HEK293 cells containing the adenovirus gene E1+ to produce infectious AAV particles. Alternatively, the Rep, Cap, and adenovirus helper genes may be combined into a single plasmid. Similar packaging cells and methods can be used for other viruses, such as retroviruses.

[00368] Multiple serotypes of AAV have been identified. These serotypes differ in the types of cells they infect (i.e., their tropism), allowing preferential transduction of specific cell types. Serotypes for photoreceptor cells include AAV2, AAV5, and AAV8. Serotypes for retinal pigment epithelium tissue include AAV1, AAV2, AAV4, AAV5, and AAV8. In a specific example, the AAV vector comprising the nucleic acid construct can be AAV2, AAV5, or AAV8.

[00369] Tropism can be further refined through pseudotyping, which is the mixing of a capsid and a genome from different viral serotypes. For example AAV2/5 indicates a virus containing the genome of serotype 2 packaged in the capsid from serotype 5. Use of pseudotyped viruses can improve transduction efficiency, as well as alter tropism. Hybrid capsids derived from different serotypes can also be used to alter viral tropism. For example, AAV-DJ contains a hybrid capsid from eight serotypes and displays high infectivity across a broad range of cell types *in vivo*. AAV-DJ8 is another example that displays the properties of AAV-DJ but with enhanced brain uptake. AAV serotypes can also be modified through mutations. Examples of mutational modifications of AAV2 include Y444F, Y500F, Y730F, and S662V. Examples of mutational modifications of AAV3 include Y705F, Y731F, and T492V. Examples of mutational modifications of AAV6 include S663V and T492V. Other pseudotyped/modified AAV variants include AAV2/1, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV2.5, AAV8.2, and AAV/SASTG. In a specific example, the AAV is AAV7m8, an AAV variant that mediates highly efficient

delivery to all retinal layers and to photoreceptors. *See, e.g.*, Dalkara et al. (2013) *Sci. Transl. Med.* 5:189ra76, herein incorporated by reference in its entirety for all purposes.

[00370] To accelerate transgene expression, self-complementary AAV (scAAV) variants can be used. Because AAV depends on the cell's DNA replication machinery to synthesize the complementary strand of the AAV's single-stranded DNA genome, transgene expression may be delayed. To address this delay, scAAV containing complementary sequences that are capable of spontaneously annealing upon infection can be used, eliminating the requirement for host cell DNA synthesis. However, single-stranded AAV (ssAAV) vectors can also be used.

[00371] To increase packaging capacity, longer transgenes may be split between two AAV transfer plasmids, the first with a 3' splice donor and the second with a 5' splice acceptor. Upon co-infection of a cell, these viruses form concatemers, are spliced together, and the full-length transgene can be expressed. Although this allows for longer transgene expression, expression is less efficient. Similar methods for increasing capacity utilize homologous recombination. For example, a transgene can be divided between two transfer plasmids but with substantial sequence overlap such that co-expression induces homologous recombination and expression of the full-length transgene.

[00372] In certain AAVs, the cargo can include a nuclease agent (i.e., nucleic acid encoding a nuclease agent). In certain AAVs, the cargo can include a guide RNA or a nucleic acid encoding a guide RNA. In certain AAVs, the cargo can include an mRNA encoding a Cas nuclease, such as Cas9, and a guide RNA or a nucleic acid encoding a guide RNA. In certain AAVs, the cargo can include a nucleic acid construct as described elsewhere herein. In certain AAVs, the cargo can include a nuclease agent and a nucleic acid construct as described elsewhere herein. In certain AAVs, the cargo can include an mRNA encoding a Cas nuclease, such as Cas9, a guide RNA or a nucleic acid encoding a guide RNA, and a nucleic acid construct as described elsewhere herein.

[00373] Introduction of nucleic acids and proteins can also be accomplished by lipid nanoparticle (LNP)-mediated delivery. For example, LNP-mediated delivery can be used to deliver a guide RNA in the form of RNA. Delivery through such methods results in transient presence of the guide RNA, and the biodegradable lipids improve clearance, improve tolerability, and decrease immunogenicity. Lipid formulations can protect biological molecules from degradation while improving their cellular uptake. Lipid nanoparticles are particles

comprising a plurality of lipid molecules physically associated with each other by intermolecular forces. These include microspheres (including unilamellar and multilamellar vesicles, e.g., liposomes), a dispersed phase in an emulsion, micelles, or an internal phase in a suspension. Such lipid nanoparticles can be used to encapsulate one or more nucleic acids or proteins for delivery. Formulations which contain cationic lipids are useful for delivering polyanions such as nucleic acids. Other lipids that can be included are neutral lipids (i.e., uncharged or zwitterionic lipids), anionic lipids, helper lipids that enhance transfection, and stealth lipids that increase the length of time for which nanoparticles can exist *in vivo*. Examples of suitable cationic lipids, neutral lipids, anionic lipids, helper lipids, and stealth lipids can be found in WO 2016/010840 A1 and WO 2017/173054 A1, each of which is herein incorporated by reference in its entirety for all purposes. An exemplary lipid nanoparticle can comprise a cationic lipid and one or more other components. In one example, the other component can comprise a helper lipid such as cholesterol. In another example, the other components can comprise a helper lipid such as cholesterol and a neutral lipid such as DSPC. In another example, the other components can comprise a helper lipid such as cholesterol, an optional neutral lipid such as DSPC, and a stealth lipid such as S010, S024, S027, S031, or S033.

[00374] The LNP may contain one or more or all of the following: (i) a lipid for encapsulation and for endosomal escape; (ii) a neutral lipid for stabilization; (iii) a helper lipid for stabilization; and (iv) a stealth lipid. *See, e.g.*, Finn et al. (2018) *Cell Rep.* 22(9):2227-2235 and WO 2017/173054 A1, each of which is herein incorporated by reference in its entirety for all purposes. In certain LNPs, the cargo can include a nuclease agent. In certain LNPs, the cargo can include a guide RNA or a nucleic acid encoding a guide RNA. In certain LNPs, the cargo can include an mRNA encoding a Cas nuclease, such as Cas9, and a guide RNA or a nucleic acid encoding a guide RNA. In certain LNPs, the cargo can include an exogenous donor sequence. In certain LNPs, the cargo can include a nuclease agent and an exogenous donor sequence. In certain LNPs, the cargo can include an mRNA encoding a Cas nuclease, such as Cas9, a guide RNA or a nucleic acid encoding a guide RNA, and an exogenous donor sequence.

[00375] The lipid for encapsulation and endosomal escape can be a cationic lipid. The lipid can also be a biodegradable lipid, such as a biodegradable ionizable lipid. One example of a suitable lipid is Lipid A or LP01, which is (9*Z*,12*Z*)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-

bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. *See, e.g.*, Finn et al. (2018) *Cell Rep.* 22(9):2227-2235 and WO 2017/173054 A1, each of which is herein incorporated by reference in its entirety for all purposes. Another example of a suitable lipid is Lipid B, which is ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate), also called ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate). Another example of a suitable lipid is Lipid C, which is 2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1,3-diyl(9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate). Another example of a suitable lipid is Lipid D, which is 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl 3-octylundecanoate. Other suitable lipids include heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (also known as Dlin-MC3-DMA (MC3))).

[00376] Some such lipids suitable for use in the LNPs described herein are biodegradable *in vivo*. For example, LNPs comprising such a lipid include those where at least 75% of the lipid is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. As another example, at least 50% of the LNP is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days.

[00377] Such lipids may be ionizable depending upon the pH of the medium they are in. For example, in a slightly acidic medium, the lipids may be protonated and thus bear a positive charge. Conversely, in a slightly basic medium, such as, for example, blood where pH is approximately 7.35, the lipids may not be protonated and thus bear no charge. In some embodiments, the lipids may be protonated at a pH of at least about 9, 9.5, or 10. The ability of such a lipid to bear a charge is related to its intrinsic pKa. For example, the lipid may, independently, have a pKa in the range of from about 5.8 to about 6.2.

[00378] Neutral lipids function to stabilize and improve processing of the LNPs. Examples of suitable neutral lipids include a variety of neutral, uncharged or zwitterionic lipids. Examples of neutral phospholipids suitable for use in the present disclosure include, but are not limited to, 5-heptadecylbenzene-1,3-diol (resorcinol), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), phosphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine (PLPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine

(EPC), dilauryloylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DBPC), 1-stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine, and combinations thereof. For example, the neutral phospholipid may be selected from the group consisting of distearoylphosphatidylcholine (DSPC) and dimyristoyl phosphatidyl ethanolamine (DMPE).

[00379] Helper lipids include lipids that enhance transfection. The mechanism by which the helper lipid enhances transfection can include enhancing particle stability. In certain cases, the helper lipid can enhance membrane fusogenicity. Helper lipids include steroids, sterols, and alkyl resorcinols. Examples of suitable helper lipids suitable include cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. In one example, the helper lipid may be cholesterol or cholesterol hemisuccinate.

[00380] Stealth lipids include lipids that alter the length of time the nanoparticles can exist in vivo. Stealth lipids may assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. Stealth lipids may modulate pharmacokinetic properties of the LNP. Suitable stealth lipids include lipids having a hydrophilic head group linked to a lipid moiety.

[00381] The hydrophilic head group of stealth lipid can comprise, for example, a polymer moiety selected from polymers based on PEG (sometimes referred to as poly(ethylene oxide)), poly(oxazoline), poly(vinyl alcohol), poly(glycerol), poly(N- vinylpyrrolidone), polyaminoacids, and poly N-(2-hydroxypropyl)methacrylamide. The term PEG means any polyethylene glycol or other polyalkylene ether polymer. In certain LNP formulations, the PEG, is a PEG-2K, also termed PEG 2000, which has an average molecular weight of about 2,000 daltons. *See, e.g.,* WO 2017/173054 A1, herein incorporated by reference in its entirety for all purposes.

[00382] The lipid moiety of the stealth lipid may be derived, for example, from diacylglycerol

or diacylglycamide, including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C4 to about C40 saturated or unsaturated carbon atoms, wherein the chain may comprise one or more functional groups such as, for example, an amide or ester. The dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups.

[00383] As one example, the stealth lipid may be selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG), PEG-dipalmitoylglycerol, PEG-distearoylglycerol (PEG-DSPE), PEG-dilaurylglycamide, PEG- dimyristylglycamide, PEG-dipalmitoylglycamide, and PEG-distearoylglycamide, PEG- cholesterol (l-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-sn- glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k- DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE), 1,2-distearoyl-sn-glycerol, methoxypoly ethylene glycol (PEG2k-DSG), poly(ethylene glycol)-2000-dimethacrylate (PEG2k-DMA), and 1,2- distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA). In one particular example, the stealth lipid may be PEG2k-DMG.

[00384] The LNPs can comprise different respective molar ratios of the component lipids in the formulation. The mol-% of the CCD lipid may be, for example, from about 30 mol-% to about 60 mol-%, from about 35 mol-% to about 55 mol-%, from about 40 mol-% to about 50 mol-%, from about 42 mol-% to about 47 mol-%, or about 45%. The mol-% of the helper lipid may be, for example, from about 30 mol-% to about 60 mol-%, from about 35 mol-% to about 55 mol-%, from about 40 mol-% to about 50 mol-%, from about 41 mol-% to about 46 mol-%, or about 44 mol-%. The mol-% of the neutral lipid may be, for example, from about 1 mol-% to about 20 mol-%, from about 5 mol-% to about 15 mol-%, from about 7 mol-% to about 12 mol-%, or about 9 mol-%. The mol-% of the stealth lipid may be, for example, from about 1 mol-% to about 10 mol-%, from about 1 mol-% to about 5 mol-%, from about 1 mol-% to about 3 mol-%, about 2 mol-%, or about 1 mol-%.

[00385] The LNPs can have different ratios between the positively charged amine groups of the biodegradable lipid (N) and the negatively charged phosphate groups (P) of the nucleic acid to be encapsulated. This may be mathematically represented by the equation N/P. For example,

the N/P ratio may be from about 0.5 to about 100, from about 1 to about 50, from about 1 to about 25, from about 1 to about 10, from about 1 to about 7, from about 3 to about 5, from about 4 to about 5, about 4, about 4.5, or about 5.

[00386] In some LNPs, the cargo can comprise Cas mRNA and gRNA. The Cas mRNA and gRNAs can be in different ratios. For example, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid ranging from about 25:1 to about 1:25, ranging from about 10:1 to about 1:10, ranging from about 5:1 to about 1:5, or about 1:1. Alternatively, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid from about 1:1 to about 1:5, or about 10:1. Alternatively, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid of about 1:10, 25:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, or 1:25. Alternatively, the LNP formulation can include a ratio of Cas mRNA to gRNA nucleic acid of from about 1:1 to about 1:2. In specific examples, the ratio of Cas mRNA to gRNA can be about 1:1 or about 1:2.

[00387] Exemplary dosing of LNPs includes about 0.1, about 0.25, about 0.3, about 0.5, about 1, about 2, about 3, about 4, about 5, about 6, about 8, or about 10 mg/kg body weight (mpk) or about 0.1 to about 10, about 0.25 to about 10, about 0.3 to about 10, about 0.5 to about 10, about 1 to about 10, about 2 to about 10, about 3 to about 10, about 4 to about 10, about 5 to about 10, about 6 to about 10, about 8 to about 10, about 0.1 to about 8, about 0.1 to about 6, about 0.1 to about 5, about 0.1 to about 4, about 0.1 to about 3, about 0.1 to about 2, about 0.1 to about 1, about 0.1 to about 0.5, about 0.1 to about 0.3, about 0.1 to about 0.25, about 0.25 to about 8, about 0.3 to about 6, about 0.5 to about 5, about 1 to about 5, or about 2 to about 3 mg/kg body weight with respect to total RNA (Cas9 mRNA and gRNA) cargo content. Such LNPs can be administered, for example, intravenously. In one example, LNP doses between about 0.01 mg/kg and about 10 mg/kg, between about 0.1 and about 10 mg/kg, or between about 0.01 and about 0.3 mg/kg can be used. For example, LNP doses of about 0.01, about 0.03, about 0.1, about 0.3, about 1, about 3, or about 10 mg/kg can be used. Additional exemplary dosing of LNPs includes about 0.1, about 0.25, about 0.3, about 0.5, about 1, about 2, about 3, about 4, about 5, about 6, about 8, or about 10 mg/kg (mpk) body weight or about 0.1 to about 10, about 0.25 to about 10, about 0.3 to about 10, about 0.5 to about 10, about 1 to about 10, about 2 to about 10, about 3 to about 10, about 4 to about 10, about 5 to about 10, about 6 to about 10, about 8 to about 10, about 0.1 to about 8, about 0.1 to about 6, about 0.1 to about 5, about 0.1 to about 4, about 0.1 to about 3, about 0.1 to about 2, about 0.1 to about 1, about 0.1 to about 0.5, about 0.1 to about 0.3,

about 0.1 to about 0.25, about 0.25 to about 8, about 0.3 to about 6, about 0.5 to about 5, about 1 to about 5, or about 2 to about 3 mg/kg body weight with respect to total RNA (Cas9 mRNA and gRNA) cargo content. Such LNPs can be administered, for example, intravenously. In one example, LNP doses between about 0.01 mg/kg and about 10 mg/kg, between about 0.1 and about 10 mg/kg, or between about 0.01 and about 0.3 mg/kg can be used. For example, LNP doses of about 0.01, about 0.03, about 0.1, about 0.3, about 0.5, about 1, about 2, about 3, or about 10 mg/kg can be used. In another example, LNP doses between about 0.5 and about 10, between about 0.5 and about 5, between about 0.5 and about 3, between about 1 and about 10, between about 1 and about 5, between about 1 and about 3, or between about 1 and about 2 mg/kg can be used.

[00388] The mode of delivery can be selected to decrease immunogenicity. For example, different components may be delivered by different modes (e.g., bi-modal delivery). These different modes may confer different pharmacodynamics or pharmacokinetic properties on the subject delivered molecule. For example, the different modes can result in different tissue distribution, different half-life, or different temporal distribution. Some modes of delivery (e.g., delivery of a nucleic acid vector that persists in a cell by autonomous replication or genomic integration) result in more persistent expression and presence of the molecule, whereas other modes of delivery are transient and less persistent (e.g., delivery of an RNA or a protein). Delivery of components in a more transient manner, for example as RNA, can ensure that the Cas/gRNA complex is only present and active for a short period of time and can reduce immunogenicity. Such transient delivery can also reduce the possibility of off-target modifications.

[00389] Administration *in vivo* can be by any suitable route including, for example, via intravitreal injection or subretinal injection. Significantly smaller amounts of the components (compared with systemic approaches) may exert an effect when administered locally (for example, subretinal or intravitreal) compared to when administered systemically (for example, intravenously). Local modes of administration may also reduce or eliminate the incidence of potentially toxic side effects that may occur when therapeutically effective amounts of a component are administered systemically.

[00390] Compositions comprising the nuclease agents (e.g., Cas9 mRNAs and guide RNAs or nucleic acids encoding the guide RNAs) and/or nucleic acid constructs disclosed herein can be

formulated using one or more physiologically and pharmaceutically acceptable carriers, diluents, excipients or auxiliaries. The formulation can depend on the route of administration chosen. The term "pharmaceutically acceptable" means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

[00391] The frequency of administration and the number of dosages can depend on the half-life of the nucleic acid constructs or nuclease agents (or nucleic acids encoding the nuclease agents) and the route of administration among other factors. The introduction of nucleic acids or proteins into the cell or animal can be performed one time or multiple times over a period of time. For example, the introduction can be performed only once over a period of time, at least two times over a period of time, at least three times over a period of time, at least four times over a period of time, at least five times over a period of time, at least six times over a period of time, at least seven times over a period of time, at least eight times over a period of time, at least nine times over a period of times, at least ten times over a period of time, at least eleven times, at least twelve times over a period of time, at least thirteen times over a period of time, at least fourteen times over a period of time, at least fifteen times over a period of time, at least sixteen times over a period of time, at least seventeen times over a period of time, at least eighteen times over a period of time, at least nineteen times over a period of time, or at least twenty times over a period of time.

[00392] Optionally, such methods can further comprise assessing expression and/or activity of the inserted retinoschisin coding sequence or the encoded retinoschisin protein in the animal or in a cell. Various methods can be used to identify cells having a targeted genetic modification. The screening can comprise a quantitative assay for assessing modification of allele (MOA) of a parental chromosome. For example, the quantitative assay can be carried out via a quantitative PCR, such as a real-time PCR (qPCR). The real-time PCR can utilize a first primer set that recognizes the target locus and a second primer set that recognizes a non-targeted reference locus. The primer set can comprise a fluorescent probe that recognizes the amplified sequence. Other examples of suitable quantitative assays include fluorescence-mediated in situ hybridization (FISH), comparative genomic hybridization, isothermic DNA amplification, quantitative hybridization to an immobilized probe(s), INVADER® Probes, TAQMAN®

Molecular Beacon probes, or ECLIPSE™ probe technology (*see, e.g.*, US 2005/0144655, herein incorporated by reference in its entirety for all purposes).

[00393] Next-generation sequencing (NGS) can also be used for screening. Next-generation sequencing can also be referred to as "NGS" or "massively parallel sequencing" or "high throughput sequencing." NGS can be used as a screening tool in addition to the MOA assays to define the exact nature of the targeted genetic modification and whether it is consistent across cell types or tissue types or organ types.

[00394] Assessing modification of the target genomic locus in an animal can be in any cell type from any tissue or organ. For example, the assessment can be in multiple cell types from the same tissue or organ (e.g., eye) or in cells from multiple locations within the tissue or organ. This can provide information about which cell types within a target tissue or organ are being targeted or which sections of a tissue or organ are being reached by the nucleic acid construct. As another example, the assessment can be in multiple types of tissue or in multiple organs. In methods in which a particular tissue, organ, or cell type is being targeted, this can provide information about how effectively that tissue or organ is being targeted and whether there are off-target effects in other tissues or organs.

[00395] Methods for measuring expression of retinoschisin can include, for example, measuring protein expression. Such methods are well-known. Such methods can also comprise assessing expression of the mRNA encoded by the retinoschisin coding sequence. This measuring can be within the eye or particular cell types or regions within the eye (e.g., retinal cells such as photoreceptors).

[00396] One example of an assay that can be used is the BASESCOPE™ RNA *in situ* hybridization (ISH) assay, which a method that can quantify cell-specific edited transcripts, including single nucleotide changes, in the context of intact fixed tissue. The BASESCOPE™ RNA ISH assay can complement NGS and qPCR in characterization of gene editing. Whereas NGS/qPCR can provide quantitative average values of wild type and edited sequences, they provide no information on heterogeneity or percentage of edited cells within a tissue. The BASESCOPE™ ISH assay can provide a landscape view of an entire tissue and quantification of wild type versus edited transcripts with single-cell resolution, where the actual number of cells within the target tissue containing the edited mRNA transcript can be quantified. The BASESCOPE™ assay achieves single-molecule RNA detection using paired oligo ("ZZ")

probes to amplify signal without non-specific background. However, the BASESCOPE™ probe design and signal amplification system enables single-molecule RNA detection with a 1 ZZ probe and it can differentially detect single nucleotide edits and mutations in intact fixed tissue.

[00397] Assays for measuring activity of a retinoschisin protein can include, for example, optical coherence tomography (OCT) and electroretinogram (ERG) testing. OCT scans can be used to score retinal cavities and/or to measure retinal photoreceptor thickness). Other assays include optokinetic tests. Such methods are well-known. For example, if the cell or animal being modified is a cell with a mutation that is associated with or causes X-linked juvenile retinoschisis (e.g., an R141C mutation), such functional assays can be used to assess rescue of the disease phenotype. Optical coherence tomography (OCT) provides high-resolution cross-sectional images of the macular region. In individuals with XLRS, OCT reveals cystic spaces primarily in the inner nuclear and outer plexiform layers of the retina. Such functional testing can also include electroretinogram (ERG) testing, which are diagnostic tests that measure the electrical activity generated by neural and non-neuronal cells in the retina in response to a light stimulus. XLRS is associated with reduced phototopic and scotopic b-wave amplitudes in ERGs.

[00398] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise, if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

BRIEF DESCRIPTION OF THE SEQUENCES

[00399] The nucleotide and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three-letter code for amino acids. The nucleotide sequences follow the standard convention of beginning at the 5' end of the sequence and proceeding forward (i.e., from left to right in each line) to the 3' end. Only one strand of each nucleotide sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand. When a nucleotide sequence encoding an amino acid sequence is provided, it is understood that codon degenerate variants thereof that encode the same amino acid sequence are also provided. The amino acid sequences follow the standard convention of beginning at the amino terminus of the sequence and proceeding forward (i.e., from left to right in each line) to the carboxy terminus.

[00400] Table 4. Description of Sequences.

SEQ ID NO	Type	Description
1	Protein	Mouse Retinoschisin (UniProt Q9Z1L4)
2	Protein	Human Retinoschisin (UniProt O15537)
3	Protein	Human Retinoschisin R141C
4	Protein	Chimeric Mouse-Human Retinoschisin
5	Protein	Human Portion of Chimeric Human Retinoschisin
6	DNA	Human *RS1* Coding Sequence (NCBI CCDS14187.1)
7	DNA	Mouse *Rs1* Sequence in Hybrid cDNA
8	DNA	Human *RS1* cDNA Exons 2-6
9	DNA	Codon-Optimized Human *RS1* cDNA Exons 2-6
10	DNA	Reverse Complement of Codon-Optimized Human *RS1* cDNA Exons 2-6
11	DNA	T^{h} Hybrid *RS1* cDNA
12	DNA	T^{mh} Hybrid *RS1* cDNA
13	DNA	T^{h} Nucleotide Sequence (SA, *hRS1*, polyA)
14	DNA	T^{mh} Hybrid *RS1* Sequence (SA, *hRS1*, polyA)
15	DNA	Fragment of Human *RS1* Intron 1 with Splice Acceptor (V1)
16	DNA	Fragment of Human *RS1* Intron 1 with Splice Acceptor (V1)
17	DNA	Mouse Splice Acceptor V1
18	DNA	Mouse Splice Acceptor V2
19	DNA	Reverse Complement of Mouse Splice Acceptor V1
20	DNA	Reverse Complement of Mouse Splice Acceptor V2
21	DNA	ADML Splice Acceptor
22	DNA	bGH PolyA
23	DNA	SV40 PolyA
24	DNA	Reverse Complement of SV40 PolyA
25	DNA	Human *RS1* PolyA
26	DNA	Cas9 DNA
27	Protein	Cas9 Protein
28	DNA	Cas9 cDNA
29	RNA	crRNA Tail
30	RNA	TracrRNA V1
31	RNA	TracrRNA V2

SEQ ID NO	Type	Description
32	RNA	TracrRNA V3
33	RNA	Guide RNA Scaffold V1
34	RNA	Guide RNA Scaffold V2
35	RNA	Guide RNA Scaffold V3
36	RNA	Guide RNA Scaffold V4
37	RNA	Guide RNA Scaffold V5
38	RNA	Guide RNA Scaffold V6
39	RNA	Guide RNA Scaffold V7
40	DNA	Guide RNA Target Sequence Plus PAM V1
41	DNA	Guide RNA Target Sequence Plus PAM V2
42	DNA	Guide RNA Target Sequence Plus PAM V3
43	RNA	Mouse *Rs1* Intron 1 Guide RNA
44	DNA	Generic Modified sgRNA
45	DNA	pAAV hRs1_cDNA, HITI
46	DNA	pscAAV_Rs1_tandem.sgU
47	DNA	pAAV_mhRs1_sgU
48	Protein	Mouse Retinoschisin R141C
49	Protein	NLS v1
50	Protein	NLS v2
51	Protein	NLS v3
52	RNA	crRNA Tail v2
53	RNA	Guide RNA Scaffold V8
54-1895	DNA	Human *RS1* Intron 1 Guide RNA Target Sequences
1896-3147	DNA	Mouse *Rs1* Intron 1 Guide RNA Target Sequences
3148-4989	RNA	Human *RS1* Intron 1 Guide Sequences
4990-6241	RNA	Mouse *Rs1* Intron 1 Guide Sequences
6242	Protein	Cas9 Protein
6243	RNA	Cas9 mRNA
6244	DNA	Cas9 DNA
6245	RNA	Cas9 mRNA
6246	Protein	Cas9 Protein
6247	DNA	HDR-1800 Construct
6248	DNA	HDR-900 Construct
6249	DNA	HDR-450 Construct

EXAMPLES

Example 1. Insertion of *RS1* Coding Sequence into Mouse *Rs1* Locus in XLRS Mouse Model

[00401] In order to model potential X-linked juvenile retinoschisis therapy (XLRS) CRISPR therapeutics strategies *in vivo,* we generated a mouse line with constitutive expression of Cas9 protein in all tissues ($Rosa^{Cas9/+}$; *see, e.g.*, US 2019/0032155 and WO 2019/028032, each of which is herein incorporated by reference in its entirety for all purposes) and crossed it to mice containing a mutant copy of murine *retinoschisin (Rs1)* ($Rs1^{R141C/Y}$) to generate $Rosa^{Cas9/+}$; $Rs1^{R141C/Y}$ mice. The mice with the *Rs1* R141C mutation, which causes intracellular retention of

retinoschisin to replicate key features of the human condition, including intraretinal schisis, retinal degeneration, and a reduced ERG b-wave.

[00402] The *Rs1* gene is a highly conserved extracellular protein involved in the cellular organization of the retina. It is assembled and secreted from photoreceptors and bipolar cells as a homo-oligomeric protein complex. Over 200 mutations have been detected in *Rs1,* many leading to early onset of macular degeneration due to a non-functional protein or the absence of protein secretion. Lack of functional *Rs1* expression causes schisis within the retinal layers leading to the early and progressive vision loss associated with XLRS. To date, there are no publications reporting pre-clinical or clinical studies using Cas9 technology for XLRS treatment. Here, we describe an approach for the use of Cas9 in combination with adeno-associated virus (AAV) to rescue the non-secreting R141C variant of *Rs1* in mice. The constructs are designed to be integrated into the endogenous *Rs1* locus in the retina (e.g., photoreceptors), where the hybrid mouse-human *RS1* transcript will be expressed through the endogenous *Rs1* promoter.

[00403] Subretinal delivery of AAV serotype 7m8 has led to successful expression of transgenes (data not shown). While AAVs have the potential to persist ectopically for a year or more, we endeavor to develop a strategy for permanent rescue of mutant *Rs1* phenotypes. To this end, three viral vectors were designed encoding WT human *RS1* exons 2-6 (without a promoter) as well as a guide RNA targeting mouse *Rs1* intron 1. The sequence of the guide RNA is set forth in SEQ ID NO: 43, and the guide RNA target sequence in mouse *Rs1* intron 1 is set forth in SEQ ID NO: 2383. Upon injection of any one of these viruses into *RosaCas9*/+ ; *Rs1R141C/Y* mice, we expected the guide RNA expression to lead to cutting at the *Rs1* locus and integration of the viral genome containing the human *RS1* cDNA fragment. The mouse *Rs1* locus is shown in **Figure 1**. Mouse exon 1 would splice into human exon 2-6 cDNA (**Figure 2**), and expression of the hybrid mouse human protein should prevent transcription of mutant mouse *Rs1*.

[00404] Viral vector version 1 (SEQ ID NO: 47) was generated by bidirectional insertion in a single stranded AAV (ssAAV) vector. Two hundred bases of *RS1* human intron 1 (containing exon 2 splice signals) were placed in front of a cDNA encoding exons 2-6 of human *RS1* transcript followed by bovine growth hormone (bGH) polyA (T^h). A second human cDNA was generated with mouse codon optimization and integrated into the vector in the opposite orientation. This sequence was flanked by 200 bp containing the mouse splice signals and an SV40 polyA (T^{mh}). The sequences were designed to be divergent enough so as to reduce the

likelihood of viral transcripts self-annealing. Further, an sgRNA expression unit was added in between the transcripts (**Figure 3A**). The benefit of this strategy is that the viral genome can be inserted in either orientation and still form a viable reading frame for allele rescue.

[00405] Viral vector version 2 (SEQ ID NO: 46) was generated by bidirectional insertion in a self-complimentary AAV (scAAV) vector. This version is configured just as version 1, with two differences. First, the sequence was incorporated into a self-complimentary AAV vector. Second, to comply with the maximum self-complimentary genome size of 2100 bp, the splice signal regions were reduced to 60 bp (**Figure 3A**).

[00406] Viral vector version 3 (SEQ ID NO: 45) was designed for homology-independent targeted integration (HITI). HITI leverages the need for the Cas9 recognition sequence to have a protospacer adjacent motif (PAM). The construct was designed to express T^h and the same guide RNA targeting the *Rs1* intron 1. However, where versions 1 and 2 do not contain guide RNA target sequences, version 3 is flanked by an inverted guide RNA target sequence on each side (**Figure 3B**). Once the viral genome is double-stranded, it will express one guide RNA sequence that should simultaneously cause a double-strand break in mouse *Rs1* intron 1 as well as on both sides of the viral genome, effectively cleaving off the ITR sequences. The liberated viral genome is now free to insert into the mouse genome in either orientation. However, if the cDNA integrates in the reverse orientation, the guide RNA target sequences should be reconstituted and available for further Cas9 cleavage. If the cDNA integrates in the desired orientation, the guide RNA target sequences will be destroyed, and the sequence locked in place.

[00407] While the *Rs1* sequences of version 1 and 2 are near identical, the viral backbones may change the timing of double-stranded viral production and thus impact gene insertion efficiencies. An scAAV will become double stranded faster than an ssAAV due to differences in the mechanism by which the second strand is synthesized. Both ITRs in ssAAVs can act as replication origins and contain single-stranded nuclease domains that cut newly synthesized strands. Replication can be initiated from either ITR, so a mixture of the + strand and – strand will be generated. Once the polymerase gets to the other ITR, the synthesized strand is cleaved and released. The primary mechanism to synthesize the compliment strand is to use host-cell machinery. However, at a low level, the + and – strands synthesized by the virus may spontaneously anneal to create the double-stranded virus. In the case of scAAV, one of the two ITR sequences is mutated to remove the nuclease domain. The replication fork starts at the active

ITR and moves through the mutant ITR, without being cleaved, and the synthesis continues on the opposite strand. The single-stranded synthesis containing both the + and – strand self-compliment to make the double-stranded virus.

[00408] Versions 1-3 were each able to mediate gene insertion at the mouse *Rs1* locus in the Cas9 mouse background. In the pilot study, a small amount of virus was injected into right eye of each mouse, and the left eye was not injected as a control. Both retinas were harvested and cut in half. Half of each retina was used for non-homologous end joining (NHEJ) characterization at the intron 1 of mouse *Rs1* locus. The other half was used to generate cDNA for NGS amplicon sequencing over the mutant region. Due to PCR efficiencies and bias, we were able to amplify each transcript variant, but we could not quantify the actual expression profiles. NHEJ characterization provides a clue to guide RNA activity as well as insertion activity. Detection of NHEJ implies an allele without gene insertion that will likely express the mutant mouse transcript. Detection of either modified transcript indicates that gene insertion has occurred.

[00409] Thirteen mice were injected in the right eyes: three for version 1, and five each for versions 2 and 3. All injected eyes had improved retinal organization as seen in optical coherence tomography (OCT) imaging. *See* **Figure 4**. Scoring of retinal cavities shown in OCT scans (containing 61 images each) from 3 different locations of each eye was conducted based on the following preset criteria by three independent readers. A score of 1 was assigned if there were 1-4 cavities on at least one individual image. A score of 2 was assigned if there were ≥ 4 cavities on at least one individual image, but the cavities were not fused. A score of 3 was assigned if there were fused cavities on at least one individual image. A score of 4 was assigned if there were fused cavities on at least one individual image and the retina was stretched. The average scores for each treatment group were compared with a control group containing the pooled non-treated eyes by nonparametric Kruskal–Wallis one-way analysis of variance and Post hoc test Dunn's multiple comparisons test.

[00410] Next generation targeted resequencing amplicons were designed for the regions depicted in **Figure 3** (horizontal arrows) as these regions are distinct in the four expected sequence variants ((1) WT mouse, the mouse reference sequence not containing the R141C mutant; (2) mutant mouse, the mouse reference sequence harboring the R141C mutant; (3) humanized transcript 1, the human reference sequence; and (4) humanized transcript 2, the mouse codon-optimized human reference sequence). Mouse retinas were harvested from study

mice and total mRNA was extracted from the tissues. mRNA was used to generate cDNA to act as a template for next generation sequencing (NGS) amplification. Target-specific oligos with incorporated barcodes for sequence identification were used to amplify all four variants per tissue. The PCR products from each mouse were normalized and pooled into a single tube for further preparation. The finalized library was loaded on to a MiSeq and the 2x300 program was used to sequence samples. Informatic codes were then used to deconvolute the samples and create a de novo sequence reference. The number of reads that matched each variant were quantified and the percentage was obtained by dividing the variant read count by the total read count. As expected, WT mouse sequences were only found in female $Rosa^{Cas9/+};Rs1^{R141C/+}$ mice. The NGS results for the four expected sequence variants are shown in **Figure 5**. PCR bias and efficiency are not accounted for. A separate amplicon was used to amplify the *Rs1* intron 1 guide RNA target sequence. Reads that matched the mouse reference sequence or contained non-homologous end joining were quantified to assess how often the guide RNA cut without making an insertion. *See* **Figures 6A** and **6B**. Bar graphs showing some of the data from the tables in **Figure 5** are shown in **Figures 7A-7C** for versions 1-3, respectively.

[00411] All mice showed varying levels of all transcripts, with more T^{h} reads than T^{mh} reads in all mice. As expected, mice with very high NHEJ rates had lower amounts of human transcripts present. While versions 1 and 2 had similar sequence profiles, version 3 had a greater presence of residual mutant mouse transcript. This was likely due to the need for a specific insertion orientation. While the guide RNA design should help to push the insertion towards the desired insertion, it is likely that Cas9 cleavage damaged the recognition sequences beyond the possibility of re-targeting. Additionally, a large insertion/deletion (indel) on the viral genome may have disrupted the splice signals and prevented proper expression.

Example 2. Insertion of *RS1* Coding Sequence into Human *RS1* Locus in Human Retinoblastoma Cells

[00412] We next tested insertion of *RS1* coding sequence into the human *RS1* locus in WERI-Rb1 human retinoblastoma cells *in vitro*. The WERI-Rb1 cell line (ATCC® HTB 169TM) was derived from human retinoblastoma. Retinoblastoma cells are precursors of photoreceptors and are an appropriate *in vitro* photoreceptor cell model because photoreceptor cell lines are not available. The cells were characterized in-house with retinal specific cell markers by ICC and RT-PCR, which showed only cone-specific mRNAs/proteins but not their rod counterparts were

found, suggesting that this neoplasm was of cone cell lineage. In addition, RS1 was reported to be expressed and released by WERI-Rb1 cells. We also detected RS1 expression at mRNA level and protein levels (data not shown). Lipid nanoparticles (LNPs) were formulated with one of six guide RNAs targeting intron 1 of the human *RS1* and Cas9 mRNA (sequence set forth in SEQ ID NO: 6245).

[00413] Table 5. Human *RS1* Guide RNAs.

gRNA	Guide RNA Target Sequence (5' to 3')	Direction	SEQ ID NO
hG1	CTGTATCTTCTTAGCCAGTA	+	57
hG2	CCCTTTTGTACGTTTTTTAA	+	56
hG3	AAAAAAGGACTTGACATGCA	+	65
hG4	AGAGCTTTGGCCGAGGACGA	+	1210
hG5	TCTAGGACTTCTTCGCATCG	-	1203
hG6	AGTCTGAGGCTGGGGCAACT	-	581

[00414] In a first experiment, human retinoblastoma cells in suspension were transduced with approximately 5e5 MOI of AAV donor (viral vector versions 1 and 2 from Example 1), and CRISPR/Cas9 LNPs were administered 2 hours post-infection. All LNPs were dosed at 500 ng per well (48-well plate), except the "high" samples, which were dosed at 1000 ng LNP. Complete gene expression analysis by RT-qPCR was then done. RT-qPCR was performed by harvesting the total RNA from samples and treating them with DNAse to degrade all DNA contained in the samples. Next, the RNA samples were subjected to reverse transcription to create cDNA for all mRNA contained in the sample. Target specific TaqMan assays were then used to quantify the unique *RS1* sequences expressed in the cells. RNA was harvested 72 hours post-infection. A no reverse transcription control was completed by harvesting the total RNA and DNAase treating as above. For the control, the reverse transcription polymerase was replaced with water such that no cDNA should be generated. Reverse transcribed and control non-reverse transcribed samples were run with a housekeeping gene (DROSHA). Results for viral vector version 1 (bidirectional insertion in a single stranded AAV (ssAAV) vector) are shown in **Figure 8A**. Results for viral vector version 1 (bidirectional insertion in a self-complimentary AAV (scAAV) vector) are shown in **Figure 8B**. These results are shown as delta Ct in order to normalize differences in RNA quantity and quality. The lower the number, the higher the expression of the target sequence. These results indicate robust expression of the introduced sequences. TaqMan assays and viral sequences were optimized to reduce cross-detection of the

endogenous human *RS1* expression. In a second experiment, human retinoblastoma cells in suspension were transduced with approximately 5e5 MOI of AAV donor (viral vector versions 1 and 2 from Example 1), and CRISPR/Cas9 LNPs were administered 2 hours prior to infection. All LNPs were dosed at 500 ng per well (48-well plate), except the "high" samples, which were dosed at 1000 ng LNP. Complete gene expression analysis by RT-qPCR was then done. Results for viral vector version 1 (bidirectional insertion in a single stranded AAV (ssAAV) vector) are shown in **Figure 9A**. Results for viral vector version 1 (bidirectional insertion in a self-complimentary AAV (scAAV) vector) are shown in **Figure 9B**. These results are shown as delta Ct in order to normalize differences in RNA quantity and quality. The lower the number, the higher the expression of the target sequence. These results indicate robust expression of the introduced sequences. TaqMan assays and viral sequences were optimized to reduce cross-detection of the endogenous human *RS1* expression.

Example 3. Insertion of *RS1* Coding Sequence into Mouse *Rs1* Locus in XLRS Mouse Model via Homologous Recombination

[00415] In order to model potential X-linked juvenile retinoschisis therapy (XLRS) CRISPR therapeutics strategies *in vivo,* we use the mouse line described in Example 1 with constitutive expression of Cas9 protein in all tissues crossed to mice containing a mutant copy of murine *retinoschisin (Rs1)* (*Rosa*$^{Cas9/+}$; *Rs1*$^{R141C/Y}$ mice). CRISPR/Cas9 is used in combination with adeno-associated virus (AAV) to rescue the non-secreting R141C variant of *Rs1* in mice. The constructs are designed to be integrated into the endogenous *Rs1* locus in the retina (e.g., photoreceptors) via homologous recombination, where the hybrid mouse-human *RS1* transcript will be expressed through the endogenous *Rs1* promoter. The mice are at the age of P14 when the retina is still undergoing mitosis, cellular differentiation, and maturation. Injection is during mitosis of the retina, and the evaluation is post-mitosis at 2 months post-injection.

[00416] Three viral vectors were designed encoding WT human *RS1* exons 2-6 (without a promoter) as well as a guide RNA targeting mouse *Rs1* intron 1 (guide RNA target sequence set forth in SEQ ID NO: 2887), with all of these elements flanked by homology arms. *See* **Figure 10**. These elements are flanked by an inverted guide RNA target sequence on each side. Once the viral genome is double-stranded, it will express one guide RNA sequence that simultaneously causes a double-strand break in mouse *Rs1* intron 1 as well as on both sides of the viral genome,

effectively cleaving off the ITR sequences. The homology arms in the first viral vector are approximately 1800 bp each, the homology arms in the second viral vector are approximately 900 bp each, and the homology arms in the third viral vector are approximately 450 bp each. The sequences for the three vectors are set forth in SEQ ID NOS: 6247-6249, respectively. Upon injection of any one of these viruses into the eye of *RosaCas9/+* ; *Rs1R141C/Y* mice, guide RNA expression leads to cutting at the *Rs1* locus and homologous recombination with the viral genome containing the human *RS1* cDNA fragment. The mouse *Rs1* locus is shown in **Figure 1**. Mouse exon 1 splices into human exon 2-6 cDNA (**Figure 2**), and expression of the hybrid mouse human protein prevents transcription of mutant mouse *Rs1*.

[00417] Retinas are harvested and cut in half. Half of each retina is used for non-homologous end joining (NHEJ) characterization at the intron 1 of mouse *Rs1* locus. The other half is used to generate cDNA for NGS amplicon sequencing over the mutant region. NHEJ characterization provides information on guide RNA activity as well as insertion activity. Detection of NHEJ implies an allele without gene insertion that will likely express the mutant mouse transcript. Detection of the inserted transcript indicates that gene insertion has occurred.

[00418] Retinal organization is assessed by optical coherence tomography (OCT) imaging. Scoring of retinal cavities in OCT scans is conducted based on the following preset criteria by three independent readers. A score of 1 is assigned if there are 1-4 cavities on at least one individual image. A score of 2 is assigned if there are ≥ 4 cavities on at least one individual image, but the cavities are not fused. A score of 3 is assigned if there are fused cavities on at least one individual image. A score of 4 is assigned if there are fused cavities on at least one individual image and the retina is stretched. The average scores for each treatment group are compared with a control group containing the pooled non-treated eyes by nonparametric Kruskal–Wallis one-way analysis of variance and Post hoc test Dunn's multiple comparisons test.

[00419] Mouse retinas are harvested from study mice, and total mRNA is extracted from the tissues. mRNA is used to generate cDNA to act as a template for next generation sequencing (NGS) amplification. Target-specific oligos with incorporated barcodes for sequence identification are used to amplify all variants per tissue. The PCR products from each mouse are normalized and pooled into a single tube for further preparation. The finalized library is loaded on to a MiSeq, and the 2x300 program is used to sequence samples. Informatic codes are then

used to deconvolute the samples and create a de novo sequence reference. The number of reads that match each variant are quantified, and the percentage is obtained by dividing the variant read count by the total read count.

Example 4. Insertion of *RS1* Coding Sequence into Human *RS1* Locus in Human Retinoblastoma Cells via Homologous Recombination

[00420] We next test insertion of *RS1* coding sequence into the human *RS1* locus via homologous recombination in human retinoblastoma cells *in vitro*. Lipid nanoparticles (LNPs) are formulated with guide RNAs targeting intron 1 of the human *RS1* and Cas9 mRNA. In a first experiment, human retinoblastoma cells in suspension are transduced with approximately 5e5 MOI of AAV donor (viral vectors from Example 3, but with human *RS1* homology arms), and CRISPR/Cas9 LNPs are administered 2 hours post-infection. All LNPs are dosed at 500 ng per well or 1000 ng per well. Complete gene expression analysis by RT-qPCR is then done. In a second experiment, human retinoblastoma cells in suspension are transduced with approximately 5e5 MOI of AAV donor (viral vectors from Example 3, but with human *RS1* homology arms), and CRISPR/Cas9 LNPs were administered 2 hours prior to infection. All LNPs were dosed at 500 ng per well or 1000 ng per well. Complete gene expression analysis by RT-qPCR is then done.

We claim:

1. A composition for use in treating X-linked juvenile retinoschisis in a subject, comprising:

(a) a nucleic acid construct comprising a coding sequence for a retinoschisin protein or functional fragment thereof for integration into a target genomic locus, wherein the coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or functional fragment thereof, wherein:

(I) the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, and

(II) the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, optionally wherein the splice acceptor site is from intron 1 of human *RS1*; and

(b) a nuclease agent comprising a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus, and

wherein the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct is to be expressed in a retinal cell,

wherein the target genomic locus is in an endogenous *RS1* locus comprising an endogenous *RS1* gene,

wherein integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site, and

wherein the integration of the nucleic acid construct into the endogenous *RS1* locus in the retinal cell reduces or eliminates expression of endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct.

2. The composition for use of claim 1, wherein the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence.

3. The composition for use of claim 1 or 2, wherein the nucleic acid construct is for homology-independent targeted integration into the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent, optionally:

(I) wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation; or

(II) wherein the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and

wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

4. The composition for use of claim 1 or 2, wherein the nucleic acid construct is for homologous recombination with the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a nuclease target sequence for a nuclease agent, and optionally wherein each homology arm is between 25 nucleotides and 2.5 kb in length,

optionally wherein the retinoschisin protein or functional fragment thereof is a human retinoschisin protein or functional fragment thereof, the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and each homology arm is between 25 nucleotides and 2.5 kb in length.

5. The composition for use of claim 1 or 2, wherein the nucleic acid construct is bidirectional and comprises:

(a) a first segment comprising a first coding sequence for a first retinoschisin protein or functional fragment thereof, wherein the first coding sequence encodes the protein encoded by exons 2-6 of human *RS1*; and

(b) a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or functional fragment thereof, wherein the second coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

optionally:

(I) wherein the nucleic acid construct does not comprise a homology arm; or

(II) wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof, and wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence; or

(III) wherein the second segment is located 3' of the first segment,

wherein both the first retinoschisin protein or functional fragment thereof and the second retinoschisin protein or functional fragment thereof are a human retinoschisin protein or functional fragment thereof,

wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof,

wherein both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence,

wherein the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence,

wherein the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or functional fragment thereof or the second retinoschisin protein or functional fragment thereof, and

wherein the nucleic acid construct does not comprise a homology arm.

6. The composition for use of any one of claims 1-5, wherein the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene at the endogenous *RS1* locus.

7. The composition for use of any one of claims 1-6, wherein the nucleic acid construct is in a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) viral vector, and optionally wherein the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, and AAV7m8.

8. The composition for use of any one of claims 1-7, wherein the Cas protein is a Cas9 protein, optionally wherein:

(I) the composition comprises a DNA encoding the Cas protein, and wherein the composition comprises the DNA encoding the guide RNA, and optionally wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors; or

(II) the composition comprises a messenger RNA encoding the Cas protein, wherein the composition comprises the guide RNA in the form of RNA, and optionally wherein the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle.

9. The composition for use of any one of claims 1-8, wherein the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis.

10. The composition for use of any one of claims 1-9, wherein the retinal cell is a human retinal cell.

11. Use of a nucleic acid construct and a nuclease agent in the manufacture of a medicament for treating X-linked juvenile retinoschisis in a subject, wherein:

(a) the nucleic acid construct comprises a coding sequence for a retinoschisin protein or functional fragment thereof for integration into a target genomic locus, wherein the coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or functional fragment thereof, and wherein:

(I) the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, and

(II) the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, optionally wherein the splice acceptor site is from intron 1 of human *RS1*; and

(b) the nuclease agent comprises a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus,

wherein the target genomic locus is in an endogenous *RS1* locus comprising an endogenous *RS1* gene,

wherein integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site,

wherein the integration of the nucleic acid construct into the endogenous *RS1* locus in the retinal cell reduces or eliminates expression of endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct, and

wherein (a) and (b) are to be administered sequentially, in any order.

12. The use of claim 11, wherein the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence.

13. The use of claim 11 or 12, wherein the nucleic acid construct is for homology-independent targeted integration into the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent, optionally:

(I) wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation; or

(II) wherein the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and

wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

14. The use of claim 11 or 12, wherein the nucleic acid construct is for homologous recombination with the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a nuclease target sequence for a nuclease agent, and optionally wherein each homology arm is between 25 nucleotides and 2.5 kb in length,

optionally wherein the retinoschisin protein or functional fragment thereof is a human retinoschisin protein or functional fragment thereof, the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and each homology arm is between 25 nucleotides and 2.5 kb in length.

15. The use of claim 11 or 12, wherein the nucleic acid construct is bidirectional and comprises:

(a) a first segment comprising a first coding sequence for a first retinoschisin protein or functional fragment thereof, wherein the first coding sequence encodes the protein encoded by exons 2-6 of human *RS1*; and

(b) a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or functional fragment thereof, wherein the second coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

optionally:

(I) wherein the nucleic acid construct does not comprise a homology arm; or

(II) wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof, and wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence; or

(III) wherein the second segment is located 3' of the first segment,

wherein both the first retinoschisin protein or functional fragment thereof and the second retinoschisin protein or functional fragment thereof are a human retinoschisin protein or functional fragment thereof,

wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof,

wherein both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence,

wherein the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence,

wherein the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement

of a second splice acceptor site located 3' of the reverse complement of the second coding sequence,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or functional fragment thereof or the second retinoschisin protein or functional fragment thereof, and

wherein the nucleic acid construct does not comprise a homology arm.

16. The use of any one of claims 11-15, wherein the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene at the endogenous *RS1* locus.

17. The use of any one of claims 11-16, wherein the nucleic acid construct is in a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) viral vector, and optionally wherein the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, and AAV7m8.

18. The use of any one of claims 11-17, wherein the Cas protein is a Cas9 protein, optionally wherein:

(I) the combination comprises a DNA encoding the Cas protein, and wherein the combination comprises the DNA encoding the guide RNA, and optionally wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors; or

(II) the combination comprises a messenger RNA encoding the Cas protein, wherein the combination comprises the guide RNA in the form of RNA, and optionally wherein the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle.

19. The use of any one of claims 11-18, wherein the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis.

20. The use of any one of claims 11-19, wherein the retinal cell is a human retinal cell.

21. A combination for integrating a coding sequence for a retinoschisin protein or functional fragment thereof into a target genomic locus in a retinal cell, wherein the combination comprises:

(a) a nucleic acid construct comprising the coding sequence for the retinoschisin protein or functional fragment thereof for integration into the target genomic locus, wherein the coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or functional fragment thereof, and wherein:

(I) the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, and

(II) the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, optionally wherein the splice acceptor site is from intron 1 of human *RS1*; and

(b) a nuclease agent comprising a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus,

wherein the target genomic locus is in an endogenous *RS1* locus comprising an endogenous *RS1* gene,

wherein integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site, and

wherein integration of the nucleic acid construct into the endogenous *RS1* locus in the retinal cell reduces or eliminates expression of endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct.

22. The combination of claim 21, wherein the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence.

23. The combination of claim 21 or 22, wherein the nucleic acid construct is for homology-independent targeted integration into the target genomic locus, wherein the nucleic

acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent, optionally:

(I) wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation; or

(II) wherein the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and

wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

24. The combination of claim 21 or 22, wherein the nucleic acid construct is for homologous recombination with the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a nuclease target sequence for a nuclease agent, and optionally wherein each homology arm is between 25 nucleotides and 2.5 kb in length,

optionally wherein the retinoschisin protein or functional fragment thereof is a human retinoschisin protein or functional fragment thereof, the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and each homology arm is between 25 nucleotides and 2.5 kb in length.

25. The combination of claim 21 or 22, wherein the nucleic acid construct is bidirectional and comprises:

(a) a first segment comprising a first coding sequence for a first retinoschisin protein or functional fragment thereof, wherein the first coding sequence encodes the protein encoded by exons 2-6 of human *RS1*; and

(b) a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or functional fragment thereof, wherein the second coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

optionally:

(I) wherein the nucleic acid construct does not comprise a homology arm; or

(II) wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof, and wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence; or

(III) wherein the second segment is located 3' of the first segment,

wherein both the first retinoschisin protein or functional fragment thereof and the second retinoschisin protein or functional fragment thereof are a human retinoschisin protein or functional fragment thereof,

wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof,

wherein both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence,

wherein the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence,

wherein the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement

of a second splice acceptor site located 3' of the reverse complement of the second coding sequence,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or functional fragment thereof or the second retinoschisin protein or functional fragment thereof, and

wherein the nucleic acid construct does not comprise a homology arm.

26. The combination of any one of claims 21-25, wherein the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene at the endogenous *RS1* locus.

27. The combination of any one of claims 21-26, wherein the nucleic acid construct is in a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) viral vector, and optionally wherein the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, and AAV7m8.

28. The combination of any one of claims 21-27, wherein the Cas protein is a Cas9 protein, optionally wherein:

(I) the combination comprises a DNA encoding the Cas protein, and wherein the combination comprises the DNA encoding the guide RNA, and optionally wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors; or

(II) the combination comprises a messenger RNA encoding the Cas protein, wherein the combination comprises the guide RNA in the form of RNA, and optionally wherein the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle.

29. The combination of any one of claims 21-28, wherein the nucleic acid construct and the nuclease agent are in the same delivery vehicle.

30. The combination of any one of claims 21-28, wherein the nucleic acid construct and the nuclease agent are in different delivery vehicles.