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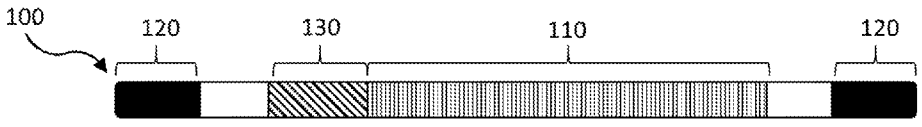
(54) Title: COMPOSITIONS AND METHODS OF TREATING AMYOTROPHIC LATERAL SCLEROSIS (ALS)

FIG. 1



(57) Abstract: The present invention relates to adeno-associated viral (AAV) particles encoding siRNA molecules and methods for treating amyotrophic lateral sclerosis (ALS). The present invention relates to compositions, methods and processes for the design, preparation, manufacture, use and/or formulation of AAV particles comprising modulatory polynucleotides, e.g., polynucleotides encoding at least one small interfering RNA (siRNA) molecules which target the superoxide dismutase 1 (SOD1) gene. Methods for using the AAV particles to inhibit the expression of the SOD1 gene in a subject with a neurodegenerative disease (e.g., amyotrophic lateral sclerosis (ALS)) are also disclosed.

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COMPOSITIONS AND METHODS OF TREATING AMYOTROPHIC LATERAL SCLEROSIS (ALS)

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/501,788, filed May 5, 2017, entitled Compositions and Methods of Treating Amyotrophic Lateral Sclerosis (ALS), U.S. Provisional Patent Application No. 62/507,927, filed May 18, 2017, entitled Compositions and Methods of Treating Amyotrophic Lateral Sclerosis (ALS), U.S. Provisional Patent Application No. 62/520,100, filed June 15, 2017, entitled Compositions and Methods of Treating Amyotrophic Lateral Sclerosis (ALS), and U.S. Provisional Patent Application No. 62/566,609, filed October 2, 2017, entitled Compositions and Methods of Treating Amyotrophic Lateral Sclerosis (ALS), the contents of each of which is incorporated herein by reference in its entirety.

REFERENCE TO THE SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format as an ASCII text file. The Sequence Listing is provided as an ASCII text file entitled 14482_0164_228_SEQ_LISTING.txt, created on April 30, 2018, which is 6,635,467 bytes in size. The Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to compositions, methods and processes for the design, preparation, manufacture, use and/or formulation of AAV particles comprising modulatory polynucleotides, e.g., polynucleotides encoding at least one small interfering RNA (siRNA) molecules which target the superoxide dismutase 1 (SOD1) gene. Targeting of the SOD1 gene may interfere with SOD1 gene expression and the resultant SOD1 protein production. The AAV particles comprising modulatory polynucleotides encoding at least one siRNA molecules may be inserted into recombinant adeno-associated virus (AAV) vectors. Methods for using the AAV particles to inhibit the expression of the SOD1 gene in a subject with a neurodegenerative disease (e.g., amyotrophic lateral sclerosis (ALS)) are also disclosed.

BACKGROUND OF THE INVENTION

[0004] Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is the most fatal progressive neurodegenerative disease, characterized by the predominant loss of motor neurons (MNs) in primary motor cortex, the brainstem, and the spinal cord. The loss of motor neurons devastates basic, fundamental movements, such as breathing, and typically causes death to patients within 2~5 years after diagnosis. Progressive deterioration of motor function in

patients severely disrupts their breathing ability, requiring some form of breathing aid for survival of the patients. Other symptoms also include muscle weakness in hands, arms, legs or the muscles of swallowing. Some patients (e.g., FTD-ALS) may also develop frontotemporal dementia.

[0005] According to the ALS Association, approximately 5,600 people in the United States of America are diagnosed with ALS each year. The incidence of ALS is two per 100,000 people, and it is estimated that as many as 30,000 Americans may have the disease at any given time.

[0006] Two forms of ALS have been described: one is sporadic ALS (sALS), which is the most common form of ALS in the United States of America and accounts for 90 to 95% of all cases diagnosed; the other is familial ALS (fALS), which occurs in a family lineage mainly with a dominant inheritance and only accounts for about 5 to 10% of all cases in the United States of America. sALS and fALS are clinically indistinguishable.

[0007] Pathological studies found that disturbance of some cellular processes occur after disease onset, including increased ER stress, generation of free radicals (i.e., reactive oxygen species (ROS)), mitochondrial dysfunction, protein aggregation, apoptosis, inflammation and glutamate excitotoxicity, specifically in the motor neurons (MNs).

[0008] The causes of ALS are complicated and heterogeneous. In general, ALS is considered to be a complex genetic disorder in which multiple genes in combination with environmental exposures combine to render a person susceptible. More than a dozen genes associated with ALS have been discovered, including, SOD-1 (Cu^{2+}/Zn^{2+} superoxide dismutase), TDP-43 (TARDBP, TAR DNA binding protein-43), FUS (Fused in Sarcoma/Translocated in Sarcoma), ANG (Angiogenin), ATXN2 (Ataxin-2), valosin containing protein (VCP), OPTN (Optineurin) and an expansion of the noncoding GGGGCC hexanucleotide repeat in the chromosome 9, open reading frame 72 (C9ORF72). However, the exact mechanisms of motor neuron degeneration are still elusive.

[0009] Currently, there is no curative treatment for ALS. The only FDA approved drug is Riluzole, which antagonizes the glutamate response to reduce the pathological development of ALS. However, only about a three-month life span expansion for ALS patients in the early stages has been reported, and no therapeutic benefit for ALS patients in the late stages has been observed, indicating a lack of therapeutic options for the patients (Bensimon G et al., *J Neurol.* 2002, 249, 609–615). Therefore, a new treatment strategy that can effectively prevent the disease progression is still in demand.

[0010] Many different strategies are under investigation for potential treatment of both sporadic and familial ALS. One strategy is based on the neuroprotective and/or regenerative effect of neurotrophic factors, such as Insulin-like growth factor I (IGF-I), Glial cell line-derived neurotrophic factor (GDNF), Vascular endothelial growth factor (VEGF), Colivelin and Activity dependent neurotrophic factor (ADNF) derived peptide, which can promote neuronal survival. Several studies demonstrated that neurotrophic factors can preserve motor neuron functionality, therefore improving motor performance in the SOD1 transgenic mice. However, such treatment often fails to prolong the survival of SOD1 mice, suggesting that neurotrophic factors are not sufficient to prolong neuronal survival (See a review by Yacila and Sari, *Curr Med Chem.*, 2014, 21(31), 3583-3593).

[0011] Another strategy for ALS treatment has focused on stem cell based therapy. Stem cells have the potential to generate motor neurons, thereby replacing degenerating motor neurons in the ALS –affected CNS such as primary motor cortex, brainstem and spinal cord. Stem cells derived from multiple sources have been investigated, including induced pluripotent stem cells (iPSCs), mesenchymal stromal cells (MSCs) (e.g. bone marrow mesenchymal stromal cells (BMSCs) and adipocyte stem cells (ASCs)) and neural tissue origin neural stem cells (e.g., fetal spinal neural stem cells (NSCs), multipotent neural progenitor cells (NPCs)) (e.g., reviewed by Kim C et al., *Exp. Neurobiol.*, 2014, 23(3), 207-214).

[0012] Mutations in the gene of superoxide dismutase type I (SOD1; Cu^{2+}/Zn^{2+} superoxide dismutase type I) are the most common cause of fALS, accounting for about 20 to 30% of all fALS cases. Recent reports indicate that SOD1 mutations may also be linked to about 4% of all sALS cases (Robberecht and Philip, *Nat. Rev. Neurosci.*, 2013, 14, 248-264). SOD1-linked fALS is most likely not caused by loss of the normal SOD1 activity, but rather by a gain of a toxic function. One of the hypotheses for mutant SOD1-linked fALS toxicity proposes that an aberrant SOD1 enzyme causes small molecules such as peroxynitrite or hydrogen peroxide to produce damaging free radicals. Other hypotheses for mutant SOD1 neurotoxicity include inhibition of the proteasome activity, mitochondrial damage, disruption of RNA processing and formation of intracellular aggregates. Abnormal accumulation of mutant SOD1 variants and/or wild-type SOD1 in ALS forms insoluble fibrillar aggregates which are identified as pathological inclusions. Aggregated SOD1 protein can induce mitochondria stress (Vehvilainen P et al., *Front Cell Neurosci.*, 2014, 8, 126) and other toxicity to cells, particularly to motor neurons.

[0013] These findings indicate that SOD1 can be a potential therapeutic target for both familial and sporadic ALS. A therapy that can reduce the SOD1 protein produced in the central

nervous system of ALS patients may ameliorate the symptoms of ALS in patients such as motor neuron degeneration and muscle weakness and atrophy. Agents and methods that aim to prevent the formation of wild type and/or mutant SOD1 protein aggregation may prevent disease progression and allow for amelioration of ALS symptoms. RNA interfering (RNAi) mediated gene silencing has drawn researchers' interest in recent years. Small double stranded RNA (small interfering RNA) molecules that target the SOD1 gene haven been taught in the art for their potential in treating ALS (See, e.g., U.S. Pat. No. 7,632,938 and U.S. Patent Publication No. 20060229268, the contents of which is herein incorporated by reference in its entirety).

[0014] The present invention develops an RNA interference based approach to inhibit or prevent the expression of SOD1 in ALS patients for treatment of the disease.

[0015] The present invention provides novel double stranded RNA (dsRNA) constructs and siRNA constructs and methods of their design. In addition, these novel siRNA constructs may be synthetic molecules or be encoded in an expression vector (one or both strands) for delivery into cells. Such vectors include, but are not limited to adeno-associated viral vectors such as vector genomes of any of the AAV serotypes or other viral delivery vehicles such as lentivirus, etc.

SUMMARY OF THE INVENTION

[0016] Described herein are methods, processes, compositions kits and devices for the administration of AAV particles comprising modulatory polynucleotides encoding at least one siRNA molecules for the treatment, prophylaxis, palliation and/or amelioration of a disease and/or disorder (e.g., amyotrophic lateral sclerosis (ALS)).

[0017] The present invention relates to RNA molecule mediated gene specific interference with gene expression and protein production. Methods for treating motor neuron degeneration diseases such as amyotrophic lateral sclerosis are also included in the present invention. The siRNA included in the compositions featured herein encompass a dsRNA having an antisense strand (the antisense strand) having a region that is 30 nucleotides or less, generally 19-24 nucleotides in length, that is substantially complementary to at least part of an mRNA transcript of the SOD1 gene.

[0018] The present invention provides short double stranded RNA molecules such as small interfering RNA (siRNA) duplexes that target SOD1 mRNA to interfere with SOD1 gene expression and/or SOD1 protein production. The siRNA duplexes of the present invention may interfere with both alleles of the SOD1 gene irrespective of any particular mutation in the SOD1 gene, and may particularly interact with those found in ALS disease.

[0019] In some embodiments, such siRNA molecules, or a single strand of the siRNA molecules, are inserted into adeno-associated viral (AAV) vectors to be introduced into cells, specifically motor neurons and/or other surrounding cells in the central nervous system. The AAV vector may comprise sequences encoding 1, 2, 3, 4, or more than 4 siRNA duplexes.

[0020] The siRNA duplex of the present invention comprises an antisense strand and a sense strand hybridized together forming a duplex structure, wherein the antisense strand is complementary to the nucleic acid sequence of the targeted SOD1 gene, and wherein the sense strand is homologous to the nucleic acid sequence of the targeted SOD1 gene. In some aspects, the 5'end of the antisense strand has a 5' phosphate group and the 3'end of the sense strand contains a 3'hydroxyl group. In other aspects, there are none, one or 2 nucleotides overhangs at the 3'end of each strand.

[0021] According to the present invention, each strand of the siRNA duplex targeting the SOD1 gene is about 19-25 nucleotides in length, preferably about 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides in length. In some aspects, the siRNAs may be unmodified RNA molecules.

[0022] In other aspects, the siRNAs may contain at least one modified nucleotide, such as base, sugar or backbone modification.

[0023] In one embodiment, an siRNA or dsRNA includes at least two sequences that are complementary to each other. The dsRNA includes a sense strand having a first sequence and an antisense strand having a second sequence. The antisense strand includes a nucleotide sequence that is substantially complementary to at least part of an mRNA encoding SOD1, and the region of complementarity is 30 nucleotides or less, and at least 15 nucleotides in length. Generally, the dsRNA is 19 to 24, e.g., 19 to 21 nucleotides in length. In some embodiments the dsRNA is from about 15 to about 25 nucleotides in length, and in other embodiments the dsRNA is from about 25 to about 30 nucleotides in length.

[0024] The dsRNA, either upon contacting with a cell expressing SOD1 or upon transcription within a cell expressing SOD1, inhibits or suppresses the expression of a SOD1 gene by at least 10%, at least 20%, at least 25%, at least 30%, at least 35% or at least 40% or more, such as when assayed by a method as described herein.

[0025] According to the present invention, AAV vectors comprising the nucleic acids encoding the siRNA duplexes, one strand of the siRNA duplex or the dsRNA targeting SOD1 gene are produced, the AAV vector serotype may be AAV1, AAV2, AAV3, AAV4, AAV5,

AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ8 and/or AAV-DJ, and variants thereof.

[0026] The present invention also provides pharmaceutical compositions comprising at least one siRNA duplex targeting the SOD1 gene and a pharmaceutically acceptable carrier. In some aspects, a nucleic acid sequence encoding the siRNA duplex is inserted into an AAV vector.

[0027] In some embodiments, the present invention provides methods for inhibiting/silencing SOD1 gene expression in a cell. Accordingly, the siRNA duplexes or dsRNA can be used to substantially inhibit SOD1 gene expression in a cell, in particular in a motor neuron. In some aspects, the inhibition of SOD1 gene expression refers to an inhibition by at least about 20%, preferably by at least about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. Accordingly, the protein product of the targeted gene may be inhibited by at least about 20%, preferably by at least about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. The SOD1 gene can be either a wild type gene or a mutated SOD1 gene with at least one mutation. Accordingly, the SOD1 protein is either wild type protein or a mutated polypeptide with at least one mutation.

[0028] In some embodiments, the present invention provides methods for treating, or ameliorating amyotrophic lateral sclerosis associated with abnormal SOD1 gene and/or SOD1 protein in a subject in need of treatment, the method comprising administering to the subject a pharmaceutically effective amount of at least one siRNA duplex targeting the SOD1 gene, delivering said siRNA duplex into targeted cells, inhibiting SOD1 gene expression and protein production, and ameliorating symptoms of ALS in the subject.

[0029] In some embodiments, an AAV vector comprising the nucleic acid sequence encoding at least one siRNA duplex targeting the SOD1 gene is administered to the subject in need for treating and/or ameliorating ALS. The AAV vector serotype may be selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10 and AAV-DJ, and variants thereof.

[0030] In some aspects, ALS is familial ALS linked to SOD1 mutations. In other aspects, ALS is sporadic ALS which is characterized by abnormal aggregation of SOD1 protein or disruption of SOD1 protein function or localization, though not necessarily as a result of genetic mutation. The symptoms of ALS ameliorated by the present method may include motor neuron degeneration, muscle weakness, stiffness of muscles, slurred speech and /or difficulty in breathing.

[0031] In some embodiments, the siRNA duplexes or dsRNA targeting SOD1 gene or the AAV vectors comprising such siRNA-encoding molecules may be introduced directly into the central nervous system of the subject, for example, by intracranial injection.

[0032] In some embodiments, the pharmaceutical composition of the present invention is used as a solo therapy. In other embodiments, the pharmaceutical composition of the present invention is used in combination therapy. The combination therapy may be in combination with one or more neuroprotective agents such as small molecule compounds, growth factors and hormones which have been tested for their neuroprotective effect on motor neuron degeneration.

[0033] In some embodiments, the present invention provides methods for treating, or ameliorating amyotrophic lateral sclerosis by administering to a subject in need thereof a therapeutically effective amount of a plasmid or AAV vector described herein. The ALS may be familial ALS or sporadic ALS.

[0034] The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

[0036] FIG. 1 is a schematic of a viral genome of the invention.

[0037] FIG. 2 is a schematic of a viral genome of the invention.

[0038] FIG. 3 is a schematic of a viral genome of the invention.

[0039] FIG. 4 is a schematic of a viral genome of the invention.

[0040] FIG. 5 is a schematic of a viral genome of the invention.

[0041] FIG. 6 is a schematic of a viral genome of the invention.

[0042] FIG. 7 is a schematic of a viral genome of the invention.

[0043] FIG. 8 is a schematic of a viral genome of the invention.

[0044] FIG. 9 is a schematic of a viral genome of the invention.

[0045] The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any materials and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred materials and methods are now described. Other features, objects and advantages of the

invention will be apparent from the description. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present description will control.

DETAILED DESCRIPTION OF THE INVENTION

I. COMPOSITIONS OF THE INVENTION

[0046] According to the present invention, compositions for delivering modulatory polynucleotides and/or modulatory polynucleotide-based compositions by adeno-associated viruses (AAVs) are provided. AAV particles of the invention may be provided via any of several routes of administration, to a cell, tissue, organ, or organism, *in vivo*, *ex vivo* or *in vitro*.

[0047] As used herein, an "AAV particle" is a virus which comprises a viral genome with at least one payload region and at least one inverted terminal repeat (ITR) region.

[0048] As used herein, "viral genome" or "vector genome" or "viral vector" refers to the nucleic acid sequence(s) encapsulated in an AAV particle. Viral genomes comprise at least one payload region encoding polypeptides or fragments thereof.

[0049] As used herein, a "payload" or "payload region" is any nucleic acid molecule which encodes one or more polypeptides of the invention. At a minimum, a payload region comprises nucleic acid sequences that encode a sense and antisense sequence, an siRNA-based composition, or a fragment thereof, but may also optionally comprise one or more functional or regulatory elements to facilitate transcriptional expression and/or polypeptide translation.

[0050] The nucleic acid sequences and polypeptides disclosed herein may be engineered to contain modular elements and/or sequence motifs assembled to enable expression of the modulatory polynucleotides and/or modulatory polynucleotide-based compositions of the invention. In some embodiments, the nucleic acid sequence comprising the payload region may comprise one or more of a promoter region, an intron, a Kozak sequence, an enhancer or a polyadenylation sequence. Payload regions of the invention typically encode at least one sense and antisense sequence, an siRNA-based compositions, or fragments of the foregoing in combination with each other or in combination with other polypeptide moieties.

[0051] The payload regions of the invention may be delivered to one or more target cells, tissues, organs or organisms within the viral genome of an AAV particle.

Adeno-associated viruses (AAVs) and AAV particles

[0052] Viruses of the Parvoviridae family are small non-enveloped icosahedral capsid viruses characterized by a single stranded DNA genome. Parvoviridae family viruses consist of two subfamilies: Parvovirinae, which infect vertebrates, and Densovirinae, which infect invertebrates. Due to its relatively simple structure, and due to the fact that it is easily manipulated using standard molecular biology techniques, this virus family is useful as a biological tool. The genome of the virus may be modified to contain a minimum of components for the assembly of a functional recombinant virus, or viral particle, which is loaded with or engineered to express or deliver a desired payload, which may be delivered to a target cell, tissue, organ, or organism.

[0053] The parvoviruses and other members of the Parvoviridae family are generally described in Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication," Chapter 69 in FIELDS VIROLOGY (3d Ed. 1996), the contents of which are incorporated by reference in their entirety.

[0054] The Parvoviridae family comprises the Dependovirus genus which includes adeno-associated viruses (AAV) capable of replication in vertebrate hosts including, but not limited to, human, primate, bovine, canine, equine, and ovine species.

[0055] The AAV viral genome is a linear, single-stranded DNA (ssDNA) molecule approximately 5,000 nucleotides (nt) in length. The AAV viral genome can comprise a payload region and at least one inverted terminal repeat (ITR) or ITR region. ITRs traditionally flank the coding nucleotide sequences for the non-structural proteins (encoded by Rep genes) and the structural proteins (encoded by capsid genes or Cap genes). While not wishing to be bound by theory, an AAV viral genome typically comprises two ITR sequences. The AAV viral genome comprises a characteristic T-shaped hairpin structure defined by the self-complementary terminal 145 nt of the 5' and 3' ends of the ssDNA which form an energetically stable double stranded region. The double stranded hairpin structures comprise multiple functions including, but not limited to, acting as an origin for DNA replication by functioning as primers for the endogenous DNA polymerase complex of the host viral replication cell.

[0056] In addition to the encoded heterologous payload, AAV vectors may comprise the viral genome, in whole or in part, of any naturally occurring and/or recombinant AAV serotype nucleotide sequence or variant. AAV variants may have sequences of significant homology at the nucleic acid (genome or capsid) and amino acid levels (capsids), to produce constructs which are generally physical and functional equivalents, replicate by similar mechanisms, and assemble by similar mechanisms. Chiorini et al., J. Vir. 71: 6823-33(1997); Srivastava et al., J.

Vir. 45:555-64 (1983); Chiorini et al., J. Vir. 73:1309-1319 (1999); Rutledge et al., J. Vir. 72:309-319 (1998); and Wu et al., J. Vir. 74: 8635-47 (2000), the contents of each of which are incorporated herein by reference in their entirety.

[0057] In one embodiment, AAV particles of the present invention are recombinant AAV vectors which are replication defective, lacking sequences encoding functional Rep and Cap proteins within their viral genome. These defective AAV vectors may lack most or all parental coding sequences and essentially carry only one or two AAV ITR sequences and the nucleic acid of interest for delivery to a cell, a tissue, an organ or an organism.

[0058] In one embodiment, the viral genome of the AAV particles of the present invention comprise at least one control element which provides for the replication, transcription and translation of a coding sequence encoded therein. Not all of the control elements need always be present as long as the coding sequence is capable of being replicated, transcribed and/or translated in an appropriate host cell. Non-limiting examples of expression control elements include sequences for transcription initiation and/or termination, promoter and/or enhancer sequences, efficient RNA processing signals such as splicing and polyadenylation signals, sequences that stabilize cytoplasmic mRNA, sequences that enhance translation efficacy (e.g., Kozak consensus sequence), sequences that enhance protein stability, and/or sequences that enhance protein processing and/or secretion.

[0059] According to the present invention, AAV particles for use in therapeutics and/or diagnostics comprise a virus that has been distilled or reduced to the minimum components necessary for transduction of a nucleic acid payload or cargo of interest. In this manner, AAV particles are engineered as vehicles for specific delivery while lacking the deleterious replication and/or integration features found in wild-type viruses.

[0060] AAV vectors of the present invention may be produced recombinantly and may be based on adeno-associated virus (AAV) parent or reference sequences. As used herein, a "vector" is any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule such as the nucleic acids described herein.

[0061] In addition to single stranded AAV viral genomes (e.g., ssAAVs), the present invention also provides for self-complementary AAV (scAAVs) viral genomes. scAAV viral genomes contain DNA strands which anneal together to form double stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the cell.

[0062] In one embodiment, the AAV particle of the present invention is an scAAV.

[0063] In one embodiment, the AAV particle of the present invention is an ssAAV.

[0064] Methods for producing and/or modifying AAV particles are disclosed in the art such as pseudotyped AAV vectors (PCT Patent Publication Nos. WO200028004; WO200123001; WO2004112727; WO 2005005610 and WO 2005072364, the content of each of which is incorporated herein by reference in its entirety).

[0065] AAV particles may be modified to enhance the efficiency of delivery. Such modified AAV particles can be packaged efficiently and be used to successfully infect the target cells at high frequency and with minimal toxicity. In some embodiments the capsids of the AAV particles are engineered according to the methods described in US Publication Number US 20130195801, the contents of which are incorporated herein by reference in their entirety.

[0066] In one embodiment, the AAV particles comprising a payload region encoding the polypeptides of the invention may be introduced into mammalian cells.

AAV serotypes

[0067] AAV particles of the present invention may comprise or be derived from any natural or recombinant AAV serotype. According to the present invention, the AAV particles may utilize or be based on a serotype selected from any of the following AAV1, AAV2, AAV2G9, AAV3, AAV3a, AAV3b, AAV3-3, AAV4, AAV4-4, AAV5, AAV6, AAV6.1, AAV6.2, AAV6.1.2, AAV7, AAV7.2, AAV8, AAV9, AAV9.11, AAV9.13, AAV9.16, AAV9.24, AAV9.45, AAV9.47, AAV9.61, AAV9.68, AAV9.84, AAV9.9, AAV10, AAV11, AAV12, AAV16.3, AAV24.1, AAV27.3, AAV42.12, AAV42-1b, AAV42-2, AAV42-3a, AAV42-3b, AAV42-4, AAV42-5a, AAV42-5b, AAV42-6b, AAV42-8, AAV42-10, AAV42-11, AAV42-12, AAV42-13, AAV42-15, AAV42-aa, AAV43-1, AAV43-12, AAV43-20, AAV43-21, AAV43-23, AAV43-25, AAV43-5, AAV44.1, AAV44.2, AAV44.5, AAV223.1, AAV223.2, AAV223.4, AAV223.5, AAV223.6, AAV223.7, AAV1-7/rh.48, AAV1-8/rh.49, AAV2-15/rh.62, AAV2-3/rh.61, AAV2-4/rh.50, AAV2-5/rh.51, AAV3.1/hu.6, AAV3.1/hu.9, AAV3-9/rh.52, AAV3-11/rh.53, AAV4-8/r11.64, AAV4-9/rh.54, AAV4-19/rh.55, AAV5-3/rh.57, AAV5-22/rh.58, AAV7.3/hu.7, AAV16.8/hu.10, AAV16.12/hu.11, AAV29.3/bb.1, AAV29.5/bb.2, AAV106.1/hu.37, AAV114.3/hu.40, AAV127.2/hu.41, AAV127.5/hu.42, AAV128.3/hu.44, AAV130.4/hu.48, AAV145.1/hu.53, AAV145.5/hu.54, AAV145.6/hu.55, AAV161.10/hu.60, AAV161.6/hu.61, AAV33.12/hu.17, AAV33.4/hu.15, AAV33.8/hu.16, AAV52/hu.19, AAV52.1/hu.20, AAV58.2/hu.25, AAVA3.3, AAVA3.4, AAVA3.5, AAVA3.7, AAVC1, AAVC2, AAVC5, AAV-DJ, AAV-DJ8, AAVF3, AAVF5, AAVH2, AAVrh.72, AAVhu.8,

AAVrh.68, AAVrh.70, AAVpi.1, AAVpi.3, AAVpi.2, AAVrh.60, AAVrh.44, AAVrh.65, AAVrh.55, AAVrh.47, AAVrh.69, AAVrh.45, AAVrh.59, AAVhu.12, AAVH6, AAVLK03, AAVH-1/hu.1, AAVH-5/hu.3, AAVLG-10/rh.40, AAVLG-4/rh.38, AAVLG-9/hu.39, AAVN721-8/rh.43, AAVCh.5, AAVCh.5R1, AAVcy.2, AAVcy.3, AAVcy.4, AAVcy.5, AAVCy.5R1, AAVCy.5R2, AAVCy.5R3, AAVCy.5R4, AAVcy.6, AAVhu.1, AAVhu.2, AAVhu.3, AAVhu.4, AAVhu.5, AAVhu.6, AAVhu.7, AAVhu.9, AAVhu.10, AAVhu.11, AAVhu.13, AAVhu.15, AAVhu.16, AAVhu.17, AAVhu.18, AAVhu.20, AAVhu.21, AAVhu.22, AAVhu.23.2, AAVhu.24, AAVhu.25, AAVhu.27, AAVhu.28, AAVhu.29, AAVhu.29R, AAVhu.31, AAVhu.32, AAVhu.34, AAVhu.35, AAVhu.37, AAVhu.39, AAVhu.40, AAVhu.41, AAVhu.42, AAVhu.43, AAVhu.44, AAVhu.44R1, AAVhu.44R2, AAVhu.44R3, AAVhu.45, AAVhu.46, AAVhu.47, AAVhu.48, AAVhu.48R1, AAVhu.48R2, AAVhu.48R3, AAVhu.49, AAVhu.51, AAVhu.52, AAVhu.54, AAVhu.55, AAVhu.56, AAVhu.57, AAVhu.58, AAVhu.60, AAVhu.61, AAVhu.63, AAVhu.64, AAVhu.66, AAVhu.67, AAVhu.14/9, AAVhu.t 19, AAVrh.2, AAVrh.2R, AAVrh.8, AAVrh.8R, AAVrh.10, AAVrh.12, AAVrh.13, AAVrh.13R, AAVrh.14, AAVrh.17, AAVrh.18, AAVrh.19, AAVrh.20, AAVrh.21, AAVrh.22, AAVrh.23, AAVrh.24, AAVrh.25, AAVrh.31, AAVrh.32, AAVrh.33, AAVrh.34, AAVrh.35, AAVrh.36, AAVrh.37, AAVrh.37R2, AAVrh.38, AAVrh.39, AAVrh.40, AAVrh.46, AAVrh.48, AAVrh.48.1, AAVrh.48.1.2, AAVrh.48.2, AAVrh.49, AAVrh.51, AAVrh.52, AAVrh.53, AAVrh.54, AAVrh.56, AAVrh.57, AAVrh.58, AAVrh.61, AAVrh.64, AAVrh.64R1, AAVrh.64R2, AAVrh.67, AAVrh.73, AAVrh.74, AAVrh8R, AAVrh8R A586R mutant, AAVrh8R R533A mutant, AAAV, BAAV, caprine AAV, bovine AAV, AAVhE1.1, AAVhEr1.5, AAVhER1.14, AAVhEr1.8, AAVhEr1.16, AAVhEr1.18, AAVhEr1.35, AAVhEr1.7, AAVhEr1.36, AAVhEr2.29, AAVhEr2.4, AAVhEr2.16, AAVhEr2.30, AAVhEr2.31, AAVhEr2.36, AAVhER1.23, AAVhEr3.1, AAV2.5T , AAV-PAEC, AAV-LK01, AAV-LK02, AAV-LK03, AAV-LK04, AAV-LK05, AAV-LK06, AAV-LK07, AAV-LK08, AAV-LK09, AAV-LK10, AAV-LK11, AAV-LK12, AAV-LK13, AAV-LK14, AAV-LK15, AAV-LK16, AAV-LK17, AAV-LK18, AAV-LK19, AAV-PAEC2, AAV-PAEC4, AAV-PAEC6, AAV-PAEC7, AAV-PAEC8, AAV-PAEC11, AAV-PAEC12, AAV-2-pre-miRNA-101 , AAV-8h, AAV-8b, AAV-h, AAV-b, AAV SM 10-2 , AAV Shuffle 100-1 , AAV Shuffle 100-3, AAV Shuffle 100-7, AAV Shuffle 10-2, AAV Shuffle 10-6, AAV Shuffle 10-8, AAV Shuffle 100-2, AAV SM 10-1, AAV SM 10-8 , AAV SM 100-3, AAV SM 100-10, BNP61 AAV, BNP62 AAV, BNP63 AAV, AAVrh.50, AAVrh.43, AAVrh.62, AAVrh.48, AAVhu.19, AAVhu.11, AAVhu.53, AAV4-8/rh.64, AAVLG-9/hu.39, AAV54.5/hu.23, AAV54.2/hu.22,

AAV54.7/hu.24, AAV54.1/hu.21, AAV54.4R/hu.27, AAV46.2/hu.28, AAV46.6/hu.29, AAV128.1/hu.43, true type AAV (ttAAV), UPENN AAV 10, Japanese AAV 10 serotypes, AAV CBr-7.1, AAV CBr-7.10, AAV CBr-7.2, AAV CBr-7.3, AAV CBr-7.4, AAV CBr-7.5, AAV CBr-7.7, AAV CBr-7.8, AAV CBr-B7.3, AAV CBr-B7.4, AAV CBr-E1, AAV CBr-E2, AAV CBr-E3, AAV CBr-E4, AAV CBr-E5, AAV CBr-e5, AAV CBr-E6, AAV CBr-E7, AAV CBr-E8, AAV CHt-1, AAV CHt-2, AAV CHt-3, AAV CHt-6.1, AAV CHt-6.10, AAV CHt-6.5, AAV CHt-6.6, AAV CHt-6.7, AAV CHt-6.8, AAV CHt-P1, AAV CHt-P2, AAV CHt-P5, AAV CHt-P6, AAV CHt-P8, AAV CHt-P9, AAV CKd-1, AAV CKd-10, AAV CKd-2, AAV CKd-3, AAV CKd-4, AAV CKd-6, AAV CKd-7, AAV CKd-8, AAV CKd-B1, AAV CKd-B2, AAV CKd-B3, AAV CKd-B4, AAV CKd-B5, AAV CKd-B6, AAV CKd-B7, AAV CKd-B8, AAV CKd-H1, AAV CKd-H2, AAV CKd-H3, AAV CKd-H4, AAV CKd-H5, AAV CKd-H6, AAV CKd-N3, AAV CKd-N4, AAV CKd-N9, AAV CLg-F1, AAV CLg-F2, AAV CLg-F3, AAV CLg-F4, AAV CLg-F5, AAV CLg-F6, AAV CLg-F7, AAV CLg-F8, AAV CLv-1, AAV CLv1-1, AAV Clv1-10, AAV CLv1-2, AAV CLv-12, AAV CLv1-3, AAV CLv-13, AAV CLv1-4, AAV Clv1-7, AAV Clv1-8, AAV Clv1-9, AAV CLv-2, AAV CLv-3, AAV CLv-4, AAV CLv-6, AAV CLv-8, AAV CLv-D1, AAV CLv-D2, AAV CLv-D3, AAV CLv-D4, AAV CLv-D5, AAV CLv-D6, AAV CLv-D7, AAV CLv-D8, AAV CLv-E1, AAV CLv-K1, AAV CLv-K3, AAV CLv-K6, AAV CLv-L4, AAV CLv-L5, AAV CLv-L6, AAV CLv-M1, AAV CLv-M11, AAV CLv-M2, AAV CLv-M5, AAV CLv-M6, AAV CLv-M7, AAV CLv-M8, AAV CLv-M9, AAV CLv-R1, AAV CLv-R2, AAV CLv-R3, AAV CLv-R4, AAV CLv-R5, AAV CLv-R6, AAV CLv-R7, AAV CLv-R8, AAV CLv-R9, AAV CSp-1, AAV CSp-10, AAV CSp-11, AAV CSp-2, AAV CSp-3, AAV CSp-4, AAV CSp-6, AAV CSp-7, AAV CSp-8, AAV CSp-8.10, AAV CSp-8.2, AAV CSp-8.4, AAV CSp-8.5, AAV CSp-8.6, AAV CSp-8.7, AAV CSp-8.8, AAV CSp-8.9, AAV CSp-9, AAV.hu.48R3, AAV.VR-355, AAV3B, AAV4, AAV5, AAVF1/HSC1, AAVF11/HSC11, AAVF12/HSC12, AAVF13/HSC13, AAVF14/HSC14, AAVF15/HSC15, AAVF16/HSC16, AAVF17/HSC17, AAVF2/HSC2, AAVF3/HSC3, AAVF4/HSC4, AAVF5/HSC5, AAVF6/HSC6, AAVF7/HSC7, AAVF8/HSC8, AAVF9/HSC9, AAV-PHP.B (PHP.B), AAV-PHP.A (PHP.A), G2B-26, G2B-13, TH1.1-32, TH1.1-35, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B-EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B-SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B-STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-

QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A15/G2A3, AAVG2B4, AAVG2B5 and variants thereof.

[0068] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Publication No. US20030138772, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV1 (SEQ ID NO: 6 and 64 of US20030138772), AAV2 (SEQ ID NO: 7 and 70 of US20030138772), AAV3 (SEQ ID NO: 8 and 71 of US20030138772), AAV4 (SEQ ID NO: 63 of US20030138772), AAV5 (SEQ ID NO: 114 of US20030138772), AAV6 (SEQ ID NO: 65 of US20030138772), AAV7 (SEQ ID NO: 1-3 of US20030138772), AAV8 (SEQ ID NO: 4 and 95 of US20030138772), AAV9 (SEQ ID NO: 5 and 100 of US20030138772), AAV10 (SEQ ID NO: 117 of US20030138772), AAV11 (SEQ ID NO: 118 of US20030138772), AAV12 (SEQ ID NO: 119 of US20030138772), AAVrh10 (amino acids 1 to 738 of SEQ ID NO: 81 of US20030138772), AAV16.3 (US20030138772 SEQ ID NO: 10), AAV29.3/bb.1 (US20030138772 SEQ ID NO: 11), AAV29.4 (US20030138772 SEQ ID NO: 12), AAV29.5/bb.2 (US20030138772 SEQ ID NO: 13), AAV1.3 (US20030138772 SEQ ID NO: 14), AAV13.3 (US20030138772 SEQ ID NO: 15), AAV24.1 (US20030138772 SEQ ID NO: 16), AAV27.3 (US20030138772 SEQ ID NO: 17), AAV7.2 (US20030138772 SEQ ID NO: 18), AAVC1 (US20030138772 SEQ ID NO: 19), AAVC3 (US20030138772 SEQ ID NO: 20), AAVC5 (US20030138772 SEQ ID NO: 21), AAVF1 (US20030138772 SEQ ID NO: 22), AAVF3 (US20030138772 SEQ ID NO: 23), AAVF5 (US20030138772 SEQ ID NO: 24), AAVH6 (US20030138772 SEQ ID NO: 25), AAVH2 (US20030138772 SEQ ID NO: 26), AAV42-8 (US20030138772 SEQ ID NO: 27), AAV42-15 (US20030138772 SEQ ID NO: 28), AAV42-5b (US20030138772 SEQ ID NO: 29), AAV42-1b (US20030138772 SEQ ID NO: 30), AAV42-13 (US20030138772 SEQ ID NO: 31), AAV42-3a (US20030138772 SEQ ID NO: 32), AAV42-4 (US20030138772 SEQ ID NO: 33), AAV42-5a (US20030138772 SEQ ID NO: 34), AAV42-10 (US20030138772 SEQ ID NO: 35), AAV42-3b (US20030138772 SEQ ID NO: 36), AAV42-11 (US20030138772 SEQ ID NO: 37), AAV42-6b (US20030138772 SEQ ID NO: 38), AAV43-1 (US20030138772 SEQ ID NO: 39), AAV43-5 (US20030138772 SEQ ID NO: 40), AAV43-12 (US20030138772 SEQ ID NO: 41), AAV43-20 (US20030138772 SEQ ID NO: 42), AAV43-21 (US20030138772 SEQ ID NO: 43), AAV43-23 (US20030138772 SEQ ID NO: 44), AAV43-25 (US20030138772 SEQ ID NO: 45), AAV44.1 (US20030138772 SEQ ID NO: 46), AAV44.5 (US20030138772 SEQ ID NO: 47), AAV223.1 (US20030138772 SEQ ID NO: 48), AAV223.2 (US20030138772 SEQ ID NO: 49), AAV223.4 (US20030138772 SEQ ID NO: 50), AAV223.5 (US20030138772 SEQ ID NO: 51), AAV223.6 (US20030138772 SEQ ID NO: 52),

AAV223.7 (US20030138772 SEQ ID NO: 53), AAVA3.4 (US20030138772 SEQ ID NO: 54), AAVA3.5 (US20030138772 SEQ ID NO: 55), AAVA3.7 (US20030138772 SEQ ID NO: 56), AAVA3.3 (US20030138772 SEQ ID NO: 57), AAV42.12 (US20030138772 SEQ ID NO: 58), AAV44.2 (US20030138772 SEQ ID NO: 59), AAV42-2 (US20030138772 SEQ ID NO: 9), or variants thereof.

[0069] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Publication No. US20150159173, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV2 (SEQ ID NO: 7 and 23 of US20150159173), rh20 (SEQ ID NO: 1 of US20150159173), rh32/33 (SEQ ID NO: 2 of US20150159173), rh39 (SEQ ID NO: 3, 20 and 36 of US20150159173), rh46 (SEQ ID NO: 4 and 22 of US20150159173), rh73 (SEQ ID NO: 5 of US20150159173), rh74 (SEQ ID NO: 6 of US20150159173), AAV6.1 (SEQ ID NO: 29 of US20150159173), rh.8 (SEQ ID NO: 41 of US20150159173), rh.48.1 (SEQ ID NO: 44 of US20150159173), hu.44 (SEQ ID NO: 45 of US20150159173), hu.29 (SEQ ID NO: 42 of US20150159173), hu.48 (SEQ ID NO: 38 of US20150159173), rh54 (SEQ ID NO: 49 of US20150159173), AAV2 (SEQ ID NO: 7 of US20150159173), cy.5 (SEQ ID NO: 8 and 24 of US20150159173), rh.10 (SEQ ID NO: 9 and 25 of US20150159173), rh.13 (SEQ ID NO: 10 and 26 of US20150159173), AAV1 (SEQ ID NO: 11 and 27 of US20150159173), AAV3 (SEQ ID NO: 12 and 28 of US20150159173), AAV6 (SEQ ID NO: 13 and 29 of US20150159173), AAV7 (SEQ ID NO: 14 and 30 of US20150159173), AAV8 (SEQ ID NO: 15 and 31 of US20150159173), hu.13 (SEQ ID NO: 16 and 32 of US20150159173), hu.26 (SEQ ID NO: 17 and 33 of US20150159173), hu.37 (SEQ ID NO: 18 and 34 of US20150159173), hu.53 (SEQ ID NO: 19 and 35 of US20150159173), rh.43 (SEQ ID NO: 21 and 37 of US20150159173), rh2 (SEQ ID NO: 39 of US20150159173), rh.37 (SEQ ID NO: 40 of US20150159173), rh.64 (SEQ ID NO: 43 of US20150159173), rh.48 (SEQ ID NO: 44 of US20150159173), ch.5 (SEQ ID NO 46 of US20150159173), rh.67 (SEQ ID NO: 47 of US20150159173), rh.58 (SEQ ID NO: 48 of US20150159173), or variants thereof including, but not limited to Cy5R1, Cy5R2, Cy5R3, Cy5R4, rh.13R, rh.37R2, rh.2R, rh.8R, rh.48.1, rh.48.2, rh.48.1.2, hu.44R1, hu.44R2, hu.44R3, hu.29R, ch.5R1, rh64R1, rh64R2, AAV6.2, AAV6.1, AAV6.12, hu.48R1, hu.48R2, and hu.48R3.

[0070] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US 7198951, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV9 (SEQ ID NO: 1-3 of US 7198951), AAV2

(SEQ ID NO: 4 of US 7198951), AAV1 (SEQ ID NO: 5 of US 7198951), AAV3 (SEQ ID NO: 6 of US 7198951), and AAV8 (SEQ ID NO: 7 of US7198951).

[0071] In some embodiments, the AAV serotype may be, or have, a mutation in the AAV9 sequence as described by N Pulicherla et al. (Molecular Therapy 19(6):1070-1078 (2011), herein incorporated by reference in its entirety), such as but not limited to, AAV9.9, AAV9.11, AAV9.13, AAV9.16, AAV9.24, AAV9.45, AAV9.47, AAV9.61, AAV9.68, AAV9.84.

[0072] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US 6156303, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV3B (SEQ ID NO: 1 and 10 of US 6156303), AAV6 (SEQ ID NO: 2, 7 and 11 of US 6156303), AAV2 (SEQ ID NO: 3 and 8 of US 6156303), AAV3A (SEQ ID NO: 4 and 9, of US 6156303), or derivatives thereof.

[0073] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Publication No. US20140359799, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV8 (SEQ ID NO: 1 of US20140359799), AAVDJ (SEQ ID NO: 2 and 3 of US20140359799), or variants thereof.

[0074] In some embodiments, the serotype may be AAVDJ (or AAV-DJ) or a variant thereof, such as AAVDJ8 (or AAV-DJ8), as described by Grimm et al. (Journal of Virology 82(12): 5887-5911 (2008), herein incorporated by reference in its entirety). The amino acid sequence of AAVDJ8 may comprise two or more mutations in order to remove the heparin binding domain (HBD). As a non-limiting example, the AAV-DJ sequence described as SEQ ID NO: 1 in US Patent No. 7,588,772, the contents of which are herein incorporated by reference in their entirety, may comprise two mutations: (1) R587Q where arginine (R; Arg) at amino acid 587 is changed to glutamine (Q; Gln) and (2) R590T where arginine (R; Arg) at amino acid 590 is changed to threonine (T; Thr). As another non-limiting example, may comprise three mutations: (1) K406R where lysine (K; Lys) at amino acid 406 is changed to arginine (R; Arg), (2) R587Q where arginine (R; Arg) at amino acid 587 is changed to glutamine (Q; Gln) and (3) R590T where arginine (R; Arg) at amino acid 590 is changed to threonine (T; Thr).

[0075] In some embodiments, the AAV serotype may be, or have, a sequence of AAV4 as described in International Publication No. WO1998011244, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAV4 (SEQ ID NO: 1-20 of WO1998011244).

[0076] In some embodiments, the AAV serotype may be, or have, a mutation in the AAV2 sequence to generate AAV2G9 as described in International Publication No. WO2014144229 and herein incorporated by reference in its entirety.

[0077] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2005033321, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAV3-3 (SEQ ID NO: 217 of WO2005033321), AAV1 (SEQ ID NO: 219 and 202 of WO2005033321), AAV106.1/hu.37 (SEQ ID No: 10 of WO2005033321), AAV114.3/hu.40 (SEQ ID No: 11 of WO2005033321), AAV127.2/hu.41 (SEQ ID NO:6 and 8 of WO2005033321), AAV128.3/hu.44 (SEQ ID No: 81 of WO2005033321), AAV130.4/hu.48 (SEQ ID NO: 78 of WO2005033321), AAV145.1/hu.53 (SEQ ID No: 176 and 177 of WO2005033321), AAV145.6/hu.56 (SEQ ID NO: 168 and 192 of WO2005033321), AAV16.12/hu.11 (SEQ ID NO:: 153 and 57 of WO2005033321), AAV16.8/hu.10 (SEQ ID NO:: 156 and 56 of WO2005033321), AAV161.10/hu.60 (SEQ ID No: 170 of WO2005033321), AAV161.6/hu.61 (SEQ ID No: 174 of WO2005033321), AAV1-7/rh.48 (SEQ ID NO: 32 of WO2005033321), AAV1-8/rh.49 (SEQ ID NOs: 103 and 25 of WO2005033321), AAV2 (SEQ ID NO: 211 and 221 of WO2005033321), AAV2-15/rh.62 (SEQ ID No: 33 and 114 of WO2005033321), AAV2-3/rh.61 (SEQ ID NO: 21 of WO2005033321), AAV2-4/rh.50 (SEQ ID No: 23 and 108 of WO2005033321), AAV2-5/rh.51 (SEQ ID NO: 104 and 22 of WO2005033321), AAV3.1/hu.6 (SEQ ID NO: 5 and 84 of WO2005033321), AAV3.1/hu.9 (SEQ ID NO: 155 and 58 of WO2005033321), AAV3-11/rh.53 (SEQ ID NO: 186 and 176 of WO2005033321), AAV3-3 (SEQ ID NO: 200 of WO2005033321), AAV33.12/hu.17 (SEQ ID NO:4 of WO2005033321), AAV33.4/hu.15 (SEQ ID No: 50 of WO2005033321), AAV33.8/hu.16 (SEQ ID No: 51 of WO2005033321), AAV3-9/rh.52 (SEQ ID NO: 96 and 18 of WO2005033321), AAV4-19/rh.55 (SEQ ID NO: 117 of WO2005033321), AAV4-4 (SEQ ID NO: 201 and 218 of WO2005033321), AAV4-9/rh.54 (SEQ ID NO: 116 of WO2005033321), AAV5 (SEQ ID NO: 199 and 216 of WO2005033321), AAV52.1/hu.20 (SEQ ID NO: 63 of WO2005033321), AAV52/hu.19 (SEQ ID NO: 133 of WO2005033321), AAV5-22/rh.58 (SEQ ID No: 27 of WO2005033321), AAV5-3/rh.57 (SEQ ID NO: 105 of WO2005033321), AAV5-3/rh.57 (SEQ ID No: 26 of WO2005033321), AAV58.2/hu.25 (SEQ ID No: 49 of WO2005033321), AAV6 (SEQ ID NO: 203 and 220 of WO2005033321), AAV7 (SEQ ID NO: 222 and 213 of WO2005033321), AAV7.3/hu.7 (SEQ ID No: 55 of WO2005033321), AAV8 (SEQ ID NO: 223 and 214 of WO2005033321), AAVH-1/hu.1 (SEQ ID No: 46 of WO2005033321), AAVH-5/hu.3 (SEQ ID No: 44 of WO2005033321), AAVhu.1 (SEQ ID NO:

144 of WO2005033321), AAVhu.10 (SEQ ID NO: 156 of WO2005033321), AAVhu.11 (SEQ ID NO: 153 of WO2005033321), AAVhu.12 (WO2005033321 SEQ ID NO: 59), AAVhu.13 (SEQ ID NO: 129 of WO2005033321), AAVhu.14/AAV9 (SEQ ID NO: 123 and 3 of WO2005033321), AAVhu.15 (SEQ ID NO: 147 of WO2005033321), AAVhu.16 (SEQ ID NO: 148 of WO2005033321), AAVhu.17 (SEQ ID NO: 83 of WO2005033321), AAVhu.18 (SEQ ID NO: 149 of WO2005033321), AAVhu.19 (SEQ ID NO: 133 of WO2005033321), AAVhu.2 (SEQ ID NO: 143 of WO2005033321), AAVhu.20 (SEQ ID NO: 134 of WO2005033321), AAVhu.21 (SEQ ID NO: 135 of WO2005033321), AAVhu.22 (SEQ ID NO: 138 of WO2005033321), AAVhu.23.2 (SEQ ID NO: 137 of WO2005033321), AAVhu.24 (SEQ ID NO: 136 of WO2005033321), AAVhu.25 (SEQ ID NO: 146 of WO2005033321), AAVhu.27 (SEQ ID NO: 140 of WO2005033321), AAVhu.29 (SEQ ID NO: 132 of WO2005033321), AAVhu.3 (SEQ ID NO: 145 of WO2005033321), AAVhu.31 (SEQ ID NO: 121 of WO2005033321), AAVhu.32 (SEQ ID NO: 122 of WO2005033321), AAVhu.34 (SEQ ID NO: 125 of WO2005033321), AAVhu.35 (SEQ ID NO: 164 of WO2005033321), AAVhu.37 (SEQ ID NO: 88 of WO2005033321), AAVhu.39 (SEQ ID NO: 102 of WO2005033321), AAVhu.4 (SEQ ID NO: 141 of WO2005033321), AAVhu.40 (SEQ ID NO: 87 of WO2005033321), AAVhu.41 (SEQ ID NO: 91 of WO2005033321), AAVhu.42 (SEQ ID NO: 85 of WO2005033321), AAVhu.43 (SEQ ID NO: 160 of WO2005033321), AAVhu.44 (SEQ ID NO: 144 of WO2005033321), AAVhu.45 (SEQ ID NO: 127 of WO2005033321), AAVhu.46 (SEQ ID NO: 159 of WO2005033321), AAVhu.47 (SEQ ID NO: 128 of WO2005033321), AAVhu.48 (SEQ ID NO: 157 of WO2005033321), AAVhu.49 (SEQ ID NO: 189 of WO2005033321), AAVhu.51 (SEQ ID NO: 190 of WO2005033321), AAVhu.52 (SEQ ID NO: 191 of WO2005033321), AAVhu.53 (SEQ ID NO: 186 of WO2005033321), AAVhu.54 (SEQ ID NO: 188 of WO2005033321), AAVhu.55 (SEQ ID NO: 187 of WO2005033321), AAVhu.56 (SEQ ID NO: 192 of WO2005033321), AAVhu.57 (SEQ ID NO: 193 of WO2005033321), AAVhu.58 (SEQ ID NO: 194 of WO2005033321), AAVhu.6 (SEQ ID NO: 84 of WO2005033321), AAVhu.60 (SEQ ID NO: 184 of WO2005033321), AAVhu.61 (SEQ ID NO: 185 of WO2005033321), AAVhu.63 (SEQ ID NO: 195 of WO2005033321), AAVhu.64 (SEQ ID NO: 196 of WO2005033321), AAVhu.66 (SEQ ID NO: 197 of WO2005033321), AAVhu.67 (SEQ ID NO: 198 of WO2005033321), AAVhu.7 (SEQ ID NO: 150 of WO2005033321), AAVhu.8 (WO2005033321 SEQ ID NO: 12), AAVhu.9 (SEQ ID NO: 155 of WO2005033321), AAVLG-10/rh.40 (SEQ ID No: 14 of WO2005033321), AAVLG-4/rh.38 (SEQ ID NO: 86 of WO2005033321), AAVLG-4/rh.38 (SEQ ID No: 7 of WO2005033321), AAVN721-8/rh.43

(SEQ ID NO: 163 of WO2005033321), AAVN721-8/rh.43 (SEQ ID No: 43 of WO2005033321), AAVpi.1 (WO2005033321 SEQ ID NO: 28), AAVpi.2 (WO2005033321 SEQ ID NO: 30), AAVpi.3 (WO2005033321 SEQ ID NO: 29), AAVrh.38 (SEQ ID NO: 86 of WO2005033321), AAVrh.40 (SEQ ID NO: 92 of WO2005033321), AAVrh.43 (SEQ ID NO: 163 of WO2005033321), AAVrh.44 (WO2005033321 SEQ ID NO: 34), AAVrh.45 (WO2005033321 SEQ ID NO: 41), AAVrh.47 (WO2005033321 SEQ ID NO: 38), AAVrh.48 (SEQ ID NO: 115 of WO2005033321), AAVrh.49 (SEQ ID NO: 103 of WO2005033321), AAVrh.50 (SEQ ID NO: 108 of WO2005033321), AAVrh.51 (SEQ ID NO: 104 of WO2005033321), AAVrh.52 (SEQ ID NO: 96 of WO2005033321), AAVrh.53 (SEQ ID NO: 97 of WO2005033321), AAVrh.55 (WO2005033321 SEQ ID NO: 37), AAVrh.56 (SEQ ID NO: 152 of WO2005033321), AAVrh.57 (SEQ ID NO: 105 of WO2005033321), AAVrh.58 (SEQ ID NO: 106 of WO2005033321), AAVrh.59 (WO2005033321 SEQ ID NO: 42), AAVrh.60 (WO2005033321 SEQ ID NO: 31), AAVrh.61 (SEQ ID NO: 107 of WO2005033321), AAVrh.62 (SEQ ID NO: 114 of WO2005033321), AAVrh.64 (SEQ ID NO: 99 of WO2005033321), AAVrh.65 (WO2005033321 SEQ ID NO: 35), AAVrh.68 (WO2005033321 SEQ ID NO: 16), AAVrh.69 (WO2005033321 SEQ ID NO: 39), AAVrh.70 (WO2005033321 SEQ ID NO: 20), AAVrh.72 (WO2005033321 SEQ ID NO: 9), or variants thereof including, but not limited to, AAVcy.2, AAVcy.3, AAVcy.4, AAVcy.5, AAVcy.6, AAVrh.12, AAVrh.17, AAVrh.18, AAVrh.19, AAVrh.21, AAVrh.22, AAVrh.23, AAVrh.24, AAVrh.25, AAVrh.25/42 15, AAVrh.31, AAVrh.32, AAVrh.33, AAVrh.34, AAVrh.35, AAVrh.36, AAVrh.37, AAVrh14. Non limiting examples of variants include SEQ ID NO: 13, 15, 17, 19, 24, 36, 40, 45, 47, 48, 51-54, 60-62, 64-77, 79, 80, 82, 89, 90, 93-95, 98, 100, 101, 109-113, 118-120, 124, 126, 131, 139, 142, 151,154, 158, 161, 162, 165-183, 202, 204-212, 215, 219, 224-236, of WO2005033321, the contents of which are herein incorporated by reference in their entirety.

[0078] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2015168666, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAVrh8R (SEQ ID NO: 9 of WO2015168666), AAVrh8R A586R mutant (SEQ ID NO: 10 of WO2015168666), AAVrh8R R533A mutant (SEQ ID NO: 11 of WO2015168666), or variants thereof.

[0079] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US9233131, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAVhE1.1 (SEQ ID NO:44 of US9233131), AAVhEr1.5 (SEQ ID NO:45 of US9233131), AAVhER1.14 (SEQ ID NO:46 of US9233131),

AAVhEr1.8 (SEQ ID NO:47 of US9233131), AAVhEr1.16 (SEQ ID NO:48 of US9233131), AAVhEr1.18 (SEQ ID NO:49 of US9233131), AAVhEr1.35 (SEQ ID NO:50 of US9233131), AAVhEr1.7 (SEQ ID NO:51 of US9233131), AAVhEr1.36 (SEQ ID NO:52 of US9233131), AAVhEr2.29 (SEQ ID NO:53 of US9233131), AAVhEr2.4 (SEQ ID NO:54 of US9233131), AAVhEr2.16 (SEQ ID NO:55 of US9233131), AAVhEr2.30 (SEQ ID NO:56 of US9233131), AAVhEr2.31 (SEQ ID NO:58 of US9233131), AAVhEr2.36 (SEQ ID NO:57 of US9233131), AAVhER1.23 (SEQ ID NO:53 of US9233131), AAVhEr3.1 (SEQ ID NO:59 of US9233131), AAV2.5T (SEQ ID NO:42 of US9233131), or variants thereof.

[0080] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20150376607, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV-PAEC (SEQ ID NO:1 of US20150376607), AAV-LK01 (SEQ ID NO:2 of US20150376607), AAV-LK02 (SEQ ID NO:3 of US20150376607), AAV-LK03 (SEQ ID NO:4 of US20150376607), AAV-LK04 (SEQ ID NO:5 of US20150376607), AAV-LK05 (SEQ ID NO:6 of US20150376607), AAV-LK06 (SEQ ID NO:7 of US20150376607), AAV-LK07 (SEQ ID NO:8 of US20150376607), AAV-LK08 (SEQ ID NO:9 of US20150376607), AAV-LK09 (SEQ ID NO:10 of US20150376607), AAV-LK10 (SEQ ID NO:11 of US20150376607), AAV-LK11 (SEQ ID NO:12 of US20150376607), AAV-LK12 (SEQ ID NO:13 of US20150376607), AAV-LK13 (SEQ ID NO:14 of US20150376607), AAV-LK14 (SEQ ID NO:15 of US20150376607), AAV-LK15 (SEQ ID NO:16 of US20150376607), AAV-LK16 (SEQ ID NO:17 of US20150376607), AAV-LK17 (SEQ ID NO:18 of US20150376607), AAV-LK18 (SEQ ID NO:19 of US20150376607), AAV-LK19 (SEQ ID NO:20 of US20150376607), AAV-PAEC2 (SEQ ID NO:21 of US20150376607), AAV-PAEC4 (SEQ ID NO:22 of US20150376607), AAV-PAEC6 (SEQ ID NO:23 of US20150376607), AAV-PAEC7 (SEQ ID NO:24 of US20150376607), AAV-PAEC8 (SEQ ID NO:25 of US20150376607), AAV-PAEC11 (SEQ ID NO:26 of US20150376607), AAV-PAEC12 (SEQ ID NO:27, of US20150376607), or variants thereof.

[0081] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US9163261, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV-2-pre-miRNA-101 (SEQ ID NO: 1 US9163261), or variants thereof.

[0082] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20150376240, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV-8h (SEQ ID NO: 6

of US20150376240), AAV-8b (SEQ ID NO: 5 of US20150376240), AAV-h (SEQ ID NO: 2 of US20150376240), AAV-b (SEQ ID NO: 1 of US20150376240), or variants thereof.

[0083] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20160017295, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV SM 10-2 (SEQ ID NO: 22 of US20160017295), AAV Shuffle 100-1 (SEQ ID NO: 23 of US20160017295), AAV Shuffle 100-3 (SEQ ID NO: 24 of US20160017295), AAV Shuffle 100-7 (SEQ ID NO: 25 of US20160017295), AAV Shuffle 10-2 (SEQ ID NO: 34 of US20160017295), AAV Shuffle 10-6 (SEQ ID NO: 35 of US20160017295), AAV Shuffle 10-8 (SEQ ID NO: 36 of US20160017295), AAV Shuffle 100-2 (SEQ ID NO: 37 of US20160017295), AAV SM 10-1 (SEQ ID NO: 38 of US20160017295), AAV SM 10-8 (SEQ ID NO: 39 of US20160017295), AAV SM 100-3 (SEQ ID NO: 40 of US20160017295), AAV SM 100-10 (SEQ ID NO: 41 of US20160017295), or variants thereof.

[0084] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20150238550, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, BNP61 AAV (SEQ ID NO: 1 of US20150238550), BNP62 AAV (SEQ ID NO: 3 of US20150238550), BNP63 AAV (SEQ ID NO: 4 of US20150238550), or variants thereof.

[0085] In some embodiments, the AAV serotype may be or may have a sequence as described in United States Patent Publication No. US20150315612, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAVrh.50 (SEQ ID NO: 108 of US20150315612), AAVrh.43 (SEQ ID NO: 163 of US20150315612), AAVrh.62 (SEQ ID NO: 114 of US20150315612), AAVrh.48 (SEQ ID NO: 115 of US20150315612), AAVhu.19 (SEQ ID NO: 133 of US20150315612), AAVhu.11 (SEQ ID NO: 153 of US20150315612), AAVhu.53 (SEQ ID NO: 186 of US20150315612), AAV4-8/rh.64 (SEQ ID No: 15 of US20150315612), AAVLG-9/hu.39 (SEQ ID No: 24 of US20150315612), AAV54.5/hu.23 (SEQ ID No: 60 of US20150315612), AAV54.2/hu.22 (SEQ ID No: 67 of US20150315612), AAV54.7/hu.24 (SEQ ID No: 66 of US20150315612), AAV54.1/hu.21 (SEQ ID No: 65 of US20150315612), AAV54.4R/hu.27 (SEQ ID No: 64 of US20150315612), AAV46.2/hu.28 (SEQ ID No: 68 of US20150315612), AAV46.6/hu.29 (SEQ ID No: 69 of US20150315612), AAV128.1/hu.43 (SEQ ID No: 80 of US20150315612), or variants thereof.

[0086] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2015121501, the contents of which are herein incorporated by

reference in their entirety, such as, but not limited to, true type AAV (ttAAV) (SEQ ID NO: 2 of WO2015121501), "UPenn AAV10" (SEQ ID NO: 8 of WO2015121501), "Japanese AAV10" (SEQ ID NO: 9 of WO2015121501), or variants thereof.

[0087] According to the present invention, AAV capsid serotype selection or use may be from a variety of species. In one embodiment, the AAV may be an avian AAV (AAAV). The AAAV serotype may be, or have, a sequence as described in United States Patent No. US 9238800, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAAV (SEQ ID NO: 1, 2, 4, 6, 8, 10, 12, and 14 of US 9,238,800), or variants thereof.

[0088] In one embodiment, the AAV may be a bovine AAV (BAAV). The BAAV serotype may be, or have, a sequence as described in United States Patent No. US 9,193,769, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, BAAV (SEQ ID NO: 1 and 6 of US 9193769), or variants thereof. The BAAV serotype may be or have a sequence as described in United States Patent No. US7427396, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, BAAV (SEQ ID NO: 5 and 6 of US7427396), or variants thereof.

[0089] In one embodiment, the AAV may be a caprine AAV. The caprine AAV serotype may be, or have, a sequence as described in United States Patent No. US7427396, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, caprine AAV (SEQ ID NO: 3 of US7427396), or variants thereof.

[0090] In other embodiments the AAV may be engineered as a hybrid AAV from two or more parental serotypes. In one embodiment, the AAV may be AAV2G9 which comprises sequences from AAV2 and AAV9. The AAV2G9 AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20160017005, the contents of which are herein incorporated by reference in its entirety.

[0091] In one embodiment, the AAV may be a serotype generated by the AAV9 capsid library with mutations in amino acids 390-627 (VP1 numbering) as described by Pulicherla et al. (Molecular Therapy 19(6):1070-1078 (2011), the contents of which are herein incorporated by reference in their entirety. The serotype and corresponding nucleotide and amino acid substitutions may be, but is not limited to, AAV9.1 (G1594C; D532H), AAV6.2 (T1418A and T1436X; V473D and I479K), AAV9.3 (T1238A; F413Y), AAV9.4 (T1250C and A1617T; F417S), AAV9.5 (A1235G, A1314T, A1642G, C1760T; Q412R, T548A, A587V), AAV9.6 (T1231A; F411I), AAV9.9 (G1203A, G1785T; W595C), AAV9.10 (A1500G, T1676C;

M559T), AAV9.11 (A1425T, A1702C, A1769T; T568P, Q590L), AAV9.13 (A1369C, A1720T; N457H, T574S), AAV9.14 (T1340A, T1362C, T1560C, G1713A; L447H), AAV9.16 (A1775T; Q592L), AAV9.24 (T1507C, T1521G; W503R), AAV9.26 (A1337G, A1769C; Y446C, Q590P), AAV9.33 (A1667C; D556A), AAV9.34 (A1534G, C1794T; N512D), AAV9.35 (A1289T, T1450A, C1494T, A1515T, C1794A, G1816A; Q430L, Y484N, N98K, V606I), AAV9.40 (A1694T, E565V), AAV9.41 (A1348T, T1362C; T450S), AAV9.44 (A1684C, A1701T, A1737G; N562H, K567N), AAV9.45 (A1492T, C1804T; N498Y, L602F), AAV9.46 (G1441C, T1525C, T1549G; G481R, W509R, L517V), 9.47 (G1241A, G1358A, A1669G, C1745T; S414N, G453D, K557E, T582I), AAV9.48 (C1445T, A1736T; P482L, Q579L), AAV9.50 (A1638T, C1683T, T1805A; Q546H, L602H), AAV9.53 (G1301A, A1405C, C1664T, G1811T; R134Q, S469R, A555V, G604V), AAV9.54 (C1531A, T1609A; L511I, L537M), AAV9.55 (T1605A; F535L), AAV9.58 (C1475T, C1579A; T492I, H527N), AAV.59 (T1336C; Y446H), AAV9.61 (A1493T; N498I), AAV9.64 (C1531A, A1617T; L511I), AAV9.65 (C1335T, T1530C, C1568A; A523D), AAV9.68 (C1510A; P504T), AAV9.80 (G1441A,;G481R), AAV9.83 (C1402A, A1500T; P468T, E500D), AAV9.87 (T1464C, T1468C; S490P), AAV9.90 (A1196T; Y399F), AAV9.91 (T1316G, A1583T, C1782G, T1806C; L439R, K528I), AAV9.93 (A1273G, A1421G, A1638C, C1712T, G1732A, A1744T, A1832T; S425G, Q474R, Q546H, P571L, G578R, T582S, D611V), AAV9.94 (A1675T; M559L) and AAV9.95 (T1605A; F535L).

[0092] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2016049230, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAVF1/HSC1 (SEQ ID NO: 2 and 20 of WO2016049230), AAVF2/HSC2 (SEQ ID NO: 3 and 21 of WO2016049230), AAVF3/HSC3 (SEQ ID NO: 5 and 22 of WO2016049230), AAVF4/HSC4 (SEQ ID NO: 6 and 23 of WO2016049230), AAVF5/HSC5 (SEQ ID NO: 11 and 25 of WO2016049230), AAVF6/HSC6 (SEQ ID NO: 7 and 24 of WO2016049230), AAVF7/HSC7 (SEQ ID NO: 8 and 27 of WO2016049230), AAVF8/HSC8 (SEQ ID NO: 9 and 28 of WO2016049230), AAVF9/HSC9 (SEQ ID NO: 10 and 29 of WO2016049230), AAVF11/HSC11 (SEQ ID NO: 4 and 26 of WO2016049230), AAVF12/HSC12 (SEQ ID NO: 12 and 30 of WO2016049230), AAVF13/HSC13 (SEQ ID NO: 14 and 31 of WO2016049230), AAVF14/HSC14 (SEQ ID NO: 15 and 32 of WO2016049230), AAVF15/HSC15 (SEQ ID NO: 16 and 33 of WO2016049230), AAVF16/HSC16 (SEQ ID NO: 17 and 34 of WO2016049230), AAVF17/HSC17 (SEQ ID NO: 13 and 35 of WO2016049230), or variants or derivatives thereof.

[0093] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US 8734809, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV CBr-E1 (SEQ ID NO: 13 and 87 of US8734809), AAV CBr-E2 (SEQ ID NO: 14 and 88 of US8734809), AAV CBr-E3 (SEQ ID NO: 15 and 89 of US8734809), AAV CBr-E4 (SEQ ID NO: 16 and 90 of US8734809), AAV CBr-E5 (SEQ ID NO: 17 and 91 of US8734809), AAV CBr-e5 (SEQ ID NO: 18 and 92 of US8734809), AAV CBr-E6 (SEQ ID NO: 19 and 93 of US8734809), AAV CBr-E7 (SEQ ID NO: 20 and 94 of US8734809), AAV CBr-E8 (SEQ ID NO: 21 and 95 of US8734809), AAV CLv-D1 (SEQ ID NO: 22 and 96 of US8734809), AAV CLv-D2 (SEQ ID NO: 23 and 97 of US8734809), AAV CLv-D3 (SEQ ID NO: 24 and 98 of US8734809), AAV CLv-D4 (SEQ ID NO: 25 and 99 of US8734809), AAV CLv-D5 (SEQ ID NO: 26 and 100 of US8734809), AAV CLv-D6 (SEQ ID NO: 27 and 101 of US8734809), AAV CLv-D7 (SEQ ID NO: 28 and 102 of US8734809), AAV CLv-D8 (SEQ ID NO: 29 and 103 of US8734809), AAV CLv-E1 (SEQ ID NO: 13 and 87 of US8734809), AAV CLv-R1 (SEQ ID NO: 30 and 104 of US8734809), AAV CLv-R2 (SEQ ID NO: 31 and 105 of US8734809), AAV CLv-R3 (SEQ ID NO: 32 and 106 of US8734809), AAV CLv-R4 (SEQ ID NO: 33 and 107 of US8734809), AAV CLv-R5 (SEQ ID NO: 34 and 108 of US8734809), AAV CLv-R6 (SEQ ID NO: 35 and 109 of US8734809), AAV CLv-R7 (SEQ ID NO: 36 and 110 of US8734809), AAV CLv-R8 (SEQ ID NO: 37 and 111 of US8734809), AAV CLv-R9 (SEQ ID NO: 38 and 112 of US8734809), AAV CLg-F1 (SEQ ID NO: 39 and 113 of US8734809), AAV CLg-F2 (SEQ ID NO: 40 and 114 of US8734809), AAV CLg-F3 (SEQ ID NO: 41 and 115 of US8734809), AAV CLg-F4 (SEQ ID NO: 42 and 116 of US8734809), AAV CLg-F5 (SEQ ID NO: 43 and 117 of US8734809), AAV CLg-F6 (SEQ ID NO: 43 and 117 of US8734809), AAV CLg-F7 (SEQ ID NO: 44 and 118 of US8734809), AAV CLg-F8 (SEQ ID NO: 43 and 117 of US8734809), AAV CSp-1 (SEQ ID NO: 45 and 119 of US8734809), AAV CSp-10 (SEQ ID NO: 46 and 120 of US8734809), AAV CSp-11 (SEQ ID NO: 47 and 121 of US8734809), AAV CSp-2 (SEQ ID NO: 48 and 122 of US8734809), AAV CSp-3 (SEQ ID NO: 49 and 123 of US8734809), AAV CSp-4 (SEQ ID NO: 50 and 124 of US8734809), AAV CSp-6 (SEQ ID NO: 51 and 125 of US8734809), AAV CSp-7 (SEQ ID NO: 52 and 126 of US8734809), AAV CSp-8 (SEQ ID NO: 53 and 127 of US8734809), AAV CSp-9 (SEQ ID NO: 54 and 128 of US8734809), AAV CHt-2 (SEQ ID NO: 55 and 129 of US8734809), AAV CHt-3 (SEQ ID NO: 56 and 130 of US8734809), AAV CKd-1 (SEQ ID NO: 57 and 131 of US8734809), AAV CKd-10 (SEQ ID NO: 58 and 132 of US8734809), AAV CKd-2 (SEQ ID NO: 59 and 133 of US8734809), AAV CKd-3 (SEQ ID NO: 60 and 134 of

US8734809), AAV CKd-4 (SEQ ID NO: 61 and 135 of US8734809), AAV CKd-6 (SEQ ID NO: 62 and 136 of US8734809), AAV CKd-7 (SEQ ID NO: 63 and 137 of US8734809), AAV CKd-8 (SEQ ID NO: 64 and 138 of US8734809), AAV CLv-1 (SEQ ID NO: 35 and 139 of US8734809), AAV CLv-12 (SEQ ID NO: 66 and 140 of US8734809), AAV CLv-13 (SEQ ID NO: 67 and 141 of US8734809), AAV CLv-2 (SEQ ID NO: 68 and 142 of US8734809), AAV CLv-3 (SEQ ID NO: 69 and 143 of US8734809), AAV CLv-4 (SEQ ID NO: 70 and 144 of US8734809), AAV CLv-6 (SEQ ID NO: 71 and 145 of US8734809), AAV CLv-8 (SEQ ID NO: 72 and 146 of US8734809), AAV CKd-B1 (SEQ ID NO: 73 and 147 of US8734809), AAV CKd-B2 (SEQ ID NO: 74 and 148 of US8734809), AAV CKd-B3 (SEQ ID NO: 75 and 149 of US8734809), AAV CKd-B4 (SEQ ID NO: 76 and 150 of US8734809), AAV CKd-B5 (SEQ ID NO: 77 and 151 of US8734809), AAV CKd-B6 (SEQ ID NO: 78 and 152 of US8734809), AAV CKd-B7 (SEQ ID NO: 79 and 153 of US8734809), AAV CKd-B8 (SEQ ID NO: 80 and 154 of US8734809), AAV CKd-H1 (SEQ ID NO: 81 and 155 of US8734809), AAV CKd-H2 (SEQ ID NO: 82 and 156 of US8734809), AAV CKd-H3 (SEQ ID NO: 83 and 157 of US8734809), AAV CKd-H4 (SEQ ID NO: 84 and 158 of US8734809), AAV CKd-H5 (SEQ ID NO: 85 and 159 of US8734809), AAV CKd-H6 (SEQ ID NO: 77 and 151 of US8734809), AAV CHt-1 (SEQ ID NO: 86 and 160 of US8734809), AAV CLv1-1 (SEQ ID NO: 171 of US8734809), AAV CLv1-2 (SEQ ID NO: 172 of US8734809), AAV CLv1-3 (SEQ ID NO: 173 of US8734809), AAV CLv1-4 (SEQ ID NO: 174 of US8734809), AAV Clv1-7 (SEQ ID NO: 175 of US8734809), AAV Clv1-8 (SEQ ID NO: 176 of US8734809), AAV Clv1-9 (SEQ ID NO: 177 of US8734809), AAV Clv1-10 (SEQ ID NO: 178 of US8734809), AAV.VR-355 (SEQ ID NO: 181 of US8734809), AAV.hu.48R3 (SEQ ID NO: 183 of US8734809), or variants or derivatives thereof.

[0094] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2016065001, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAV CHt-P2 (SEQ ID NO: 1 and 51 of WO2016065001), AAV CHt-P5 (SEQ ID NO: 2 and 52 of WO2016065001), AAV CHt-P9 (SEQ ID NO: 3 and 53 of WO2016065001), AAV CBr-7.1 (SEQ ID NO: 4 and 54 of WO2016065001), AAV CBr-7.2 (SEQ ID NO: 5 and 55 of WO2016065001), AAV CBr-7.3 (SEQ ID NO: 6 and 56 of WO2016065001), AAV CBr-7.4 (SEQ ID NO: 7 and 57 of WO2016065001), AAV CBr-7.5 (SEQ ID NO: 8 and 58 of WO2016065001), AAV CBr-7.7 (SEQ ID NO: 9 and 59 of WO2016065001), AAV CBr-7.8 (SEQ ID NO: 10 and 60 of WO2016065001), AAV CBr-7.10 (SEQ ID NO: 11 and 61 of WO2016065001), AAV CKd-N3

(SEQ ID NO: 12 and 62 of WO2016065001), AAV CKd-N4 (SEQ ID NO: 13 and 63 of WO2016065001), AAV CKd-N9 (SEQ ID NO: 14 and 64 of WO2016065001), AAV CLv-L4 (SEQ ID NO: 15 and 65 of WO2016065001), AAV CLv-L5 (SEQ ID NO: 16 and 66 of WO2016065001), AAV CLv-L6 (SEQ ID NO: 17 and 67 of WO2016065001), AAV CLv-K1 (SEQ ID NO: 18 and 68 of WO2016065001), AAV CLv-K3 (SEQ ID NO: 19 and 69 of WO2016065001), AAV CLv-K6 (SEQ ID NO: 20 and 70 of WO2016065001), AAV CLv-M1 (SEQ ID NO: 21 and 71 of WO2016065001), AAV CLv-M11 (SEQ ID NO: 22 and 72 of WO2016065001), AAV CLv-M2 (SEQ ID NO: 23 and 73 of WO2016065001), AAV CLv-M5 (SEQ ID NO: 24 and 74 of WO2016065001), AAV CLv-M6 (SEQ ID NO: 25 and 75 of WO2016065001), AAV CLv-M7 (SEQ ID NO: 26 and 76 of WO2016065001), AAV CLv-M8 (SEQ ID NO: 27 and 77 of WO2016065001), AAV CLv-M9 (SEQ ID NO: 28 and 78 of WO2016065001), AAV CHt-P1 (SEQ ID NO: 29 and 79 of WO2016065001), AAV CHt-P6 (SEQ ID NO: 30 and 80 of WO2016065001), AAV CHt-P8 (SEQ ID NO: 31 and 81 of WO2016065001), AAV CHt-6.1 (SEQ ID NO: 32 and 82 of WO2016065001), AAV CHt-6.10 (SEQ ID NO: 33 and 83 of WO2016065001), AAV CHt-6.5 (SEQ ID NO: 34 and 84 of WO2016065001), AAV CHt-6.6 (SEQ ID NO: 35 and 85 of WO2016065001), AAV CHt-6.7 (SEQ ID NO: 36 and 86 of WO2016065001), AAV CHt-6.8 (SEQ ID NO: 37 and 87 of WO2016065001), AAV CSp-8.10 (SEQ ID NO: 38 and 88 of WO2016065001), AAV CSp-8.2 (SEQ ID NO: 39 and 89 of WO2016065001), AAV CSp-8.4 (SEQ ID NO: 40 and 90 of WO2016065001), AAV CSp-8.5 (SEQ ID NO: 41 and 91 of WO2016065001), AAV CSp-8.6 (SEQ ID NO: 42 and 92 of WO2016065001), AAV CSp-8.7 (SEQ ID NO: 43 and 93 of WO2016065001), AAV CSp-8.8 (SEQ ID NO: 44 and 94 of WO2016065001), AAV CSp-8.9 (SEQ ID NO: 45 and 95 of WO2016065001), AAV CBr-B7.3 (SEQ ID NO: 46 and 96 of WO2016065001), AAV CBr-B7.4 (SEQ ID NO: 47 and 97 of WO2016065001), AAV3B (SEQ ID NO: 48 and 98 of WO2016065001), AAV4 (SEQ ID NO: 49 and 99 of WO2016065001), AAV5 (SEQ ID NO: 50 and 100 of WO2016065001), or variants or derivatives thereof.

[0095] In one embodiment, the AAV may be a serotype selected from any of those found in Table 1.

[0096] In one embodiment, the AAV may comprise a sequence, fragment or variant thereof, of the sequences in Table 1.

[0097] In one embodiment, the AAV may be encoded by a sequence, fragment or variant as described in Table 1.

Table 1. AAV Serotypes

Serotype	SEQ ID NO	Reference Information
AAV1	1	US20150159173 SEQ ID NO: 11, US20150315612 SEQ ID NO: 202
AAV1	2	US20160017295 SEQ ID NO: 1US20030138772 SEQ ID NO: 64, US20150159173 SEQ ID NO: 27, US20150315612 SEQ ID NO: 219, US7198951 SEQ ID NO: 5
AAV1	3	US20030138772 SEQ ID NO: 6
AAV1.3	4	US20030138772 SEQ ID NO: 14
AAV10	5	US20030138772 SEQ ID NO: 117
AAV10	6	WO2015121501 SEQ ID NO: 9
AAV10	7	WO2015121501 SEQ ID NO: 8
AAV11	8	US20030138772 SEQ ID NO: 118
AAV12	9	US20030138772 SEQ ID NO: 119
AAV2	10	US20150159173 SEQ ID NO: 7, US20150315612 SEQ ID NO: 211
AAV2	11	US20030138772 SEQ ID NO: 70, US20150159173 SEQ ID NO: 23, US20150315612 SEQ ID NO: 221, US20160017295 SEQ ID NO: 2, US6156303 SEQ ID NO: 4, US7198951 SEQ ID NO: 4, WO2015121501 SEQ ID NO: 1
AAV2	12	US6156303 SEQ ID NO: 8
AAV2	13	US20030138772 SEQ ID NO: 7
AAV2	14	US6156303 SEQ ID NO: 3
AAV2.5T	15	US9233131 SEQ ID NO: 42
AAV223.10	16	US20030138772 SEQ ID NO: 75
AAV223.2	17	US20030138772 SEQ ID NO: 49
AAV223.2	18	US20030138772 SEQ ID NO: 76
AAV223.4	19	US20030138772 SEQ ID NO: 50
AAV223.4	20	US20030138772 SEQ ID NO: 73
AAV223.5	21	US20030138772 SEQ ID NO: 51
AAV223.5	22	US20030138772 SEQ ID NO: 74
AAV223.6	23	US20030138772 SEQ ID NO: 52
AAV223.6	24	US20030138772 SEQ ID NO: 78
AAV223.7	25	US20030138772 SEQ ID NO: 53
AAV223.7	26	US20030138772 SEQ ID NO: 77
AAV29.3	27	US20030138772 SEQ ID NO: 82
AAV29.4	28	US20030138772 SEQ ID NO: 12
AAV29.5	29	US20030138772 SEQ ID NO: 83
AAV29.5 (AAVbb.2)	30	US20030138772 SEQ ID NO: 13
AAV3	31	US20150159173 SEQ ID NO: 12
AAV3	32	US20030138772 SEQ ID NO: 71, US20150159173 SEQ ID NO: 28, US20160017295 SEQ ID NO: 3, US7198951 SEQ ID NO: 6
AAV3	33	US20030138772 SEQ ID NO: 8
AAV3.3b	34	US20030138772 SEQ ID NO: 72
AAV3-3	35	US20150315612 SEQ ID NO: 200
AAV3-3	36	US20150315612 SEQ ID NO: 217
AAV3a	37	US6156303 SEQ ID NO: 5
AAV3a	38	US6156303 SEQ ID NO: 9
AAV3b	39	US6156303 SEQ ID NO: 6

AAV3b	40	US6156303 SEQ ID NO: 10
AAV3b	41	US6156303 SEQ ID NO: 1
AAV4	42	US20140348794 SEQ ID NO: 17
AAV4	43	US20140348794 SEQ ID NO: 5
AAV4	44	US20140348794 SEQ ID NO: 3
AAV4	45	US20140348794 SEQ ID NO: 14
AAV4	46	US20140348794 SEQ ID NO: 15
AAV4	47	US20140348794 SEQ ID NO: 19
AAV4	48	US20140348794 SEQ ID NO: 12
AAV4	49	US20140348794 SEQ ID NO: 13
AAV4	50	US20140348794 SEQ ID NO: 7
AAV4	51	US20140348794 SEQ ID NO: 8
AAV4	52	US20140348794 SEQ ID NO: 9
AAV4	53	US20140348794 SEQ ID NO: 2
AAV4	54	US20140348794 SEQ ID NO: 10
AAV4	55	US20140348794 SEQ ID NO: 11
AAV4	56	US20140348794 SEQ ID NO: 18
AAV4	57	US20030138772 SEQ ID NO: 63, US20160017295 SEQ ID NO: 4, US20140348794 SEQ ID NO: 4
AAV4	58	US20140348794 SEQ ID NO: 16
AAV4	59	US20140348794 SEQ ID NO: 20
AAV4	60	US20140348794 SEQ ID NO: 6
AAV4	61	US20140348794 SEQ ID NO: 1
AAV42.2	62	US20030138772 SEQ ID NO: 9
AAV42.2	63	US20030138772 SEQ ID NO: 102
AAV42.3b	64	US20030138772 SEQ ID NO: 36
AAV42.3B	65	US20030138772 SEQ ID NO: 107
AAV42.4	66	US20030138772 SEQ ID NO: 33
AAV42.4	67	US20030138772 SEQ ID NO: 88
AAV42.8	68	US20030138772 SEQ ID NO: 27
AAV42.8	69	US20030138772 SEQ ID NO: 85
AAV43.1	70	US20030138772 SEQ ID NO: 39
AAV43.1	71	US20030138772 SEQ ID NO: 92
AAV43.12	72	US20030138772 SEQ ID NO: 41
AAV43.12	73	US20030138772 SEQ ID NO: 93
AAV43.20	74	US20030138772 SEQ ID NO: 42
AAV43.20	75	US20030138772 SEQ ID NO: 99
AAV43.21	76	US20030138772 SEQ ID NO: 43
AAV43.21	77	US20030138772 SEQ ID NO: 96
AAV43.23	78	US20030138772 SEQ ID NO: 44
AAV43.23	79	US20030138772 SEQ ID NO: 98
AAV43.25	80	US20030138772 SEQ ID NO: 45
AAV43.25	81	US20030138772 SEQ ID NO: 97
AAV43.5	82	US20030138772 SEQ ID NO: 40

AAV43.5	83	US20030138772 SEQ ID NO: 94
AAV4-4	84	US20150315612 SEQ ID NO: 201
AAV4-4	85	US20150315612 SEQ ID NO: 218
AAV44.1	86	US20030138772 SEQ ID NO: 46
AAV44.1	87	US20030138772 SEQ ID NO: 79
AAV44.5	88	US20030138772 SEQ ID NO: 47
AAV44.5	89	US20030138772 SEQ ID NO: 80
AAV4407	90	US20150315612 SEQ ID NO: 90
AAV5	91	US7427396 SEQ ID NO: 1
AAV5	92	US20030138772 SEQ ID NO: 114
AAV5	93	US20160017295 SEQ ID NO: 5, US7427396 SEQ ID NO: 2, US20150315612 SEQ ID NO: 216
AAV5	94	US20150315612 SEQ ID NO: 199
AAV6	95	US20150159173 SEQ ID NO: 13
AAV6	96	US20030138772 SEQ ID NO: 65, US20150159173 SEQ ID NO: 29, US20160017295 SEQ ID NO: 6, US6156303 SEQ ID NO: 7
AAV6	97	US6156303 SEQ ID NO: 11
AAV6	98	US6156303 SEQ ID NO: 2
AAV6	99	US20150315612 SEQ ID NO: 203
AAV6	100	US20150315612 SEQ ID NO: 220
AAV6.1	101	US20150159173
AAV6.12	102	US20150159173
AAV6.2	103	US20150159173
AAV7	104	US20150159173 SEQ ID NO: 14
AAV7	105	US20150315612 SEQ ID NO: 183
AAV7	106	US20030138772 SEQ ID NO: 2, US20150159173 SEQ ID NO: 30, US20150315612 SEQ ID NO: 181, US20160017295 SEQ ID NO: 7
AAV7	107	US20030138772 SEQ ID NO: 3
AAV7	108	US20030138772 SEQ ID NO: 1, US20150315612 SEQ ID NO: 180
AAV7	109	US20150315612 SEQ ID NO: 213
AAV7	110	US20150315612 SEQ ID NO: 222
AAV8	111	US20150159173 SEQ ID NO: 15
AAV8	112	US20150376240 SEQ ID NO: 7
AAV8	113	US20030138772 SEQ ID NO: 4, US20150315612 SEQ ID NO: 182
AAV8	114	US20030138772 SEQ ID NO: 95, US20140359799 SEQ ID NO: 1, US20150159173 SEQ ID NO: 31, US20160017295 SEQ ID NO: 8, US7198951 SEQ ID NO: 7, US20150315612 SEQ ID NO: 223
AAV8	115	US20150376240 SEQ ID NO: 8
AAV8	116	US20150315612 SEQ ID NO: 214
AAV-8b	117	US20150376240 SEQ ID NO: 5
AAV-8b	118	US20150376240 SEQ ID NO: 3
AAV-8h	119	US20150376240 SEQ ID NO: 6
AAV-8h	120	US20150376240 SEQ ID NO: 4
AAV9	121	US20030138772 SEQ ID NO: 5
AAV9	122	US7198951 SEQ ID NO: 1
AAV9	123	US20160017295 SEQ ID NO: 9

AAV9	124	US20030138772 SEQ ID NO: 100, US7198951 SEQ ID NO: 2
AAV9	125	US7198951 SEQ ID NO: 3
AAV9 (AAVhu.14)	126	US7906111 SEQ ID NO: 3; WO2015038958 SEQ ID NO: 11
AAV9 (AAVhu.14)	127	US7906111 SEQ ID NO: 123; WO2015038958 SEQ ID NO: 2
AAVA3.1	128	US20030138772 SEQ ID NO: 120
AAVA3.3	129	US20030138772 SEQ ID NO: 57
AAVA3.3	130	US20030138772 SEQ ID NO: 66
AAVA3.4	131	US20030138772 SEQ ID NO: 54
AAVA3.4	132	US20030138772 SEQ ID NO: 68
AAVA3.5	133	US20030138772 SEQ ID NO: 55
AAVA3.5	134	US20030138772 SEQ ID NO: 69
AAVA3.7	135	US20030138772 SEQ ID NO: 56
AAVA3.7	136	US20030138772 SEQ ID NO: 67
AAV29.3 (AAVbb.1)	137	US20030138772 SEQ ID NO: 11
AAVC2	138	US20030138772 SEQ ID NO: 61
AAVCh.5	139	US20150159173 SEQ ID NO: 46, US20150315612 SEQ ID NO: 234
AAVcy.2 (AAV13.3)	140	US20030138772 SEQ ID NO: 15
AAV24.1	141	US20030138772 SEQ ID NO: 101
AAVcy.3 (AAV24.1)	142	US20030138772 SEQ ID NO: 16
AAV27.3	143	US20030138772 SEQ ID NO: 104
AAVcy.4 (AAV27.3)	144	US20030138772 SEQ ID NO: 17
AAVcy.5	145	US20150315612 SEQ ID NO: 227
AAV7.2	146	US20030138772 SEQ ID NO: 103
AAVcy.5 (AAV7.2)	147	US20030138772 SEQ ID NO: 18
AAV16.3	148	US20030138772 SEQ ID NO: 105
AAVcy.6 (AAV16.3)	149	US20030138772 SEQ ID NO: 10
AAVcy.5	150	US20150159173 SEQ ID NO: 8
AAVcy.5	151	US20150159173 SEQ ID NO: 24
AAVCy.5R1	152	US20150159173
AAVCy.5R2	153	US20150159173
AAVCy.5R3	154	US20150159173
AAVCy.5R4	155	US20150159173
AAVDJ	156	US20140359799 SEQ ID NO: 3, US7588772 SEQ ID NO: 2
AAVDJ	157	US20140359799 SEQ ID NO: 2, US7588772 SEQ ID NO: 1
AAVDJ-8	158	US7588772; Grimm et al 2008
AAVDJ-8	159	US7588772; Grimm et al 2008
AAVF5	160	US20030138772 SEQ ID NO: 110
AAVH2	161	US20030138772 SEQ ID NO: 26
AAVH6	162	US20030138772 SEQ ID NO: 25
AAVhE1.1	163	US9233131 SEQ ID NO: 44

AAVhEr1.14	164	US9233131 SEQ ID NO: 46
AAVhEr1.16	165	US9233131 SEQ ID NO: 48
AAVhEr1.18	166	US9233131 SEQ ID NO: 49
AAVhEr1.23 (AAVhEr2.29)	167	US9233131 SEQ ID NO: 53
AAVhEr1.35	168	US9233131 SEQ ID NO: 50
AAVhEr1.36	169	US9233131 SEQ ID NO: 52
AAVhEr1.5	170	US9233131 SEQ ID NO: 45
AAVhEr1.7	171	US9233131 SEQ ID NO: 51
AAVhEr1.8	172	US9233131 SEQ ID NO: 47
AAVhEr2.16	173	US9233131 SEQ ID NO: 55
AAVhEr2.30	174	US9233131 SEQ ID NO: 56
AAVhEr2.31	175	US9233131 SEQ ID NO: 58
AAVhEr2.36	176	US9233131 SEQ ID NO: 57
AAVhEr2.4	177	US9233131 SEQ ID NO: 54
AAVhEr3.1	178	US9233131 SEQ ID NO: 59
AAVhu.1	179	US20150315612 SEQ ID NO: 46
AAVhu.1	180	US20150315612 SEQ ID NO: 144
AAVhu.10 (AAV16.8)	181	US20150315612 SEQ ID NO: 56
AAVhu.10 (AAV16.8)	182	US20150315612 SEQ ID NO: 156
AAVhu.11 (AAV16.12)	183	US20150315612 SEQ ID NO: 57
AAVhu.11 (AAV16.12)	184	US20150315612 SEQ ID NO: 153
AAVhu.12	185	US20150315612 SEQ ID NO: 59
AAVhu.12	186	US20150315612 SEQ ID NO: 154
AAVhu.13	187	US20150159173 SEQ ID NO: 16, US20150315612 SEQ ID NO: 71
AAVhu.13	188	US20150159173 SEQ ID NO: 32, US20150315612 SEQ ID NO: 129
AAVhu.136.1	189	US20150315612 SEQ ID NO: 165
AAVhu.140.1	190	US20150315612 SEQ ID NO: 166
AAVhu.140.2	191	US20150315612 SEQ ID NO: 167
AAVhu.145.6	192	US20150315612 SEQ ID No: 178
AAVhu.15	193	US20150315612 SEQ ID NO: 147
AAVhu.15 (AAV33.4)	194	US20150315612 SEQ ID NO: 50
AAVhu.156.1	195	US20150315612 SEQ ID No: 179
AAVhu.16	196	US20150315612 SEQ ID NO: 148
AAVhu.16 (AAV33.8)	197	US20150315612 SEQ ID NO: 51
AAVhu.17	198	US20150315612 SEQ ID NO: 83
AAVhu.17 (AAV33.12)	199	US20150315612 SEQ ID NO: 4

AAVhu.172.1	200	US20150315612 SEQ ID NO: 171
AAVhu.172.2	201	US20150315612 SEQ ID NO: 172
AAVhu.173.4	202	US20150315612 SEQ ID NO: 173
AAVhu.173.8	203	US20150315612 SEQ ID NO: 175
AAVhu.18	204	US20150315612 SEQ ID NO: 52
AAVhu.18	205	US20150315612 SEQ ID NO: 149
AAVhu.19	206	US20150315612 SEQ ID NO: 62
AAVhu.19	207	US20150315612 SEQ ID NO: 133
AAVhu.2	208	US20150315612 SEQ ID NO: 48
AAVhu.2	209	US20150315612 SEQ ID NO: 143
AAVhu.20	210	US20150315612 SEQ ID NO: 63
AAVhu.20	211	US20150315612 SEQ ID NO: 134
AAVhu.21	212	US20150315612 SEQ ID NO: 65
AAVhu.21	213	US20150315612 SEQ ID NO: 135
AAVhu.22	214	US20150315612 SEQ ID NO: 67
AAVhu.22	215	US20150315612 SEQ ID NO: 138
AAVhu.23	216	US20150315612 SEQ ID NO: 60
AAVhu.23.2	217	US20150315612 SEQ ID NO: 137
AAVhu.24	218	US20150315612 SEQ ID NO: 66
AAVhu.24	219	US20150315612 SEQ ID NO: 136
AAVhu.25	220	US20150315612 SEQ ID NO: 49
AAVhu.25	221	US20150315612 SEQ ID NO: 146
AAVhu.26	222	US20150159173 SEQ ID NO: 17, US20150315612 SEQ ID NO: 61
AAVhu.26	223	US20150159173 SEQ ID NO: 33, US20150315612 SEQ ID NO: 139
AAVhu.27	224	US20150315612 SEQ ID NO: 64
AAVhu.27	225	US20150315612 SEQ ID NO: 140
AAVhu.28	226	US20150315612 SEQ ID NO: 68
AAVhu.28	227	US20150315612 SEQ ID NO: 130
AAVhu.29	228	US20150315612 SEQ ID NO: 69
AAVhu.29	229	US20150159173 SEQ ID NO: 42, US20150315612 SEQ ID NO: 132
AAVhu.29	230	US20150315612 SEQ ID NO: 225
AAVhu.29R	231	US20150159173
AAVhu.3	232	US20150315612 SEQ ID NO: 44
AAVhu.3	233	US20150315612 SEQ ID NO: 145
AAVhu.30	234	US20150315612 SEQ ID NO: 70
AAVhu.30	235	US20150315612 SEQ ID NO: 131
AAVhu.31	236	US20150315612 SEQ ID NO: 1
AAVhu.31	237	US20150315612 SEQ ID NO: 121
AAVhu.32	238	US20150315612 SEQ ID NO: 2
AAVhu.32	239	US20150315612 SEQ ID NO: 122
AAVhu.33	240	US20150315612 SEQ ID NO: 75
AAVhu.33	241	US20150315612 SEQ ID NO: 124

AAVhu.34	242	US20150315612 SEQ ID NO: 72
AAVhu.34	243	US20150315612 SEQ ID NO: 125
AAVhu.35	244	US20150315612 SEQ ID NO: 73
AAVhu.35	245	US20150315612 SEQ ID NO: 164
AAVhu.36	246	US20150315612 SEQ ID NO: 74
AAVhu.36	247	US20150315612 SEQ ID NO: 126
AAVhu.37	248	US20150159173 SEQ ID NO: 34, US20150315612 SEQ ID NO: 88
AAVhu.37 (AAV106.1)	249	US20150315612 SEQ ID NO: 10, US20150159173 SEQ ID NO: 18
AAVhu.38	250	US20150315612 SEQ ID NO: 161
AAVhu.39	251	US20150315612 SEQ ID NO: 102
AAVhu.39 (AAVLG-9)	252	US20150315612 SEQ ID NO: 24
AAVhu.4	253	US20150315612 SEQ ID NO: 47
AAVhu.4	254	US20150315612 SEQ ID NO: 141
AAVhu.40	255	US20150315612 SEQ ID NO: 87
AAVhu.40 (AAV114.3)	256	US20150315612 SEQ ID No: 11
AAVhu.41	257	US20150315612 SEQ ID NO: 91
AAVhu.41 (AAV127.2)	258	US20150315612 SEQ ID NO: 6
AAVhu.42	259	US20150315612 SEQ ID NO: 85
AAVhu.42 (AAV127.5)	260	US20150315612 SEQ ID NO: 8
AAVhu.43	261	US20150315612 SEQ ID NO: 160
AAVhu.43	262	US20150315612 SEQ ID NO: 236
AAVhu.43 (AAV128.1)	263	US20150315612 SEQ ID NO: 80
AAVhu.44	264	US20150159173 SEQ ID NO: 45, US20150315612 SEQ ID NO: 158
AAVhu.44 (AAV128.3)	265	US20150315612 SEQ ID NO: 81
AAVhu.44R1	266	US20150159173
AAVhu.44R2	267	US20150159173
AAVhu.44R3	268	US20150159173
AAVhu.45	269	US20150315612 SEQ ID NO: 76
AAVhu.45	270	US20150315612 SEQ ID NO: 127
AAVhu.46	271	US20150315612 SEQ ID NO: 82
AAVhu.46	272	US20150315612 SEQ ID NO: 159
AAVhu.46	273	US20150315612 SEQ ID NO: 224
AAVhu.47	274	US20150315612 SEQ ID NO: 77
AAVhu.47	275	US20150315612 SEQ ID NO: 128
AAVhu.48	276	US20150159173 SEQ ID NO: 38
AAVhu.48	277	US20150315612 SEQ ID NO: 157
AAVhu.48 (AAV130.4)	278	US20150315612 SEQ ID NO: 78
AAVhu.48R1	279	US20150159173
AAVhu.48R2	280	US20150159173
AAVhu.48R3	281	US20150159173

AAVhu.49	282	US20150315612 SEQ ID NO: 209
AAVhu.49	283	US20150315612 SEQ ID NO: 189
AAVhu.5	284	US20150315612 SEQ ID NO: 45
AAVhu.5	285	US20150315612 SEQ ID NO: 142
AAVhu.51	286	US20150315612 SEQ ID NO: 208
AAVhu.51	287	US20150315612 SEQ ID NO: 190
AAVhu.52	288	US20150315612 SEQ ID NO: 210
AAVhu.52	289	US20150315612 SEQ ID NO: 191
AAVhu.53	290	US20150159173 SEQ ID NO: 19
AAVhu.53	291	US20150159173 SEQ ID NO: 35
AAVhu.53 (AAV145.1)	292	US20150315612 SEQ ID NO: 176
AAVhu.54	293	US20150315612 SEQ ID NO: 188
AAVhu.54 (AAV145.5)	294	US20150315612 SEQ ID No: 177
AAVhu.55	295	US20150315612 SEQ ID NO: 187
AAVhu.56	296	US20150315612 SEQ ID NO: 205
AAVhu.56 (AAV145.6)	297	US20150315612 SEQ ID NO: 168
AAVhu.56 (AAV145.6)	298	US20150315612 SEQ ID NO: 192
AAVhu.57	299	US20150315612 SEQ ID NO: 206
AAVhu.57	300	US20150315612 SEQ ID NO: 169
AAVhu.57	301	US20150315612 SEQ ID NO: 193
AAVhu.58	302	US20150315612 SEQ ID NO: 207
AAVhu.58	303	US20150315612 SEQ ID NO: 194
AAVhu.6 (AAV3.1)	304	US20150315612 SEQ ID NO: 5
AAVhu.6 (AAV3.1)	305	US20150315612 SEQ ID NO: 84
AAVhu.60	306	US20150315612 SEQ ID NO: 184
AAVhu.60 (AAV161.10)	307	US20150315612 SEQ ID NO: 170
AAVhu.61	308	US20150315612 SEQ ID NO: 185
AAVhu.61 (AAV161.6)	309	US20150315612 SEQ ID NO: 174
AAVhu.63	310	US20150315612 SEQ ID NO: 204
AAVhu.63	311	US20150315612 SEQ ID NO: 195
AAVhu.64	312	US20150315612 SEQ ID NO: 212
AAVhu.64	313	US20150315612 SEQ ID NO: 196
AAVhu.66	314	US20150315612 SEQ ID NO: 197
AAVhu.67	315	US20150315612 SEQ ID NO: 215
AAVhu.67	316	US20150315612 SEQ ID NO: 198
AAVhu.7	317	US20150315612 SEQ ID NO: 226
AAVhu.7	318	US20150315612 SEQ ID NO: 150
AAVhu.7 (AAV7.3)	319	US20150315612 SEQ ID NO: 55
AAVhu.71	320	US20150315612 SEQ ID NO: 79

AAVhu.8	321	US20150315612 SEQ ID NO: 53
AAVhu.8	322	US20150315612 SEQ ID NO: 12
AAVhu.8	323	US20150315612 SEQ ID NO: 151
AAVhu.9 (AAV3.1)	324	US20150315612 SEQ ID NO: 58
AAVhu.9 (AAV3.1)	325	US20150315612 SEQ ID NO: 155
AAV-LK01	326	US20150376607 SEQ ID NO: 2
AAV-LK01	327	US20150376607 SEQ ID NO: 29
AAV-LK02	328	US20150376607 SEQ ID NO: 3
AAV-LK02	329	US20150376607 SEQ ID NO: 30
AAV-LK03	330	US20150376607 SEQ ID NO: 4
AAV-LK03	331	WO2015121501 SEQ ID NO: 12, US20150376607 SEQ ID NO: 31
AAV-LK04	332	US20150376607 SEQ ID NO: 5
AAV-LK04	333	US20150376607 SEQ ID NO: 32
AAV-LK05	334	US20150376607 SEQ ID NO: 6
AAV-LK05	335	US20150376607 SEQ ID NO: 33
AAV-LK06	336	US20150376607 SEQ ID NO: 7
AAV-LK06	337	US20150376607 SEQ ID NO: 34
AAV-LK07	338	US20150376607 SEQ ID NO: 8
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AAV-LK08	340	US20150376607 SEQ ID NO: 9
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AAV-LK11	346	US20150376607 SEQ ID NO: 12
AAV-LK11	347	US20150376607 SEQ ID NO: 39
AAV-LK12	348	US20150376607 SEQ ID NO: 13
AAV-LK12	349	US20150376607 SEQ ID NO: 40
AAV-LK13	350	US20150376607 SEQ ID NO: 14
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AAV-LK15	354	US20150376607 SEQ ID NO: 16
AAV-LK15	355	US20150376607 SEQ ID NO: 43
AAV-LK16	356	US20150376607 SEQ ID NO: 17
AAV-LK16	357	US20150376607 SEQ ID NO: 44
AAV-LK17	358	US20150376607 SEQ ID NO: 18
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AAV-LK18	360	US20150376607 SEQ ID NO: 19
AAV-LK18	361	US20150376607 SEQ ID NO: 46
AAV-LK19	362	US20150376607 SEQ ID NO: 20
AAV-LK19	363	US20150376607 SEQ ID NO: 47

AAV-PAEC	364	US20150376607 SEQ ID NO: 1
AAV-PAEC	365	US20150376607 SEQ ID NO: 48
AAV-PAEC11	366	US20150376607 SEQ ID NO: 26
AAV-PAEC11	367	US20150376607 SEQ ID NO: 54
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AAV-PAEC12	369	US20150376607 SEQ ID NO: 51
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AAV-PAEC2	372	US20150376607 SEQ ID NO: 21
AAV-PAEC2	373	US20150376607 SEQ ID NO: 56
AAV-PAEC4	374	US20150376607 SEQ ID NO: 22
AAV-PAEC4	375	US20150376607 SEQ ID NO: 55
AAV-PAEC6	376	US20150376607 SEQ ID NO: 23
AAV-PAEC6	377	US20150376607 SEQ ID NO: 52
AAV-PAEC7	378	US20150376607 SEQ ID NO: 24
AAV-PAEC7	379	US20150376607 SEQ ID NO: 53
AAV-PAEC8	380	US20150376607 SEQ ID NO: 25
AAV-PAEC8	381	US20150376607 SEQ ID NO: 50
AAVpi.1	382	US20150315612 SEQ ID NO: 28
AAVpi.1	383	US20150315612 SEQ ID NO: 93
AAVpi.2	384	US20150315612 SEQ ID NO: 30
AAVpi.2	385	US20150315612 SEQ ID NO: 95
AAVpi.3	386	US20150315612 SEQ ID NO: 29
AAVpi.3	387	US20150315612 SEQ ID NO: 94
AAVrh.10	388	US20150159173 SEQ ID NO: 9
AAVrh.10	389	US20150159173 SEQ ID NO: 25
AAV44.2	390	US20030138772 SEQ ID NO: 59
AAVrh.10 (AAV44.2)	391	US20030138772 SEQ ID NO: 81
AAV42.1B	392	US20030138772 SEQ ID NO: 90
AAVrh.12 (AAV42.1b)	393	US20030138772 SEQ ID NO: 30
AAVrh.13	394	US20150159173 SEQ ID NO: 10
AAVrh.13	395	US20150159173 SEQ ID NO: 26
AAVrh.13	396	US20150315612 SEQ ID NO: 228
AAVrh.13R	397	US20150159173
AAV42.3A	398	US20030138772 SEQ ID NO: 87
AAVrh.14 (AAV42.3a)	399	US20030138772 SEQ ID NO: 32
AAV42.5A	400	US20030138772 SEQ ID NO: 89
AAVrh.17 (AAV42.5a)	401	US20030138772 SEQ ID NO: 34
AAV42.5B	402	US20030138772 SEQ ID NO: 91

AAVrh.18 (AAV42.5b)	403	US20030138772 SEQ ID NO: 29
AAV42.6B	404	US20030138772 SEQ ID NO: 112
AAVrh.19 (AAV42.6b)	405	US20030138772 SEQ ID NO: 38
AAVrh.2	406	US20150159173 SEQ ID NO: 39
AAVrh.2	407	US20150315612 SEQ ID NO: 231
AAVrh.20	408	US20150159173 SEQ ID NO: 1
AAV42.10	409	US20030138772 SEQ ID NO: 106
AAVrh.21 (AAV42.10)	410	US20030138772 SEQ ID NO: 35
AAV42.11	411	US20030138772 SEQ ID NO: 108
AAVrh.22 (AAV42.11)	412	US20030138772 SEQ ID NO: 37
AAV42.12	413	US20030138772 SEQ ID NO: 113
AAVrh.23 (AAV42.12)	414	US20030138772 SEQ ID NO: 58
AAV42.13	415	US20030138772 SEQ ID NO: 86
AAVrh.24 (AAV42.13)	416	US20030138772 SEQ ID NO: 31
AAV42.15	417	US20030138772 SEQ ID NO: 84
AAVrh.25 (AAV42.15)	418	US20030138772 SEQ ID NO: 28
AAVrh.2R	419	US20150159173
AAVrh.31 (AAV223.1)	420	US20030138772 SEQ ID NO: 48
AAVC1	421	US20030138772 SEQ ID NO: 60
AAVrh.32 (AAVC1)	422	US20030138772 SEQ ID NO: 19
AAVrh.32/33	423	US20150159173 SEQ ID NO: 2
AAVrh.33 (AAVC3)	424	US20030138772 SEQ ID NO: 20
AAVC5	425	US20030138772 SEQ ID NO: 62
AAVrh.34 (AAVC5)	426	US20030138772 SEQ ID NO: 21
AAVF1	427	US20030138772 SEQ ID NO: 109
AAVrh.35 (AAVF1)	428	US20030138772 SEQ ID NO: 22
AAVF3	429	US20030138772 SEQ ID NO: 111
AAVrh.36 (AAVF3)	430	US20030138772 SEQ ID NO: 23
AAVrh.37	431	US20030138772 SEQ ID NO: 24
AAVrh.37	432	US20150159173 SEQ ID NO: 40
AAVrh.37	433	US20150315612 SEQ ID NO: 229
AAVrh.37R2	434	US20150159173
AAVrh.38 (AAVLG-4)	435	US20150315612 SEQ ID NO: 7
AAVrh.38 (AAVLG-4)	436	US20150315612 SEQ ID NO: 86
AAVrh.39	437	US20150159173 SEQ ID NO: 20, US20150315612 SEQ ID NO: 13
AAVrh.39	438	US20150159173 SEQ ID NO: 3, US20150159173 SEQ ID NO: 36, US20150315612 SEQ ID NO: 89

AAVrh.40	439	US20150315612 SEQ ID NO: 92
AAVrh.40 (AAVLG-10)	440	US20150315612 SEQ ID No: 14
AAVrh.43 (AAVN721-8)	441	US20150315612 SEQ ID NO: 43, US20150159173 SEQ ID NO: 21
AAVrh.43 (AAVN721-8)	442	US20150315612 SEQ ID NO: 163, US20150159173 SEQ ID NO: 37
AAVrh.44	443	US20150315612 SEQ ID NO: 34
AAVrh.44	444	US20150315612 SEQ ID NO: 111
AAVrh.45	445	US20150315612 SEQ ID NO: 41
AAVrh.45	446	US20150315612 SEQ ID NO: 109
AAVrh.46	447	US20150159173 SEQ ID NO: 22, US20150315612 SEQ ID NO: 19
AAVrh.46	448	US20150159173 SEQ ID NO: 4, US20150315612 SEQ ID NO: 101
AAVrh.47	449	US20150315612 SEQ ID NO: 38
AAVrh.47	450	US20150315612 SEQ ID NO: 118
AAVrh.48	451	US20150159173 SEQ ID NO: 44, US20150315612 SEQ ID NO: 115
AAVrh.48.1	452	US20150159173
AAVrh.48.1.2	453	US20150159173
AAVrh.48.2	454	US20150159173
AAVrh.48 (AAV1-7)	455	US20150315612 SEQ ID NO: 32
AAVrh.49 (AAV1-8)	456	US20150315612 SEQ ID NO: 25
AAVrh.49 (AAV1-8)	457	US20150315612 SEQ ID NO: 103
AAVrh.50 (AAV2-4)	458	US20150315612 SEQ ID NO: 23
AAVrh.50 (AAV2-4)	459	US20150315612 SEQ ID NO: 108
AAVrh.51 (AAV2-5)	460	US20150315612 SEQ ID No: 22
AAVrh.51 (AAV2-5)	461	US20150315612 SEQ ID NO: 104
AAVrh.52 (AAV3-9)	462	US20150315612 SEQ ID NO: 18
AAVrh.52 (AAV3-9)	463	US20150315612 SEQ ID NO: 96
AAVrh.53	464	US20150315612 SEQ ID NO: 97
AAVrh.53 (AAV3-11)	465	US20150315612 SEQ ID NO: 17
AAVrh.53 (AAV3-11)	466	US20150315612 SEQ ID NO: 186
AAVrh.54	467	US20150315612 SEQ ID NO: 40
AAVrh.54	468	US20150159173 SEQ ID NO: 49, US20150315612 SEQ ID NO: 116
AAVrh.55	469	US20150315612 SEQ ID NO: 37
AAVrh.55 (AAV4-19)	470	US20150315612 SEQ ID NO: 117
AAVrh.56	471	US20150315612 SEQ ID NO: 54
AAVrh.56	472	US20150315612 SEQ ID NO: 152

AAVrh.57	473	US20150315612 SEQ ID NO: 26
AAVrh.57	474	US20150315612 SEQ ID NO: 105
AAVrh.58	475	US20150315612 SEQ ID NO: 27
AAVrh.58	476	US20150159173 SEQ ID NO: 48, US20150315612 SEQ ID NO: 106
AAVrh.58	477	US20150315612 SEQ ID NO: 232
AAVrh.59	478	US20150315612 SEQ ID NO: 42
AAVrh.59	479	US20150315612 SEQ ID NO: 110
AAVrh.60	480	US20150315612 SEQ ID NO: 31
AAVrh.60	481	US20150315612 SEQ ID NO: 120
AAVrh.61	482	US20150315612 SEQ ID NO: 107
AAVrh.61 (AAV2-3)	483	US20150315612 SEQ ID NO: 21
AAVrh.62 (AAV2-15)	484	US20150315612 SEQ ID No: 33
AAVrh.62 (AAV2-15)	485	US20150315612 SEQ ID NO: 114
AAVrh.64	486	US20150315612 SEQ ID No: 15
AAVrh.64	487	US20150159173 SEQ ID NO: 43, US20150315612 SEQ ID NO: 99
AAVrh.64	488	US20150315612 SEQ ID NO: 233
AAVRh.64R 1	489	US20150159173
AAVRh.64R 2	490	US20150159173
AAVrh.65	491	US20150315612 SEQ ID NO: 35
AAVrh.65	492	US20150315612 SEQ ID NO: 112
AAVrh.67	493	US20150315612 SEQ ID NO: 36
AAVrh.67	494	US20150315612 SEQ ID NO: 230
AAVrh.67	495	US20150159173 SEQ ID NO: 47, US20150315612 SEQ ID NO: 113
AAVrh.68	496	US20150315612 SEQ ID NO: 16
AAVrh.68	497	US20150315612 SEQ ID NO: 100
AAVrh.69	498	US20150315612 SEQ ID NO: 39
AAVrh.69	499	US20150315612 SEQ ID NO: 119
AAVrh.70	500	US20150315612 SEQ ID NO: 20
AAVrh.70	501	US20150315612 SEQ ID NO: 98
AAVrh.71	502	US20150315612 SEQ ID NO: 162
AAVrh.72	503	US20150315612 SEQ ID NO: 9
AAVrh.73	504	US20150159173 SEQ ID NO: 5
AAVrh.74	505	US20150159173 SEQ ID NO: 6
AAVrh.8	506	US20150159173 SEQ ID NO: 41
AAVrh.8	507	US20150315612 SEQ ID NO: 235
AAVrh.8R	508	US20150159173, WO2015168666 SEQ ID NO: 9
AAVrh.8R A586R mutant	509	WO2015168666 SEQ ID NO: 10
AAVrh.8R R533A mutant	510	WO2015168666 SEQ ID NO: 11

BAAV (bovine AAV)	511	US9193769 SEQ ID NO: 8
BAAV (bovine AAV)	512	US9193769 SEQ ID NO: 10
BAAV (bovine AAV)	513	US9193769 SEQ ID NO: 4
BAAV (bovine AAV)	514	US9193769 SEQ ID NO: 2
BAAV (bovine AAV)	515	US9193769 SEQ ID NO: 6
BAAV (bovine AAV)	516	US9193769 SEQ ID NO: 1
BAAV (bovine AAV)	517	US9193769 SEQ ID NO: 5
BAAV (bovine AAV)	518	US9193769 SEQ ID NO: 3
BAAV (bovine AAV)	519	US9193769 SEQ ID NO: 11
BAAV (bovine AAV)	520	US7427396 SEQ ID NO: 5
BAAV (bovine AAV)	521	US7427396 SEQ ID NO: 6
BAAV (bovine AAV)	522	US9193769 SEQ ID NO: 7
BAAV (bovine AAV)	523	US9193769 SEQ ID NO: 9
BNP61 AAV	524	US20150238550 SEQ ID NO: 1
BNP61 AAV	525	US20150238550 SEQ ID NO: 2
BNP62 AAV	526	US20150238550 SEQ ID NO: 3
BNP63 AAV	527	US20150238550 SEQ ID NO: 4
caprine AAV	528	US7427396 SEQ ID NO: 3
caprine AAV	529	US7427396 SEQ ID NO: 4
true type AAV (ttAAV)	530	WO2015121501 SEQ ID NO: 2
AAAV (Avian AAV)	531	US9238800 SEQ ID NO: 12
AAAV (Avian AAV)	532	US9238800 SEQ ID NO: 2
AAAV (Avian AAV)	533	US9238800 SEQ ID NO: 6
AAAV (Avian AAV)	534	US9238800 SEQ ID NO: 4

AAAV (Avian AAV)	535	US9238800 SEQ ID NO: 8
AAAV (Avian AAV)	536	US9238800 SEQ ID NO: 14
AAAV (Avian AAV)	537	US9238800 SEQ ID NO: 10
AAAV (Avian AAV)	538	US9238800 SEQ ID NO: 15
AAAV (Avian AAV)	539	US9238800 SEQ ID NO: 5
AAAV (Avian AAV)	540	US9238800 SEQ ID NO: 9
AAAV (Avian AAV)	541	US9238800 SEQ ID NO: 3
AAAV (Avian AAV)	542	US9238800 SEQ ID NO: 7
AAAV (Avian AAV)	543	US9238800 SEQ ID NO: 11
AAAV (Avian AAV)	544	US9238800 SEQ ID NO: 13
AAAV (Avian AAV)	545	US9238800 SEQ ID NO: 1
AAV Shuffle 100-1	546	US20160017295 SEQ ID NO: 23
AAV Shuffle 100-1	547	US20160017295 SEQ ID NO: 11
AAV Shuffle 100-2	548	US20160017295 SEQ ID NO: 37
AAV Shuffle 100-2	549	US20160017295 SEQ ID NO: 29
AAV Shuffle 100-3	550	US20160017295 SEQ ID NO: 24
AAV Shuffle 100-3	551	US20160017295 SEQ ID NO: 12
AAV Shuffle 100-7	552	US20160017295 SEQ ID NO: 25
AAV Shuffle 100-7	553	US20160017295 SEQ ID NO: 13
AAV Shuffle 10-2	554	US20160017295 SEQ ID NO: 34
AAV Shuffle 10-2	555	US20160017295 SEQ ID NO: 26
AAV Shuffle 10-6	556	US20160017295 SEQ ID NO: 35
AAV Shuffle 10-6	557	US20160017295 SEQ ID NO: 27
AAV Shuffle 10-8	558	US20160017295 SEQ ID NO: 36
AAV Shuffle 10-8	559	US20160017295 SEQ ID NO: 28
AAV SM 100-10	560	US20160017295 SEQ ID NO: 41
AAV SM 100-10	561	US20160017295 SEQ ID NO: 33
AAV SM 100-3	562	US20160017295 SEQ ID NO: 40
AAV SM 100-3	563	US20160017295 SEQ ID NO: 32

AAV SM 10-1	564	US20160017295 SEQ ID NO: 38
AAV SM 10-1	565	US20160017295 SEQ ID NO: 30
AAV SM 10-2	566	US20160017295 SEQ ID NO: 10
AAV SM 10-2	567	US20160017295 SEQ ID NO: 22
AAV SM 10-8	568	US20160017295 SEQ ID NO: 39
AAV SM 10-8	569	US20160017295 SEQ ID NO: 31
AAV SM 100-10	560	US20160017295 SEQ ID NO: 41
AAV SM 100-10	561	US20160017295 SEQ ID NO: 33
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AAV SM 10-2	566	US20160017295 SEQ ID NO: 10
AAV SM 10-2	567	US20160017295 SEQ ID NO: 22
AAV SM 10-8	568	US20160017295 SEQ ID NO: 39
AAV SM 10-8	569	US20160017295 SEQ ID NO: 31
AAVF1/HSC 1	570	WO2016049230 SEQ ID NO: 20
AAVF2/HSC 2	571	WO2016049230 SEQ ID NO: 21
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AAVF5/HSC 5	574	WO2016049230 SEQ ID NO: 25
AAVF6/HSC 6	575	WO2016049230 SEQ ID NO: 24
AAVF7/HSC 7	576	WO2016049230 SEQ ID NO: 27
AAVF8/HSC 8	577	WO2016049230 SEQ ID NO: 28
AAVF9/HSC 9	578	WO2016049230 SEQ ID NO: 29
AAVF11/HSC11	579	WO2016049230 SEQ ID NO: 26
AAVF12/HSC12	580	WO2016049230 SEQ ID NO: 30
AAVF13/HSC13	581	WO2016049230 SEQ ID NO: 31
AAVF14/HSC14	582	WO2016049230 SEQ ID NO: 32

AAVF15/HSC15	583	WO2016049230 SEQ ID NO: 33
AAVF16/HSC16	584	WO2016049230 SEQ ID NO: 34
AAVF17/HSC17	585	WO2016049230 SEQ ID NO: 35
AAVF1/HSC1	586	WO2016049230 SEQ ID NO: 2
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AAV CLv-D4	614	US8734809 SEQ ID NO: 25

AAV CLv-D5	615	US8734809 SEQ ID NO: 26
AAV CLv-D6	616	US8734809 SEQ ID NO: 27
AAV CLv-D7	617	US8734809 SEQ ID NO: 28
AAV CLv-D8	618	US8734809 SEQ ID NO: 29
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AAV CLv-R2	621	US8734809 SEQ ID NO: 31
AAV CLv-R3	622	US8734809 SEQ ID NO: 32
AAV CLv-R4	623	US8734809 SEQ ID NO: 33
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AAV CLv-R7	626	US8734809 SEQ ID NO: 36
AAV CLv-R8	627	US8734809 SEQ ID NO: 37
AAV CLv-R9	628	US8734809 SEQ ID NO: 38
AAV CLg-F1	629	US8734809 SEQ ID NO: 39
AAV CLg-F2	630	US8734809 SEQ ID NO: 40
AAV CLg-F3	631	US8734809 SEQ ID NO: 41
AAV CLg-F4	632	US8734809 SEQ ID NO: 42
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AAV CSp-11	639	US8734809 SEQ ID NO: 47
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AAV CSp-6	643	US8734809 SEQ ID NO: 51
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AAV CSp-8	645	US8734809 SEQ ID NO: 53
AAV CSp-9	646	US8734809 SEQ ID NO: 54
AAV CHt-2	647	US8734809 SEQ ID NO: 55
AAV CHt-3	648	US8734809 SEQ ID NO: 56
AAV CKd-1	649	US8734809 SEQ ID NO: 57
AAV CKd-10	650	US8734809 SEQ ID NO: 58
AAV CKd-2	651	US8734809 SEQ ID NO: 59

AAV CKd-3	652	US8734809 SEQ ID NO: 60
AAV CKd-4	653	US8734809 SEQ ID NO: 61
AAV CKd-6	654	US8734809 SEQ ID NO: 62
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AAV CKd-8	656	US8734809 SEQ ID NO: 64
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AAV CLv-6	663	US8734809 SEQ ID NO: 71
AAV CLv-8	664	US8734809 SEQ ID NO: 72
AAV CKd-B1	665	US8734809 SEQ ID NO: 73
AAV CKd-B2	666	US8734809 SEQ ID NO: 74
AAV CKd-B3	667	US8734809 SEQ ID NO: 75
AAV CKd-B4	668	US8734809 SEQ ID NO: 76
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AAV CKd-B6	670	US8734809 SEQ ID NO: 78
AAV CKd-B7	671	US8734809 SEQ ID NO: 79
AAV CKd-B8	672	US8734809 SEQ ID NO: 80
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AAV CKd-H2	674	US8734809 SEQ ID NO: 82
AAV CKd-H3	675	US8734809 SEQ ID NO: 83
AAV CKd-H4	676	US8734809 SEQ ID NO: 84
AAV CKd-H5	677	US8734809 SEQ ID NO: 85
AAV CKd-H6	678	US8734809 SEQ ID NO: 77
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AAV CLv1-3	682	US8734809 SEQ ID NO: 173
AAV CLv1-4	683	US8734809 SEQ ID NO: 174
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AAV Clv1-8	685	US8734809 SEQ ID NO: 176
AAV Clv1-9	686	US8734809 SEQ ID NO: 177
AAV Clv1-10	687	US8734809 SEQ ID NO: 178

AAV.VR-355	688	US8734809 SEQ ID NO: 181
AAV.hu.48R3	689	US8734809 SEQ ID NO: 183
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AAV CBr-E2	691	US8734809 SEQ ID NO: 88
AAV CBr-E3	692	US8734809 SEQ ID NO: 89
AAV CBr-E4	693	US8734809 SEQ ID NO: 90
AAV CBr-E5	694	US8734809 SEQ ID NO: 91
AAV CBr-e5	695	US8734809 SEQ ID NO: 92
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AAV CBr-E7	697	US8734809 SEQ ID NO: 94
AAV CBr-E8	698	US8734809 SEQ ID NO: 95
AAV CLv-D1	699	US8734809 SEQ ID NO: 96
AAV CLv-D2	700	US8734809 SEQ ID NO: 97
AAV CLv-D3	701	US8734809 SEQ ID NO: 98
AAV CLv-D4	702	US8734809 SEQ ID NO: 99
AAV CLv-D5	703	US8734809 SEQ ID NO: 100
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AAV CLv-D7	705	US8734809 SEQ ID NO: 102
AAV CLv-D8	706	US8734809 SEQ ID NO: 103
AAV CLv-E1	707	US8734809 SEQ ID NO: 87
AAV CLv-R1	708	US8734809 SEQ ID NO: 104
AAV CLv-R2	709	US8734809 SEQ ID NO: 105
AAV CLv-R3	710	US8734809 SEQ ID NO: 106
AAV CLv-R4	711	US8734809 SEQ ID NO: 107
AAV CLv-R5	712	US8734809 SEQ ID NO: 108
AAV CLv-R6	713	US8734809 SEQ ID NO: 109
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AAV CLv-R9	716	US8734809 SEQ ID NO: 112
AAV CLg-F1	717	US8734809 SEQ ID NO: 113
AAV CLg-F2	718	US8734809 SEQ ID NO: 114
AAV CLg-F3	719	US8734809 SEQ ID NO: 115
AAV CLg-F4	720	US8734809 SEQ ID NO: 116
AAV CLg-F5	721	US8734809 SEQ ID NO: 117
AAV CLg-F6	722	US8734809 SEQ ID NO: 117

AAV CLg-F7	723	US8734809 SEQ ID NO: 118
AAV CLg-F8	724	US8734809 SEQ ID NO: 117
AAV CSp-1	725	US8734809 SEQ ID NO: 119
AAV CSp-10	726	US8734809 SEQ ID NO: 120
AAV CSp-11	727	US8734809 SEQ ID NO: 121
AAV CSp-2	728	US8734809 SEQ ID NO: 122
AAV CSp-3	729	US8734809 SEQ ID NO: 123
AAV CSp-4	730	US8734809 SEQ ID NO: 124
AAV CSp-6	731	US8734809 SEQ ID NO: 125
AAV CSp-7	732	US8734809 SEQ ID NO: 126
AAV CSp-8	733	US8734809 SEQ ID NO: 127
AAV CSp-9	734	US8734809 SEQ ID NO: 128
AAV CHt-2	735	US8734809 SEQ ID NO: 129
AAV CHt-3	736	US8734809 SEQ ID NO: 130
AAV CKd-1	737	US8734809 SEQ ID NO: 131
AAV CKd-10	738	US8734809 SEQ ID NO: 132
AAV CKd-2	739	US8734809 SEQ ID NO: 133
AAV CKd-3	740	US8734809 SEQ ID NO: 134
AAV CKd-4	741	US8734809 SEQ ID NO: 135
AAV CKd-6	742	US8734809 SEQ ID NO: 136
AAV CKd-7	743	US8734809 SEQ ID NO: 137
AAV CKd-8	744	US8734809 SEQ ID NO: 138
AAV CLv-1	745	US8734809 SEQ ID NO: 139
AAV CLv-12	746	US8734809 SEQ ID NO: 140
AAV CLv-13	747	US8734809 SEQ ID NO: 141
AAV CLv-2	748	US8734809 SEQ ID NO: 142
AAV CLv-3	749	US8734809 SEQ ID NO: 143
AAV CLv-4	750	US8734809 SEQ ID NO: 144
AAV CLv-6	751	US8734809 SEQ ID NO: 145
AAV CLv-8	752	US8734809 SEQ ID NO: 146
AAV CKd-B1	753	US8734809 SEQ ID NO: 147
AAV CKd-B2	754	US8734809 SEQ ID NO: 148
AAV CKd-B3	755	US8734809 SEQ ID NO: 149
AAV CKd-B4	756	US8734809 SEQ ID NO: 150
AAV CKd-B5	757	US8734809 SEQ ID NO: 151
AAV CKd-B6	758	US8734809 SEQ ID NO: 152
AAV CKd-B7	759	US8734809 SEQ ID NO: 153
AAV CKd-B8	760	US8734809 SEQ ID NO: 154
AAV CKd-H1	761	US8734809 SEQ ID NO: 155

AAV CKd-H2	762	US8734809 SEQ ID NO: 156
AAV CKd-H3	763	US8734809 SEQ ID NO: 157
AAV CKd-H4	764	US8734809 SEQ ID NO: 158
AAV CKd-H5	765	US8734809 SEQ ID NO: 159
AAV CKd-H6	766	US8734809 SEQ ID NO: 151
AAV CHt-1	767	US8734809 SEQ ID NO: 160
AAV CHt-P2	768	WO2016065001 SEQ ID NO: 1
AAV CHt-P5	769	WO2016065001 SEQ ID NO: 2
AAV CHt-P9	770	WO2016065001 SEQ ID NO: 3
AAV CBr-7.1	771	WO2016065001 SEQ ID NO: 4
AAV CBr-7.2	772	WO2016065001 SEQ ID NO: 5
AAV CBr-7.3	773	WO2016065001 SEQ ID NO: 6
AAV CBr-7.4	774	WO2016065001 SEQ ID NO: 7
AAV CBr-7.5	775	WO2016065001 SEQ ID NO: 8
AAV CBr-7.7	776	WO2016065001 SEQ ID NO: 9
AAV CBr-7.8	777	WO2016065001 SEQ ID NO: 10
AAV CBr-7.10	778	WO2016065001 SEQ ID NO: 11
AAV CKd-N3	779	WO2016065001 SEQ ID NO: 12
AAV CKd-N4	780	WO2016065001 SEQ ID NO: 13
AAV CKd-N9	781	WO2016065001 SEQ ID NO: 14
AAV CLv-L4	782	WO2016065001 SEQ ID NO: 15
AAV CLv-L5	783	WO2016065001 SEQ ID NO: 16
AAV CLv-L6	784	WO2016065001 SEQ ID NO: 17
AAV CLv-K1	785	WO2016065001 SEQ ID NO: 18
AAV CLv-K3	786	WO2016065001 SEQ ID NO: 19
AAV CLv-K6	787	WO2016065001 SEQ ID NO: 20
AAV CLv-M1	788	WO2016065001 SEQ ID NO: 21
AAV CLv-M11	789	WO2016065001 SEQ ID NO: 22
AAV CLv-M2	790	WO2016065001 SEQ ID NO: 23
AAV CLv-M5	791	WO2016065001 SEQ ID NO: 24
AAV CLv-M6	792	WO2016065001 SEQ ID NO: 25

AAV CLv-M7	793	WO2016065001 SEQ ID NO: 26
AAV CLv-M8	794	WO2016065001 SEQ ID NO: 27
AAV CLv-M9	795	WO2016065001 SEQ ID NO: 28
AAV CHt-P1	796	WO2016065001 SEQ ID NO: 29
AAV CHt-P6	797	WO2016065001 SEQ ID NO: 30
AAV CHt-P8	798	WO2016065001 SEQ ID NO: 31
AAV CHt-6.1	799	WO2016065001 SEQ ID NO: 32
AAV CHt-6.10	800	WO2016065001 SEQ ID NO: 33
AAV CHt-6.5	801	WO2016065001 SEQ ID NO: 34
AAV CHt-6.6	802	WO2016065001 SEQ ID NO: 35
AAV CHt-6.7	803	WO2016065001 SEQ ID NO: 36
AAV CHt-6.8	804	WO2016065001 SEQ ID NO: 37
AAV CSp-8.10	805	WO2016065001 SEQ ID NO: 38
AAV CSp-8.2	806	WO2016065001 SEQ ID NO: 39
AAV CSp-8.4	807	WO2016065001 SEQ ID NO: 40
AAV CSp-8.5	808	WO2016065001 SEQ ID NO: 41
AAV CSp-8.6	809	WO2016065001 SEQ ID NO: 42
AAV CSp-8.7	810	WO2016065001 SEQ ID NO: 43
AAV CSp-8.8	811	WO2016065001 SEQ ID NO: 44
AAV CSp-8.9	812	WO2016065001 SEQ ID NO: 45
AAV CBr-B7.3	813	WO2016065001 SEQ ID NO: 46
AAV CBr-B7.4	814	WO2016065001 SEQ ID NO: 47
AAV3B	815	WO2016065001 SEQ ID NO: 48
AAV4	816	WO2016065001 SEQ ID NO: 49
AAV5	817	WO2016065001 SEQ ID NO: 50
AAV CHt-P2	818	WO2016065001 SEQ ID NO: 51
AAV CHt-P5	819	WO2016065001 SEQ ID NO: 52
AAV CHt-P9	820	WO2016065001 SEQ ID NO: 53
AAV CBr-7.1	821	WO2016065001 SEQ ID NO: 54
AAV CBr-7.2	822	WO2016065001 SEQ ID NO: 55
AAV CBr-7.3	823	WO2016065001 SEQ ID NO: 56
AAV CBr-7.4	824	WO2016065001 SEQ ID NO: 57

AAV CBr-7.5	825	WO2016065001 SEQ ID NO: 58
AAV CBr-7.7	826	WO2016065001 SEQ ID NO: 59
AAV CBr-7.8	827	WO2016065001 SEQ ID NO: 60
AAV CBr-7.10	828	WO2016065001 SEQ ID NO: 61
AAV CKd-N3	829	WO2016065001 SEQ ID NO: 62
AAV CKd-N4	830	WO2016065001 SEQ ID NO: 63
AAV CKd-N9	831	WO2016065001 SEQ ID NO: 64
AAV CLv-L4	832	WO2016065001 SEQ ID NO: 65
AAV CLv-L5	833	WO2016065001 SEQ ID NO: 66
AAV CLv-L6	834	WO2016065001 SEQ ID NO: 67
AAV CLv-K1	835	WO2016065001 SEQ ID NO: 68
AAV CLv-K3	836	WO2016065001 SEQ ID NO: 69
AAV CLv-K6	837	WO2016065001 SEQ ID NO: 70
AAV CLv-M1	838	WO2016065001 SEQ ID NO: 71
AAV CLv-M11	839	WO2016065001 SEQ ID NO: 72
AAV CLv-M2	840	WO2016065001 SEQ ID NO: 73
AAV CLv-M5	841	WO2016065001 SEQ ID NO: 74
AAV CLv-M6	842	WO2016065001 SEQ ID NO: 75
AAV CLv-M7	843	WO2016065001 SEQ ID NO: 76
AAV CLv-M8	844	WO2016065001 SEQ ID NO: 77
AAV CLv-M9	845	WO2016065001 SEQ ID NO: 78
AAV CHt-P1	846	WO2016065001 SEQ ID NO: 79
AAV CHt-P6	847	WO2016065001 SEQ ID NO: 80
AAV CHt-P8	848	WO2016065001 SEQ ID NO: 81
AAV CHt-6.1	849	WO2016065001 SEQ ID NO: 82
AAV CHt-6.10	850	WO2016065001 SEQ ID NO: 83
AAV CHt-6.5	851	WO2016065001 SEQ ID NO: 84
AAV CHt-6.6	852	WO2016065001 SEQ ID NO: 85
AAV CHt-6.7	853	WO2016065001 SEQ ID NO: 86
AAV CHt-6.8	854	WO2016065001 SEQ ID NO: 87
AAV CSp-8.10	855	WO2016065001 SEQ ID NO: 88

AAV CSp-8.2	856	WO2016065001 SEQ ID NO: 89
AAV CSp-8.4	857	WO2016065001 SEQ ID NO: 90
AAV CSp-8.5	858	WO2016065001 SEQ ID NO: 91
AAV CSp-8.6	859	WO2016065001 SEQ ID NO: 92
AAV CSp-8.7	860	WO2016065001 SEQ ID NO: 93
AAV CSp-8.8	861	WO2016065001 SEQ ID NO: 94
AAV CSp-8.9	862	WO2016065001 SEQ ID NO: 95
AAV CBr-B7.3	863	WO2016065001 SEQ ID NO: 96
AAV CBr-B7.4	864	WO2016065001 SEQ ID NO: 97
AAV3B	865	WO2016065001 SEQ ID NO: 98
AAV4	866	WO2016065001 SEQ ID NO: 99
AAV5	867	WO2016065001 SEQ ID NO: 100
AAVPHP.B or G2B-26	868	WO2015038958 SEQ ID NO: 8 and 13; GenBankALU85156.1
AAVPHP.B	869	WO2015038958 SEQ ID NO: 9
AAVG2B-13	870	WO2015038958 SEQ ID NO: 12
AAVTH1.1-32	871	WO2015038958 SEQ ID NO: 14
AAVTH1.1-35	872	WO2015038958 SEQ ID NO: 15
PHP.N/PHP.B-DGT	1418	WO2017100671 SEQ ID NO: 46
PHP.S/G2A12	1419	WO2017100671 SEQ ID NO: 47
AAV9/hu.14 K449R	1420	WO2017100671 SEQ ID NO: 45
GPV	1421	US9624274B2 SEQ ID NO: 192
B19	1422	US9624274B2 SEQ ID NO: 193
MVM	1423	US9624274B2 SEQ ID NO: 194
FPV	1424	US9624274B2 SEQ ID NO: 195
CPV	1425	US9624274B2 SEQ ID NO: 196
AAV6	1426	US9546112B2 SEQ ID NO: 5
AAV6	1427	US9457103B2 SEQ ID NO: 1
AAV2	1428	US9457103B2 SEQ ID NO: 2
ShH10	1429	US9457103B2 SEQ ID NO: 3
ShH13	1430	US9457103B2 SEQ ID NO: 4
ShH10	1431	US9457103B2 SEQ ID NO: 5
ShH10	1432	US9457103B2 SEQ ID NO: 6
ShH10	1433	US9457103B2 SEQ ID NO: 7
ShH10	1434	US9457103B2 SEQ ID NO: 8
ShH10	1435	US9457103B2 SEQ ID NO: 9
rh74	1436	US9434928B2 SEQ ID NO: 1, US2015023924A1 SEQ ID NO: 2

rh74	1437	US9434928B2 SEQ ID NO: 2, US2015023924A1 SEQ ID NO: 1
AAV8	1438	US9434928B2 SEQ ID NO: 4
rh74	1439	US9434928B2 SEQ ID NO: 5
rh74 (RHM4-1)	1440	US2015023924A1 SEQ ID NO: 5, US20160375110A1 SEQ ID NO: 4
rh74 (RHM15-1)	1441	US2015023924A1 SEQ ID NO: 6, US20160375110A1 SEQ ID NO: 5
rh74 (RHM15-2)	1442	US2015023924A1 SEQ ID NO: 7, US20160375110A1 SEQ ID NO: 6
rh74 (RHM15-3/RHM15-5)	1443	US2015023924A1 SEQ ID NO: 8, US20160375110A1 SEQ ID NO: 7
rh74 (RHM15-4)	1444	US2015023924A1 SEQ ID NO: 9, US20160375110A1 SEQ ID NO: 8
rh74 (RHM15-6)	1445	US2015023924A1 SEQ ID NO: 10, US20160375110A1 SEQ ID NO: 9
rh74 (RHM4-1)	1446	US2015023924A1 SEQ ID NO: 11
rh74 (RHM15-1)	1447	US2015023924A1 SEQ ID NO: 12
rh74 (RHM15-2)	1448	US2015023924A1 SEQ ID NO: 13
rh74 (RHM15-3/RHM15-5)	1449	US2015023924A1 SEQ ID NO: 14
rh74 (RHM15-4)	1450	US2015023924A1 SEQ ID NO: 15
rh74 (RHM15-6)	1451	US2015023924A1 SEQ ID NO: 16
AAV2 (comprising lung specific polypeptide)	1452	US20160175389A1 SEQ ID NO: 9
AAV2 (comprising lung specific polypeptide)	1453	US20160175389A1 SEQ ID NO: 10
Anc80	1454	US20170051257A1 SEQ ID NO: 1
Anc80	1455	US20170051257A1 SEQ ID NO: 2
Anc81	1456	US20170051257A1 SEQ ID NO: 3
Anc80	1457	US20170051257A1 SEQ ID NO: 4
Anc82	1458	US20170051257A1 SEQ ID NO: 5
Anc82	1459	US20170051257A1 SEQ ID NO: 6
Anc83	1460	US20170051257A1 SEQ ID NO: 7
Anc83	1461	US20170051257A1 SEQ ID NO: 8
Anc84	1462	US20170051257A1 SEQ ID NO: 9
Anc84	1463	US20170051257A1 SEQ ID NO: 10
Anc94	1464	US20170051257A1 SEQ ID NO: 11
Anc94	1465	US20170051257A1 SEQ ID NO: 12
Anc113	1466	US20170051257A1 SEQ ID NO: 13
Anc113	1467	US20170051257A1 SEQ ID NO: 14
Anc126	1468	US20170051257A1 SEQ ID NO: 15

Anc126	1469	US20170051257A1 SEQ ID NO: 16
Anc127	1470	US20170051257A1 SEQ ID NO: 17
Anc127	1471	US20170051257A1 SEQ ID NO: 18
Anc80L27	1472	US20170051257A1 SEQ ID NO: 19
Anc80L59	1473	US20170051257A1 SEQ ID NO: 20
Anc80L60	1474	US20170051257A1 SEQ ID NO: 21
Anc80L62	1475	US20170051257A1 SEQ ID NO: 22
Anc80L65	1476	US20170051257A1 SEQ ID NO: 23
Anc80L33	1477	US20170051257A1 SEQ ID NO: 24
Anc80L36	1478	US20170051257A1 SEQ ID NO: 25
Anc80L44	1479	US20170051257A1 SEQ ID NO: 26
Anc80L1	1480	US20170051257A1 SEQ ID NO: 35
Anc80L1	1481	US20170051257A1 SEQ ID NO: 36
AAV-X1	1482	US8283151B2 SEQ ID NO: 11
AAV-X1b	1483	US8283151B2 SEQ ID NO: 12
AAV-X5	1484	US8283151B2 SEQ ID NO: 13
AAV-X19	1485	US8283151B2 SEQ ID NO: 14
AAV-X21	1486	US8283151B2 SEQ ID NO: 15
AAV-X22	1487	US8283151B2 SEQ ID NO: 16
AAV-X23	1488	US8283151B2 SEQ ID NO: 17
AAV-X24	1489	US8283151B2 SEQ ID NO: 18
AAV-X25	1490	US8283151B2 SEQ ID NO: 19
AAV-X26	1491	US8283151B2 SEQ ID NO: 20
AAV-X1	1492	US8283151B2 SEQ ID NO: 21
AAV-X1b	1493	US8283151B2 SEQ ID NO: 22
AAV-X5	1494	US8283151B2 SEQ ID NO: 23
AAV-X19	1495	US8283151B2 SEQ ID NO: 24
AAV-X21	1496	US8283151B2 SEQ ID NO: 25
AAV-X22	1497	US8283151B2 SEQ ID NO: 26
AAV-X23	1498	US8283151B2 SEQ ID NO: 27
AAV-X24	1499	US8283151B2 SEQ ID NO: 28
AAV-X25	1500	US8283151B2 SEQ ID NO: 29
AAV-X26	1501	US8283151B2 SEQ ID NO: 30
AAVrh8	1502	WO2016054554A1 SEQ ID NO: 8
AAVrh8VP2 FC5	1503	WO2016054554A1 SEQ ID NO: 9
AAVrh8VP2 FC44	1504	WO2016054554A1 SEQ ID NO: 10
AAVrh8VP2 ApoB100	1505	WO2016054554A1 SEQ ID NO: 11
AAVrh8VP2 RVG	1506	WO2016054554A1 SEQ ID NO: 12
AAVrh8VP2 Angiopep-2 VP2	1507	WO2016054554A1 SEQ ID NO: 13
AAV9.47VP 1.3	1508	WO2016054554A1 SEQ ID NO: 14

AAV9.47VP 2ICAMg3	1509	WO2016054554A1 SEQ ID NO: 15
AAV9.47VP 2RVG	1510	WO2016054554A1 SEQ ID NO: 16
AAV9.47VP 2Angiopep-2	1511	WO2016054554A1 SEQ ID NO: 17
AAV9.47VP 2A-string	1512	WO2016054554A1 SEQ ID NO: 18
AAVrh8VP2 FC5 VP2	1513	WO2016054554A1 SEQ ID NO: 19
AAVrh8VP2 FC44 VP2	1514	WO2016054554A1 SEQ ID NO: 20
AAVrh8VP2 ApoB100 VP2	1515	WO2016054554A1 SEQ ID NO: 21
AAVrh8VP2 RVG VP2	1516	WO2016054554A1 SEQ ID NO: 22
AAVrh8VP2 Angiopep-2 VP2	1517	WO2016054554A1 SEQ ID NO: 23
AAV9.47VP 2ICAMg3 VP2	1518	WO2016054554A1 SEQ ID NO: 24
AAV9.47VP 2RVG VP2	1519	WO2016054554A1 SEQ ID NO: 25
AAV9.47VP 2Angiopep-2 VP2	1520	WO2016054554A1 SEQ ID NO: 26
AAV9.47VP 2A-string VP2	1521	WO2016054554A1 SEQ ID NO: 27
rAAV-B1	1522	WO2016054557A1 SEQ ID NO: 1
rAAV-B2	1523	WO2016054557A1 SEQ ID NO: 2
rAAV-B3	1524	WO2016054557A1 SEQ ID NO: 3
rAAV-B4	1525	WO2016054557A1 SEQ ID NO: 4
rAAV-B1	1526	WO2016054557A1 SEQ ID NO: 5
rAAV-B2	1527	WO2016054557A1 SEQ ID NO: 6
rAAV-B3	1528	WO2016054557A1 SEQ ID NO: 7
rAAV-B4	1529	WO2016054557A1 SEQ ID NO: 8
rAAV-L1	1530	WO2016054557A1 SEQ ID NO: 9
rAAV-L2	1531	WO2016054557A1 SEQ ID NO: 10
rAAV-L3	1532	WO2016054557A1 SEQ ID NO: 11
rAAV-L4	1533	WO2016054557A1 SEQ ID NO: 12
rAAV-L1	1534	WO2016054557A1 SEQ ID NO: 13
rAAV-L2	1535	WO2016054557A1 SEQ ID NO: 14
rAAV-L3	1536	WO2016054557A1 SEQ ID NO: 15
rAAV-L4	1537	WO2016054557A1 SEQ ID NO: 16
AAV9	1538	WO2016073739A1 SEQ ID NO: 3
rAAV	1539	WO2016081811A1 SEQ ID NO: 1
rAAV	1540	WO2016081811A1 SEQ ID NO: 2
rAAV	1541	WO2016081811A1 SEQ ID NO: 3
rAAV	1542	WO2016081811A1 SEQ ID NO: 4

rAAV	1543	WO2016081811A1 SEQ ID NO: 5
rAAV	1544	WO2016081811A1 SEQ ID NO: 6
rAAV	1545	WO2016081811A1 SEQ ID NO: 7
rAAV	1546	WO2016081811A1 SEQ ID NO: 8
rAAV	1547	WO2016081811A1 SEQ ID NO: 9
rAAV	1548	WO2016081811A1 SEQ ID NO: 10
rAAV	1549	WO2016081811A1 SEQ ID NO: 11
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rAAV	1558	WO2016081811A1 SEQ ID NO: 20
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rAAV	1560	WO2016081811A1 SEQ ID NO: 22
rAAV	1561	WO2016081811A1 SEQ ID NO: 23
rAAV	1562	WO2016081811A1 SEQ ID NO: 24
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rAAV	1568	WO2016081811A1 SEQ ID NO: 30
rAAV	1569	WO2016081811A1 SEQ ID NO: 31
rAAV	1570	WO2016081811A1 SEQ ID NO: 32
rAAV	1571	WO2016081811A1 SEQ ID NO: 33
rAAV	1572	WO2016081811A1 SEQ ID NO: 34
rAAV	1573	WO2016081811A1 SEQ ID NO: 35
rAAV	1574	WO2016081811A1 SEQ ID NO: 36
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rAAV	1583	WO2016081811A1 SEQ ID NO: 45
rAAV	1584	WO2016081811A1 SEQ ID NO: 46
rAAV	1585	WO2016081811A1 SEQ ID NO: 47
rAAV	1586	WO2016081811A1 SEQ ID NO: 48

rAAV	1587	WO2016081811A1 SEQ ID NO: 49
rAAV	1588	WO2016081811A1 SEQ ID NO: 50
rAAV	1589	WO2016081811A1 SEQ ID NO: 51
rAAV	1590	WO2016081811A1 SEQ ID NO: 52
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rAAV	1592	WO2016081811A1 SEQ ID NO: 54
rAAV	1593	WO2016081811A1 SEQ ID NO: 55
rAAV	1594	WO2016081811A1 SEQ ID NO: 56
rAAV	1595	WO2016081811A1 SEQ ID NO: 57
rAAV	1596	WO2016081811A1 SEQ ID NO: 58
rAAV	1597	WO2016081811A1 SEQ ID NO: 59
rAAV	1598	WO2016081811A1 SEQ ID NO: 60
rAAV	1599	WO2016081811A1 SEQ ID NO: 61
rAAV	1600	WO2016081811A1 SEQ ID NO: 62
rAAV	1601	WO2016081811A1 SEQ ID NO: 63
rAAV	1602	WO2016081811A1 SEQ ID NO: 64
rAAV	1603	WO2016081811A1 SEQ ID NO: 65
rAAV	1604	WO2016081811A1 SEQ ID NO: 66
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rAAV	1606	WO2016081811A1 SEQ ID NO: 68
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rAAV	1608	WO2016081811A1 SEQ ID NO: 70
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rAAV	1610	WO2016081811A1 SEQ ID NO: 72
rAAV	1611	WO2016081811A1 SEQ ID NO: 73
rAAV	1612	WO2016081811A1 SEQ ID NO: 74
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rAAV	1614	WO2016081811A1 SEQ ID NO: 76
rAAV	1615	WO2016081811A1 SEQ ID NO: 77
rAAV	1616	WO2016081811A1 SEQ ID NO: 78
rAAV	1617	WO2016081811A1 SEQ ID NO: 79
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rAAV	1623	WO2016081811A1 SEQ ID NO: 85
rAAV	1624	WO2016081811A1 SEQ ID NO: 86
rAAV	1625	WO2016081811A1 SEQ ID NO: 87
rAAV	1626	WO2016081811A1 SEQ ID NO: 88
rAAV	1627	WO2016081811A1 SEQ ID NO: 89
rAAV	1628	WO2016081811A1 SEQ ID NO: 90
rAAV	1629	WO2016081811A1 SEQ ID NO: 91
rAAV	1630	WO2016081811A1 SEQ ID NO: 92

rAAV	1631	WO2016081811A1 SEQ ID NO: 93
rAAV	1632	WO2016081811A1 SEQ ID NO: 94
rAAV	1633	WO2016081811A1 SEQ ID NO: 95
rAAV	1634	WO2016081811A1 SEQ ID NO: 96
rAAV	1635	WO2016081811A1 SEQ ID NO: 97
rAAV	1636	WO2016081811A1 SEQ ID NO: 98
rAAV	1637	WO2016081811A1 SEQ ID NO: 99
rAAV	1638	WO2016081811A1 SEQ ID NO: 100
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rAAV	1649	WO2016081811A1 SEQ ID NO: 111
rAAV	1650	WO2016081811A1 SEQ ID NO: 112
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rAAV	1652	WO2016081811A1 SEQ ID NO: 114
rAAV	1653	WO2016081811A1 SEQ ID NO: 115
rAAV	1654	WO2016081811A1 SEQ ID NO: 116
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rAAV	1656	WO2016081811A1 SEQ ID NO: 118
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rAAV	1662	WO2016081811A1 SEQ ID NO: 124
rAAV	1663	WO2016081811A1 SEQ ID NO: 125
rAAV	1664	WO2016081811A1 SEQ ID NO: 126
rAAV	1665	WO2016081811A1 SEQ ID NO: 127
rAAV	1666	WO2016081811A1 SEQ ID NO: 128
AAV8 E532K	1667	WO2016081811A1 SEQ ID NO: 133
AAV8 E532K	1668	WO2016081811A1 SEQ ID NO: 134
rAAV4	1669	WO2016115382A1 SEQ ID NO: 2
rAAV4	1670	WO2016115382A1 SEQ ID NO: 3
rAAV4	1671	WO2016115382A1 SEQ ID NO: 4
rAAV4	1672	WO2016115382A1 SEQ ID NO: 5
rAAV4	1673	WO2016115382A1 SEQ ID NO: 6

rAAV4	1674	WO2016115382A1 SEQ ID NO: 7
rAAV4	1675	WO2016115382A1 SEQ ID NO: 8
rAAV4	1676	WO2016115382A1 SEQ ID NO: 9
rAAV4	1677	WO2016115382A1 SEQ ID NO: 10
rAAV4	1678	WO2016115382A1 SEQ ID NO: 11
rAAV4	1679	WO2016115382A1 SEQ ID NO: 12
rAAV4	1680	WO2016115382A1 SEQ ID NO: 13
rAAV4	1681	WO2016115382A1 SEQ ID NO: 14
rAAV4	1682	WO2016115382A1 SEQ ID NO: 15
rAAV4	1683	WO2016115382A1 SEQ ID NO: 16
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rAAV4	1685	WO2016115382A1 SEQ ID NO: 18
rAAV4	1686	WO2016115382A1 SEQ ID NO: 19
rAAV4	1687	WO2016115382A1 SEQ ID NO: 20
rAAV4	1688	WO2016115382A1 SEQ ID NO: 21
AAV11	1689	WO2016115382A1 SEQ ID NO: 22
AAV12	1690	WO2016115382A1 SEQ ID NO: 23
rh32	1691	WO2016115382A1 SEQ ID NO: 25
rh33	1692	WO2016115382A1 SEQ ID NO: 26
rh34	1693	WO2016115382A1 SEQ ID NO: 27
rAAV4	1694	WO2016115382A1 SEQ ID NO: 28
rAAV4	1695	WO2016115382A1 SEQ ID NO: 29
rAAV4	1696	WO2016115382A1 SEQ ID NO: 30
rAAV4	1697	WO2016115382A1 SEQ ID NO: 31
rAAV4	1698	WO2016115382A1 SEQ ID NO: 32
rAAV4	1699	WO2016115382A1 SEQ ID NO: 33
AAV2/8	1700	WO2016131981A1 SEQ ID NO: 47
AAV2/8	1701	WO2016131981A1 SEQ ID NO: 48
ancestral AAV	1702	WO2016154344A1 SEQ ID NO: 7
ancestral AAV variant C4	1703	WO2016154344A1 SEQ ID NO: 13
ancestral AAV variant C7	1704	WO2016154344A1 SEQ ID NO: 14
ancestral AAV variant G4	1705	WO2016154344A1 SEQ ID NO: 15
consensus amino acid sequence of ancestral AAV variants, C4, C7 and G4	1706	WO2016154344A1 SEQ ID NO: 16
consensus amino acid sequence of	1707	WO2016154344A1 SEQ ID NO: 17

ancestral AAV variants, C4 and C7		
AAV8 (with a AAV2 phospholipase domain)	1708	WO2016150403A1 SEQ ID NO: 13
AAV VR-942n	1709	US20160289275A1 SEQ ID NO: 10
AAV5-A (M569V)	1710	US20160289275A1 SEQ ID NO: 13
AAV5-A (M569V)	1711	US20160289275A1 SEQ ID NO: 14
AAV5-A (Y585V)	1712	US20160289275A1 SEQ ID NO: 16
AAV5-A (Y585V)	1713	US20160289275A1 SEQ ID NO: 17
AAV5-A (L587T)	1714	US20160289275A1 SEQ ID NO: 19
AAV5-A (L587T)	1715	US20160289275A1 SEQ ID NO: 20
AAV5-A (Y585V/L587T)	1716	US20160289275A1 SEQ ID NO: 22
AAV5-A (Y585V/L587T)	1717	US20160289275A1 SEQ ID NO: 23
AAV5-B (D652A)	1718	US20160289275A1 SEQ ID NO: 25
AAV5-B (D652A)	1719	US20160289275A1 SEQ ID NO: 26
AAV5-B (T362M)	1720	US20160289275A1 SEQ ID NO: 28
AAV5-B (T362M)	1721	US20160289275A1 SEQ ID NO: 29
AAV5-B (Q359D)	1722	US20160289275A1 SEQ ID NO: 31
AAV5-B (Q359D)	1723	US20160289275A1 SEQ ID NO: 32
AAV5-B (E350Q)	1724	US20160289275A1 SEQ ID NO: 34
AAV5-B (E350Q)	1725	US20160289275A1 SEQ ID NO: 35
AAV5-B (P533S)	1726	US20160289275A1 SEQ ID NO: 37
AAV5-B (P533S)	1727	US20160289275A1 SEQ ID NO: 38
AAV5-B (P533G)	1728	US20160289275A1 SEQ ID NO: 40
AAV5-B (P533G)	1729	US20160289275A1 SEQ ID NO: 41
AAV5-mutation in loop VII	1730	US20160289275A1 SEQ ID NO: 43
AAV5-mutation in loop VII	1731	US20160289275A1 SEQ ID NO: 44

AAV8	1732	US20160289275A1 SEQ ID NO: 47
Mut A (LK03/AAV8)	1733	WO2016181123A1 SEQ ID NO: 1
Mut B (LK03/AAV5)	1734	WO2016181123A1 SEQ ID NO: 2
Mut C (AAV8/AAV 3B)	1735	WO2016181123A1 SEQ ID NO: 3
Mut D (AAV5/AAV 3B)	1736	WO2016181123A1 SEQ ID NO: 4
Mut E (AAV8/AAV 3B)	1737	WO2016181123A1 SEQ ID NO: 5
Mut F (AAV3B/AA V8)	1738	WO2016181123A1 SEQ ID NO: 6
AAV44.9	1739	WO2016183297A1 SEQ ID NO: 4
AAV44.9	1740	WO2016183297A1 SEQ ID NO: 5
AAVrh8	1741	WO2016183297A1 SEQ ID NO: 6
AAV44.9 (S470N)	1742	WO2016183297A1 SEQ ID NO: 9
rh74 VP1	1743	US20160375110A1 SEQ ID NO: 1
AAV-LK03 (L125I)	1744	WO2017015102A1 SEQ ID NO: 5
AAV3B (S663V+T49 2V)	1745	WO2017015102A1 SEQ ID NO: 6
Anc80	1746	WO2017019994A2 SEQ ID NO: 1
Anc80	1747	WO2017019994A2 SEQ ID NO: 2
Anc81	1748	WO2017019994A2 SEQ ID NO: 3
Anc81	1749	WO2017019994A2 SEQ ID NO: 4
Anc82	1750	WO2017019994A2 SEQ ID NO: 5
Anc82	1751	WO2017019994A2 SEQ ID NO: 6
Anc83	1752	WO2017019994A2 SEQ ID NO: 7
Anc83	1753	WO2017019994A2 SEQ ID NO: 8
Anc84	1754	WO2017019994A2 SEQ ID NO: 9
Anc84	1755	WO2017019994A2 SEQ ID NO: 10
Anc94	1756	WO2017019994A2 SEQ ID NO: 11
Anc94	1757	WO2017019994A2 SEQ ID NO: 12
Anc113	1758	WO2017019994A2 SEQ ID NO: 13
Anc113	1759	WO2017019994A2 SEQ ID NO: 14
Anc126	1760	WO2017019994A2 SEQ ID NO: 15
Anc126	1761	WO2017019994A2 SEQ ID NO: 16
Anc127	1762	WO2017019994A2 SEQ ID NO: 17
Anc127	1763	WO2017019994A2 SEQ ID NO: 18
Anc80L27	1764	WO2017019994A2 SEQ ID NO: 19
Anc80L59	1765	WO2017019994A2 SEQ ID NO: 20

Anc80L60	1766	WO2017019994A2 SEQ ID NO: 21
Anc80L62	1767	WO2017019994A2 SEQ ID NO: 22
Anc80L65	1768	WO2017019994A2 SEQ ID NO: 23
Anc80L33	1769	WO2017019994A2 SEQ ID NO: 24
Anc80L36	1770	WO2017019994A2 SEQ ID NO: 25
Anc80L44	1771	WO2017019994A2 SEQ ID NO: 26
Anc80L1	1772	WO2017019994A2 SEQ ID NO: 35
Anc80L1	1773	WO2017019994A2 SEQ ID NO: 36
AAVrh10	1774	WO2017019994A2 SEQ ID NO: 41
Anc110	1775	WO2017019994A2 SEQ ID NO: 42
Anc110	1776	WO2017019994A2 SEQ ID NO: 43
AAVrh32.33	1777	WO2017019994A2 SEQ ID NO: 45
AAVrh74	1778	WO2017049031A1 SEQ ID NO: 1
AAV2	1779	WO2017053629A2 SEQ ID NO: 49
AAV2	1780	WO2017053629A2 SEQ ID NO: 50
AAV2	1781	WO2017053629A2 SEQ ID NO: 82
Parvo-like virus	1782	WO2017070476A2 SEQ ID NO: 1
Parvo-like virus	1783	WO2017070476A2 SEQ ID NO: 2
Parvo-like virus	1784	WO2017070476A2 SEQ ID NO: 3
Parvo-like virus	1785	WO2017070476A2 SEQ ID NO: 4
Parvo-like virus	1786	WO2017070476A2 SEQ ID NO: 5
Parvo-like virus	1787	WO2017070476A2 SEQ ID NO: 6
AAVrh.10	1788	WO2017070516A1 SEQ ID NO: 7
AAVrh.10	1789	WO2017070516A1 SEQ ID NO: 14
AAV2tYF	1790	WO2017070491A1 SEQ ID NO: 1
AAV-SPK	1791	WO2017075619A1 SEQ ID NO:28
AAV2.5	1792	US20170128528A1 SEQ ID NO: 13
AAV1.1	1793	US20170128528A1 SEQ ID NO: 15
AAV6.1	1794	US20170128528A1 SEQ ID NO: 17
AAV6.3.1	1795	US20170128528A1 SEQ ID NO: 18
AAV2i8	1796	US20170128528A1 SEQ ID NO: 28
AAV2i8	1797	US20170128528A1 SEQ ID NO: 29
ttAAV	1798	US20170128528A1 SEQ ID NO: 30
ttAAV-S312N	1799	US20170128528A1 SEQ ID NO: 32
ttAAV-S312N	1800	US20170128528A1 SEQ ID NO: 33
AAV6 (Y705, Y731, and T492)	1801	WO2016134337A1 SEQ ID NO: 24
AAV2	1802	WO2016134375A1 SEQ ID NO: 9
AAV2	1803	WO2016134375A1 SEQ ID NO: 10

[0098] Each of the patents, applications and/or publications listed in Table 1 are hereby incorporated by reference in their entirety.

[0099] In one embodiment, the AAV serotype may be, or may have a sequence as described in International Patent Publication WO2015038958, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV9 (SEQ ID NO: 2 and 11 of WO2015038958 or SEQ ID NO: 127 and 126 respectively herein), PHP.B (SEQ ID NO: 8 and 9 of WO2015038958, herein SEQ ID NO: 868 and 869), G2B-13 (SEQ ID NO: 12 of WO2015038958, herein SEQ ID NO: 870), G2B-26 (SEQ ID NO: 13 of WO2015038958, herein SEQ ID NO: 868 and 869), TH1.1-32 (SEQ ID NO: 14 of WO2015038958, herein SEQ ID NO: 871), TH1.1-35 (SEQ ID NO: 15 of WO2015038958, herein SEQ ID NO: 872) or variants thereof. Further, any of the targeting peptides or amino acid inserts described in WO2015038958, may be inserted into any parent AAV serotype, such as, but not limited to, AAV9 (SEQ ID NO: 126 for the DNA sequence and SEQ ID NO: 127 for the amino acid sequence). In one embodiment, the amino acid insert is inserted between amino acids 586-592 of the parent AAV (e.g., AAV9). In another embodiment, the amino acid insert is inserted between amino acids 588-589 of the parent AAV sequence. The amino acid insert may be, but is not limited to, any of the following amino acid sequences, TLAVPFK (SEQ ID NO: 1 of WO2015038958; herein SEQ ID NO: 873), KFPVALT (SEQ ID NO: 3 of WO2015038958; herein SEQ ID NO: 874), LAVPFK (SEQ ID NO: 31 of WO2015038958; herein SEQ ID NO: 875), AVPFK (SEQ ID NO: 32 of WO2015038958; herein SEQ ID NO: 876), VPFK (SEQ ID NO: 33 of WO2015038958; herein SEQ ID NO: 877), TLAVPF (SEQ ID NO: 34 of WO2015038958; herein SEQ ID NO: 878), TLAVP (SEQ ID NO: 35 of WO2015038958; herein SEQ ID NO: 879), TLAV (SEQ ID NO: 36 of WO2015038958; herein SEQ ID NO: 880), SVSKPFL (SEQ ID NO: 28 of WO2015038958; herein SEQ ID NO: 881), FTLTTPK (SEQ ID NO: 29 of WO2015038958; herein SEQ ID NO: 882), MNATKNV (SEQ ID NO: 30 of WO2015038958; herein SEQ ID NO: 883), QSSQTPR (SEQ ID NO: 54 of WO2015038958; herein SEQ ID NO: 884), ILGTGTS (SEQ ID NO: 55 of WO2015038958; herein SEQ ID NO: 885), TRTNPEA (SEQ ID NO: 56 of WO2015038958; herein SEQ ID NO: 886), NGGTSSS (SEQ ID NO: 58 of WO2015038958; herein SEQ ID NO: 887), or YTLSQGW (SEQ ID NO: 60 of WO2015038958; herein SEQ ID NO: 888). Non-limiting examples of nucleotide sequences that may encode the amino acid inserts include the following, AAGTTTCCTGTGGCGTTGACT (for SEQ ID NO: 3 of WO2015038958; herein SEQ ID NO: 889), ACTTTGGCGGTGCCTTTTAAG (SEQ ID NO: 24 and 49 of WO2015038958; herein SEQ ID

NO: 890), AGTGTGAGTAAGCCTTTTTTG (SEQ ID NO: 25 of WO2015038958; herein SEQ ID NO: 891), TTTACGTTGACGACGCCTAAG (SEQ ID NO: 26 of WO2015038958; herein SEQ ID NO: 892), ATGAATGCTACGAAGAATGTG (SEQ ID NO: 27 of WO2015038958; herein SEQ ID NO: 893), CAGTCGTCGCAGACGCCTAGG (SEQ ID NO: 48 of WO2015038958; herein SEQ ID NO: 894), ATTCTGGGGACTGGTACTTCG (SEQ ID NO: 50 and 52 of WO2015038958; herein SEQ ID NO: 895), ACGCGGACTAATCCTGAGGCT (SEQ ID NO: 51 of WO2015038958; herein SEQ ID NO: 896), AATGGGGGGACTAGTAGTTCT (SEQ ID NO: 53 of WO2015038958; herein SEQ ID NO: 897), or TATACTTTGTCGCAGGGTTGG (SEQ ID NO: 59 of WO2015038958; herein SEQ ID NO: 898).

[00100] In one embodiment, the AAV serotype may be engineered to comprise at least one AAV capsid CD8+ T-cell epitope for AAV2 such as, but not limited to, SADNNNSEY (SEQ ID NO: 899), LIDQYLYYL (SEQ ID NO: 900), VPQYGYLTL (SEQ ID NO: 901), TTSTRTWAL (SEQ ID NO: 902), YHLNGRDSL (SEQ ID NO: 903), SQAVGRSSF (SEQ ID NO: 904), VPANPSTTF (SEQ ID NO: 905), FPQSGVLIF (SEQ ID NO: 906), YFDFNRFHCHFSPRD (SEQ ID NO: 907), VGNSSGNWHCDSTWM (SEQ ID NO: 908), QFSQAGASDIRDQSR (SEQ ID NO: 909), GASDIRQSRNWLP (SEQ ID NO: 910) and GNRQAATADVNTQGV (SEQ ID NO: 911).

[00101] In one embodiment, the AAV serotype may be engineered to comprise at least one AAV capsid CD8+ T-cell epitope for AAV1 such as, but not limited to, LDRLMNPLI (SEQ ID NO: 912), TTSTRTWAL (SEQ ID NO: 902), and QPAKKRLNF (SEQ ID NO: 913)).

[00102] In one embodiment, the AAV serotype may be, or may have a sequence as described in International Patent Publication WO2017100671, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV9 (SEQ ID NO: 45 of WO2017100671, herein SEQ ID NO: 1420), PHP.N (SEQ ID NO: 46 of WO2017100671, herein SEQ ID NO: 1418), PHP.S (SEQ ID NO: 47 of WO2017100671, herein SEQ ID NO: 1419), or variants thereof. Further, any of the targeting peptides or amino acid inserts described in WO2017100671 may be inserted into any parent AAV serotype, such as, but not limited to, AAV9 (SEQ ID NO: 127 or SEQ ID NO: 1420). In one embodiment, the amino acid insert is inserted between amino acids 586-592 of the parent AAV (e.g., AAV9). In another embodiment, the amino acid insert is inserted between amino acids 588-589 of the parent AAV sequence. The amino acid insert may be, but is not limited to, any of the following amino acid sequences, AQTLAVPFKAQ (SEQ ID NO: 1 of WO2017100671; herein SEQ ID NO: 1804),

AQSVSKPFLAQ (SEQ ID NO: 2 of WO2017100671; herein SEQ ID NO: 1805), AQFTLTTPKAQ (SEQ ID NO: 3 in the sequence listing of WO2017100671; herein SEQ ID NO: 1806), DGTLAVPFKAQ (SEQ ID NO: 4 in the sequence listing of WO2017100671; herein SEQ ID NO: 1807), ESTLAVPFKAQ (SEQ ID NO: 5 of WO2017100671; herein SEQ ID NO: 1808), GGTLAVPFKAQ (SEQ ID NO: 6 of WO2017100671; herein SEQ ID NO: 1809), AQTLATPFKAQ (SEQ ID NO: 7 and 33 of WO2017100671; herein SEQ ID NO: 1810), ATTLATPFKAQ (SEQ ID NO: 8 of WO2017100671; herein SEQ ID NO: 1811), DGTLATPFKAQ (SEQ ID NO: 9 of WO2017100671; herein SEQ ID NO: 1812), GGTLATPFKAQ (SEQ ID NO: 10 of WO2017100671; herein SEQ ID NO: 1813), SGSLAVPFKAQ (SEQ ID NO: 11 of WO2017100671; herein SEQ ID NO: 1814), AQTLAQPFKAQ (SEQ ID NO: 12 of WO2017100671; herein SEQ ID NO: 1815), AQTLQQPFKAQ (SEQ ID NO: 13 of WO2017100671; herein SEQ ID NO: 1816), AQTLSNPFKAQ (SEQ ID NO: 14 of WO2017100671; herein SEQ ID NO: 1817), AQTLAVPFSNP (SEQ ID NO: 15 of WO2017100671; herein SEQ ID NO: 1818), QGTLAVPFKAQ (SEQ ID NO: 16 of WO2017100671; herein SEQ ID NO: 1819), NQTLAVPFKAQ (SEQ ID NO: 17 of WO2017100671; herein SEQ ID NO: 1820), EGSLAVPFKAQ (SEQ ID NO: 18 of WO2017100671; herein SEQ ID NO: 1821), SGNLAVPFKAQ (SEQ ID NO: 19 of WO2017100671; herein SEQ ID NO: 1822), EGTLAVPFKAQ (SEQ ID NO: 20 of WO2017100671; herein SEQ ID NO: 1823), DSTLAVPFKAQ (SEQ ID NO: 21 in Table 1 of WO2017100671; herein SEQ ID NO: 1824), AVTLAVPFKAQ (SEQ ID NO: 22 of WO2017100671; herein SEQ ID NO: 1825), AQTLSTPFKAQ (SEQ ID NO: 23 of WO2017100671; herein SEQ ID NO: 1826), AQTLPQPFKAQ (SEQ ID NO: 24 and 32 of WO2017100671; herein SEQ ID NO: 1827), AQTLSQPFKAQ (SEQ ID NO: 25 of WO2017100671; herein SEQ ID NO: 1828), AQTLQLPFKAQ (SEQ ID NO: 26 of WO2017100671; herein SEQ ID NO: 1829), AQTLTMPFKAQ (SEQ ID NO: 27, and 34 of WO2017100671 and SEQ ID NO: 35 in the sequence listing of WO2017100671; herein SEQ ID NO: 1830), AQTLTTPFKAQ (SEQ ID NO: 28 of WO2017100671; herein SEQ ID NO: 1831), AQYTLSQGWAQ (SEQ ID NO: 29 of WO2017100671; herein SEQ ID NO: 1832), AQMNATKNVAQ (SEQ ID NO: 30 of WO2017100671; herein SEQ ID NO: 1833), AQVSGGHHSAQ (SEQ ID NO: 31 of WO2017100671; herein SEQ ID NO: 1834), AQTLTAPFKAQ (SEQ ID NO: 35 in Table 1 of WO2017100671; herein SEQ ID NO: 1835), AQTLSKPFKAQ (SEQ ID NO: 36 of WO2017100671; herein SEQ ID NO: 1836), QAVRTSL (SEQ ID NO: 37 of WO2017100671;

herein SEQ ID NO: 1837), YTLSQGW (SEQ ID NO: 38 of WO2017100671; herein SEQ ID NO: 888), LAKERLS (SEQ ID NO: 39 of WO2017100671; herein SEQ ID NO: 1838), TLAVPFK (SEQ ID NO: 40 in the sequence listing of WO2017100671; herein SEQ ID NO: 873), SVSKPFL (SEQ ID NO: 41 of WO2017100671; herein SEQ ID NO: 881), FTLTTPK (SEQ ID NO: 42 of WO2017100671; herein SEQ ID NO: 882), MNSTKNV (SEQ ID NO: 43 of WO2017100671; herein SEQ ID NO: 1839), VSGGHHS (SEQ ID NO: 44 of WO2017100671; herein SEQ ID NO: 1840), SAQTLAVPFKAQAQ (SEQ ID NO: 48 of WO2017100671; herein SEQ ID NO: 1841), SXXXLAVPFKAQAQ (SEQ ID NO: 49 of WO2017100671 wherein X may be any amino acid; herein SEQ ID NO: 1842), SAQXXXVPFKAQAQ (SEQ ID NO: 50 of WO2017100671 wherein X may be any amino acid; herein SEQ ID NO: 1843), SAQTLXXXFKAQAQ (SEQ ID NO: 51 of WO2017100671 wherein X may be any amino acid; herein SEQ ID NO: 1844), SAQTLAVXXXAQAQ (SEQ ID NO: 52 of WO2017100671 wherein X may be any amino acid; herein SEQ ID NO: 1845), SAQTLAVPFXXXAQ (SEQ ID NO: 53 of WO2017100671 wherein X may be any amino acid; herein SEQ ID NO: 1846), TNHQSAQ (SEQ ID NO: 65 of WO2017100671; herein SEQ ID NO: 1847), AQAQTGW (SEQ ID NO: 66 of WO2017100671; herein SEQ ID NO: 1848), DGTLATPFK (SEQ ID NO: 67 of WO2017100671; herein SEQ ID NO: 1849), DGTLATPFKXX (SEQ ID NO: 68 of WO2017100671 wherein X may be any amino acid; herein SEQ ID NO: 1850), LAVPFKAQ (SEQ ID NO: 80 of WO2017100671; herein SEQ ID NO: 1851), VPFKAQ (SEQ ID NO: 81 of WO2017100671; herein SEQ ID NO: 1852), FKAQ (SEQ ID NO: 82 of WO2017100671; herein SEQ ID NO: 1853), AQTLAV (SEQ ID NO: 83 of WO2017100671; herein SEQ ID NO: 1854), AQTLAVPF (SEQ ID NO: 84 of WO2017100671; herein SEQ ID NO: 1855), QAVR (SEQ ID NO: 85 of WO2017100671; herein SEQ ID NO: 1856), AVRT (SEQ ID NO: 86 of WO2017100671; herein SEQ ID NO: 1857), VRTS (SEQ ID NO: 87 of WO2017100671; herein SEQ ID NO: 1858), RTSL (SEQ ID NO: 88 of WO2017100671; herein SEQ ID NO: 1859), QAVRT (SEQ ID NO: 89 of WO2017100671; herein SEQ ID NO: 1860), AVRTS (SEQ ID NO: 90 of WO2017100671; herein SEQ ID NO: 1861), VRTSL (SEQ ID NO: 91 of WO2017100671; herein SEQ ID NO: 1862), QAVRTS (SEQ ID NO: 92 of WO2017100671; herein SEQ ID NO: 1863), or AVRTSL (SEQ ID NO: 93 of WO2017100671; herein SEQ ID NO: 1864).

[00103] Non-limiting examples of nucleotide sequences that may encode the amino acid inserts include the following, GATGGGACTTTGGCGGTGCCTTTTAAGGCACAG (SEQ ID NO: 54 of WO2017100671; herein SEQ ID NO: 1865),

GATGGGACGTTGGCGGTGCCTTTTAAGGCACAG (SEQ ID NO: 55 of WO2017100671; herein SEQ ID NO: 1866), CAGGCGGTTAGGACGTCTTTG (SEQ ID NO: 56 of WO2017100671; herein SEQ ID NO: 1867), CAGGTCTTCACGGACTCAGACTATCAG (SEQ ID NO: 57 and 78 of WO2017100671; herein SEQ ID NO: 1868), CAAGTAAAACCTCTACAAATGTGGTAAAATCG (SEQ ID NO: 58 of WO2017100671; herein SEQ ID NO: 1869), ACTCATCGACCAATACTTGTACTATCTCTCTAGAAC (SEQ ID NO: 59 of WO2017100671; herein SEQ ID NO: 1870), GGAAGTATTCCTTGGTTTTGAACCCA (SEQ ID NO: 60 of WO2017100671; herein SEQ ID NO: 1871), GGTCGCGGTTCTTGTTTGTGGAT (SEQ ID NO: 61 of WO2017100671; herein SEQ ID NO: 1872), CGACCTTGAAGCGCATGAACTCCT (SEQ ID NO: 62 of WO2017100671; herein SEQ ID NO: 1873), GTATTCCTTGGTTTTGAACCCAACCGGTCTGCGCCTGTGCMNNMNNMNNMNNMNNMNNMNNTTGGGCACTCTGGTGGTTTGTC (SEQ ID NO: 63 of WO2017100671 wherein N may be A, C, T, or G; herein SEQ ID NO: 1874), GTATTCCTTGGTTTTGAACCCAACCGGTCTGCGCMNNMNNMNNAAAAGGCACCGCCAAAGTTTG (SEQ ID NO: 69 of WO2017100671 wherein N may be A, C, T, or G; herein SEQ ID NO: 1875), GTATTCCTTGGTTTTGAACCCAACCGGTCTGCGCCTGTGCMNNMNNMNNCACCGCCAAAGTTTGGGCACT (SEQ ID NO: 70 of WO2017100671 wherein N may be A, C, T, or G; herein SEQ ID NO: 1876), GTATTCCTTGGTTTTGAACCCAACCGGTCTGCGCCTGTGCCTTAAAMNNMNNMNNCAAAGTTTGGGCACTCTGGTGG (SEQ ID NO: 71 of WO2017100671 wherein N may be A, C, T, or G; herein SEQ ID NO: 1877), GTATTCCTTGGTTTTGAACCCAACCGGTCTGCGCCTGTGCCTTAAAAGGCACMNNMNNNMNNTTGGGCACTCTGGTGGTTTGTG (SEQ ID NO: 72 of WO2017100671 wherein N may be A, C, T, or G; herein SEQ ID NO: 1878), ACTTTGGCGGTGCCTTTTAAG (SEQ ID NO: 74 of WO2017100671; herein SEQ ID NO: 890), AGTGTGAGTAAGCCTTTTTTG (SEQ ID NO: 75 of WO2017100671; herein SEQ ID NO: 891), TTTACGTTGACGACGCCTAAG (SEQ ID NO: 76 of WO2017100671; herein SEQ ID NO: 892), TATACTTTGTCGCAGGGTTGG (SEQ ID NO: 77 of WO2017100671; herein SEQ ID NO: 898), or CTTGCGAAGGAGCGGCTTTCG (SEQ ID NO: 79 of WO2017100671; herein SEQ ID NO: 1879).

[00104] In one embodiment, the AAV serotype may be, or may have a sequence as described in United States Patent No. US 9624274, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV1 (SEQ ID NO: 181 of US9624274), AAV6 (SEQ ID NO: 182 of US9624274), AAV2 (SEQ ID NO: 183 of US9624274), AAV3b (SEQ ID NO: 184 of US9624274), AAV7 (SEQ ID NO: 185 of US9624274), AAV8 (SEQ ID NO: 186 of US9624274), AAV10 (SEQ ID NO: 187 of US9624274), AAV4 (SEQ ID NO: 188 of US9624274), AAV11 (SEQ ID NO: 189 of US9624274), bAAV (SEQ ID NO: 190 of US9624274), AAV5 (SEQ ID NO: 191 of US9624274), GPV (SEQ ID NO: 192 of US9624274; herein SEQ ID NO: 1421), B19 (SEQ ID NO: 193 of US9624274; herein SEQ ID NO: 1422), MVM (SEQ ID NO: 194 of US9624274; herein SEQ ID NO: 1423), FPV (SEQ ID NO: 195 of US9624274; herein SEQ ID NO: 1424), CPV (SEQ ID NO: 196 of US9624274; herein SEQ ID NO: 1425) or variants thereof. Further, any of the structural protein inserts described in US 9624274, may be inserted into, but not limited to, I-453 and I-587 of any parent AAV serotype, such as, but not limited to, AAV2 (SEQ ID NO: 183 of US9624274). The amino acid insert may be, but is not limited to, any of the following amino acid sequences, VNLTWSRASG (SEQ ID NO: 50 of US9624274; herein SEQ ID NO: 1880), EFCINHRGYWVCGD (SEQ ID NO:55 of US9624274; herein SEQ ID NO: 1881), EDGQVMDVDLS (SEQ ID NO: 85 of US9624274; herein SEQ ID NO: 1882), EKQRNGTLT (SEQ ID NO: 86 of US9624274; herein SEQ ID NO: 1883), TYQCRVTHPHLPRALMR (SEQ ID NO: 87 of US9624274; herein SEQ ID NO: 1884), RHSTTQPRKTKGSG (SEQ ID NO: 88 of US9624274; herein SEQ ID NO: 1885), DSNPRGVSAYLSR (SEQ ID NO: 89 of US9624274; herein SEQ ID NO: 1886), TITCLWDLAPSK (SEQ ID NO: 90 of US9624274; herein SEQ ID NO: 1887), KTKGSGFFVF (SEQ ID NO: 91 of US9624274; herein SEQ ID NO: 1888), THPHLPRALMRS (SEQ ID NO: 92 of US9624274; herein SEQ ID NO: 1889), GETYQCRVTHPHLPRALMRSTTK (SEQ ID NO: 93 of US9624274; herein SEQ ID NO: 1890), LPRALMRS (SEQ ID NO: 94 of US9624274; herein SEQ ID NO: 1891), INHRGYWV (SEQ ID NO: 95 of US9624274; herein SEQ ID NO: 1892), CDAGSVRTNAPD (SEQ ID NO: 60 of US9624274; herein SEQ ID NO: 1893), AKAVSNLTESRSESLQS (SEQ ID NO: 96 of US9624274; herein SEQ ID NO: 1894), SLTGDEFKKVLET (SEQ ID NO: 97 of US9624274; herein SEQ ID NO: 1895), REAVAYRFEED (SEQ ID NO: 98 of US9624274; herein SEQ ID NO: 1896), INPEIITLDG (SEQ ID NO: 99 of US9624274; herein SEQ ID NO: 1897), DISVTGAPVITATYL (SEQ ID NO: 100 of US9624274; herein SEQ ID NO: 1898), DISVTGAPVITA (SEQ ID NO: 101 of US9624274; herein SEQ ID NO: 1899), PKTVSNLTESSSESVQS (SEQ ID NO: 102 of

US9624274; herein SEQ ID NO: 1900), SLMGDEFKAVLET (SEQ ID NO: 103 of US9624274; herein SEQ ID NO: 1901), QHSVAYTFEED (SEQ ID NO: 104 of US9624274; herein SEQ ID NO: 1902), INPEIITRDG (SEQ ID NO: 105 of US9624274; herein SEQ ID NO: 1903), DISLTGDPVITASYL (SEQ ID NO: 106 of US9624274; herein SEQ ID NO: 1904), DISLTGDPVITA (SEQ ID NO: 107 of US9624274; herein SEQ ID NO: 1905), DQSIDFEIDSA (SEQ ID NO: 108 of US9624274; herein SEQ ID NO: 1906), KNVSEDLPLPTFSPTLLGDS (SEQ ID NO: 109 of US9624274; herein SEQ ID NO: 1907), KNVSEDLPLPT (SEQ ID NO: 110 of US9624274; herein SEQ ID NO: 1908), CDSGRVRTDAPD (SEQ ID NO: 111 of US9624274; herein SEQ ID NO: 1909), FPEHLLVDFLQSLS (SEQ ID NO: 112 of US9624274; herein SEQ ID NO: 1910), DAEFRHDSG (SEQ ID NO: 65 of US9624274; herein SEQ ID NO: 1911), HYAAAQWDFGNTMCQL (SEQ ID NO: 113 of US9624274; herein SEQ ID NO: 1912), YAAQWDFGNTMCQ (SEQ ID NO: 114 of US9624274; herein SEQ ID NO: 1913), RSQKEGLHYT (SEQ ID NO: 115 of US9624274; herein SEQ ID NO: 1914), SSRTPSDKPVAHWANPQAE (SEQ ID NO: 116 of US9624274; herein SEQ ID NO: 1915), SRTPSDKPVAHWANP (SEQ ID NO: 117 of US9624274; herein SEQ ID NO: 1916), SSRTPSDKP (SEQ ID NO: 118 of US9624274; herein SEQ ID NO: 1917), NADGNVDYHMNSVP (SEQ ID NO: 119 of US9624274; herein SEQ ID NO: 1918), DGNVDYHMNSV (SEQ ID NO: 120 of US9624274; herein SEQ ID NO: 1919), RSFKEFLQSSLRALRQ (SEQ ID NO: 121 of US9624274; herein SEQ ID NO: 1920); FKEFLQSSLRA (SEQ ID NO: 122 of US9624274; herein SEQ ID NO: 1921), or QMWAPQWGPD (SEQ ID NO: 123 of US9624274; herein SEQ ID NO: 1922).

[00105] In one embodiment, the AAV serotype may be, or may have a sequence as described in United States Patent No. US 9475845, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV capsid proteins comprising modification of one or more amino acids at amino acid positions 585 to 590 of the native AAV2 capsid protein. Further the modification may result in, but not limited to, the amino acid sequence RGNRQA (SEQ ID NO: 3 of US9475845; herein SEQ ID NO: 1923), SSSTDP (SEQ ID NO: 4 of US9475845; herein SEQ ID NO: 1924), SSNTAP (SEQ ID NO: 5 of US9475845; herein SEQ ID NO: 1925), SNSNLP (SEQ ID NO: 6 of US9475845; herein SEQ ID NO: 1926), SSTTAP (SEQ ID NO: 7 of US9475845; herein SEQ ID NO: 1927), AANTAA (SEQ ID NO: 8 of US9475845; herein SEQ ID NO: 1928), QQNTAP (SEQ ID NO: 9 of US9475845; herein SEQ ID NO: 1929), SAQAQA (SEQ ID NO: 10 of US9475845; herein SEQ ID NO: 1930), QANTGP (SEQ ID NO: 11 of US9475845; herein SEQ ID NO: 1931), NATTAP (SEQ ID NO:

12 of US9475845; herein SEQ ID NO: 1932), SSTAGP (SEQ ID NO: 13 and 20 of US9475845; herein SEQ ID NO: 1933), QQNTAA (SEQ ID NO: 14 of US9475845; herein SEQ ID NO: 1934), PSTAGP (SEQ ID NO: 15 of US9475845; herein SEQ ID NO: 1935), NQNTAP (SEQ ID NO: 16 of US9475845; herein SEQ ID NO: 1936), QAANAP (SEQ ID NO: 17 of US9475845; herein SEQ ID NO: 1937), SIVGLP (SEQ ID NO: 18 of US9475845; herein SEQ ID NO: 1938), AASTAA (SEQ ID NO: 19, and 27 of US9475845; herein SEQ ID NO: 1939), SQNTTA (SEQ ID NO: 21 of US9475845; herein SEQ ID NO: 1940), QQDTAP (SEQ ID NO: 22 of US9475845; herein SEQ ID NO: 1941), QTNTGP (SEQ ID NO: 23 of US9475845; herein SEQ ID NO: 1942), QTNGAP (SEQ ID NO: 24 of US9475845; herein SEQ ID NO: 1943), QQNAAP (SEQ ID NO: 25 of US9475845; herein SEQ ID NO: 1944), or AANTQA (SEQ ID NO: 26 of US9475845; herein SEQ ID NO: 1945). In one embodiment, the amino acid modification is a substitution at amino acid positions 262 through 265 in the native AAV2 capsid protein or the corresponding position in the capsid protein of another AAV with a targeting sequence. The targeting sequence may be, but is not limited to, any of the amino acid sequences, NGRAHA (SEQ ID NO: 38 of US9475845; herein SEQ ID NO: 1946), QPEHSST (SEQ ID NO: 39 and 50 of US9475845; herein SEQ ID NO: 1947), VNTANST (SEQ ID NO: 40 of US9475845; herein SEQ ID NO: 1948), HGPMQKS (SEQ ID NO: 41 of US9475845; herein SEQ ID NO: 1949), PHKPPLA (SEQ ID NO: 42 of US9475845; herein SEQ ID NO: 1950), IKNNEMW (SEQ ID NO: 43 of US9475845; herein SEQ ID NO: 1951), RNLDTPM (SEQ ID NO: 44 of US9475845; herein SEQ ID NO: 1952), VDSHRQS (SEQ ID NO: 45 of US9475845; herein SEQ ID NO: 1953), YDSKTKT (SEQ ID NO: 46 of US9475845; herein SEQ ID NO: 1954), SQLPHQK (SEQ ID NO: 47 of US9475845; herein SEQ ID NO: 1955), STMQQNT (SEQ ID NO: 48 of US9475845; herein SEQ ID NO: 1956), TERYMTQ (SEQ ID NO: 49 of US9475845; herein SEQ ID NO: 1957), DASLSTS (SEQ ID NO: 51 of US9475845; herein SEQ ID NO: 1958), DLPNKKT (SEQ ID NO: 52 of US9475845; herein SEQ ID NO: 1959), DLTAARL (SEQ ID NO: 53 of US9475845; herein SEQ ID NO: 1960), EPHQFNY (SEQ ID NO: 54 of US9475845; herein SEQ ID NO: 1961), EPQSNHT (SEQ ID NO: 55 of US9475845; herein SEQ ID NO: 1962), MSSWPSQ (SEQ ID NO: 56 of US9475845; herein SEQ ID NO: 1963), NPKHNAT (SEQ ID NO: 57 of US9475845; herein SEQ ID NO: 1964), PDGMRTT (SEQ ID NO: 58 of US9475845; herein SEQ ID NO: 1965), PNNNKTT (SEQ ID NO: 59 of US9475845; herein SEQ ID NO: 1966), QSTTHDS (SEQ ID NO: 60 of US9475845; herein SEQ ID NO: 1967), TGSKQKQ (SEQ ID NO: 61 of US9475845; herein SEQ ID NO: 1968), SLKHQAL (SEQ ID NO: 62 of US9475845; herein SEQ ID NO: 1969), SPIDGEQ (SEQ ID

NO: 63 of US9475845; herein SEQ ID NO: 1970), WIFPWIQL (SEQ ID NO: 64 and 112 of US9475845; herein SEQ ID NO: 1971), CDCRGDCFC (SEQ ID NO: 65 of US9475845; herein SEQ ID NO: 1972), CNGRC (SEQ ID NO: 66 of US9475845; herein SEQ ID NO: 1973), CPRECES (SEQ ID NO: 67 of US9475845; herein SEQ ID NO: 1974), CTTHWGFTLC (SEQ ID NO: 68 and 123 of US9475845; herein SEQ ID NO: 1975), CGRRAGGSC (SEQ ID NO: 69 of US9475845; herein SEQ ID NO: 1976), CKGGRAKDC (SEQ ID NO: 70 of US9475845; herein SEQ ID NO: 1977), CVPELGHEC (SEQ ID NO: 71 and 115 of US9475845; herein SEQ ID NO: 1978), CRRETAWAK (SEQ ID NO: 72 of US9475845; herein SEQ ID NO: 1979), VSWFSHRYSPFAVS (SEQ ID NO: 73 of US9475845; herein SEQ ID NO: 1980), GYRDGYAGPILYN (SEQ ID NO: 74 of US9475845; herein SEQ ID NO: 1981), XXXYXXX (SEQ ID NO: 75 of US9475845; herein SEQ ID NO: 1982), YXNW (SEQ ID NO: 76 of US9475845; herein SEQ ID NO: 1983), RPLPPLP (SEQ ID NO: 77 of US9475845; herein SEQ ID NO: 1984), APPLPPR (SEQ ID NO: 78 of US9475845; herein SEQ ID NO: 1985), DVFYPYPYASGS (SEQ ID NO: 79 of US9475845; herein SEQ ID NO: 1986), MYWYPY (SEQ ID NO: 80 of US9475845; herein SEQ ID NO: 1987), DITWDQLWDLMK (SEQ ID NO: 81 of US9475845; herein SEQ ID NO: 1988), CWDDXWLC (SEQ ID NO: 82 of US9475845; herein SEQ ID NO: 1989), EWCEYLGGYLRCYA (SEQ ID NO: 83 of US9475845; herein SEQ ID NO: 1990), YXCXXGPXTWXCXP (SEQ ID NO: 84 of US9475845; herein SEQ ID NO: 1991), IEGPTLRQWLAARA (SEQ ID NO: 85 of US9475845; herein SEQ ID NO: 1992), LWXXX (SEQ ID NO: 86 of US9475845; herein SEQ ID NO: 1993), XFXXYLW (SEQ ID NO: 87 of US9475845; herein SEQ ID NO: 1994), SSIISHFRWGLCD (SEQ ID NO: 88 of US9475845; herein SEQ ID NO: 1995), MSRPACPPNDKYE (SEQ ID NO: 89 of US9475845; herein SEQ ID NO: 1996), CLRSGRGC (SEQ ID NO: 90 of US9475845; herein SEQ ID NO: 1997), CHWMFSPWC (SEQ ID NO: 91 of US9475845; herein SEQ ID NO: 1998), WXXF (SEQ ID NO: 92 of US9475845; herein SEQ ID NO: 1999), CSSRLDAC (SEQ ID NO: 93 of US9475845; herein SEQ ID NO: 2000), CLPVASC (SEQ ID NO: 94 of US9475845; herein SEQ ID NO: 2001), CGFECVRQCPERC (SEQ ID NO: 95 of US9475845; herein SEQ ID NO: 2002), CVALCREACGEGC (SEQ ID NO: 96 of US9475845; herein SEQ ID NO: 2003), SWCEPGWCR (SEQ ID NO: 97 of US9475845; herein SEQ ID NO: 2004), YSGKWGW (SEQ ID NO: 98 of US9475845; herein SEQ ID NO: 2005), GLSGGRS (SEQ ID NO: 99 of US9475845; herein SEQ ID NO: 2006), LMLPRAD (SEQ ID NO: 100 of US9475845; herein SEQ ID NO: 2007), CSCFRDVCC (SEQ ID NO: 101 of US9475845; herein SEQ ID NO: 2008), CRDVVSVIC (SEQ ID NO: 102 of US9475845; herein SEQ ID NO: 2009), MARSGL

(SEQ ID NO: 103 of US9475845; herein SEQ ID NO: 2010), MARAKE (SEQ ID NO: 104 of US9475845; herein SEQ ID NO: 2011), MSRTMS (SEQ ID NO: 105 of US9475845; herein SEQ ID NO: 2012), KCCYSL (SEQ ID NO: 106 of US9475845; herein SEQ ID NO: 2013), MYWGDSHWLQYWYE (SEQ ID NO: 107 of US9475845; herein SEQ ID NO: 2014), MQLPLAT (SEQ ID NO: 108 of US9475845; herein SEQ ID NO: 2015), EWLS (SEQ ID NO: 109 of US9475845; herein SEQ ID NO: 2016), SNEW (SEQ ID NO: 110 of US9475845; herein SEQ ID NO: 2017), TNYL (SEQ ID NO: 111 of US9475845; herein SEQ ID NO: 2018), WDLAWMFRLPVG (SEQ ID NO: 113 of US9475845; herein SEQ ID NO: 2019), CTVALPGGYVRVC (SEQ ID NO: 114 of US9475845; herein SEQ ID NO: 2020), CVAYCIEHHCWTC (SEQ ID NO: 116 of US9475845; herein SEQ ID NO: 2021), CVFAHNYDYLVC (SEQ ID NO: 117 of US9475845; herein SEQ ID NO: 2022), CVFTSNYAFC (SEQ ID NO: 118 of US9475845; herein SEQ ID NO: 2023), VHSPNKK (SEQ ID NO: 119 of US9475845; herein SEQ ID NO: 2024), CRGDGWC (SEQ ID NO: 120 of US9475845; herein SEQ ID NO: 2025), XRGCDX (SEQ ID NO: 121 of US9475845; herein SEQ ID NO: 2026), PXXX (SEQ ID NO: 122 of US9475845; herein SEQ ID NO: 2027), SGKGPRQITAL (SEQ ID NO: 124 of US9475845; herein SEQ ID NO: 2028), AAAAAAAAAXXXXX (SEQ ID NO: 125 of US9475845; herein SEQ ID NO: 2029), VYMSPF (SEQ ID NO: 126 of US9475845; herein SEQ ID NO: 2030), ATWLPPR (SEQ ID NO: 127 of US9475845; herein SEQ ID NO: 2031), HTMYYHHYQHHL (SEQ ID NO: 128 of US9475845; herein SEQ ID NO: 2032), SEVGCRAGPLQWLCEKYFG (SEQ ID NO: 129 of US9475845; herein SEQ ID NO: 2033), CGLLPVGRPDRNVWRWLC (SEQ ID NO: 130 of US9475845; herein SEQ ID NO: 2034), CKGQCDRFKGLPWEC (SEQ ID NO: 131 of US9475845; herein SEQ ID NO: 2035), SGRSA (SEQ ID NO: 132 of US9475845; herein SEQ ID NO: 2036), WGFP (SEQ ID NO: 133 of US9475845; herein SEQ ID NO: 2037), AEPMPHSLNFSQYLWYT (SEQ ID NO: 134 of US9475845; herein SEQ ID NO: 2038), WAYXSP (SEQ ID NO: 135 of US9475845; herein SEQ ID NO: 2039), IELLQAR (SEQ ID NO: 136 of US9475845; herein SEQ ID NO: 2040), AYTKCSRQWRTCMTTH (SEQ ID NO: 137 of US9475845; herein SEQ ID NO: 2041), PQNSKIPGPTFLDPH (SEQ ID NO: 138 of US9475845; herein SEQ ID NO: 2042), SMEPALPDWWWKMFK (SEQ ID NO: 139 of US9475845; herein SEQ ID NO: 2043), ANTPCGPYTHDCPVKR (SEQ ID NO: 140 of US9475845; herein SEQ ID NO: 2044), TACHQHVRMVRP (SEQ ID NO: 141 of US9475845; herein SEQ ID NO: 2045), VPWMEPAYQRFL (SEQ ID NO: 142 of US9475845; herein SEQ ID NO: 2046), DPRATPGS (SEQ ID NO: 143 of US9475845; herein SEQ ID NO: 2047),

FRPNRAQDYNTN (SEQ ID NO: 144 of US9475845; herein SEQ ID NO: 2048), CTKNSYLMC (SEQ ID NO: 145 of US9475845; herein SEQ ID NO: 2049), CXXTXXXGXGC (SEQ ID NO: 146 of US9475845; herein SEQ ID NO: 2050), CPIEDRPMC (SEQ ID NO: 147 of US9475845; herein SEQ ID NO: 2051), HEWSYLAPYPWF (SEQ ID NO: 148 of US9475845; herein SEQ ID NO: 2052), MCPKHPLGC (SEQ ID NO: 149 of US9475845; herein SEQ ID NO: 2053), RMWPSSTVNLSAGRR (SEQ ID NO: 150 of US9475845; herein SEQ ID NO: 2054), SAKTAVSQRVWLPSHRGGEP (SEQ ID NO: 151 of US9475845; herein SEQ ID NO: 2055), KSREHVNNSACPSKRITAAL (SEQ ID NO: 152 of US9475845; herein SEQ ID NO: 2056), EGFR (SEQ ID NO: 153 of US9475845; herein SEQ ID NO: 2057), AGLGVR (SEQ ID NO: 154 of US9475845; herein SEQ ID NO: 2058), GTRQGHTMRLGVSDG (SEQ ID NO: 155 of US9475845; herein SEQ ID NO: 2059), IAGLATPGWSHWLAL (SEQ ID NO: 156 of US9475845; herein SEQ ID NO: 2060), SMSIARL (SEQ ID NO: 157 of US9475845; herein SEQ ID NO: 2061), HTFEPGV (SEQ ID NO: 158 of US9475845; herein SEQ ID NO: 2062), NTSLKRISNKRIRRK (SEQ ID NO: 159 of US9475845; herein SEQ ID NO: 2063), LRIKRKRRKRKKTRK (SEQ ID NO: 160 of US9475845; herein SEQ ID NO: 2064), GGG, GFS, LWS, EGG, LLV, LSP, LBS, AGG, GRR, GGH and GTV.

[00106] In one embodiment, the AAV serotype may be, or may have a sequence as described in United States Publication No. US 20160369298, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, site-specific mutated capsid protein of AAV2 (SEQ ID NO: 97 of US 20160369298; herein SEQ ID NO: 2065) or variants thereof, wherein the specific site is at least one site selected from sites R447, G453, S578, N587, N587+1, S662 of VP1 or fragment thereof.

[00107] Further, any of the mutated sequences described in US 20160369298, may be or may have, but not limited to, any of the following sequences SDSGASN (SEQ ID NO: 1 and SEQ ID NO: 231 of US20160369298; herein SEQ ID NO: 2066), SPSGASN (SEQ ID NO: 2 of US20160369298; herein SEQ ID NO: 2067), SHSGASN (SEQ ID NO: 3 of US20160369298; herein SEQ ID NO: 2068), SRSGASN (SEQ ID NO: 4 of US20160369298; herein SEQ ID NO: 2069), SKSGASN (SEQ ID NO: 5 of US20160369298; herein SEQ ID NO: 2070), SNSGASN (SEQ ID NO: 6 of US20160369298; herein SEQ ID NO: 2071), SGSGASN (SEQ ID NO: 7 of US20160369298; herein SEQ ID NO: 2072), SASGASN (SEQ ID NO: 8, 175, and 221 of US20160369298; herein SEQ ID NO: 2073), SESGTSN (SEQ ID NO: 9 of US20160369298; herein SEQ ID NO: 2074), STTGGSN (SEQ ID NO: 10 of US20160369298; herein SEQ ID

NO: 2075), SSAGSTN (SEQ ID NO: 11 of US20160369298; herein SEQ ID NO: 2076), NNDSQA (SEQ ID NO: 12 of US20160369298; herein SEQ ID NO: 2077), NNRNQA (SEQ ID NO: 13 of US20160369298; herein SEQ ID NO: 2078), NNNKQA (SEQ ID NO: 14 of US20160369298; herein SEQ ID NO: 2079), NAKRQA (SEQ ID NO: 15 of US20160369298; herein SEQ ID NO: 2080), NDEHQA (SEQ ID NO: 16 of US20160369298; herein SEQ ID NO: 2081), NTSQKA (SEQ ID NO: 17 of US20160369298; herein SEQ ID NO: 2082), YYLSRTNTPSGTDTQSRLVFSQAGA (SEQ ID NO: 18 of US20160369298; herein SEQ ID NO: 2083), YYLSRTNTDSGTETQSGLDFSQAGA (SEQ ID NO: 19 of US20160369298; herein SEQ ID NO: 2084), YYLSRTNTESGTPTQSALEFSQAGA (SEQ ID NO: 20 of US20160369298; herein SEQ ID NO: 2085), YYLSRTNTHSGTHTQSPLHFSQAGA (SEQ ID NO: 21 of US20160369298; herein SEQ ID NO: 2086), YYLSRTNTSSGTITISHLIFSQAGA (SEQ ID NO: 22 of US20160369298; herein SEQ ID NO: 2087), YYLSRTNTRSGIMTKSSLMFSQAGA (SEQ ID NO: 23 of US20160369298; herein SEQ ID NO: 2088), YYLSRTNTKSGRKTLSNLSFSQAGA (SEQ ID NO: 24 of US20160369298; herein SEQ ID NO: 2089), YYLSRTNDGSGPVTPSKLRFSQRGA (SEQ ID NO: 25 of US20160369298; herein SEQ ID NO: 2090), YYLSRTNAASGHATHSDLKFSQPGA (SEQ ID NO: 26 of US20160369298; herein SEQ ID NO: 2091), YYLSRTNGQAGSLTMSELGFSQVGA (SEQ ID NO: 27 of US20160369298; herein SEQ ID NO: 2092), YYLSRTNSTGGNQTTSQLLFSQLSA (SEQ ID NO: 28 of US20160369298; herein SEQ ID NO: 2093), YFLSRTNNNTGLNTNSTLNFSQGRA (SEQ ID NO: 29 of US20160369298; herein SEQ ID NO: 2094), SKTGADNNNSEYSWTG (SEQ ID NO: 30 of US20160369298; herein SEQ ID NO: 2095), SKTDADNNNSEYSWTG (SEQ ID NO: 31 of US20160369298; herein SEQ ID NO: 2096), SKTEADNNNSEYSWTG (SEQ ID NO: 32 of US20160369298; herein SEQ ID NO: 2097), SKTPADNNNSEYSWTG (SEQ ID NO: 33 of US20160369298; herein SEQ ID NO: 2098), SKTHADNNNSEYSWTG (SEQ ID NO: 34 of US20160369298; herein SEQ ID NO: 2099), SKTQADNNNSEYSWTG (SEQ ID NO: 35 of US20160369298; herein SEQ ID NO: 2100), SKTIADNNNSEYSWTG (SEQ ID NO: 36 of US20160369298; herein SEQ ID NO: 2101), SKTMADNNNSEYSWTG (SEQ ID NO: 37 of US20160369298; herein SEQ ID NO: 2102), SKTRADNNNSEYSWTG (SEQ ID NO: 38 of US20160369298; herein SEQ ID NO: 2103), SKTNADNNNSEYSWTG (SEQ ID NO: 39 of US20160369298; herein SEQ ID NO: 2104), SKTVGRNNNSEYSWTG (SEQ ID NO: 40 of US20160369298; herein SEQ ID NO: 2105), SKTADRNNNSEYSWTG (SEQ ID NO: 41 of US20160369298; herein SEQ ID NO: 2106), SKKLSQNNNSKYSWQG (SEQ ID NO: 42 of

US20160369298; herein SEQ ID NO: 2107), SKPTTGNNNSDYSWPG (SEQ ID NO: 43 of US20160369298; herein SEQ ID NO: 2108), STQKNENNNSNYSWPG (SEQ ID NO: 44 of US20160369298; herein SEQ ID NO: 2109), HKDDEGKF (SEQ ID NO: 45 of US20160369298; herein SEQ ID NO: 2110), HKDDNRKF (SEQ ID NO: 46 of US20160369298; herein SEQ ID NO: 2111), HKDDTNKF (SEQ ID NO: 47 of US20160369298; herein SEQ ID NO: 2112), HEDSDKNF (SEQ ID NO: 48 of US20160369298; herein SEQ ID NO: 2113), HRDGADSF (SEQ ID NO: 49 of US20160369298; herein SEQ ID NO: 2114), HGDNKSRF (SEQ ID NO: 50 of US20160369298; herein SEQ ID NO: 2115), KQGSEKTNVDFEEV (SEQ ID NO: 51 of US20160369298; herein SEQ ID NO: 2116), KQGSEKTNVDSEEV (SEQ ID NO: 52 of US20160369298; herein SEQ ID NO: 2117), KQGSEKTNVDVEEV (SEQ ID NO: 53 of US20160369298; herein SEQ ID NO: 2118), KQGSDKTNVDDAGV (SEQ ID NO: 54 of US20160369298; herein SEQ ID NO: 2119), KQGSSKTNVDPREV (SEQ ID NO: 55 of US20160369298; herein SEQ ID NO: 2120), KQGSRKTNVDHKQV (SEQ ID NO: 56 of US20160369298; herein SEQ ID NO: 2121), KQGSKGGNVDTNRV (SEQ ID NO: 57 of US20160369298; herein SEQ ID NO: 2122), KQGSGEANVDNGDV (SEQ ID NO: 58 of US20160369298; herein SEQ ID NO: 2123), KQDAAADNIDYDHV (SEQ ID NO: 59 of US20160369298; herein SEQ ID NO: 2124), KQSGTRSNAAASSV (SEQ ID NO: 60 of US20160369298; herein SEQ ID NO: 2125), KENTNTNDTELTNV (SEQ ID NO: 61 of US20160369298; herein SEQ ID NO: 2126), QRGNNVAATADVNT (SEQ ID NO: 62 of US20160369298; herein SEQ ID NO: 2127), QRGNNEAATADVNT (SEQ ID NO: 63 of US20160369298; herein SEQ ID NO: 2128), QRGNNPAATADVNT (SEQ ID NO: 64 of US20160369298; herein SEQ ID NO: 2129), QRGNNHAATADVNT (SEQ ID NO: 65 of US20160369298; herein SEQ ID NO: 2130), QEENNIAATPGVNT (SEQ ID NO: 66 of US20160369298; herein SEQ ID NO: 2131), QPPNNMAATHEVNT (SEQ ID NO: 67 of US20160369298; herein SEQ ID NO: 2132), QHHNNSAATTIVNT (SEQ ID NO: 68 of US20160369298; herein SEQ ID NO: 2133), QTTNNRAAFNMVET (SEQ ID NO: 69 of US20160369298; herein SEQ ID NO: 2134), QKKNNNAASKKVAT (SEQ ID NO: 70 of US20160369298; herein SEQ ID NO: 2135), QGGNNKAADDAVKT (SEQ ID NO: 71 of US20160369298; herein SEQ ID NO: 2136), QAAKGGAADDAVKT (SEQ ID NO: 72 of US20160369298; herein SEQ ID NO: 2137), QDDRAAAANESVDT (SEQ ID NO: 73 of US20160369298; herein SEQ ID NO: 2138), QQQHDDAAYQRVHT (SEQ ID NO: 74 of US20160369298; herein SEQ ID NO: 2139), QSSSSLAAVSTVQT (SEQ ID NO: 75 of

US20160369298; herein SEQ ID NO: 2140), QNNQTTAAIRNVTT (SEQ ID NO: 76 of US20160369298; herein SEQ ID NO: 2141), NYNKKSDNVDFT (SEQ ID NO: 77 of US20160369298; herein SEQ ID NO: 2142), NYNKKSENVDFT (SEQ ID NO: 78 of US20160369298; herein SEQ ID NO: 2143), NYNKKSLNVDFT (SEQ ID NO: 79 of US20160369298; herein SEQ ID NO: 2144), NYNKKSPNVDFT (SEQ ID NO: 80 of US20160369298; herein SEQ ID NO: 2145), NYSKKSHCVDFT (SEQ ID NO: 81 of US20160369298; herein SEQ ID NO: 2146), NYRKTIYVDFT (SEQ ID NO: 82 of US20160369298; herein SEQ ID NO: 2147), NYKEKKDVHFT (SEQ ID NO: 83 of US20160369298; herein SEQ ID NO: 2148), NYGHRAIVQFT (SEQ ID NO: 84 of US20160369298; herein SEQ ID NO: 2149), NYANHQFVVCT (SEQ ID NO: 85 of US20160369298; herein SEQ ID NO: 2150), NYDDDPTGVLLT (SEQ ID NO: 86 of US20160369298; herein SEQ ID NO: 2151), NYDDPTGVLLT (SEQ ID NO: 87 of US20160369298; herein SEQ ID NO: 2152), NFEQQNSVEWT (SEQ ID NO: 88 of US20160369298; herein SEQ ID NO: 2153), SQSGASN (SEQ ID NO: 89 and SEQ ID NO: 241 of US20160369298; herein SEQ ID NO: 2154), NNGSQA (SEQ ID NO: 90 of US20160369298; herein SEQ ID NO: 2155), YYLSRTNTPSGTTTWSRLQFSQAGA (SEQ ID NO: 91 of US20160369298; herein SEQ ID NO: 2156), SKTSADNNNSEYSWTG (SEQ ID NO: 92 of US20160369298; herein SEQ ID NO: 2157), HKDDEEKF (SEQ ID NO: 93, 209, 214, 219, 224, 234, 239, and 244 of US20160369298; herein SEQ ID NO: 2158), KQGSEKTNVDIEEV (SEQ ID NO: 94 of US20160369298; herein SEQ ID NO: 2159), QRGNNQAATADVNT (SEQ ID NO: 95 of US20160369298; herein SEQ ID NO: 2160), NYNKKSVNVDFT (SEQ ID NO: 96 of US20160369298; herein SEQ ID NO: 2161), SQSGASNYNTPSGTTTQSRLQFSTSADNNNSEYSWTGATKYH (SEQ ID NO: 106 of US20160369298; herein SEQ ID NO: 2162), SASGASNFNSEGGSLTQSSLGFSTDGENNNSDFSWTGATKYH (SEQ ID NO: 107 of US20160369298; herein SEQ ID NO: 2163), SQSGASNYNTPSGTTTQSRLQFSTDGENNNSDFSWTGATKYH (SEQ ID NO: 108 of US20160369298; herein SEQ ID NO: 2164), SASGASNYNTPSGTTTQSRLQFSTSADNNNSEFSWPGATTYH (SEQ ID NO: 109 of US20160369298; herein SEQ ID NO: 2165), SQSGASNFNSEGGSLTQSSLGFSTDGENNNSDFSWTGATKYH (SEQ ID NO: 110 of US20160369298; herein SEQ ID NO: 2166), SASGASNYNTPSGSLTQSSLGFSTDGENNNSDFSWTGATKYH (SEQ ID NO: 111 of

US20160369298; herein SEQ ID NO: 2167), SQSGASNYNTPSGTTTQSRLQFSTSADNNNSDFSWTGATKYH (SEQ ID NO: 112 of US20160369298; herein SEQ ID NO: 2168), SGAGASNFNSEGGSLTQSSLGFSTDGENNNSDFSWTGATKYH (SEQ ID NO: 113 of US20160369298; herein SEQ ID NO: 2169), SGAGASN (SEQ ID NO: 176 of US20160369298; herein SEQ ID NO: 2170), NSEGGSLTQSSLGFS (SEQ ID NO: 177, 185, 193 and 202 of US20160369298; herein SEQ ID NO: 2171), TDGENNNSDFS (SEQ ID NO: 178 of US20160369298; herein SEQ ID NO: 2172), SEFSWPGATT (SEQ ID NO: 179 of US20160369298; herein SEQ ID NO: 2173), TSADNNNSDFSWT (SEQ ID NO: 180 of US20160369298; herein SEQ ID NO: 2174), SQSGASNY (SEQ ID NO: 181, 187, and 198 of US20160369298; herein SEQ ID NO: 2175), NTPSGTTTQSRLQFS (SEQ ID NO: 182, 188, 191, and 199 of US20160369298; herein SEQ ID NO: 2176), TSADNNNSEYSWTGATKYH (SEQ ID NO: 183 of US20160369298; herein SEQ ID NO: 2177), SASGASNF (SEQ ID NO: 184 of US20160369298; herein SEQ ID NO: 2178), TDGENNNSDFSWTGATKYH (SEQ ID NO: 186, 189, 194, 197, and 203 of US20160369298; herein SEQ ID NO: 2179), SASGASNY (SEQ ID NO: 190 and SEQ ID NO: 195 of US20160369298; herein SEQ ID NO: 2180), TSADNNNSEFSWPGATTYH (SEQ ID NO: 192 of US20160369298; herein SEQ ID NO: 2181), NTPSGSLTQSSLGFS (SEQ ID NO: 196 of US20160369298; herein SEQ ID NO: 2182), TSADNNNSDFSWTGATKYH (SEQ ID NO: 200 of US20160369298; herein SEQ ID NO: 2183), SGAGASNF (SEQ ID NO: 201 of US20160369298; herein SEQ ID NO: 2184), CTCCAGVVSVVSMRSRVCVNSGCAGCTDHCVVSRNSGTCVMSACACAA (SEQ ID NO: 204 of US20160369298; herein SEQ ID NO: 2185), CTCCAGAGAGGCAACAGACAAGCAGCTACCGCAGATGTCAACACACAA (SEQ ID NO: 205 of US20160369298; herein SEQ ID NO: 2186), SAAGASN (SEQ ID NO: 206 of US20160369298; herein SEQ ID NO: 2187), YFLSRTNTESGSTTQSTLRFSQAG (SEQ ID NO: 207 of US20160369298; herein SEQ ID NO: 2188), SKTSADNNNSDFS (SEQ ID NO: 208, 228, and 253 of US20160369298; herein SEQ ID NO: 2189), KQGSEKTDVDIDKV (SEQ ID NO: 210 of US20160369298; herein SEQ ID NO: 2190), STAGASN (SEQ ID NO: 211 of US20160369298; herein SEQ ID NO: 2191), YFLSRTNTTSGIETQSTLRFSQAG (SEQ ID NO: 212 and SEQ ID NO: 247 of US20160369298; herein SEQ ID NO: 2192), SKTDGENNNSDFS (SEQ ID NO: 213 and SEQ ID NO: 248 of US20160369298; herein SEQ ID NO: 2193), KQGAAADDVEIDGV (SEQ ID NO: 215 and SEQ ID NO: 250 of US20160369298; herein SEQ ID NO: 2194), SEAGASN (SEQ ID NO: 216 of US20160369298;

herein SEQ ID NO: 2195), YYLSRTNTPSGTTTQSRLQFSQAG (SEQ ID NO: 217, 232 and 242 of US20160369298; herein SEQ ID NO: 2196), SKTSADNNNSEYS (SEQ ID NO: 218, 233, 238, and 243 of US20160369298; herein SEQ ID NO: 2197), KQGSEKTNVDIEKV (SEQ ID NO: 220, 225 and 245 of US20160369298; herein SEQ ID NO: 2198), YFLSRTNDASGSDTKSTLLFSQAG (SEQ ID NO: 222 of US20160369298; herein SEQ ID NO: 2199), STTPSENNNSEYS (SEQ ID NO: 223 of US20160369298; herein SEQ ID NO: 2200), SAAGATN (SEQ ID NO: 226 and SEQ ID NO: 251 of US20160369298; herein SEQ ID NO: 2201), YFLSRTNGEAGSATLSELRFSQAG (SEQ ID NO: 227 of US20160369298; herein SEQ ID NO: 2202), HGDDADRF (SEQ ID NO: 229 and SEQ ID NO: 254 of US20160369298; herein SEQ ID NO: 2203), KQGAEKSDVEVDRV (SEQ ID NO: 230 and SEQ ID NO: 255 of US20160369298; herein SEQ ID NO: 2204), KQDSGGDNIDIDQV (SEQ ID NO: 235 of US20160369298; herein SEQ ID NO: 2205), SDAGASN (SEQ ID NO: 236 of US20160369298; herein SEQ ID NO: 2206), YFLSRTNTEGGHDTQSTLRFSQAG (SEQ ID NO: 237 of US20160369298; herein SEQ ID NO: 2207), KEDGGGSDVAIDEV (SEQ ID NO: 240 of US20160369298; herein SEQ ID NO: 2208), SNAGASN (SEQ ID NO: 246 of US20160369298; herein SEQ ID NO: 2209), and YFLSRTNGEAGSATLSELRFSQPG (SEQ ID NO: 252 of US20160369298; herein SEQ ID NO: 2210). Non-limiting examples of nucleotide sequences that may encode the amino acid mutated sites include the following, AGCVVMDCAGGARSCASCAAC (SEQ ID NO: 97 of US20160369298; herein SEQ ID NO: 2211), AACRACRRSMRSMAGGCA (SEQ ID NO: 98 of US20160369298; herein SEQ ID NO: 2212), CACRRGGACRRCRMSRRSARSTTT (SEQ ID NO: 99 of US20160369298; herein SEQ ID NO: 2213), TATTTCTTGAGCAGAACAAACRVCVVSRSCGGAMNCVHSACGMHSTCAVVSCTTVDS TTTTCTCAGSBCRGSGCG (SEQ ID NO: 100 of US20160369298; herein SEQ ID NO: 2214), TCAAMAMMAVNSRVCSRSAACAACAACAGTRASTTCTCGTGGMMAGGA (SEQ ID NO: 101 of US20160369298; herein SEQ ID NO: 2215), AAGSAARRCRSCRVSRVARVCRATRYCGMSNHCRVMVRSGTC (SEQ ID NO: 102 of US20160369298; herein SEQ ID NO: 2216), CAGVVSVVSMRSRVCVNSGCAGCTDHCVVSRNSGTCVMSACA (SEQ ID NO: 103 of US20160369298; herein SEQ ID NO: 2217), AACTWCRVSVASMVSVHSDDTGTGSWSTKSACT (SEQ ID NO: 104 of US20160369298; herein SEQ ID NO: 2218), TTGTTGAACATCACCACGTGACGCACGTTC (SEQ ID NO: 256 of US20160369298; herein SEQ ID NO: 2219),

TCCCCGTGGTTCTACTACATAATGTGGCCG (SEQ ID NO: 257 of US20160369298; herein SEQ ID NO: 2220), TTCCACACTCCGTTTTGGATAATGTTGAAC (SEQ ID NO: 258 of US20160369298; herein SEQ ID NO: 2221), AGGGACATCCCCAGCTCCATGCTGTGGTCG (SEQ ID NO: 259 of US20160369298; herein SEQ ID NO: 2222), AGGGACAACCCCTCCGACTCGCCCTAATCC (SEQ ID NO: 260 of US20160369298; herein SEQ ID NO: 2223), TCCTAGTAGAAGACACCCTCTCACTGCCCG (SEQ ID NO: 261 of US20160369298; herein SEQ ID NO: 2224), AGTACCATGTACACCCACTCTCCCAGTGCC (SEQ ID NO: 262 of US20160369298; herein SEQ ID NO: 2225), ATATGGACGTTCATGCTGATCACCATACCG (SEQ ID NO: 263 of US20160369298; herein SEQ ID NO: 2226), AGCAGGAGCTCCTTGGCCTCAGCGTGCGAG (SEQ ID NO: 264 of US20160369298; herein SEQ ID NO: 2227), ACAAGCAGCTTCACTATGACAACCACTGAC (SEQ ID NO: 265 of US20160369298; herein SEQ ID NO: 2228), CAGCCTAGGAACTGGCTTCCTGGACCCTGTTACCGCCAGCAGAGAGTCTCAAMAMM AVNSRVCSRSAACAACAACAGTRASTTCTCCTGGMMAGGAGCTACCAAGTACCACC TCAATGGCAGAGACTCTCTGGTGAATCCCGGACCAGCTATGGCAAGCCACRRGGAC RRCRMSRRSARSTTTTTTCCTCAGAGCGGGGTTCTCATCTTTGGGAAGSAARRCRSCR VSRVARVCRATRYCGMSNHCRVMVRSGTCATGATTACAGACGAAGAGGAGATCTGG AC (SEQ ID NO: 266 of US20160369298; herein SEQ ID NO: 2229), TGGGACAATGGCGGTCGTCTCTCAGAGTTKTKKT (SEQ ID NO: 267 of US20160369298; herein SEQ ID NO: 2230), AGAGGACCKKTCCTCGATGGTTCATGGTGGAGTTA (SEQ ID NO: 268 of US20160369298; herein SEQ ID NO: 2231), CCACTTAGGGCCTGGTCGATACCGTTCGGTG (SEQ ID NO: 269 of US20160369298; herein SEQ ID NO: 2232), and TCTCGCCCCAAGAGTAGAAACCCTTCSTTYYG (SEQ ID NO: 270 of US20160369298; herein SEQ ID NO: 2233).

[00108] In some embodiments, the AAV serotype may comprise an ocular cell targeting peptide as described in International Patent Publication WO2016134375, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to SEQ ID NO: 9, and SEQ ID NO:10 of WO2016134375. Further, any of the ocular cell targeting peptides or amino acids described in WO2016134375, may be inserted into any parent AAV serotype, such as, but not limited to, AAV2 (SEQ ID NO:8 of WO2016134375; herein SEQ ID NO: 2234), or AAV9 (SEQ ID NO: 11 of WO2016134375; herein SEQ ID NO: 2235). In some embodiments,

modifications, such as insertions are made in AAV2 proteins at P34-A35, T138-A139, A139-P140, G453- T454, N587-R588, and/or R588-Q589. In certain embodiments, insertions are made at D384, G385, 1560, T561, N562, E563, E564, E565, N704, and/or Y705 of AAV9. The ocular cell targeting peptide may be, but is not limited to, any of the following amino acid sequences, GSTPPPM (SEQ ID NO: 1 of WO2016134375; herein SEQ ID NO: 2236), or GETRAPL (SEQ ID NO: 4 of WO2016134375; herein SEQ ID NO: 2237).

[00109] In some embodiments, the AAV serotype may be modified as described in the United States Publication US 20170145405 the contents of which are herein incorporated by reference in their entirety. AAV serotypes may include, modified AAV2(e.g., modifications at Y444F, Y500F, Y730F and/or S662V), modified AAV3 (e.g., modifications at Y705F, Y731F and/or T492V), and modified AAV6 (e.g., modifications at S663V and/or T492V).

[00110] In some embodiments, the AAV serotype may be modified as described in the International Publication WO2017083722 the contents of which are herein incorporated by reference in their entirety. AAV serotypes may include, AAV1 (Y705+731F+T492V), AAV2 (Y444+500+730F+T491V), AAV3 (Y705+731F), AAV5, AAV 5(Y436+693+719F), AAV6 (VP3 variant Y705F/Y731F/T492V), AAV8 (Y733F), AAV9, AAV9 (VP3 variant Y731F), and AAV10 (Y733F).

[00111] In some embodiments, the AAV serotype may comprise, as described in International Patent Publication WO2017015102, the contents of which are herein incorporated by reference in their entirety, an engineered epitope comprising the amino acids SPAKFA (SEQ ID NO: 24 of WO2017015102; herein SEQ ID NO: 2238) or NKDKLN (SEQ ID NO:2 of WO2017015102; herein SEQ ID NO: 2239). The epitope may be inserted in the region of amino acids 665 to 670 based on the numbering of the VP1 capsid of AAV8 (SEQ ID NO:3 of WO2017015102) and/or residues 664 to 668 of AAV3B (SEQ ID NO:3).

[00112] In one embodiment, the AAV serotype may be, or may have a sequence as described in International Patent Publication WO2017058892, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV variants with capsid proteins that may comprise a substitution at one or more (e.g., 2, 3, 4, 5, 6, or 7) of amino acid residues 262-268, 370- 379, 451 -459, 472-473, 493-500, 528-534, 547-552, 588- 597, 709-710, 716-722 of AAV1, in any combination, or the equivalent amino acid residues in AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, bovine AAV or avian AAV. The amino acid substitution may be, but is not limited to, any of the amino acid sequences described in WO2017058892. In one embodiment, the AAV

may comprise an amino acid substitution at residues 256L, 258K, 259Q, 261S, 263A, 264S, 265T, 266G, 272H, 385S, 386Q, S472R, V473D, N500E 547S, 709A, 710N, 716D, 717N, 718N, 720L, A456T, Q457T, N458Q, K459S, T492S, K493A, S586R, S587G, S588N, T589R and/or 722T of AAV1 (SEQ ID NO: 1 of WO2017058892) in any combination, 244N, 246Q, 248R, 249E, 250I, 251K, 252S, 253G, 254S, 255V, 256D, 263Y, 377E, 378N, 453L, 456R, 532Q, 533P, 535N, 536P, 537G, 538T, 539T, 540A, 541T, 542Y, 543L, 546N, 653V, 654P, 656S, 697Q, 698F, 704D, 705S, 706T, 707G, 708E, 709Y and/or 710R of AAV5 (SEQ ID NO:5 of WO2017058892) in any combination, 248R, 316V, 317Q, 318D, 319S, 443N, 530N, 531S, 532Q 533P, 534A, 535N, 540A, 541 T, 542Y, 543L, 545G, 546N, 697Q, 704D, 706T, 708E, 709Yand/or 710R of AAV5 (SEQ ID NO: 5 of WO2017058892) in any combination, 264S, 266G, 269N, 272H, 457Q, 588S and/or 589I of AAV6 (SEQ ID NO:6 WO2017058892) in any combination, 457T, 459N, 496G, 499N, 500N, 589Q, 590N and/or 592A of AAV8 (SEQ ID NO: 8 WO2017058892) in any combination,451I, 452N, 453G, 454S, 455G, 456Q, 457N and/or 458Q of AAV9 (SEQ ID NO: 9 WO2017058892) in any combination.

[00113] In some embodiments, the AAV may include a sequence of amino acids at positions 155, 156 and 157 of VP1 or at positions 17, 18, 19 and 20 of VP2, as described in International Publication No. WO 2017066764, the contents of which are herein incorporated by reference in their entirety. The sequences of amino acid may be, but not limited to, N-S-S, S-X-S, S-S-Y, N-X-S, N-S-Y, S-X-Y and N-X-Y, where N, X and Y are, but not limited to, independently non-serine, or non-threonine amino acids, wherein the AAV may be, but not limited to AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12. In some embodiments, the AAV may include a deletion of at least one amino acid at positions 156, 157 or 158 of VP1 or at positions 19, 20 or 21 of VP2, wherein the AAV may be, but not limited to AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.

[00114] In one embodiment, peptides for inclusion in an AAV serotype may be identified using the methods described by Hui et al. (Molecular Therapy – Methods & Clinical Development (2015) 2, 15029 doi:10.1038/mtm.2015.29; the contents of which are herein incorporated by reference in its entirety). As a non-limiting example, the procedure includes isolating human splenocytes, restimulating the splenocytes *in vitro* using individual peptides spanning the amino acid sequence of the AAV capsid protein, IFN-gamma ELISpot with the individual peptides used for the *in vitro* restimulation, bioinformatics analysis to determine the HLA restriction of 15-mers identified by IFN-gamma ELISpot, identification of candidate

reactive 9-mer epitopes for a given HLA allele, synthesis candidate 9-mers, second IFN-gamma ELISpot screening of splenocytes from subjects carrying the HLA alleles to which identified AAV epitopes are predicted to bind, determine the AAV capsid-reactive CD8+ T cell epitopes and determine the frequency of subjects reacting to a given AAV epitope.

[00115] In one embodiment, the AAV may be a serotype generated by Cre-recombination-based AAV targeted evolution (CREATE) as described by Deverman et al., (Nature Biotechnology 34(2):204-209 (2016)), the contents of which are herein incorporated by reference in their entirety. In one embodiment, AAV serotypes generated in this manner have improved CNS transduction and/or neuronal and astrocytic tropism, as compared to other AAV serotypes. As non-limiting examples, the AAV serotype may be PHP.B, PHP.B2, PHP.B3, PHP.A, G2A12, G2A15. In one embodiment, these AAV serotypes may be AAV9 (SEQ ID NO: 126 and 127) derivatives with a 7-amino acid insert between amino acids 588-589. Non-limiting examples of these 7-amino acid inserts include TLAVPFK (SEQ ID NO: 873), SVSKPFL (SEQ ID NO: 1249), FTLTTPK (SEQ ID NO: 882), YTLSQGW (SEQ ID NO: 888), QAVRTSL (SEQ ID NO: 914) and/or LAKERLS (SEQ ID NO: 915).

[00116] In one embodiment, the AAV serotype may be as described in Jackson et al (Frontiers in Molecular Neuroscience 9:154 (2016)), the contents of which are herein incorporated by reference in their entirety. In some embodiments, the AAV serotype is PHP.B or AAV9. In some embodiments, the AAV serotype is paired with a synapsin promoter to enhance neuronal transduction, as compared to when more ubiquitous promoters are used (i.e., CBA or CMV).

[00117] In one embodiment, peptides for inclusion in an AAV serotype may be identified by isolating human splenocytes, restimulating the splenocytes *in vitro* using individual peptides spanning the amino acid sequence of the AAV capsid protein, IFN-gamma ELISpot with the individual peptides used for the *in vitro* restimulation, bioinformatics analysis to determine the given allele restriction of 15-mers identified by IFN-gamma ELISpot, identification of candidate reactive 9-mer epitopes for a given allele, synthesis candidate 9-mers, second IFN-gamma ELISpot screening of splenocytes from subjects carrying the specific alleles to which identified AAV epitopes are predicted to bind, determine the AAV capsid-reactive CD8+ T cell epitopes and determine the frequency of subjects reacting to a given AAV epitope.

[00118] AAV particles comprising a modulatory polynucleotide encoding the siRNA molecules may be prepared or derived from various serotypes of AAVs, including, but not limited to, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ8 and AAV-DJ. In some

cases, different serotypes of AAVs may be mixed together or with other types of viruses to produce chimeric AAV particles. As a non-limiting example, the AAV particle is derived from the AAV9 serotype.

Viral Genome

[00119] In one embodiment, as shown in an AAV particle comprises a viral genome with a payload region.

[00120] In one embodiment, the viral genome may comprise the components as shown in FIG. 1. The payload region **110** is located within the viral genome **100**. At the 5' and/or the 3' end of the viral genome **100** there may be at least one inverted terminal repeat (ITR) **120**. Between the 5' ITR **120** and the payload region **110**, there may be a promoter region **130**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00121] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 2. The payload region **110** is located within the viral genome **100**. At the 5' and/or the 3' end of the viral genome **100** there may be at least one inverted terminal repeat (ITR) **120**. Between the 5' ITR **120** and the payload region **110**, there may be a promoter region **130**. Between the promoter region **130** and the payload region **110**, there may be an intron region **140**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00122] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 3. At the 5' and/or the 3' end of the viral genome **100** there may be at least one inverted terminal repeat (ITR) **120**. Within the viral genome **100**, there may be an enhancer region **150**, a promoter region **130**, an intron region **140**, and a payload region **110**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00123] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 4. At the 5' and/or the 3' end of the viral genome **100** there may be at least one inverted terminal repeat (ITR) **120**. Within the viral genome **100**, there may be an enhancer region **150**, a promoter region **130**, an intron region **140**, a payload region **110**, and a polyadenylation signal sequence region **160**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00124] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 5. At the 5' and/or the 3' end of the viral genome **100** there may be at least one inverted terminal repeat (ITR) **120**. Within the viral genome **100**, there may be at least one MCS region **170**, an enhancer region **150**, a promoter region **130**, an intron region **140**, a payload region **110**,

and a polyadenylation signal sequence region **160**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00125] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 6. At the 5' and/or the 3' end of the viral genome **100** there may be at least one inverted terminal repeat (ITR) **120**. Within the viral genome **100**, there may be at least one MCS region **170**, an enhancer region **150**, a promoter region **130**, at least one exon region **180**, at least one intron region **140**, a payload region **110**, and a polyadenylation signal sequence region **160**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00126] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 7 and 8. Within the viral genome **100**, there may be at least one promoter region **130**, and a payload region **110**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

[00127] In one embodiment, the viral genome **100** may comprise the components as shown in FIG. 9. Within the viral genome **100**, there may be at least one promoter region **130**, a payload region **110**, and a polyadenylation signal sequence region **160**. In one embodiment, the payload region may comprise at least one modulatory polynucleotide.

Viral Genome Size

[00128] In one embodiment, the viral genome which comprises a payload described herein, may be single stranded or double stranded viral genome. The size of the viral genome may be small, medium, large or the maximum size. Additionally, the viral genome may comprise a promoter and a polyA tail.

[00129] In one embodiment, the viral genome which comprises a payload described herein, may be a small single stranded viral genome. A small single stranded viral genome may be 2.7 to 3.5 kb in size such as about 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, and 3.5 kb in size. As a non-limiting example, the small single stranded viral genome may be 3.2 kb in size. Additionally, the viral genome may comprise a promoter and a polyA tail.

[00130] In one embodiment, the viral genome which comprises a payload described herein, may be a small double stranded viral genome. A small double stranded viral genome may be 1.3 to 1.7 kb in size such as about 1.3, 1.4, 1.5, 1.6, and 1.7 kb in size. As a non-limiting example, the small double stranded viral genome may be 1.6 kb in size. Additionally, the viral genome may comprise a promoter and a polyA tail.

[00131] In one embodiment, the viral genome which comprises a payload described herein, may a medium single stranded viral genome. A medium single stranded viral genome may be 3.6

to 4.3 kb in size such as about 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2 and 4.3 kb in size. As a non-limiting example, the medium single stranded viral genome may be 4.0 kb in size. Additionally, the viral genome may comprise a promoter and a polyA tail.

[00132] In one embodiment, the viral genome which comprises a payload described herein, may be a medium double stranded viral genome. A medium double stranded viral genome may be 1.8 to 2.1 kb in size such as about 1.8, 1.9, 2.0, and 2.1 kb in size. As a non-limiting example, the medium double stranded viral genome may be 2.0 kb in size. Additionally, the viral genome may comprise a promoter and a polyA tail.

[00133] In one embodiment, the viral genome which comprises a payload described herein, may be a large single stranded viral genome. A large single stranded viral genome may be 4.4 to 6.0 kb in size such as about 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 and 6.0 kb in size. As a non-limiting example, the large single stranded viral genome may be 4.7 kb in size. As another non-limiting example, the large single stranded viral genome may be 4.8 kb in size. As yet another non-limiting example, the large single stranded viral genome may be 6.0 kb in size. Additionally, the viral genome may comprise a promoter and a polyA tail.

[00134] In one embodiment, the viral genome which comprises a payload described herein, may be a large double stranded viral genome. A large double stranded viral genome may be 2.2 to 3.0 kb in size such as about 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 3.0 kb in size. As a non-limiting example, the large double stranded viral genome may be 2.4 kb in size. Additionally, the viral genome may comprise a promoter and a polyA tail.

Viral Genome Component: Inverted Terminal Repeats (ITRs)

[00135] The AAV particles of the present invention comprise a viral genome with at least one ITR region and a payload region. In one embodiment the viral genome has two ITRs. These two ITRs flank the payload region at the 5' and 3' ends. The ITRs function as origins of replication comprising recognition sites for replication. ITRs comprise sequence regions which can be complementary and symmetrically arranged. ITRs incorporated into viral genomes of the invention may be comprised of naturally occurring polynucleotide sequences or recombinantly derived polynucleotide sequences.

[00136] The ITRs may be derived from the same serotype as the capsid, selected from any of the serotypes listed in Table 1, or a derivative thereof. The ITR may be of a different serotype from the capsid. In one embodiment the AAV particle has more than one ITR. In a non-limiting example, the AAV particle has a viral genome comprising two ITRs. In one embodiment the ITRs are of the same serotype as one another. In another embodiment the ITRs are of different

serotypes. Non-limiting examples include zero, one or both of the ITRs having the same serotype as the capsid. In one embodiment both ITRs of the viral genome of the AAV particle are AAV2 ITRs.

[00137] Independently, each ITR may be about 100 to about 150 nucleotides in length. An ITR may be about 100-105 nucleotides in length, 106-110 nucleotides in length, 111-115 nucleotides in length, 116-120 nucleotides in length, 121-125 nucleotides in length, 126-130 nucleotides in length, 131-135 nucleotides in length, 136-140 nucleotides in length, 141-145 nucleotides in length or 146-150 nucleotides in length. In one embodiment the ITRs are 140-142 nucleotides in length. Non limiting examples of ITR length are 102, 140, 141, 142, 145 nucleotides in length, and those having at least 95% identity thereto.

[00138] In one embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule which may be located near the 5' end of the flip ITR in an expression vector. In another embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located near the 3' end of the flip ITR in an expression vector. In yet another embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located near the 5' end of the flop ITR in an expression vector. In yet another embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located near the 3' end of the flop ITR in an expression vector. In one embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located between the 5' end of the flip ITR and the 3' end of the flop ITR in an expression vector. In one embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located between (e.g., half-way between the 5' end of the flip ITR and 3' end of the flop ITR or the 3' end of the flop ITR and the 5' end of the flip ITR), the 3' end of the flip ITR and the 5' end of the flip ITR in an expression vector. As a non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more than 30 nucleotides downstream from the 5' or 3' end of an ITR (e.g., Flip or Flop ITR) in an expression vector. As a non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more than 30 nucleotides upstream from the 5' or 3' end of an ITR (e.g., Flip or Flop ITR) in an expression vector. As another non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 5-10, 5-15, 5-20, 5-25,

5-30, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 20-25, 20-30 or 25-30 nucleotides downstream from the 5' or 3' end of an ITR (e.g., Flip or Flop ITR) in an expression vector. As another non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 5-10, 5-15, 5-20, 5-25, 5-30, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 20-25, 20-30 or 25-30 upstream from the 5' or 3' end of an ITR (e.g., Flip or Flop ITR) in an expression vector. As a non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within the first 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25% or more than 25% of the nucleotides upstream from the 5' or 3' end of an ITR (e.g., Flip or Flop ITR) in an expression vector. As another non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located with the first 1-5%, 1-10%, 1-15%, 1-20%, 1-25%, 5-10%, 5-15%, 5-20%, 5-25%, 10-15%, 10-20%, 10-25%, 15-20%, 15-25%, or 20-25% downstream from the 5' or 3' end of an ITR (e.g., Flip or Flop ITR) in an expression vector.

Viral Genome Component: Promoters

[00139] In one embodiment, the payload region of the viral genome comprises at least one element to enhance the transgene target specificity and expression (See e.g., Powell et al. Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy, 2015; the contents of which are herein incorporated by reference in its entirety). Non-limiting examples of elements to enhance the transgene target specificity and expression include promoters, endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (PolyA) signal sequences and upstream enhancers (USEs), CMV enhancers and introns.

[00140] A person skilled in the art may recognize that expression of the polypeptides of the invention in a target cell may require a specific promoter, including but not limited to, a promoter that is species specific, inducible, tissue-specific, or cell cycle-specific (Parr et al., *Nat. Med.*3:1145-9 (1997); the contents of which are herein incorporated by reference in their entirety).

[00141] In one embodiment, the promoter is deemed to be efficient when it drives expression of the polypeptide(s) encoded in the payload region of the viral genome of the AAV particle.

[00142] In one embodiment, the promoter is a promoter deemed to be efficient to drive the expression of the modulatory polynucleotide.

[00143] In one embodiment, the promoter is a promoter deemed to be efficient when it drives expression in the cell being targeted.

[00144] In one embodiment, the promoter drives expression of the payload for a period of time in targeted tissues. Expression driven by a promoter may be for a period of 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 3 weeks, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or more than 10 years. Expression may be for 1-5 hours, 1-12 hours, 1-2 days, 1-5 days, 1-2 weeks, 1-3 weeks, 1-4 weeks, 1-2 months, 1-4 months, 1-6 months, 2-6 months, 3-6 months, 3-9 months, 4-8 months, 6-12 months, 1-2 years, 1-5 years, 2-5 years, 3-6 years, 3-8 years, 4-8 years or 5-10 years.

[00145] In one embodiment, the promoter drives expression of the payload for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 21 years, 22 years, 23 years, 24 years, 25 years, 26 years, 27 years, 28 years, 29 years, 30 years, 31 years, 32 years, 33 years, 34 years, 35 years, 36 years, 37 years, 38 years, 39 years, 40 years, 41 years, 42 years, 43 years, 44 years, 45 years, 46 years, 47 years, 48 years, 49 years, 50 years, 55 years, 60 years, 65 years, or more than 65 years.

[00146] Promoters may be naturally occurring or non-naturally occurring. Non-limiting examples of promoters include viral promoters, plant promoters and mammalian promoters. In some embodiments, the promoters may be human promoters. In some embodiments, the promoter may be truncated.

[00147] Promoters which drive or promote expression in most tissues include, but are not limited to, human elongation factor 1α-subunit (EF1α), cytomegalovirus (CMV) immediate-early enhancer and/or promoter, chicken β-actin (CBA) and its derivative CAG, β glucuronidase (GUSB), or ubiquitin C (UBC). Tissue-specific expression elements can be used to restrict expression to certain cell types such as, but not limited to, muscle specific promoters, B cell

promoters, monocyte promoters, leukocyte promoters, macrophage promoters, pancreatic acinar cell promoters, endothelial cell promoters, lung tissue promoters, astrocyte promoters, or nervous system promoters which can be used to restrict expression to neurons, astrocytes, or oligodendrocytes.

[00148] Non-limiting examples of muscle-specific promoters include mammalian muscle creatine kinase (MCK) promoter, mammalian desmin (DES) promoter, mammalian troponin I (TNNI2) promoter, and mammalian skeletal alpha-actin (ASKA) promoter (see, e.g. U.S. Patent Publication US 20110212529, the contents of which are herein incorporated by reference in their entirety)

[00149] Non-limiting examples of tissue-specific expression elements for neurons include neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF-β), synapsin (Syn), methyl-CpG binding protein 2 (MeCP2), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light (NFL) or heavy (NFH), β-globin minigene nβ2, preproenkephalin (PPE), enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2) promoters. Non-limiting examples of tissue-specific expression elements for astrocytes include glial fibrillary acidic protein (GFAP) and EAAT2 promoters. A non-limiting example of a tissue-specific expression element for oligodendrocytes includes the myelin basic protein (MBP) promoter.

[00150] In one embodiment, the promoter may be less than 1 kb. The promoter may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800 nucleotides. The promoter may have a length between 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800.

[00151] In one embodiment, the promoter may be a combination of two or more components of the same or different starting or parental promoters such as, but not limited to, CMV and CBA. Each component may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800. Each component may have a length between 200-300, 200-400,

200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800. In one embodiment, the promoter is a combination of a 382 nucleotide CMV-enhancer sequence and a 260 nucleotide CBA-promoter sequence.

[00152] In one embodiment, the viral genome comprises a ubiquitous promoter. Non-limiting examples of ubiquitous promoters include CMV, CBA (including derivatives CAG, CBh, etc.), EF-1α, PGK, UBC, GUSB (hGBp), and UCOE (promoter of HNRPA2B1-CBX3).

[00153] Yu et al. (Molecular Pain 2011, 7:63; the contents of which are herein incorporated by reference in their entirety) evaluated the expression of eGFP under the CAG, EFIα, PGK and UBC promoters in rat DRG cells and primary DRG cells using lentiviral vectors and found that UBC showed weaker expression than the other 3 promoters and only 10-12% glial expression was seen for all promoters. Soderblom et al. (E. Neuro 2015; the contents of which are herein incorporated by reference in its entirety) evaluated the expression of eGFP in AAV8 with CMV and UBC promoters and AAV2 with the CMV promoter after injection in the motor cortex. Intranasal administration of a plasmid containing a UBC or EFIα promoter showed a sustained airway expression greater than the expression with the CMV promoter (See e.g., Gill et al., Gene Therapy 2001, Vol. 8, 1539-1546; the contents of which are herein incorporated by reference in their entirety). Husain et al. (Gene Therapy 2009; the contents of which are herein incorporated by reference in its entirety) evaluated an HβH construct with a hGUSB promoter, a HSV-1LAT promoter and an NSE promoter and found that the HβH construct showed weaker expression than NSE in mouse brain. Passini and Wolfe (J. Virol. 2001, 12382-12392, the contents of which are herein incorporated by reference in its entirety) evaluated the long term effects of the HβH vector following an intraventricular injection in neonatal mice and found that there was sustained expression for at least 1 year. Low expression in all brain regions was found by Xu et al. (Gene Therapy 2001, 8, 1323-1332; the contents of which are herein incorporated by reference in their entirety) when NFL and NFH promoters were used as compared to the CMV-lacZ, CMV-luc, EF, GFAP, hENK, nAChR, PPE, PPE + wpre, NSE (0.3 kb), NSE (1.8 kb) and NSE (1.8 kb + wpre). Xu et al. found that the promoter activity in descending order was NSE (1.8 kb), EF, NSE (0.3 kb), GFAP, CMV, hENK, PPE, NFL and NFH. NFL is a 650 nucleotide promoter and NFH is a 920 nucleotide promoter which are both absent in the liver but NFH is abundant in the sensory proprioceptive neurons, brain and spinal cord and NFH is present in the heart. Scn8a is a 470 nucleotide promoter which expresses throughout the DRG, spinal cord and brain with particularly high expression seen in the hippocampal neurons and cerebellar Purkinje

cells, cortex, thalamus and hypothalamus (See e.g., Drews et al. *Identification of evolutionary conserved, functional noncoding elements in the promoter region of the sodium channel gene SCN8A,* Mamm Genome (2007) 18:723-731; and Raymond et al. *Expression of Alternatively Spliced Sodium Channel α-subunit genes,* Journal of Biological Chemistry (2004) 279(44) 46234-46241; the contents of each of which are herein incorporated by reference in their entireties).

[00154] Any of promoters taught by the aforementioned Yu, Soderblom, Gill, Husain, Passini, Xu, Drews or Raymond may be used in the present inventions.

[00155] In one embodiment, the promoter is not cell specific.

[00156] In one embodiment, the promoter is an ubiquitin c (UBC) promoter. The UBC promoter may have a size of 300-350 nucleotides. As a non-limiting example, the UBC promoter is 332 nucleotides.

[00157] In one embodiment, the promoter is a β-glucuronidase (GUSB) promoter. The GUSB promoter may have a size of 350-400 nucleotides. As a non-limiting example, the GUSB promoter is 378 nucleotides.

[00158] In one embodiment, the promoter is a neurofilament light (NFL) promoter. The NFL promoter may have a size of 600-700 nucleotides. As a non-limiting example, the NFL promoter is 650 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-modulatory polynucleotide-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype.

[00159] In one embodiment, the promoter is a neurofilament heavy (NFH) promoter. The NFH promoter may have a size of 900-950 nucleotides. As a non-limiting example, the NFH promoter is 920 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-modulatory polynucleotide-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype.

[00160] In one embodiment, the promoter is a scn8a promoter. The scn8a promoter may have a size of 450-500 nucleotides. As a non-limiting example, the scn8a promoter is 470 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-modulatory polynucleotide-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype

[00161] In one embodiment, the viral genome comprises a Pol III promoter.

[00162] In one embodiment, the viral genome comprises a P1 promoter.

[00163] In one embodiment, the viral genome comprises a FXN promoter.

[00164] In one embodiment, the promoter is a phosphoglycerate kinase 1 (PGK) promoter.

[00165] In one embodiment, the promoter is a chicken β-actin (CBA) promoter.

[00166] In one embodiment, the promoter is a CAG promoter which is a construct comprising the cytomegalovirus (CMV) enhancer fused to the chicken beta-actin (CBA) promoter.

[00167] In one embodiment, the promoter is a cytomegalovirus (CMV) promoter.

[00168] In one embodiment, the viral genome comprises a H1 promoter.

[00169] In one embodiment, the viral genome comprises a U6 promoter.

[00170] In one embodiment, the promoter is a liver or a skeletal muscle promoter. Non-limiting examples of liver promoters include human α-1-antitrypsin (hAAT) and thyroxine binding globulin (TBG). Non-limiting examples of skeletal muscle promoters include Desmin, MCK or synthetic C5-12.

[00171] In one embodiment, the promoter is a RNA pol III promoter. As a non-limiting example, the RNA pol III promoter is U6. As a non-limiting example, the RNA pol III promoter is H1.

[00172] In one embodiment, the viral genome comprises two promoters. As a non-limiting example, the promoters are an EF1α promoter and a CMV promoter.

[00173] In one embodiment, the viral genome comprises an enhancer element, a promoter and/or a 5'UTR intron. The enhancer element, also referred to herein as an "enhancer," may be, but is not limited to, a CMV enhancer, the promoter may be, but is not limited to, a CMV, CBA, UBC, GUSB, NSE, Synapsin, MeCP2, and GFAP promoter and the 5'UTR/intron may be, but is not limited to, SV40, and CBA-MVM. As a non-limiting example, the enhancer, promoter and/or intron used in combination may be: (1) CMV enhancer, CMV promoter, SV40 5'UTR intron; (2) CMV enhancer, CBA promoter, SV 40 5'UTR intron; (3) CMV enhancer, CBA promoter, CBA-MVM 5'UTR intron; (4) UBC promoter; (5) GUSB promoter; (6) NSE promoter; (7) Synapsin promoter; (8) MeCP2 promoter, (9) GFAP promoter, (10) H1 promoter; and (11) U6 promoter.

[00174] In one embodiment, the viral genome comprises an engineered promoter.

[00175] In another embodiment the viral genome comprises a promoter from a naturally expressed protein.

Viral Genome Component: Untranslated Regions (UTRs)

[00176] By definition, wild type untranslated regions (UTRs) of a gene are transcribed but not translated. Generally, the 5' UTR starts at the transcription start site and ends at the start codon

and the 3' UTR starts immediately following the stop codon and continues until the termination signal for transcription.

[00177] Features typically found in abundantly expressed genes of specific target organs may be engineered into UTRs to enhance the stability and protein production. As a non-limiting example, a 5' UTR from mRNA normally expressed in the liver (e.g., albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII) may be used in the viral genomes of the AAV particles of the invention to enhance expression in hepatic cell lines or liver.

[00178] While not wishing to be bound by theory, wild-type 5′ untranslated regions (UTRs) include features which play roles in translation initiation. Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes, are usually included in 5' UTRs. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (ATG), which is followed by another 'G'.

[00179] In one embodiment, the 5'UTR in the viral genome includes a Kozak sequence.

[00180] In one embodiment, the 5'UTR in the viral genome does not include a Kozak sequence.

[00181] While not wishing to be bound by theory, wild-type 3′ UTRs are known to have stretches of Adenosines and Uridines embedded therein. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995, the contents of which are herein incorporated by reference in its entirety): Class I AREs, such as, but not limited to, c-Myc and MyoD, contain several dispersed copies of an AUUUA motif within U-rich regions. Class II AREs, such as, but not limited to, GM-CSF and TNF-a, possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Class III ARES, such as, but not limited to, c-Jun and Myogenin, are less well defined. These U rich regions do not contain an AUUUA motif. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′ UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

[00182] Introduction, removal or modification of 3′ UTR AU rich elements (AREs) can be used to modulate the stability of polynucleotides. When engineering specific polynucleotides,

e.g., payload regions of viral genomes, one or more copies of an ARE can be introduced to make polynucleotides less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

[00183] In one embodiment, the 3' UTR of the viral genome may include an oligo(dT) sequence for templated addition of a poly-A tail.

[00184] In one embodiment, the viral genome may include at least one miRNA seed, binding site or full sequence. microRNAs (or miRNA or miR) are 19-25 nucleotide noncoding RNAs that bind to the sites of nucleic acid targets and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. A microRNA sequence comprises a "seed" region, i.e., a sequence in the region of positions 2-8 of the mature microRNA, which sequence has perfect Watson-Crick complementarity to the miRNA target sequence of the nucleic acid.

[00185] In one embodiment, the viral genome may be engineered to include, alter or remove at least one miRNA binding site, sequence or seed region.

[00186] Any UTR from any gene known in the art may be incorporated into the viral genome of the AAV particle. These UTRs, or portions thereof, may be placed in the same orientation as in the gene from which they were selected or they may be altered in orientation or location. In one embodiment, the UTR used in the viral genome of the AAV particle may be inverted, shortened, lengthened, made with one or more other 5′ UTRs or 3′ UTRs known in the art. As used herein, the term "altered" as it relates to a UTR, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3′ or 5′ UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides.

[00187] In one embodiment, the viral genome of the AAV particle comprises at least one artificial UTRs which is not a variant of a wild type UTR.

[00188] In one embodiment, the viral genome of the AAV particle comprises UTRs which have been selected from a family of transcripts whose proteins share a common function, structure, feature or property.

Viral Genome Component: Polyadenylation Sequence

[00189] In one embodiment, the viral genome of the AAV particles of the present invention comprise at least one polyadenylation sequence. The viral genome of the AAV particle may

comprise a polyadenylation sequence between the 3' end of the payload coding sequence and the 5' end of the 3'ITR.

[00190] In one embodiment, the polyadenylation sequence or "polyA sequence" may range from absent to about 500 nucleotides in length. The polyadenylation sequence may be, but is not limited to, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and 500 nucleotides in length.

[00191] In one embodiment, the polyadenylation sequence is 50-100 nucleotides in length.

[00192] In one embodiment, the polyadenylation sequence is 50-150 nucleotides in length.

[00193] In one embodiment, the polyadenylation sequence is 50-160 nucleotides in length.

[00194] In one embodiment, the polyadenylation sequence is 50-200 nucleotides in length.

[00195] In one embodiment, the polyadenylation sequence is 60-100 nucleotides in length.

[00196] In one embodiment, the polyadenylation sequence is 60-150 nucleotides in length.

[00197] In one embodiment, the polyadenylation sequence is 60-160 nucleotides in length.

[00198] In one embodiment, the polyadenylation sequence is 60-200 nucleotides in length.

[00199] In one embodiment, the polyadenylation sequence is 70-100 nucleotides in length.

[00200] In one embodiment, the polyadenylation sequence is 70-150 nucleotides in length.

[00201] In one embodiment, the polyadenylation sequence is 70-160 nucleotides in length.

[00202] In one embodiment, the polyadenylation sequence is 70-200 nucleotides in length.

[00203] In one embodiment, the polyadenylation sequence is 80-100 nucleotides in length.

[00204] In one embodiment, the polyadenylation sequence is 80-150 nucleotides in length.

[00205] In one embodiment, the polyadenylation sequence is 80-160 nucleotides in length.

[00206] In one embodiment, the polyadenylation sequence is 80-200 nucleotides in length.

[00207] In one embodiment, the polyadenylation sequence is 90-100 nucleotides in length.

[00208] In one embodiment, the polyadenylation sequence is 90-150 nucleotides in length.

[00209] In one embodiment, the polyadenylation sequence is 90-160 nucleotides in length.

[00210] In one embodiment, the polyadenylation sequence is 90-200 nucleotides in length.

[00211] In one embodiment, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located upstream of the polyadenylation sequence in an expression vector. Further, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located downstream of a promoter such as, but not limited to, CMV, U6, CAG, CBA or a CBA promoter with a SV40 intron or a human betaglobin intron in an expression vector. As a non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more than 30 nucleotides downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector. As another non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 5-10, 5-15, 5-20, 5-25, 5-30, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 20-25, 20-30 or 25-30 nucleotides downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector. As a non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located within the first 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25% or more than 25% of the nucleotides downstream from

the promoter and/or upstream of the polyadenylation sequence in an expression vector. As another non-limiting example, the AAV particle comprises a nucleic acid sequence encoding an siRNA molecule may be located with the first 1-5%, 1-10%, 1-15%, 1-20%, 1-25%, 5-10%, 5-15%, 5-20%, 5-25%, 10-15%, 10-20%, 10-25%, 15-20%, 15-25%, or 20-25% downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector.

[00212] In one embodiment, the AAV particle comprises a rabbit globin polyadenylation (polyA) signal sequence.

[00213] In one embodiment, the AAV particle comprises a human growth hormone polyadenylation (polyA) signal sequence.

Viral Genome Component: Introns

[00214] In one embodiment, the payload region comprises at least one element to enhance the expression such as one or more introns or portions thereof. Non-limiting examples of introns include, MVM (67-97 bps), F.IX truncated intron 1 (300 bps), β-globin SD/immunoglobulin heavy chain splice acceptor (250 bps), adenovirus splice donor/immunoglobin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps) and hybrid adenovirus splice donor/IgG splice acceptor (230 bps).

[00215] In one embodiment, the intron or intron portion may be 100-500 nucleotides in length. The intron may have a length of 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490 or 500. The intron may have a length between 80-100, 80-120, 80-140, 80-160, 80-180, 80-200, 80-250, 80-300, 80-350, 80-400, 80-450, 80-500, 200-300, 200-400, 200-500, 300-400, 300-500, or 400-500.

[00216] In one embodiment, the AAV viral genome may comprise a promoter such as, but not limited to, CMV or U6. As a non-limiting example, the promoter for the AAV comprising the nucleic acid sequence for the siRNA molecules of the present invention is a CMV promoter. As another non-limiting example, the promoter for the AAV comprising the nucleic acid sequence for the siRNA molecules of the present invention is a U6 promoter.

[00217] In one embodiment, the AAV viral genome may comprise a CMV promoter.

[00218] In one embodiment, the AAV viral genome may comprise a U6 promoter.

[00219] In one embodiment, the AAV viral genome may comprise a CMV and a U6 promoter.

[00220] In one embodiment, the AAV viral genome may comprise a H1 promoter.

[00221] In one embodiment, the AAV viral genome may comprise a CBA promoter.

[00222] In one embodiment, the encoded siRNA molecule may be located downstream of a promoter in an expression vector such as, but not limited to, CMV, U6, H1, CBA, CAG, or a CBA promoter with an intron such as SV40 or others known in the art. Further, the encoded siRNA molecule may also be located upstream of the polyadenylation sequence in an expression vector. As a non-limiting example, the encoded siRNA molecule may be located within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more than 30 nucleotides downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector. As another non-limiting example, the encoded siRNA molecule may be located within 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 5-10, 5-15, 5-20, 5-25, 5-30, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 20-25, 20-30 or 25-30 nucleotides downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector. As a non-limiting example, the encoded siRNA molecule may be located within the first 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25% or more than 25% of the nucleotides downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector. As another non-limiting example, the encoded siRNA molecule may be located with the first 1-5%, 1-10%, 1-15%, 1-20%, 1-25%, 5-10%, 5-15%, 5-20%, 5-25%, 10-15%, 10-20%, 10-25%, 15-20%, 15-25%, or 20-25% downstream from the promoter and/or upstream of the polyadenylation sequence in an expression vector.

Viral Genome Component: Filler Sequence

[00223] In one embodiment, the viral genome comprises one or more filler sequences.

[00224] In one embodiment, the viral genome comprises one or more filler sequences in order to have the length of the viral genome be the optimal size for packaging. As a non-limiting example, the viral genome comprises at least one filler sequence in order to have the length of the viral genome be about 2.3 kb. As a non-limiting example, the viral genome comprises at least one filler sequence in order to have the length of the viral genome be about 4.6 kb.

[00225] In one embodiment, the viral genome comprises one or more filler sequences in order to reduce the likelihood that a hairpin structure of the vector genome (e.g., a modulatory polynucleotide described herein) may be read as an inverted terminal repeat (ITR) during expression and/or packaging. As a non-limiting example, the viral genome comprises at least one filler sequence in order to have the length of the viral genome be about 2.3 kb. As a non-

limiting example, the viral genome comprises at least one filler sequence in order to have the length of the viral genome be about 4.6 kb

[00226] In one embodiment, the viral genome is a single stranded (ss) viral genome and comprises one or more filler sequences which have a length about between 0.1 kb - 3.8 kb, such as, but not limited to, 0.1 kb, 0.2 kb, 0.3 kb, 0.4 kb, 0.5 kb, 0.6 kb, 0.7 kb, 0.8 kb, 0.9 kb, 1 kb, 1.1 kb, 1.2 kb, 1.3 kb, 1.4 kb, 1.5 kb, 1.6 kb, 1.7 kb, 1.8 kb, 1.9 kb, 2 kb, 2.1 kb, 2.2 kb, 2.3 kb, 2.4 kb, 2.5 kb, 2.6 kb, 2.7 kb, 2.8 kb, 2.9 kb, 3 kb, 3.1 kb, 3.2 kb, 3.3 kb, 3.4 kb, 3.5 kb, 3.6 kb, 3.7 kb, or 3.8 kb. As a non-limiting example, the total length filler sequence in the vector genome is 3.1 kb. As a non-limiting example, the total length filler sequence in the vector genome is 2.7 kb. As a non-limiting example, the total length filler sequence in the vector genome is 0.8 kb. As a non-limiting example, the total length filler sequence in the vector genome is 0.4 kb. As a non-limiting example, the length of each filler sequence in the vector genome is 0.8 kb. As a non-limiting example, the length of each filler sequence in the vector genome is 0.4 kb.

[00227] In one embodiment, the viral genome is a self-complementary (sc) viral genome and comprises one or more filler sequences which have a length about between 0.1 kb – 1.5 kb, such as, but not limited to, 0.1 kb, 0.2 kb, 0.3 kb, 0.4 kb, 0.5 kb, 0.6 kb, 0.7 kb, 0.8 kb, 0.9 kb, 1 kb, 1.1 kb, 1.2 kb, 1.3 kb, 1.4 kb, or 1.5 kb. As a non-limiting example, the total length filler sequence in the vector genome is 0.8 kb. As a non-limiting example, the total length filler sequence in the vector genome is 0.4 kb. As a non-limiting example, the length of each filler sequence in the vector genome is 0.8 kb. As a non-limiting example, the length of each filler sequence in the vector genome is 0.4 kb

[00228] In one embodiment, the viral genome comprises any portion of a filler sequence. The viral genome may comprise 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of a filler sequence.

[00229] In one embodiment, the viral genome is a single stranded (ss) viral genome and comprises one or more filler sequences in order to have the length of the viral genome be about 4.6 kb. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the 5' ITR sequence. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to a promoter sequence. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the polyadenylation signal sequence. As a non-limiting

example, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to the 3' ITR sequence. As a non-limiting example, the viral genome comprises at least one filler sequence, and the filler sequence is located between two intron sequences. As a non-limiting example, the viral genome comprises at least one filler sequence, and the filler sequence is located within an intron sequence. As a non-limiting example, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. As a non-limiting example, the viral genome comprises two filler sequences, and the first filler sequence is located 5' to a promoter sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. As a non-limiting example, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 5' to the 5' ITR sequence.

[00230] In one embodiment, the viral genome is a self-complementary (sc) viral genome and comprises one or more filler sequences in order to have the length of the viral genome be about 2.3 kb. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the 5' ITR sequence. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to a promoter sequence. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the polyadenylation signal sequence. As a non-limiting example, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to the 3' ITR sequence. As a non-limiting example, the viral genome comprises at least one filler sequence, and the filler sequence is located between two intron sequences. As a non-limiting example, the viral genome comprises at least one filler sequence, and the filler sequence is located within an intron sequence. As a non-limiting example, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. As a non-limiting example, the viral genome comprises two filler sequences, and the first filler sequence is located 5' to a promoter sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. As a non-limiting example, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 5' to the 5' ITR sequence.

[00231] In one embodiment, the viral genome may comprise one or more filler sequences between one of more regions of the viral genome. In one embodiment, the filler region may be

located before a region such as, but not limited to, a payload region, an inverted terminal repeat (ITR), a promoter region, an intron region, an enhancer region, a polyadenylation signal sequence region, a multiple cloning site (MCS) region, and/or an exon region. In one embodiment, the filler region may be located after a region such as, but not limited to, a payload region, an inverted terminal repeat (ITR), a promoter region, an intron region, an enhancer region, a polyadenylation signal sequence region, a multiple cloning site (MCS) region, and/or an exon region. In one embodiment, the filler region may be located before and after a region such as, but not limited to, a payload region, an inverted terminal repeat (ITR), a promoter region, an intron region, an enhancer region, a polyadenylation signal sequence region, a multiple cloning site (MCS) region, and/or an exon region.

[00232] In one embodiment, the viral genome may comprise one or more filler sequences which bifurcates at least one region of the viral genome. The bifurcated region of the viral genome may comprise 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the of the region to the 5' of the filler sequence region. As a non-limiting example, the filler sequence may bifurcate at least one region so that 10% of the region is located 5' to the filler sequence and 90% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 20% of the region is located 5' to the filler sequence and 80% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 30% of the region is located 5' to the filler sequence and 70% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 40% of the region is located 5' to the filler sequence and 60% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 50% of the region is located 5' to the filler sequence and 50% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 60% of the region is located 5' to the filler sequence and 40% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 70% of the region is located 5' to the filler sequence and 30% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region so that 80% of the region is located 5' to the filler sequence and 20% of the region is located 3' to the filler sequence. As a non-limiting example, the filler sequence may bifurcate at least one region

so that 90% of the region is located 5' to the filler sequence and 10% of the region is located 3' to the filler sequence.

[00233] In one embodiment, the viral genome comprises a filler sequence after the 5' ITR.

[00234] In one embodiment, the viral genome comprises a filler sequence after the promoter region. In one embodiment, the viral genome comprises a filler sequence after the payload region. In one embodiment, the viral genome comprises a filler sequence after the intron region. In one embodiment, the viral genome comprises a filler sequence after the enhancer region. In one embodiment, the viral genome comprises a filler sequence after the polyadenylation signal sequence region. In one embodiment, the viral genome comprises a filler sequence after the MCS region. In one embodiment, the viral genome comprises a filler sequence after the exon region.

[00235] In one embodiment, the viral genome comprises a filler sequence before the promoter region. In one embodiment, the viral genome comprises a filler sequence before the payload region. In one embodiment, the viral genome comprises a filler sequence before the intron region. In one embodiment, the viral genome comprises a filler sequence before the enhancer region. In one embodiment, the viral genome comprises a filler sequence before the polyadenylation signal sequence region. In one embodiment, the viral genome comprises a filler sequence before the MCS region. In one embodiment, the viral genome comprises a filler sequence before the exon region.

[00236] In one embodiment, the viral genome comprises a filler sequence before the 3' ITR.

[00237] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the promoter region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the payload region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the intron region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the enhancer region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the polyadenylation signal sequence region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the MCS region.

[00238] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the exon region.

[00239] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the payload region. In one embodiment, a filler

sequence may be located between two regions, such as, but not limited to, the promoter region and the intron region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the enhancer region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the polyadenylation signal sequence region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the MCS region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the exon region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the 3' ITR.

[00240] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the intron region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the enhancer region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the polyadenylation signal sequence region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the MCS region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the exon region.

[00241] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the 3' ITR.

[00242] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the intron region and the enhancer region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the intron region and the polyadenylation signal sequence region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the intron region and the MCS region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the intron region and the exon region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the intron region and the 3' ITR. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the enhancer region and the polyadenylation signal sequence region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the enhancer region and the MCS region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the enhancer region and the exon region. In one embodiment, a filler

sequence may be located between two regions, such as, but not limited to, the enhancer region and the 3' ITR.

[00243] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the polyadenylation signal sequence region and the MCS region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the polyadenylation signal sequence region and the exon region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the polyadenylation signal sequence region and the 3' ITR.

[00244] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the MCS region and the exon region. In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the MCS region and the 3' ITR.

[00245] In one embodiment, a filler sequence may be located between two regions, such as, but not limited to, the exon region and the 3' ITR.

[00246] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the

5' ITR and promoter region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the enhancer region and

MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and promoter region, and the second filler sequence may be located between the exon region and 3' ITR.

[00247] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a

viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located

between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and payload region, and the second filler sequence may be located between the exon region and 3' ITR.

[00248] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral

genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located

between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise

two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and intron region, and the second filler sequence may be located between the exon region and 3' ITR.

[00249] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be

located between the 5' ITR and enhancer region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region

and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and enhancer region, and the second filler sequence may be located between the exon region and 3' ITR.

[00250] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation

signal sequence region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and 3' ITR. In

one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and polyadenylation signal sequence region, and the second filler sequence may be located between the exon region and 3' ITR.

[00251] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral

genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located

between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise

two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and MCS region, and the second filler sequence may be located between the exon region and 3' ITR.

[00252] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and payload region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the promoter region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler

sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be

located between the 5' ITR and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the 5' ITR and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

[00253] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the

second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter

region and payload region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and payload region, and the second filler sequence may be located between the exon region and 3' ITR.

[00254] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the

second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and intron region, and the second filler sequence may be located between the exon region and 3' ITR.

[00255] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between

the promoter region and enhancer region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be

located between the promoter region and enhancer region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and enhancer region, and the second filler sequence may be located between the exon region and 3' ITR.

[00256] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between

the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be

located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and polyadenylation signal sequence region, and the second filler sequence may be located between the exon region and 3' ITR.

[00257] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler

sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the

polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

[00258] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be

located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region,

and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and MCS region, and the second filler sequence may be located between the exon region and 3' ITR.

[00259] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the payload region and intron region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the payload region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the payload region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the payload region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the payload region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the payload region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the intron region and

3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the promoter region and 3'ITR, and the second filler sequence may be located between the exon region and 3' ITR.

[00260] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome

may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first

filler sequence may be located between the payload region and intron region, and the second filler sequence may be located between the exon region and 3' ITR.

[00261] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome

may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and enhancer region, and the second filler sequence may be located between the exon region and 3' ITR.

[00262] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome

may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and polyadenylation signal sequence region, and the second filler sequence may be located between the exon region and 3' ITR.

[00263] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the intron region

and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and MCS region, and the second filler sequence may be located between the exon region and 3' ITR.

[00264] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment,

a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

[00265] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the intron region and enhancer region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the intron region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the intron region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the intron region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the intron region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler

sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the payload region and 3' ITR region, and the second filler sequence may be located between the exon region and 3' ITR.

[00266] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be

located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and enhancer region, and the second filler sequence may be located between the exon region and 3' ITR.

[00267] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation

signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and polyadenylation signal sequence region, and the second filler sequence may be located between the exon region and 3' ITR.

[00268] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region,

and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and MCS region, and the second filler sequence may be located between the exon region and 3' ITR.

[00269] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

[00270] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the enhancer region and polyadenylation signal sequence region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the enhancer region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the enhancer region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the enhancer region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the intron region and 3'ITR, and the second filler sequence may be located between the exon region and 3' ITR.

[00271] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and polyadenylation signal sequence region, and the second filler sequence may be located between

the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and polyadenylation signal sequence region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and polyadenylation signal sequence region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and polyadenylation signal sequence region, and the second filler sequence may be located between the exon region and 3' ITR.

[00272] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and MCS region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and MCS region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and MCS region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and MCS region, and the second filler sequence may be located between the exon region and 3' ITR.

[00273] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region.

In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and exon region, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and exon region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and exon region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

[00274] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and 3' ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and MCS region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and 3' ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and 3' ITR, and the second filler sequence may be located between the polyadenylation signal sequence region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and 3' ITR, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and 3' ITR, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the enhancer region and 3' ITR, and the second filler sequence may be located between the exon region and 3' ITR.

[00275] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and MCS region,

and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and MCS region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and MCS region, and the second filler sequence may be located between the exon region and 3' ITR.

[00276] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and exon region, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and exon region, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

[00277] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and 3' ITR, and the second filler sequence may be located between the MCS region and exon region. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and 3' ITR, and the second filler sequence may be located between the MCS region and 3' ITR. In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the polyadenylation signal sequence region and 3' ITR, and the second filler sequence may be located between the exon region and 3' ITR.

[00278] In one embodiment, a viral genome may comprise two filler sequences, the first filler sequence may be located between the MCS region and exon region, and the second filler sequence may be located between the exon region and 3' ITR.

Payloads of the Invention

[00279] The AAV particles of the present disclosure comprise at least one payload region. As used herein, "payload" or "payload region" refers to one or more polynucleotides or polynucleotide regions encoded by or within a viral genome or an expression product of such polynucleotide or polynucleotide region, e.g., a transgene, a polynucleotide encoding a

polypeptide or multi-polypeptide or a modulatory nucleic acid or regulatory nucleic acid. Payloads of the present invention typically encode modulatory polynucleotides or fragments or variants thereof.

[00280] The payload region may be constructed in such a way as to reflect a region similar to or mirroring the natural organization of an mRNA.

[00281] The payload region may comprise a combination of coding and non-coding nucleic acid sequences.

[00282] In some embodiments, the AAV payload region may encode a coding or non-coding RNA.

[00283] In one embodiment, the AAV particle comprises a viral genome with a payload region comprising nucleic acid sequences encoding a siRNA, miRNA or other RNAi agent. In such an embodiment, a viral genome encoding more than one polypeptide may be replicated and packaged into a viral particle. A target cell transduced with a viral particle may express the encoded siRNA, miRNA or other RNAi agent inside a single cell.

Modulatory Polynucleotides

[00284] In one embodiment, modulatory polynucleotides, e.g., RNA or DNA molecules, may be used to treat neurodegenerative disease, in particular, amyotrophic lateral sclerosis (ALS). As used herein, a "modulatory polynucleotide" is any nucleic acid sequence(s) which functions to modulate (either increase or decrease) the level or amount of a target gene, e.g., mRNA or protein levels.

[00285] In one embodiment, the modulatory polynucleotides may comprise at least one nucleic acid sequence encoding at least one siRNA molecule. The nucleic acids may, independently if there is more than one, encode 1, 2, 3, 4, 5, 6, 7, 8, 9, or more than 9 siRNA molecules.

[00286] In one embodiment, the molecular scaffold may be located downstream of a CMV promoter, fragment or variant thereof.

[00287] In one embodiment, the molecular scaffold may be located downstream of a CBA promoter, fragment or variant thereof.

[00288] In one embodiment, the molecular scaffold may be a natural pri-miRNA scaffold located downstream of a CMV promoter. As a non-limiting example, the natural pri-miRNA scaffold is derived from the human miR155 scaffold.

[00289] In one embodiment, the molecular scaffold may be a natural pri-miRNA scaffold located downstream of a CBA promoter.

[00290] In one embodiment, the selection of a molecular scaffold and modulatory polynucleotide is determined by a method of comparing modulatory polynucleotides in pri-miRNA (see e.g., the method described by Miniarikova et al. *Design, Characterization, and Lead Selection of Therapeutic miRNAs Targeting Huntingtin for Development of Gene Therapy for Huntington's Disease*. Molecular Therapy-Nucleic Acids (2016) 5, e297 and International Publication No. WO2016102664; the contents of each of which are herein incorporated by reference in their entireties). To evaluate the activities of the modulatory polynucleotides, the molecular scaffold used which may be used is a human pri-miRNA scaffold (e.g., miR155 scaffold) and the promoter may be CMV. The activity may be determined *in vitro* using HEK293T cells and a reporter (e.g., Luciferase).

[00291] In order to evaluate the optimal molecular scaffold for the modulatory polynucleotide, the modulatory polynucleotide is used in pri-miRNA scaffolds with a CAG promoter. The constructs are co-transfected with a reporter (e.g., luciferase reporter) at 50 ng. Constructs with greater than 80% knockdown at 50 ng co-transfection are considered efficient. In one aspect, the constructs with strong guide-strand activity are preferred. The molecular scaffolds can be processed in HEK293T cells by NGS to determine guide-passenger ratios, and processing variability.

[00292] To evaluate the molecular scaffolds and modulatory polynucleotides *in vivo* the molecular scaffolds comprising the modulatory polynucleotides are packaged in AAV (e.g., the serotype may be AAV5 (see e.g., the method and constructs described in WO2015060722, the contents of which are herein incorporated by reference in their entirety)) and administered to an *in vivo* model and the guide-passenger ratios, 5' and 3' end processing, ratio of guide to passenger strands, and knockdown can be determined in different areas of the model (e.g., tissue regions).

[00293] In one embodiment, the selection of a molecular scaffold and modulatory polynucleotide is determined by a method of comparing modulatory polynucleotides in natural pri-miRNA and synthetic pri-miRNA. The modulatory polynucleotide may, but it not limited to, targeting an exon other than exon 1. To evaluate the activities of the modulatory polynucleotides, the molecular scaffold is used with a CBA promoter. In one aspect, the activity may be determined *in vitro* using HEK293T cells, HeLa cell and a reporter (e.g., Luciferase) and knockdown efficient modulatory polynucleotides showed SOD1 knockdown of at least 80% in the cell tested. Additionally, the modulatory polynucleotides which are considered most efficient showed low to no significant passenger strand (p-strand) activity. In another aspect, the

endogenous SOD1 knockdown efficacy is evaluated by transfection *in vitro* using HEK293T cells, HeLa cell and a reporter. Efficient modulatory polynucleotides show greater than 50% endogenous SOD1 knockdown. In yet another aspect, the endogenous SOD1 knockdown efficacy is evaluated in different cell types (e.g., HEK293, HeLa, primary astrocytes, U251 astrocytes, SH-SY5Y neuron cells and fibroblasts from ALS patients) by infection (e.g., AAV2). Efficient modulatory polynucleotides show greater than 60% endogenous SOD1 knockdown.

[00294] To evaluate the molecular scaffolds and modulatory polynucleotides *in vivo* the molecular scaffolds comprising the modulatory polynucleotides are packaged in AAV and administered to an *in vivo* model and the guide-passenger ratios, 5' and 3' end processing, ratio of guide to passenger strands, and knockdown can be determined in different areas of the model (e.g., tissue regions). The molecular scaffolds can be processed from *in vivo* samples by NGS to determine guide-passenger ratios, and processing variability.

[00295] In one embodiment, the modulatory polynucleotide is designed using at least one of the following properties: loop variant, seed mismatch/bulge/wobble variant, stem mismatch, loop variant and vassal stem mismatch variant, seed mismatch and basal stem mismatch variant, stem mismatch and basal stem mismatch variant, seed wobble and basal stem wobble variant, or a stem sequence variant.

siRNA Molecules

[00296] The present invention relates to RNA interference (RNAi) induced inhibition of gene expression for treating neurodegenerative disorders. Provided herein are siRNA duplexes or encoded dsRNA that target the gene of interest (referred to herein collectively as "siRNA molecules"). Such siRNA duplexes or encoded dsRNA can reduce or silence gene expression in cells, such as but not limited to, medium spiny neurons, cortical neurons and/or astrocytes.

[00297] RNAi (also known as post-transcriptional gene silencing (PTGS), quelling, or co-suppression) is a post-transcriptional gene silencing process in which RNA molecules, in a sequence specific manner, inhibit gene expression, typically by causing the destruction of specific mRNA molecules. The active components of RNAi are short/small double stranded RNAs (dsRNAs), called small interfering RNAs (siRNAs), that typically contain 15-30 nucleotides (e.g., 19 to 25, 19 to 24 or 19-21 nucleotides) and 2 nucleotide 3' overhangs and that match the nucleic acid sequence of the target gene. These short RNA species may be naturally produced *in vivo* by Dicer-mediated cleavage of larger dsRNAs and they are functional in mammalian cells.

[00298] Naturally expressed small RNA molecules, named microRNAs (miRNAs), elicit gene silencing by regulating the expression of mRNAs. The miRNAs containing RNA Induced Silencing Complex (RISC) targets mRNAs presenting a perfect sequence complementarity with nucleotides 2-7 in the 5'region of the miRNA which is called the seed region, and other base pairs with its 3'region. miRNA mediated down regulation of gene expression may be caused by cleavage of the target mRNAs, translational inhibition of the target mRNAs, or mRNA decay. miRNA targeting sequences are usually located in the 3'-UTR of the target mRNAs. A single miRNA may target more than 100 transcripts from various genes, and one mRNA may be targeted by different miRNAs.

[00299] siRNA duplexes or dsRNA targeting a specific mRNA may be designed and synthesized *in vitro* and introduced into cells for activating RNAi processes. Elbashir et al. demonstrated that 21-nucleotide siRNA duplexes (termed small interfering RNAs) were capable of effecting potent and specific gene knockdown without inducing immune response in mammalian cells (Elbashir SM et al., *Nature*, 2001, 411, 494-498). Since this initial report, post-transcriptional gene silencing by siRNAs quickly emerged as a powerful tool for genetic analysis in mammalian cells and has the potential to produce novel therapeutics.

[00300] RNAi molecules which were designed to target against a nucleic acid sequence that encodes poly-glutamine repeat proteins which cause poly-glutamine expansion diseases such as Huntington's Disease, are described in US Patent No. 9,169,483 and 9,181,544 and International Patent Publication No. WO2015179525, the content of each of which is herein incorporated by reference in their entirety. US Patent Nos. 9,169,483 and 9,181,544 and International Patent Publication No. WO2015179525 each provide isolated RNA duplexes comprising a first strand of RNA (e.g., 15 contiguous nucleotides) and second strand of RNA (e.g., complementary to at least 12 contiguous nucleotides of the first strand) where the RNA duplex is about 15 to 30 base pairs in length. The first strand of RNA and second strand of RNA may be operably linked by an RNA loop (~4 to 50 nucleotides) to form a hairpin structure which may be inserted into an expression cassette. Non-limiting examples of loop portions include SEQ ID NO: 9-14 of US Patent No. 9,169,483, the content of which is herein incorporated by reference in its entirety. Non-limiting examples of strands of RNA which may be used, either full sequence or part of the sequence, to form RNA duplexes include SEQ ID NO: 1-8 of US Patent No. 9,169,483 and SEQ ID NO: 1-11, 33-59, 208-210, 213-215 and 218-221 of US Patent No. 9,181,544, the contents of each of which is herein incorporated by reference in its entirety. Non-limiting examples of RNAi molecules include SEQ ID NOs: 1-8 of US Patent No. 9,169,483, SEQ ID NOs: 1-11, 33-59,

208-210, 213-215 and 218-221 of US Patent No. 9,181,544 and SEQ ID NOs: 1, 6, 7, and 35-38 of International Patent Publication No. WO2015179525, the contents of each of which is herein incorporated by reference in their entirety.

[00301] *In vitro* synthetized siRNA molecules may be introduced into cells in order to activate RNAi. An exogenous siRNA duplex, when it is introduced into cells, similar to the endogenous dsRNAs, can be assembled to form the RNA Induced Silencing Complex (RISC), a multiunit complex that interacts with RNA sequences that are complementary to one of the two strands of the siRNA duplex (i.e., the antisense strand). During the process, the sense strand (or passenger strand) of the siRNA is lost from the complex, while the antisense strand (or guide strand) of the siRNA is matched with its complementary RNA. In particular, the targets of siRNA containing RISC complexes are mRNAs presenting a perfect sequence complementarity. Then, siRNA mediated gene silencing occurs by cleaving, releasing and degrading the target.

[00302] The siRNA duplex comprised of a sense strand homologous to the target mRNA and an antisense strand that is complementary to the target mRNA offers much more advantage in terms of efficiency for target RNA destruction compared to the use of the single strand (ss)-siRNAs (e.g. antisense strand RNA or antisense oligonucleotides). In many cases, it requires higher concentration of the ss-siRNA to achieve the effective gene silencing potency of the corresponding duplex.

[00303] Any of the foregoing molecules may be encoded by a viral genome.

Design and Sequences of siRNA duplexes targeting gene of interest

[00304] The present invention provides small interfering RNA (siRNA) duplexes (and modulatory polynucleotides encoding them) that target mRNA to interfere with gene expression and/or protein production.

[00305] The encoded siRNA duplex of the present invention contains an antisense strand and a sense strand hybridized together forming a duplex structure, wherein the antisense strand is complementary to the nucleic acid sequence of the targeted gene, and wherein the sense strand is homologous to the nucleic acid sequence of the targeted gene. In some aspects, the 5'end of the antisense strand has a 5' phosphate group and the 3'end of the sense strand contains a 3'hydroxyl group. In other aspects, there are none, one or 2 nucleotide overhangs at the 3'end of each strand.

[00306] Some guidelines for designing siRNAs have been proposed in the art. These guidelines generally recommend generating a 19-nucleotide duplexed region, symmetric 2-3 nucleotide 3'overhangs, 5'- phosphate and 3'- hydroxyl groups targeting a region in the gene to be silenced. Other rules that may govern siRNA sequence preference include, but are not limited

to, (i) A/U at the 5′ end of the antisense strand; (ii) G/C at the 5′ end of the sense strand; (iii) at least five A/U residues in the 5′ terminal one-third of the antisense strand; and (iv) the absence of any GC stretch of more than 9 nucleotides in length. In accordance with such consideration, together with the specific sequence of a target gene, highly effective siRNA molecules essential for suppressing mammalian target gene expression may be readily designed.

[00307] According to the present invention, siRNA molecules (e.g., siRNA duplexes or encoded dsRNA) that target the gene of interest are designed. Such siRNA molecules can specifically, suppress gene expression and protein production. In some aspects, the siRNA molecules are designed and used to selectively "knock out" gene variants in cells, i.e., mutated transcripts. In some aspects, the siRNA molecules are designed and used to selectively "knock down" gene variants in cells. In other aspects, the siRNA molecules are able to inhibit or suppress both the wild type and mutated version of the gene of interest.

[00308] In one embodiment, an siRNA molecule of the present invention comprises a sense strand and a complementary antisense strand in which both strands are hybridized together to form a duplex structure. The antisense strand has sufficient complementarity to the target mRNA sequence to direct target-specific RNAi, i.e., the siRNA molecule has a sequence sufficient to trigger the destruction of the target mRNA by the RNAi machinery or process.

[00309] In one embodiment, an siRNA molecule of the present invention comprises a sense strand and a complementary antisense strand in which both strands are hybridized together to form a duplex structure and where the start site of the hybridization to the mRNA is between nucleotide 10 and 1000 on the target mRNA sequence. As a non-limiting example, the start site may be between nucleotide 10-20, 20-30, 30-40, 40-50, 60-70, 70-80, 80-90, 90-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-70, 750-800, 800-850, 850-900, 900-950, 950-1000, on the target mRNA sequence. As yet another non-limiting example, the start site may be nucleotide 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188,

189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815,

816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, and 1000 on the target mRNA sequence.

[00310] In some embodiments, the antisense strand and target mRNA sequences have 100% complementarity. The antisense strand may be complementary to any part of the target mRNA sequence.

[00311] In other embodiments, the antisense strand and target mRNA sequences comprise at least one mismatch. As a non-limiting example, the antisense strand and the target mRNA sequence have at least 30%, 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-99%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-99%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-99%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementarity.

[00312] In one embodiment, an siRNA or dsRNA includes at least two sequences that are complementary to each other.

[00313] According to the present invention, the siRNA molecule has a length from about 10-50 or more nucleotides, i.e., each strand comprising 10-50 nucleotides (or nucleotide analogs). Preferably, the siRNA molecule has a length from about 15-30, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in each strand, wherein one of the strands is sufficiently complementarity to a target region. In one embodiment, each strand of the siRNA molecule has a length from about 19 to 25, 19 to 24 or 19 to 21 nucleotides. In one embodiment, at least one strand of the siRNA molecule is 19 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 20 nucleotides in length. In one embodiment, at least

one strand of the siRNA molecule is 21 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 22 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 23 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 24 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 25 nucleotides in length.

[00314] In some embodiments, the siRNA molecules of the present invention can be synthetic RNA duplexes comprising about 19 nucleotides to about 25 nucleotides, and two overhanging nucleotides at the 3'-end. In some aspects, the siRNA molecules may be unmodified RNA molecules. In other aspects, the siRNA molecules may contain at least one modified nucleotide, such as base, sugar or backbone modifications.

[00315] In one embodiment, the siRNA molecules of the present invention may comprise an antisense sequence and a sense sequence, or a fragment or variant thereof. As a non-limiting example, the antisense sequence and the sense sequence have at least 30%, 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-99%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-99%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-99%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementarity.

[00316] In other embodiments, the siRNA molecules of the present invention can be encoded in plasmid vectors, AAV particles, viral genome or other nucleic acid expression vectors for delivery to a cell.

[00317] DNA expression plasmids can be used to stably express the siRNA duplexes or dsRNA of the present invention in cells and achieve long-term inhibition of the target gene expression. In one aspect, the sense and antisense strands of a siRNA duplex are typically linked by a short spacer sequence leading to the expression of a stem-loop structure termed short hairpin RNA (shRNA). The hairpin is recognized and cleaved by Dicer, thus generating mature siRNA molecules.

[00318] According to the present invention, AAV particles comprising the nucleic acids encoding the siRNA molecules targeting the mRNA are produced, the AAV serotypes may be any of the serotypes listed in Table 1. Non-limiting examples of the AAV serotypes include, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ8, AAV-DJ, AAV-PHP.A, AAV-

PHP.B, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B-EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B-SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B-STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A15/G2A3, AAVG2B4, AAVG2B5, and variants thereof.

[00319] In some embodiments, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) the target mRNA. Accordingly, the siRNA duplexes or encoded dsRNA can be used to substantially inhibit the gene expression in a cell, for example a neuron. In some aspects, the inhibition of the gene expression refers to an inhibition by at least about 20%, preferably by at least about 30%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 45-50%, 45-55%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%. Accordingly, the protein product of the targeted gene may be inhibited by at least about 20%, preferably by at least about 30%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 45-50%, 45-55%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%.

[00320] In one embodiment, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) the target mRNA in spinal cord motor neurons. In some aspects, the inhibition of the gene expression refers to an inhibition by at least about 20%, preferably by at least about 30%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%,

78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 45-50%, 45-55%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%. Accordingly, the protein product of the targeted gene may be inhibited by at least about 20%, preferably by at least about 30%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 45-50%, 45-55%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%.

[00321] In one embodiment, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) the target mRNA in spinal cord motor neurons by 78%.

[00322] In one embodiment, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) the target mRNA in spinal cord motor neurons by 45-55%.

[00323] In one embodiment, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) the target mRNA in vg+ cells of motor neuron morphology. In some aspects, the inhibition of the gene expression refers to an inhibition by at least about 20%, preferably by at least about 30%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 45-50%, 45-55%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%. Accordingly, the protein product of the targeted gene may be inhibited by at least about 20%, preferably by at least about 30%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%,

80%, 81%, 82%, 83%, 84%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 45-50%, 45-55%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%.

[00324] In one embodiment, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) the target mRNA in vg+ cells of motor neuron morphology by 53%.

[00325] In one embodiment, the siRNA molecules comprise a miRNA seed match for the target located in the guide strand. In another embodiment, the siRNA molecules comprise a miRNA seed match for the target located in the passenger strand. In yet another embodiment, the siRNA duplexes or encoded dsRNA targeting the gene of interest do not comprise a seed match for the target located in the guide or passenger strand.

[00326] In one embodiment, the siRNA duplexes or encoded dsRNA targeting the gene of interest may have almost no significant full-length off target effects for the guide strand. In another embodiment, the siRNA duplexes or encoded dsRNA targeting the gene of interest may have almost no significant full-length off target effects for the passenger strand. The siRNA duplexes or encoded dsRNA targeting the gene of interest may have less than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%,11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 1-5%, 2-6%, 3-7%, 4-8%, 5-9%, 5-10%, 6-10%, 5-15%, 5-20%, 5-25% 5-30%, 10-20%, 10-30%, 10-40%, 10-50%, 15-30%, 15-40%, 15-45%, 20-40%, 20-50%, 25-50%, 30-40%, 30-50%, 35-50%, 40-50%, 45-50% full-length off target effects for the passenger strand. In yet another embodiment, the siRNA duplexes or encoded dsRNA targeting the gene of interest may have almost no significant full-length off target effects for the guide strand or the passenger strand. The siRNA duplexes or encoded dsRNA targeting the gene of interest may have less than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%,11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 1-5%, 2-6%, 3-7%, 4-8%, 5-9%, 5-10%, 6-10%, 5-15%, 5-20%, 5-25% 5-30%, 10-20%, 10-30%, 10-40%, 10-50%, 15-30%, 15-40%, 15-45%, 20-40%, 20-50%, 25-50%, 30-40%, 30-50%, 35-50%, 40-50%, 45-50% full-length off target effects for the guide or passenger strand.

[00327] In one embodiment, the siRNA duplexes or encoded dsRNA targeting the gene of interest may have high activity *in vitro*. In another embodiment, the siRNA molecules may have

low activity *in vitro*. In yet another embodiment, the siRNA duplexes or dsRNA targeting the gene of interest may have high guide strand activity and low passenger strand activity *in vitro*.

[00328] In one embodiment, the siRNA molecules have a high guide strand activity and low passenger strand activity *in vitro*. The target knock-down (KD) by the guide strand may be at least 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 99.5% or 100%. The target knock-down by the guide strand may be 40-50%, 45-50%, 50-55%, 50-60%, 60-65%, 60-70%, 60-75%, 60-80%, 60-85%, 60-90%, 60-95%, 60-99%, 60-99.5%, 60-100%, 65-70%, 65-75%, 65-80%, 65-85%, 65-90%, 65-95%, 65-99%, 65-99.5%, 65-100%, 70-75%, 70-80%, 70-85%, 70-90%, 70-95%, 70-99%, 70-99.5%, 70-100%, 75-80%, 75-85%, 75-90%, 75-95%, 75-99%, 75-99.5%, 75-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-99.5%, 80-100%, 85-90%, 85-95%, 85-99%, 85-99.5%, 85-100%, 90-95%, 90-99%, 90-99.5%, 90-100%, 95-99%, 95-99.5%, 95-100%, 99-99.5%, 99-100% or 99.5-100%. As a non-limiting example, the target knock-down (KD) by the guide strand is greater than 70%. As a non-limiting example, the target knock-down (KD) by the guide strand is greater than 60%.

[00329] In one embodiment, the siRNA duplex is designed so there is no miRNA seed match for the sense or antisense sequence to the non-gene of interest sequence.

[00330] In one embodiment, the IC_{50} of the guide strand for the nearest off target is greater than 100 multiplied by the IC_{50} of the guide strand for the on-target gene. As a non-limiting example, if the IC_{50} of the guide strand for the nearest off target is greater than 100 multiplied by the IC_{50} of the guide strand for the target then the siRNA molecule is said to have high guide strand selectivity for inhibiting the gene of interest *in vitro*.

[00331] In one embodiment, the 5' processing of the guide strand has a correct start (n) at the 5' end at least 75%, 80%, 85%, 90%, 95%, 99% or 100% of the time *in vitro* or *in vivo*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 99% of the time *in vitro*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 99% of the time *in vivo*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 90% of the time *in vitro*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 90% of the time *in vivo*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 85% of the time *in vitro*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 85% of the time *in vivo*.

[00332] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1;1, 2:10, 2:9, 2:8, 2:7, 2:6, 2:5, 2:4, 2:3, 2:2, 2:1, 3:10, 3:9, 3:8, 3:7, 3:6, 3:5, 3:4, 3:3, 3:2, 3:1, 4:10, 4:9, 4:8, 4:7, 4:6, 4:5, 4:4, 4:3, 4:2, 4:1, 5:10, 5:9, 5:8, 5:7, 5:6, 5:5, 5:4, 5:3, 5:2, 5:1, 6:10, 6:9, 6:8, 6:7, 6:6, 6:5, 6:4, 6:3, 6:2, 6:1, 7:10, 7:9, 7:8, 7:7, 7:6, 7:5, 7:4, 7:3, 7:2, 7:1, 8:10, 8:9, 8:8, 8:7, 8:6, 8:5, 8:4, 8:3, 8:2, 8:1, 9:10, 9:9, 9:8, 9:7, 9:6, 9:5, 9:4, 9:3, 9:2, 9:1, 10:10, 10:9, 10:8, 10:7, 10:6, 10:5, 10:4, 10:3, 10:2, 10:1, 1:99, 5:95, 10:90, 15:85, 20:80, 25:75, 30:70, 35:65, 40:60, 45:55, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10, 95:5, or 99:1 *in vitro* or *in vivo*. The guide to passenger ratio refers to the ratio of the guide strands to the passenger strands after intracellular processing of the pri-microRNA. For example, a 80:20 guide-to-passenger ratio would have 8 guide strands to every 2 passenger strands processed from the precursor. As a non-limiting example, the guide-to-passenger strand ratio is 8:2 *in vitro*. As a non-limiting example, the guide-to-passenger strand ratio is 8:2 *in vivo*. As a non-limiting example, the guide-to-passenger strand ratio is 9:1 *in vitro*. As a non-limiting example, the guide-to-passenger strand ratio is 9:1 *in vivo*.

[00333] In one embodiment, the guide to passenger (G:P) strand ratio is in a range of 1-99, 1.3-99, 5-99, 10-99, 15-99, 20-99, 25-99, 30-99, 35-99, 40-99, 45-99, 50-99, 55-99, 60-99, 65-99, 70-99, 75-99, 80-99, 85-99, 90-99, 95-99, 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-55, 1-60, 1-65, 1-70, 1-75, 1-80, 1-85, 1-90, 1-95, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5-50, 5-55, 5-60, 5-65, 5-70, 5-75, 5-80, 5-85, 5-90, 5-95, 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, 10-45, 10-50, 10-55, 10-60, 10-65, 10-70, 10-75, 10-80, 10-85, 10-90, 10-95, 15-20, 15-25, 15-30, 15-35, 15-40, 15-45, 15-50, 15-55, 15-60, 15-65, 15-70, 15-75, 15-80, 15-85, 15-90, 15-95, 20-25, 20-30, 20-35, 20-40, 20-45, 20-50, 20-55, 20-60, 20-65, 20-70, 20-75, 20-80, 20-85, 20-90, 20-95, 25-30, 25-35, 25-40, 25-45, 25-50, 25-55, 25-60, 25-65, 25-70, 25-75, 25-80, 25-85, 25-90, 25-95, 30-35, 30-40, 30-45, 30-50, 30-55, 30-60, 30-65, 30-70, 30-75, 30-80, 30-85, 30-90, 30-95, 35-40, 35-45, 35-50, 35-55, 35-60, 35-65, 35-70, 35-75, 35-80, 35-85, 35-90, 35-95, 40-45, 40-50, 40-55, 40-60, 40-65, 40-70, 40-75, 40-80, 40-85, 40-90, 40-95, 45-50, 45-55, 45-60, 45-65, 45-70, 45-75, 45-80, 45-85, 45-90, 45-95, 50-55, 50-60, 50-65, 50-70, 50-75, 50-80, 50-85, 50-90, 50-95, 55-60, 55-65, 55-70, 55-75, 55-80, 55-85, 55-90, 55-95, 60-65, 60-70, 60-75, 60-80, 60-85, 60-90, 60-95, 65-70, 65-75, 65-80, 65-85, 65-90, 65-95, 70-75, 70-80, 70-85, 70-90, 70-95, 75-80, 75-85, 75-90, 75-95, 80-85, 80-90, 80-95, 85-90, 85-95, or 90-95. As a non-limiting example, the guide to passenger ratio is a range of 1.3 to 99. As a non-limiting example, the guide to passenger ratio is a range of 10 to 99.

[00334] In one embodiment, the guide to passenger (G:P) strand ratio is 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, 60, 60.5, 61, 61.5, 62, 62.5, 63, 63.5, 64, 64.5, 65, 65.5, 66, 66.5, 67, 67.5, 68, 68.5, 69, 69.5, 70, 70.5, 71, 71.5, 72, 72.5, 73, 73.5, 74, 74.5, 75, 75.5, 76, 76.5, 77, 77.5, 78, 78.5, 79, 79.5, 80, 80.5, 81, 81.5, 82, 82.5, 83, 83.5, 84, 84.5, 85, 85.5, 86, 86.5, 87, 87.5, 88, 88.5, 89, 89.5, 90, 90.5, 91, 91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, or 99. As a non-limiting example, the guide to passenger (G:P) strand ratio is 11.5. As a non-limiting example, the guide to passenger (G:P) strand ratio is 99.

[00335] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is greater than 1.

[00336] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is greater than 2.

[00337] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is greater than 5.

[00338] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is greater than 10.

[00339] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is greater than 20.

[00340] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is greater than 50.

[00341] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is at least 3:1.

[00342] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is at least 5:1.

[00343] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is at least 10:1.

[00344] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is at least 20:1.

[00345] In one embodiment, the guide to passenger (G:P) (also referred to as the antisense to sense) strand ratio expressed is at least 50:1.

[00346] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1;1, 2:10, 2:9, 2:8, 2:7, 2:6, 2:5, 2:4, 2:3, 2:2, 2:1, 3:10, 3:9, 3:8, 3:7, 3:6, 3:5, 3:4, 3:3, 3:2, 3:1, 4:10, 4:9, 4:8, 4:7, 4:6, 4:5, 4:4, 4:3, 4:2, 4:1, 5:10, 5:9, 5:8, 5:7, 5:6, 5:5, 5:4, 5:3, 5:2, 5:1, 6:10, 6:9, 6:8, 6:7, 6:6, 6:5, 6:4, 6:3, 6:2, 6:1, 7:10, 7:9, 7:8, 7:7, 7:6, 7:5, 7:4, 7:3, 7:2, 7:1, 8:10, 8:9, 8:8, 8:7, 8:6, 8:5, 8:4, 8:3, 8:2, 8:1, 9:10, 9:9, 9:8, 9:7, 9:6, 9:5, 9:4, 9:3, 9:2, 9:1, 10:10, 10:9, 10:8, 10:7, 10:6, 10:5, 10:4, 10:3, 10:2, 10:1, 1:99, 5:95, 10:90, 15:85, 20:80, 25:75, 30:70, 35:65, 40:60, 45:55, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10, 95:5, or 99:1 *in vitro* or *in vivo*. The passenger to guide ratio refers to the ratio of the passenger strands to the guide strands after the intracellular processing of the pri-microRNA. For example, a 80:20 of passenger-to-guide ratio would have 8 passenger strands to every 2 guide strands processed from the precursor. As a non-limiting example, the passenger-to-guide strand ratio is 80:20 *in vitro*. As a non-limiting example, the passenger-to-guide strand ratio is 80:20 *in vivo*. As a non-limiting example, the passenger-to-guide strand ratio is 8:2 *in vitro*. As a non-limiting example, the passenger-to-guide strand ratio is 8:2 *in vivo*. As a non-limiting example, the passenger-to-guide strand ratio is 9:1 *in vitro*. As a non-limiting example, the passenger-to-guide strand ratio is 9:1 *in vivo*.

[00347] In one embodiment, the passenger to guide (P:G) strand ratio is in a range of 1-99, 1.3-99, 5-99, 10-99, 15-99, 20-99, 25-99, 30-99, 35-99, 40-99, 45-99, 50-99, 55-99, 60-99, 65-99, 70-99, 75-99, 80-99, 85-99, 90-99, 95-99, 1-10, 1-15, 1-20, 1-25, 1-30, 1-35, 1-40, 1-45, 1-50, 1-55, 1-60, 1-65, 1-70, 1-75, 1-80, 1-85, 1-90, 1-95, 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5-50, 5-55, 5-60, 5-65, 5-70, 5-75, 5-80, 5-85, 5-90, 5-95, 10-15, 10-20, 10-25, 10-30, 10-35, 10-40, 10-45, 10-50, 10-55, 10-60, 10-65, 10-70, 10-75, 10-80, 10-85, 10-90, 10-95, 15-20, 15-25, 15-30, 15-35, 15-40, 15-45, 15-50, 15-55, 15-60, 15-65, 15-70, 15-75, 15-80, 15-85, 15-90, 15-95, 20-25, 20-30, 20-35, 20-40, 20-45, 20-50, 20-55, 20-60, 20-65, 20-70, 20-75, 20-80, 20-85, 20-90, 20-95, 25-30, 25-35, 25-40, 25-45, 25-50, 25-55, 25-60, 25-65, 25-70, 25-75, 25-80, 25-85, 25-90, 25-95, 30-35, 30-40, 30-45, 30-50, 30-55, 30-60, 30-65, 30-70, 30-75, 30-80, 30-85, 30-90, 30-95, 35-40, 35-45, 35-50, 35-55, 35-60, 35-65, 35-70, 35-75, 35-80, 35-85, 35-90, 35-95, 40-45, 40-50, 40-55, 40-60, 40-65, 40-70, 40-75, 40-80, 40-85, 40-90, 40-95, 45-50, 45-55, 45-60, 45-65, 45-70, 45-75, 45-80, 45-85, 45-90, 45-95, 50-55, 50-60, 50-65, 50-70, 50-75, 50-80, 50-85, 50-90, 50-95, 55-60, 55-65, 55-70, 55-75, 55-80, 55-85, 55-90, 55-95, 60-65, 60-70, 60-75, 60-80, 60-85, 60-90, 60-95, 65-70, 65-75, 65-80, 65-85, 65-90, 65-95, 70-75, 70-

80, 70-85, 70-90, 70-95, 75-80, 75-85, 75-90, 75-95, 80-85, 80-90, 80-95, 85-90, 85-95, or 90-95.

[00348] In one embodiment, the passenger to guide (P:G) strand ratio is 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, 60, 60.5, 61, 61.5, 62, 62.5, 63, 63.5, 64, 64.5, 65, 65.5, 66, 66.5, 67, 67.5, 68, 68.5, 69, 69.5, 70, 70.5, 71, 71.5, 72, 72.5, 73, 73.5, 74, 74.5, 75, 75.5, 76, 76.5, 77, 77.5, 78, 78.5, 79, 79.5, 80, 80.5, 81, 81.5, 82, 82.5, 83, 83.5, 84, 84.5, 85, 85.5, 86, 86.5, 87, 87.5, 88, 88.5, 89, 89.5, 90, 90.5, 91, 91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, or 99.

[00349] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is greater than 1.

[00350] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is greater than 2.

[00351] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is greater than 5.

[00352] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is greater than 10.

[00353] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is greater than 20.

[00354] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is greater than 50.

[00355] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is at least 3:1.

[00356] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is at least 5:1.

[00357] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is at least 10:1.

[00358] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is at least 20:1.

[00359] In one embodiment, the passenger to guide (P:G) (also referred to as the sense to antisense) strand ratio expressed is at least 50:1.

[00360] In one embodiment, a passenger-guide strand duplex is considered effective when the pri- or pre-microRNAs demonstrate, but methods known in the art and described herein, greater than 2-fold guide to passenger strand ratio when processing is measured. As a non-limiting examples, the pri- or pre-microRNAs demonstrate great than 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, or 2 to 5-fold, 2 to 10-fold, 2 to 15-fold, 3 to 5-fold, 3 to 10-fold, 3 to 15-fold, 4 to 5-fold, 4 to 10-fold, 4 to 15-fold, 5 to 10-fold, 5 to 15-fold, 6 to 10-fold, 6 to 15-fold, 7 to 10-fold, 7 to 15-fold, 8 to 10-fold, 8 to 15-fold, 9 to 10-fold, 9 to 15-fold, 10 to 15-fold, 11 to 15-fold, 12 to 15-fold, 13 to 15-fold, or 14 to 15-fold guide to passenger strand ratio when processing is measured.

[00361] In one embodiment, the vector genome encoding the dsRNA comprises a sequence which is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more than 99% of the full length of the construct. As a non-limiting example, the vector genome comprises a sequence which is at least 80% of the full length sequence of the construct.

[00362] In one embodiment, the siRNA molecules may be used to silence wild type or mutant version of the gene of interest by targeting at least one exon on the gene of interest sequence. The exon may be exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, exon 10, exon 11, exon 12, exon 13, exon 14, exon 15, exon 16, exon 17, exon 18, exon 19, exon 20, exon 21, exon 22, exon 23, exon 24, exon 25, exon 26, exon 27, exon 28, exon 29, exon 30, exon 31, exon 32, exon 33, exon 34, exon 35, exon 36, exon 37, exon 38, exon 39, exon 40, exon 41, exon 42, exon 43, exon 44, exon 45, exon 46, exon 47, exon 48, exon 49, exon 50, exon 51, exon 52, exon 53, exon 54, exon 55, exon 56, exon 57, exon 58, exon 59, exon 60, exon 61, exon 62, exon 63, exon 64, exon 65, exon 66, and/or exon 67.

Design and Sequences of siRNA duplexes targeting SOD1 gene

[00363] The present invention provides small interfering RNA (siRNA) duplexes (and modulatory polynucleotides encoding them) that target SOD1 mRNA to interfere with SOD1 gene expression and/or SOD1 protein production.

[00364] The encoded siRNA duplex of the present invention contains an antisense strand and a sense strand hybridized together forming a duplex structure, wherein the antisense strand is complementary to the nucleic acid sequence of the targeted SOD1 gene, and wherein the sense strand is homologous to the nucleic acid sequence of the targeted SOD1 gene. In some aspects, the 5'end of the antisense strand has a 5' phosphate group and the 3'end of the sense strand

contains a 3'hydroxyl group. In other aspects, there are none, one or 2 nucleotide overhangs at the 3'end of each strand.

[00365] Some guidelines for designing siRNAs have been proposed in the art. These guidelines generally recommend generating a 19-nucleotide duplexed region, symmetric 2-3 nucleotide 3'overhangs, 5'- phosphate and 3'- hydroxyl groups targeting a region in the gene to be silenced. Other rules that may govern siRNA sequence preference include, but are not limited to, (i) A/U at the 5′ end of the antisense strand; (ii) G/C at the 5′ end of the sense strand; (iii) at least five A/U residues in the 5′ terminal one-third of the antisense strand; and (iv) the absence of any GC stretch of more than 9 nucleotides in length. In accordance with such consideration, together with the specific sequence of a target gene, highly effective siRNA molecules essential for suppressing the SOD1 gene expression may be readily designed.

[00366] According to the present invention, siRNA molecules (e.g., siRNA duplexes or encoded dsRNA) that target the SOD1 gene are designed. Such siRNA molecules can specifically, suppress SOD1 gene expression and protein production. In some aspects, the siRNA molecules are designed and used to selectively "knock out" SOD1 gene variants in cells, i.e., mutated SOD1 transcripts that are identified in patients with ALS disease. In some aspects, the siRNA molecules are designed and used to selectively "knock down" SOD1 gene variants in cells. In other aspects, the siRNA molecules are able to inhibit or suppress both the wild type and mutated SOD1 gene.

[00367] In one embodiment, an siRNA molecule of the present invention comprises a sense strand and a complementary antisense strand in which both strands are hybridized together to form a duplex structure. The antisense strand has sufficient complementarity to the SOD1 mRNA sequence to direct target-specific RNAi, i.e., the siRNA molecule has a sequence sufficient to trigger the destruction of the target mRNA by the RNAi machinery or process.

[00368] In one embodiment, an siRNA molecule of the present invention comprises a sense strand and a complementary antisense strand in which both strands are hybridized together to form a duplex structure and where the start site of the hybridization to the SOD1 mRNA is between nucleotide 15 and 1000 on the SOD1 mRNA sequence. As a non-limiting example, the start site may be between nucleotide 15-25, 15-50, 15-75, 15-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-70, 750-800, 800-850, 850-900, 900-950, and 950-1000 on the SOD1 mRNA sequence. As yet another non-limiting example, the start site may be nucleotide 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 74, 76, 77, 78, 149, 153, 157, 160, 177, 192, 193, 195, 196, 197, 198, 199, 206, 209, 210,

239, 241, 261, 263, 264, 268, 269, 276, 278, 281, 284, 290, 291, 295, 296, 316, 317, 329, 330, 337, 350, 351, 352, 354, 357, 358, 364, 375, 378, 383, 384, 390, 392, 395, 404, 406, 417, 418, 469, 470, 475, 476, 480, 487, 494, 496, 497, 501, 504, 515, 518, 522, 523, 524, 552, 554, 555, 562, 576, 577, 578, 579, 581, 583, 584, 585, 587, 588, 589, 593, 594, 595, 596, 597, 598, 599, 602, 607, 608, 609, 610, 611, 612, 613, 616, 621, 633, 635, 636, 639, 640, 641, 642, 643, 644, 645, 654, 660, 661, 666, 667, 668, 669, 673, 677, 692, 698, 699, 700, 701, 706, 749, 770, 772, 775, 781, 800, 804, 819, 829, 832, 833, 851, 854, 855, 857, 858, 859, 861, 869, 891, 892, 906, 907, 912, 913, 934, 944, and 947 on the SOD1 mRNA sequence.

[00369] In some embodiments, the antisense strand and target SOD1 mRNA sequences have 100% complementarity. The antisense strand may be complementary to any part of the target SOD1 mRNA sequence.

[00370] In other embodiments, the antisense strand and target SOD1 mRNA sequences comprise at least one mismatch. As a non-limiting example, the antisense strand and the target SOD1 mRNA sequence have at least 30%, 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-99%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-99%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-99%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementarity.

[00371] In one embodiment, an siRNA or dsRNA targeting SOD1 includes at least two sequences that are complementary to each other.

[00372] According to the present invention, the siRNA molecule targeting SOD1 has a length from about 10-50 or more nucleotides, i.e., each strand comprising 10-50 nucleotides (or nucleotide analogs). Preferably, the siRNA molecule has a length from about 15-30, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in each strand, wherein one of the strands is sufficiently complementarity to a target region. In one embodiment, each strand of the siRNA molecule has a length from about 19 to 25, 19 to 24 or 19 to 21 nucleotides. In one embodiment, at least one strand of the siRNA molecule is 19 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 20 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 21 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 22 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 23 nucleotides in length. In one

embodiment, at least one strand of the siRNA molecule is 24 nucleotides in length. In one embodiment, at least one strand of the siRNA molecule is 25 nucleotides in length.

[00373] In some embodiments, the siRNA molecules of the present invention targeting SOD1 can be synthetic RNA duplexes comprising about 19 nucleotides to about 25 nucleotides, and two overhanging nucleotides at the 3'-end. In some aspects, the siRNA molecules may be unmodified RNA molecules. In other aspects, the siRNA molecules may contain at least one modified nucleotide, such as base, sugar or backbone modifications.

[00374] In one embodiment, the siRNA molecules of the present invention targeting SOD1 may comprise a nucleotide sequence such as, but not limited to, the antisense (guide) sequences in Table 2 or a fragment or variant thereof. As a non-limiting example, the antisense sequence used in the siRNA molecule of the present invention is at least 30%, 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-99%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-99%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-99%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% of a nucleotide sequence in Table 2. As another non-limiting example, the antisense sequence used in the siRNA molecule of the present invention comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or more than 21 consecutive nucleotides of a nucleotide sequence in Table 2. As yet another non-limiting example, the antisense sequence used in the siRNA molecule of the present invention comprises nucleotides 1 to 22, 1 to 21, 1 to 20, 1 to 19, 1 to 18, 1 to 17, 1 to 16, 1 to 15, 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 2 to 22, 2 to 21, 2 to 20, 2 to 19, 2 to 18, 2 to 17, 2 to 16, 2 to 15, 2 to 14, 2 to 13, 2 to 12, 2 to 11, 2 to 10, 2 to 9, 2 to 8, 3 to 22, 3 to 21, 3 to 20, 3 to 19, 3 to 18, 3 to 17, 3 to 16, 3 to 15, 3 to 14, 3 to 13, 3 to 12, 3 to 11, 3 to 10, 3 to 9, 3 to 8, 4 to 22, 4 to 21, 4 to 20, 4 to 19, 4 to 18, 4 to 17, 4 to 16, 4 to 15, 4 to 14, 4 to 13, 4 to 12, 4 to 11, 4 to 10, 4 to 9, 4 to 8, 5 to 22, 5 to 21, 5 to 20, 5 to 19, 5 to 18, 5 to 17, 5 to 16, 5 to 15, 5 to 14, 5 to 13, 5 to 12, 5 to 11, 5 to 10, 5 to 9, 5 to 8, 6 to 22, 6 to 21, 6 to 20, 6 to 19, 6 to 18, 6 to 17, 6 to 16, 6 to 15, 6 to 14, 6 to 13, 6 to 12, 6 to 11, 6 to 10, 7 to 22, 7 to 21, 7 to 20, 7 to 19, 7 to 18, 7 to 17, 7 to 16, 7 to 15, 7 to 14, 7 to 13, 7 to 12, 8 to 22, 8 to 21, 8 to 20, 8 to 19, 8 to 18, 8 to 17, 8 to 16, 8 to 15, 8 to 14, 8 to 13, 8 to 12, 9 to 22, 9 to 21, 9 to 20, 9 to 19, 9 to 18, 9 to 17, 9 to 16, 9 to 15, 9 to 14, 10 to 22, 10 to 21, 10 to 20, 10 to 19, 10 to 18, 10 to 17, 10 to 16, 10 to 15, 10 to 14, 11 to 22, 11 to 21, 11 to 20, 11 to 19, 11 to 18, 11 to 17, 11 to 16, 11 to 15, 11 to

14, 12 to 22, 12 to 21, 12 to 20, 12 to 19, 12 to 18, 12 to 17, 12 to 16, 13 to 22, 13 to 21, 13 to 20, 13 to 19, 13 to 18, 13 to 17, 13 to 16, 14 to 22, 14 to 21, 14 to 20, 14 to 19, 14 to 18, 14 to 17, 15 to 22, 15 to 21, 15 to 20, 15 to 19, 15 to 18, 16 to 22, 16 to 21, 16 to 20, 17 to 22, 17 to 21, or 18 to 22 of the sequences in Table 2.

Table 2. Antisense Sequences

Antisense ID	Sequence	SEQ ID NO
A-3000	UUUAUAGGCCAGACCUCCGdTdT	916
A-3001	UUUUAUAGGCCAGACCUCCdTdT	917
A-3002	UCUUUAUAGGCCAGACCUCdTdT	918
A-3003	UACUUUAUAGGCCAGACCUdTdT	919
A-3004	UUACUUUAUAGGCCAGACCdTdT	920
A-3005	UACUACUUUAUAGGCCAGAdTdT	921
A-3006	UGACUACUUUAUAGGCCAGdTdT	922
A-3007	UCGACUACUUUAUAGGCCAdTdT	923
A-3008	UGCGACUACUUUAUAGGCCdTdT	924
A-3009	UCGCGACUACUUUAUAGGCdTdT	925
A-3010	UCCGCGACUACUUUAUAGGdTdT	926
A-3011	UGCUGCAGGAGACUACGACdTdT	927
A-3012	UACGCUGCAGGAGACUACGdTdT	928
A-3013	UGACGCUGCAGGAGACUACdTdT	929
A-3014	UAGACGCUGCAGGAGACUAdTdT	930
A-3015	UCACGGCCUUCGUCGCCAUdTdT	931
A-3016	UCGCACACGGCCUUCGUCGdTdT	932
A-3017	UAGCACGCACACGGCCUUCdTdT	933
A-3018	UUUCAGCACGCACACGGCCdTdT	934
A-3019	UGCACUGGGCCGUCGCCCUdTdT	935
A-3020	UAAUUGAUGAUGCCCUGCAdTdT	936
A-3021	UAAAUUGAUGAUGCCCUGCdTdT	937
A-3022	UCGAAAUUGAUGAUGCCCUdTdT	938
A-3023	UUCGAAAUUGAUGAUGCCCdTdT	939
A-3024	UCUCGAAAUUGAUGAUGCCdTdT	940
A-3025	UGCUCGAAAUUGAUGAUGCdTdT	941
A-3026	UUGCUCGAAAUUGAUGAUGdTdT	942
A-3027	UUUCCUUCUGCUCGAAAUUdTdT	943
A-3028	UACUUUCCUUCUGCUCGAAdTdT	944
A-3029	UUACUUUCCUUCUGCUCGAdTdT	945
A-3030	UAAUGCUUCCCCACACCUUdTdT	946
A-3031	UUUAAUGCUUCCCCACACCdTdT	947
A-3032	UGCAGGCCUUCAGUCAGUCdTdT	948
A-3033	UAUGCAGGCCUUCAGUCAGdTdT	949
A-3034	UCAUGCAGGCCUUCAGUCAdTdT	950
A-3035	UAAUCCAUGCAGGCCUUCAdTdT	951
A-3036	UGAAUCCAUGCAGGCCUUCdTdT	952
A-3037	UGAACAUGGAAUCCAUGCAdTdT	953
A-3038	UAUGAACAUGGAAUCCAUGdTdT	954
A-3039	UCUCAUGAACAUGGAAUCCdTdT	955
A-3040	UAAACUCAUGAACAUGGAAdTdT	956
A-3041	UAUCUCCAAACUCAUGAACdTdT	957
A-3042	UUAUCUCCAAACUCAUGAAdTdT	958
A-3043	UGUAUUAUCUCCAAACUCAdTdT	959
A-3044	UUGUAUUAUCUCCAAACUCdTdT	960
A-3045	UCCUGCACUGGUACAGCCUdTdT	961
A-3046	UACCUGCACUGGUACAGCCdTdT	962
A-3047	UAUUAAAGUGAGGACCUGCdTdT	963

A-3048	UGAUUAAAGUGAGGACCUGdTdT	964
A-3049	UGAUAGAGGAUUAAAGUGAdTdT	965
A-3050	UACCGUGUUUUCUGGAUAGdTdT	966
A-3051	UCACCGUGUUUUCUGGAUAdTdT	967
A-3052	UCCACCGUGUUUUCUGGAUdTdT	968
A-3053	UGCCCACCGUGUUUUCUGGdTdT	969
A-3054	UUUGGCCCACCGUGUUUUCdTdT	970
A-3055	UUUUGGCCCACCGUGUUUUdTdT	971
A-3056	UUCAUCCUUUGGCCCACCGdTdT	972
A-3057	UCAUGCCUCUCUUCAUCCUdTdT	973
A-3058	UCAACAUGCCUCUCUUCAUdTdT	974
A-3059	UGUCUCCAACAUGCCUCUCdTdT	975
A-3060	UAGUCUCCAACAUGCCUCUdTdT	976
A-3061	UUGCCCAAGUCUCCAACAUdTdT	977
A-3062	UAUUGCCCAAGUCUCCAACdTdT	978
A-3063	UCACAUUGCCCAAGUCUCCdTdT	979
A-3064	UGUCAGCAGUCACAUUGCCdTdT	980
A-3065	UUUGUCAGCAGUCACAUUGdTdT	981
A-3066	UCCACACCAUCUUUGUCAGdTdT	982
A-3067	UGCCACACCAUCUUUGUCAdTdT	983
A-3068	UAUGCAAUGGUCUCCUGAGdTdT	984
A-3069	UGAUGCAAUGGUCUCCUGAdTdT	985
A-3070	UCCAAUGAUGCAAUGGUCUdTdT	986
A-3071	UGCCAAUGAUGCAAUGGUCdTdT	987
A-3072	UUGCGGCCAAUGAUGCAAUdTdT	988
A-3073	UACCAGUGUGCGGCCAAUGdTdT	989
A-3074	UAUGGACCACCAGUGUGCGdTdT	990
A-3075	UUCAUGGACCACCAGUGUGdTdT	991
A-3076	UUUCAUGGACCACCAGUGUdTdT	992
A-3077	UCUUUUUCAUGGACCACCAdTdT	993
A-3078	UCUGCUUUUUCAUGGACCAdTdT	994
A-3079	UGCCCAAGUCAUCUGCUUUdTdT	995
A-3080	UUUUGCCCAAGUCAUCUGCdTdT	996
A-3081	UCACCUUUGCCCAAGUCAUdTdT	997
A-3082	UCCACCUUUGCCCAAGUCAdTdT	998
A-3083	UUCCACCUUUGCCCAAGUCdTdT	999
A-3084	UCGUUUCCUGUCUUUGUACdTdT	1000
A-3085	UAGCGUUUCCUGUCUUUGUdTdT	1001
A-3086	UCAGCGUUUCCUGUCUUUGdTdT	1002
A-3087	UCGACUUCCAGCGUUUCCUdTdT	1003
A-3088	UCACCACAAGCCAAACGACdTdT	1004
A-3089	UACACCACAAGCCAAACGAdTdT	1005
A-3090	UUACACCACAAGCCAAACGdTdT	1006
A-3091	UUUACACCACAAGCCAAACdTdT	1007
A-3092	UAAUUACACCACAAGCCAAdTdT	1008
A-3093	UCCAAUUACACCACAAGCCdTdT	1009
A-3094	UCCCAAUUACACCACAAGCdTdT	1010
A-3095	UUCCCAAUUACACCACAAGdTdT	1011
A-3096	UGAUCCCAAUUACACCACAdTdT	1012
A-3097	UCGAUCCCAAUUACACCACdTdT	1013
A-3098	UGCGAUCCCAAUUACACCAdTdT	1014
A-3099	UUUGGGCGAUCCCAAUUACdTdT	1015
A-3100	UAUUGGGCGAUCCCAAUUAdTdT	1016
A-3101	UUAUUGGGCGAUCCCAAUUdTdT	1017
A-3102	UUUAUUGGGCGAUCCCAAUdTdT	1018
A-3103	UUUUAUUGGGCGAUCCCAAdTdT	1019
A-3104	UGUUUAUUGGGCGAUCCCAdTdT	1020

A-3105	UUGUUUAUUGGGCGAUCCCdTdT	1021
A-3106	UGAAUGUUUAUUGGGCGAUdTdT	1022
A-3107	UCAAGGGAAUGUUUAUUGGdTdT	1023
A-3108	UCCAAGGGAAUGUUUAUUGdTdT	1024
A-3109	UUCCAAGGGAAUGUUUAUUdTdT	1025
A-3110	UAUCCAAGGGAAUGUUUAUdTdT	1026
A-3111	UCAUCCAAGGGAAUGUUUAdTdT	1027
A-3112	UACAUCCAAGGGAAUGUUUdTdT	1028
A-3113	UUACAUCCAAGGGAAUGUUdTdT	1029
A-3114	UGACUACAUCCAAGGGAAUdTdT	1030
A-3115	UCCUCAGACUACAUCCAAGdTdT	1031
A-3116	UUGAGUUAAGGGGCCUCAGdTdT	1032
A-3117	UGAUGAGUUAAGGGGCCUCdTdT	1033
A-3118	UAGAUGAGUUAAGGGGCCUdTdT	1034
A-3119	UAACAGAUGAGUUAAGGGGdTdT	1035
A-3120	UUAACAGAUGAGUUAAGGGdTdT	1036
A-3121	UAUAACAGAUGAGUUAAGGdTdT	1037
A-3122	UGAUAACAGAUGAGUUAAGdTdT	1038
A-3123	UGGAUAACAGAUGAGUUAAdTdT	1039
A-3124	UAGGAUAACAGAUGAGUUAdTdT	1040
A-3125	UCAGGAUAACAGAUGAGUUdTdT	1041
A-3126	UUACAGCUAGCAGGAUAACdTdT	1042
A-3127	UCAUUUCUACAGCUAGCAGdTdT	1043
A-3128	UACAUUUCUACAGCUAGCAdTdT	1044
A-3129	UAGGAUACAUUUCUACAGCdTdT	1045
A-3130	UCAGGAUACAUUUCUACAGdTdT	1046
A-3131	UUCAGGAUACAUUUCUACAdTdT	1047
A-3132	UAUCAGGAUACAUUUCUACdTdT	1048
A-3133	UGUUUAUCAGGAUACAUUUdTdT	1049
A-3134	UUAAUGUUUAUCAGGAUACdTdT	1050
A-3135	UUAAGAUUACAGUGUUUAAdTdT	1051
A-3136	UCACUUUUAAGAUUACAGUdTdT	1052
A-3137	UACACUUUUAAGAUUACAGdTdT	1053
A-3138	UUACACUUUUAAGAUUACAdTdT	1054
A-3139	UUUACACUUUUAAGAUUACdTdT	1055
A-3140	UCACAAUUACACUUUUAAGdTdT	1056
A-3141	UAGUUUCUCACUACAGGUAdTdT	1057
A-3142	UUCUUCCAAGUGAUCAUAAdTdT	1058
A-3143	UAAUCUUCCAAGUGAUCAUdTdT	1059
A-3144	UACAAAUCUUCCAAGUGAUdTdT	1060
A-3145	UAACUAUACAAAUCUUCCAdTdT	1061
A-3146	UUUUUAACUGAGUUUUAUAdTdT	1062
A-3147	UGACAUUUUAACUGAGUUUdTdT	1063
A-3148	UCAGGUCAUUGAAACAGACdTdT	1064
A-3149	UUGGCAAAAUACAGGUCAUdTdT	1065
A-3150	UGUCUGGCAAAAUACAGGUdTdT	1066
A-3151	UAGUCUGGCAAAAUACAGGdTdT	1067
A-3152	UAUACCCAUCUGUGAUUUAdTdT	1068
A-3153	UUUAAUACCCAUCUGUGAUdTdT	1069
A-3154	UUUUAAUACCCAUCUGUGAdTdT	1070
A-3155	UAGUUUAAUACCCAUCUGUdTdT	1071
A-3156	UAAGUUUAAUACCCAUCUGdTdT	1072
A-3157	UCAAGUUUAAUACCCAUCUdTdT	1073
A-3158	UGACAAGUUUAAUACCCAUdTdT	1074
A-3159	UGAAAUUCUGACAAGUUUAdTdT	1075
A-3160	UAUUCACAGGCUUGAAUGAdTdT	1076
A-3161	UUAUUCACAGGCUUGAAUGdTdT	1077

A-3162	UCCAUACAGGGUUUUUAUUdTdT	1078
A-3163	UGCCAUACAGGGUUUUUAUdTdT	1079
A-3164	UUAAGUGCCAUACAGGGUUdTdT	1080
A-3165	UAUAAGUGCCAUACAGGGUdTdT	1081
A-3166	UGAUUCUUUUAAUAGCCUCdTdT	1082
A-3167	UUUUGAAUUUGGAUUCUUUdTdT	1083
A-3168	UUAGUUUGAAUUUGGAUUCdTdT	1084

[00375] In one embodiment, the siRNA molecules of the present invention targeting SOD1 may comprise a nucleotide sequence such as, but not limited to, the sense (passenger) sequences in Table 3 or a fragment or variant thereof. As a non-limiting example, the sense sequence used in the siRNA molecule of the present invention is at least 30%, 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-99%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-99%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-99%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% of a nucleotide sequence in Table 3. As another non-limiting example, the sense sequence used in the siRNA molecule of the present invention comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or more than 21 consecutive nucleotides of a nucleotide sequence in Table 3. As yet another non-limiting example, the sense sequence used in the siRNA molecule of the present invention comprises nucleotides 1 to 22, 1 to 21, 1 to 20, 1 to 19, 1 to 18, 1 to 17, 1 to 16, 1 to 15, 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 2 to 22, 2 to 21, 2 to 20, 2 to 19, 2 to 18, 2 to 17, 2 to 16, 2 to 15, 2 to 14, 2 to 13, 2 to 12, 2 to 11, 2 to 10, 2 to 9, 2 to 8, 3 to 22, 3 to 21, 3 to 20, 3 to 19, 3 to 18, 3 to 17, 3 to 16, 3 to 15, 3 to 14, 3 to 13, 3 to 12, 3 to 11, 3 to 10, 3 to 9, 3 to 8, 4 to 22, 4 to 21, 4 to 20, 4 to 19, 4 to 18, 4 to 17, 4 to 16, 4 to 15, 4 to 14, 4 to 13, 4 to 12, 4 to 11, 4 to 10, 4 to 9, 4 to 8, 5 to 22, 5 to 21, 5 to 20, 5 to 19, 5 to 18, 5 to 17, 5 to 16, 5 to 15, 5 to 14, 5 to 13, 5 to 12, 5 to 11, 5 to 10, 5 to 9, 5 to 8, 6 to 22, 6 to 21, 6 to 20, 6 to 19, 6 to 18, 6 to 17, 6 to 16, 6 to 15, 6 to 14, 6 to 13, 6 to 12, 6 to 11, 6 to 10, 7 to 22, 7 to 21, 7 to 20, 7 to 19, 7 to 18, 7 to 17, 7 to 16, 7 to 15, 7 to 14, 7 to 13, 7 to 12, 8 to 22, 8 to 21, 8 to 20, 8 to 19, 8 to 18, 8 to 17, 8 to 16, 8 to 15, 8 to 14, 8 to 13, 8 to 12, 9 to 22, 9 to 21, 9 to 20, 9 to 19, 9 to 18, 9 to 17, 9 to 16, 9 to 15, 9 to 14, 10 to 22, 10 to 21, 10 to 20, 10 to 19, 10 to 18, 10 to 17, 10 to 16, 10 to 15, 10 to 14, 11 to 22, 11 to 21, 11 to 20, 11 to 19, 11 to 18, 11 to 17, 11 to 16, 11 to 15, 11 to 14, 12 to 22, 12 to 21, 12 to 20, 12 to 19, 12 to 18, 12 to 17, 12 to 16, 13 to 22, 13 to 21, 13 to 20, 13 to 19, 13 to 18, 13 to 17, 13 to 16, 14 to 22, 14 to 21, 14 to 20, 14 to 19, 14 to 18, 14 to

17, 15 to 22, 15 to 21, 15 to 20, 15 to 19, 15 to 18, 16 to 22, 16 to 21, 16 to 20, 17 to 22, 17 to 21, or 18 to 22 of the sequences in Table 3.

Table 3. Sense Sequences

Sense ID	Sequence	SEQ ID NO
S-3000	CGGAGGUCUGGCCUAUAACdTdT	1085
S-3001	GGAGGUCUGGCCUAUAAACdTdT	1086
S-3002	GAGGUCUGGCCUAUAAAGCdTdT	1087
S-3003	AGGUCUGGCCUAUAAAGUCdTdT	1088
S-3004	GGUCUGGCCUAUAAAGUACdTdT	1089
S-3005	UCUGGCCUAUAAAGUAGUCdTdT	1090
S-3006	CUGGCCUAUAAAGUAGUCCdTdT	1091
S-3007	UGGCCUAUAAAGUAGUCGCdTdT	1092
S-3008	GGCCUAUAAAGUAGUCGCCdTdT	1093
S-3009	GCCUAUAAAGUAGUCGCGCdTdT	1094
S-3010	CCUAUAAAGUAGUCGCGGCdTdT	1095
S-3011	GUCGUAGUCUCCUGCAGCCdTdT	1096
S-3012	CGUAGUCUCCUGCAGCGUCdTdT	1097
S-3013	GUAGUCUCCUGCAGCGUCCdTdT	1098
S-3014	UAGUCUCCUGCAGCGUCUCdTdT	1099
S-3015	AUGGCGACGAAGGCCGUGCdTdT	1100
S-3016	CGACGAAGGCCGUGUGCGCdTdT	1101
S-3017	GAAGGCCGUGUGCGUGCUCdTdT	1102
S-3018	GGCCGUGUGCGUGCUGAACdTdT	1103
S-3019	AGGGCGACGGCCCAGUGCCdTdT	1104
S-3020	UGCAGGGCAUCAUCAAUUCdTdT	1105
S-3021	GCAGGGCAUCAUCAAUUUCdTdT	1106
S-3022	AGGGCAUCAUCAAUUUCGCdTdT	1107
S-3023	GGGCAUCAUCAAUUUCGACdTdT	1108
S-3024	GGCAUCAUCAAUUUCGAGCdTdT	1109
S-3025	GCAUCAUCAAUUUCGAGCCdTdT	1110
S-3026	CAUCAUCAAUUUCGAGCACdTdT	1111
S-3027	AAUUUCGAGCAGAAGGAACdTdT	1112
S-3028	UUCGAGCAGAAGGAAAGUCdTdT	1113
S-3029	UCGAGCAGAAGGAAAGUACdTdT	1114
S-3030	AAGGUGUGGGGAAGCAUUCdTdT	1115
S-3031	GGUGUGGGGAAGCAUUAACdTdT	1116
S-3032	GACUGACUGAAGGCCUGCCdTdT	1117
S-3033	CUGACUGAAGGCCUGCAUCdTdT	1118
S-3034	UGACUGAAGGCCUGCAUGCdTdT	1119
S-3035	UGAAGGCCUGCAUGGAUUCdTdT	1120
S-3036	GAAGGCCUGCAUGGAUUCCdTdT	1121
S-3037	UGCAUGGAUUCCAUGUUCCdTdT	1122
S-3038	CAUGGAUUCCAUGUUCAUCdTdT	1123
S-3039	GGAUUCCAUGUUCAUGAGCdTdT	1124
S-3040	UUCCAUGUUCAUGAGUUUCdTdT	1125
S-3041	GUUCAUGAGUUUGGAGAUCdTdT	1126
S-3042	UUCAUGAGUUUGGAGAUACdTdT	1127
S-3043	UGAGUUUGGAGAUAAUACCdTdT	1128
S-3044	GAGUUUGGAGAUAAUACACdTdT	1129
S-3045	AGGCUGUACCAGUGCAGGCdTdT	1130
S-3046	GGCUGUACCAGUGCAGGUCdTdT	1131
S-3047	GCAGGUCCUCACUUUAAUCdTdT	1132
S-3048	CAGGUCCUCACUUUAAUCCdTdT	1133
S-3049	UCACUUUAAUCCUCUAUCCdTdT	1134
S-3050	CUAUCCAGAAAACACGGUCdTdT	1135

S-3051	UAUCCAGAAAACACGGUGCdTdT	1136
S-3052	AUCCAGAAAACACGGUGGCdTdT	1137
S-3053	CCAGAAAACACGGUGGGCCdTdT	1138
S-3054	GAAAACACGGUGGGCCAACdTdT	1139
S-3055	AAAACACGGUGGGCCAAACdTdT	1140
S-3056	CGGUGGGCCAAAGGAUGACdTdT	1141
S-3057	AGGAUGAAGAGAGGCAUGCdTdT	1142
S-3058	AUGAAGAGAGGCAUGUUGCdTdT	1143
S-3059	GAGAGGCAUGUUGGAGACCdTdT	1144
S-3060	AGAGGCAUGUUGGAGACUCdTdT	1145
S-3061	AUGUUGGAGACUUGGGCACdTdT	1146
S-3062	GUUGGAGACUUGGGCAAUCdTdT	1147
S-3063	GGAGACUUGGGCAAUGUGCdTdT	1148
S-3064	GGCAAUGUGACUGCUGACCdTdT	1149
S-3065	CAAUGUGACUGCUGACAACdTdT	1150
S-3066	CUGACAAAGAUGGUGUGGCdTdT	1151
S-3067	UGACAAAGAUGGUGUGGCCdTdT	1152
S-3068	CUCAGGAGACCAUUGCAUCdTdT	1153
S-3069	UCAGGAGACCAUUGCAUCCdTdT	1154
S-3070	AGACCAUUGCAUCAUUGGCdTdT	1155
S-3071	GACCAUUGCAUCAUUGGCCdTdT	1156
S-3072	AUUGCAUCAUUGGCCGCACdTdT	1157
S-3073	CAUUGGCCGCACACUGGUCdTdT	1158
S-3074	CGCACACUGGUGGUCCAUCdTdT	1159
S-3075	CACACUGGUGGUCCAUGACdTdT	1160
S-3076	ACACUGGUGGUCCAUGAACdTdT	1161
S-3077	UGGUGGUCCAUGAAAAAGCdTdT	1162
S-3078	UGGUCCAUGAAAAAGCAGCdTdT	1163
S-3079	AAAGCAGAUGACUUGGGCCdTdT	1164
S-3080	GCAGAUGACUUGGGCAAACdTdT	1165
S-3081	AUGACUUGGGCAAAGGUGCdTdT	1166
S-3082	UGACUUGGGCAAAGGUGGCdTdT	1167
S-3083	GACUUGGGCAAAGGUGGACdTdT	1168
S-3084	GUACAAAGACAGGAAACGCdTdT	1169
S-3085	ACAAAGACAGGAAACGCUCdTdT	1170
S-3086	CAAAGACAGGAAACGCUGCdTdT	1171
S-3087	AGGAAACGCUGGAAGUCGCdTdT	1172
S-3088	GUCGUUUGGCUUGUGGUGCdTdT	1173
S-3089	UCGUUUGGCUUGUGGUGUCdTdT	1174
S-3090	CGUUUGGCUUGUGGUGUACdTdT	1175
S-3091	GUUUGGCUUGUGGUGUAACdTdT	1176
S-3092	UUGGCUUGUGGUGUAAUUCdTdT	1177
S-3093	GGCUUGUGGUGUAAUUGGCdTdT	1178
S-3094	GCUUGUGGUGUAAUUGGGCdTdT	1179
S-3095	CUUGUGGUGUAAUUGGGACdTdT	1180
S-3096	UGUGGUGUAAUUGGGAUCCdTdT	1181
S-3097	GUGGUGUAAUUGGGAUCGCdTdT	1182
S-3098	UGGUGUAAUUGGGAUCGCCdTdT	1183
S-3099	GUAAUUGGGAUCGCCCAACdTdT	1184
S-3100	UAAUUGGGAUCGCCCAAUCdTdT	1185
S-3101	AAUUGGGAUCGCCCAAUACdTdT	1186
S-3102	AUUGGGAUCGCCCAAUAACdTdT	1187
S-3103	UUGGGAUCGCCCAAUAAACdTdT	1188
S-3104	UGGGAUCGCCCAAUAAACCdTdT	1189
S-3105	GGGAUCGCCCAAUAAACACdTdT	1190
S-3106	AUCGCCCAAUAAACAUUCCdTdT	1191
S-3107	CCAAUAAACAUUCCCUUGCdTdT	1192

S-3108	CAAUAAACAUUCCCUUGGCdTdT	1193
S-3109	AAUAAACAUUCCCUUGGACdTdT	1194
S-3110	AUAAACAUUCCCUUGGAUCdTdT	1195
S-3111	UAAACAUUCCCUUGGAUGCdTdT	1196
S-3112	AAACAUUCCCUUGGAUGUCdTdT	1197
S-3113	AACAUUCCCUUGGAUGUACdTdT	1198
S-3114	AUUCCCUUGGAUGUAGUCCdTdT	1199
S-3115	CUUGGAUGUAGUCUGAGGCdTdT	1200
S-3116	CUGAGGCCCCUUAACUCACdTdT	1201
S-3117	GAGGCCCCUUAACUCAUCCdTdT	1202
S-3118	AGGCCCCUUAACUCAUCUCdTdT	1203
S-3119	CCCCUUAACUCAUCUGUUCdTdT	1204
S-3120	CCCUUAACUCAUCUGUUACdTdT	1205
S-3121	CCUUAACUCAUCUGUUAUCdTdT	1206
S-3122	CUUAACUCAUCUGUUAUCCdTdT	1207
S-3123	UUAACUCAUCUGUUAUCCCdTdT	1208
S-3124	UAACUCAUCUGUUAUCCUCdTdT	1209
S-3125	AACUCAUCUGUUAUCCUGCdTdT	1210
S-3126	GUUAUCCUGCUAGCUGUACdTdT	1211
S-3127	CUGCUAGCUGUAGAAAUGCdTdT	1212
S-3128	UGCUAGCUGUAGAAAUGUCdTdT	1213
S-3129	GCUGUAGAAAUGUAUCCUCdTdT	1214
S-3130	CUGUAGAAAUGUAUCCUGCdTdT	1215
S-3131	UGUAGAAAUGUAUCCUGACdTdT	1216
S-3132	GUAGAAAUGUAUCCUGAUCdTdT	1217
S-3133	AAAUGUAUCCUGAUAAACCdTdT	1218
S-3134	GUAUCCUGAUAAACAUUACdTdT	1219
S-3135	UUAAACACUGUAAUCUUACdTdT	1220
S-3136	ACUGUAAUCUUAAAAGUGCdTdT	1221
S-3137	CUGUAAUCUUAAAAGUGUCdTdT	1222
S-3138	UGUAAUCUUAAAAGUGUACdTdT	1223
S-3139	GUAAUCUUAAAAGUGUAACdTdT	1224
S-3140	CUUAAAAGUGUAAUUGUGCdTdT	1225
S-3141	UACCUGUAGUGAGAAACUCdTdT	1226
S-3142	UUAUGAUCACUUGGAAGACdTdT	1227
S-3143	AUGAUCACUUGGAAGAUUCdTdT	1228
S-3144	AUCACUUGGAAGAUUUGUCdTdT	1229
S-3145	UGGAAGAUUUGUAUAGUUCdTdT	1230
S-3146	UAUAAAACUCAGUUAAAACdTdT	1231
S-3147	AAACUCAGUUAAAAUGUCCdTdT	1232
S-3148	GUCUGUUUCAAUGACCUGCdTdT	1233
S-3149	AUGACCUGUAUUUUGCCACdTdT	1234
S-3150	ACCUGUAUUUUGCCAGACCdTdT	1235
S-3151	CCUGUAUUUUGCCAGACUCdTdT	1236
S-3152	UAAAUCACAGAUGGGUAUCdTdT	1237
S-3153	AUCACAGAUGGGUAUUAACdTdT	1238
S-3154	UCACAGAUGGGUAUUAAACdTdT	1239
S-3155	ACAGAUGGGUAUUAAACUCdTdT	1240
S-3156	CAGAUGGGUAUUAAACUUCdTdT	1241
S-3157	AGAUGGGUAUUAAACUUGCdTdT	1242
S-3158	AUGGGUAUUAAACUUGUCCdTdT	1243
S-3159	UAAACUUGUCAGAAUUUCCdTdT	1244
S-3160	UCAUUCAAGCCUGUGAAUCdTdT	1245
S-3161	CAUUCAAGCCUGUGAAUACdTdT	1246
S-3162	AAUAAAAACCCUGUAUGGCdTdT	1247
S-3163	AUAAAAACCCUGUAUGGCCdTdT	1248
S-3164	AACCCUGUAUGGCACUUACdTdT	1249

S-3165	ACCCUGUAUGGCACUUAUCdTdT	1250
S-3166	GAGGCUAUUAAAAGAAUCCdTdT	1251
S-3167	AAAGAAUCCAAAUUCAAACdTdT	1252
S-3168	GAAUCCAAAUUCAAACUACdTdT	1253

[00376] In one embodiment, the siRNA molecules of the present invention targeting SOD1 may comprise an antisense sequence from Table 2 and a sense sequence from Table 3, or a fragment or variant thereof. As a non-limiting example, the antisense sequence and the sense sequence have at least 30%, 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-99%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-99%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-99%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementarity.

[00377] In one embodiment, the siRNA molecules of the present invention targeting SOD1 may comprise the sense and antisense siRNA duplex as described in Table 4. As a non-limiting example, these siRNA duplexes may be tested for *in vitro* inhibitory activity on endogenous SOD1 gene expression. The start site for the sense and antisense sequence is compared to SOD1 gene sequence known as NM_000454.4 (SEQ ID NO: 1254) from NCBI.

Table 4. Sense and antisense strand sequences of SOD1 dsRNA

siRNA Duplex ID	SS ID	Sense Strand Sequence (5'-3')	SS SEQ ID	AS ID	Antisense Strand Sequence (5'-3')	AS SEQ ID
D-2741	S-3000	CGGAGGUCUGGC CUAUAACdTdT	1085	A-3000	UUUAUAGGCCA GACCUCCGdTdT	916
D-2742	S-3001	GGAGGUCUGGCC UAUAAACdTdT	1086	A-3001	UUUUAUAGGCC AGACCUCCdTdT	917
D-2743	S-3002	GAGGUCUGGCCU AUAAAGCdTdT	1087	A-3002	UCUUUAUAGGC CAGACCUCdTdT	918
D-2744	S-3003	AGGUCUGGCCUA UAAAGUCdTdT	1088	A-3003	UACUUUAUAGG CCAGACCUdTdT	919
D-2745	S-3004	GGUCUGGCCUAU AAAGUACdTdT	1089	A-3004	UUACUUUAUAG GCCAGACCdTdT	920
D-2746	S-3005	UCUGGCCUAUAA AGUAGUCdTdT	1090	A-3005	UACUACUUUAU AGGCCAGAdTdT	921
D-2747	S-3006	CUGGCCUAUAAA GUAGUCCdTdT	1091	A-3006	UGACUACUUUA UAGGCCAGdTdT	922
D-2748	S-3007	UGGCCUAUAAAG UAGUCGCdTdT	1092	A-3007	UCGACUACUUU AUAGGCCAdTdT	923
D-2749	S-3008	GGCCUAUAAAGU AGUCGCCdTdT	1093	A-3008	UGCGACUACUU UAUAGGCCdTdT	924
D-2750	S-3009	GCCUAUAAAGUA GUCGCGCdTdT	1094	A-3009	UCGCGACUACU UUAUAGGCdTdT	925
D-2751	S-3010	CCUAUAAAGUAG UCGCGGCdTdT	1095	A-3010	UCCGCGACUAC UUUAUAGGdTdT	926

D-2752	S-3011	GUCGUAGUCUCC UGCAGCCdTdT	1096	A-3011	UGCUGCAGGAG ACUACGACdTdT	927
D-2753	S-3012	CGUAGUCUCCUG CAGCGUCdTdT	1097	A-3012	UACGCUGCAGG AGACUACGdTdT	928
D-2754	S-3013	GUAGUCUCCUGC AGCGUCCdTdT	1098	A-3013	UGACGCUGCAG GAGACUACdTdT	929
D-2755	S-3014	UAGUCUCCUGCA GCGUCUCdTdT	1099	A-3014	UAGACGCUGCA GGAGACUAdTdT	930
D-2756	S-3015	AUGGCGACGAAG GCCGUGCdTdT	1100	A-3015	UCACGGCCUUC GUCGCCAUdTdT	931
D-2757	S-3016	CGACGAAGGCCG UGUGCGCdTdT	1101	A-3016	UCGCACACGGCC UUCGUCGdTdT	932
D-2758	S-3017	GAAGGCCGUGUG CGUGCUCdTdT	1102	A-3017	UAGCACGCACA CGGCCUUCdTdT	933
D-2759	S-3018	GGCCGUGUGCGU GCUGAACdTdT	1103	A-3018	UUUCAGCACGC ACACGGCCdTdT	934
D-2760	S-3019	AGGGCGACGGCC CAGUGCCdTdT	1104	A-3019	UGCACUGGGCC GUCGCCCUdTdT	935
D-2761	S-3020	UGCAGGGCAUCA UCAAUUCdTdT	1105	A-3020	UAAUUGAUGAU GCCCUGCAdTdT	936
D-2762	S-3021	GCAGGGCAUCAU CAAUUUCdTdT	1106	A-3021	UAAAUUGAUGA UGCCCUGCdTdT	937
D-2763	S-3022	AGGGCAUCAUCA AUUUCGCdTdT	1107	A-3022	UCGAAAUUGAU GAUGCCCUdTdT	938
D-2764	S-3023	GGGCAUCAUCAA UUUCGACdTdT	1108	A-3023	UUCGAAAUUGA UGAUGCCCdTdT	939
D-2765	S-3024	GGCAUCAUCAAU UUCGAGCdTdT	1109	A-3024	UCUCGAAAUUG AUGAUGCCdTdT	940
D-2766	S-3025	GCAUCAUCAAUU UCGAGCCdTdT	1110	A-3025	UGCUCGAAAUU GAUGAUGCdTdT	941
D-2767	S-3026	CAUCAUCAAUUU CGAGCACdTdT	1111	A-3026	UUGCUCGAAAU UGAUGAUGdTdT	942
D-2768	S-3027	AAUUUCGAGCAG AAGGAACdTdT	1112	A-3027	UUUCCUUCUGC UCGAAAUUdTdT	943
D-2769	S-3028	UUCGAGCAGAAG GAAAGUCdTdT	1113	A-3028	UACUUUCCUUC UGCUCGAAdTdT	944
D-2770	S-3029	UCGAGCAGAAGG AAAGUACdTdT	1114	A-3029	UUACUUUCCUU CUGCUCGAdTdT	945
D-2771	S-3030	AAGGUGUGGGGA AGCAUUCdTdT	1115	A-3030	UAAUGCUUCCC CACACCUUdTdT	946
D-2772	S-3031	GGUGUGGGGAAG CAUUAACdTdT	1116	A-3031	UUUAAUGCUUC CCCACACCdTdT	947
D-2773	S-3032	GACUGACUGAAG GCCUGCCdTdT	1117	A-3032	UGCAGGCCUUC AGUCAGUCdTdT	948
D-2774	S-3033	CUGACUGAAGGC CUGCAUCdTdT	1118	A-3033	UAUGCAGGCCU UCAGUCAGdTdT	949
D-2775	S-3034	UGACUGAAGGCC UGCAUGCdTdT	1119	A-3034	UCAUGCAGGCC UUCAGUCAdTdT	950
D-2776	S-3035	UGAAGGCCUGCA UGGAUUCdTdT	1120	A-3035	UAAUCCAUGCA GGCCUUCAdTdT	951
D-2777	S-3036	GAAGGCCUGCAU GGAUUCCdTdT	1121	A-3036	UGAAUCCAUGC AGGCCUUCdTdT	952
D-2778	S-3037	UGCAUGGAUUCC AUGUUCCdTdT	1122	A-3037	UGAACAUGGAA UCCAUGCAdTdT	953
D-2779	S-3038	CAUGGAUUCCAU GUUCAUCdTdT	1123	A-3038	UAUGAACAUGG AAUCCAUGdTdT	954
D-2780	S-3039	GGAUUCCAUGUU CAUGAGCdTdT	1124	A-3039	UCUCAUGAACA UGGAAUCCdTdT	955

D-2781	S-3040	UUCCAUGUUCAU GAGUUUCdTdT	1125	A-3040	UAAACUCAUGA ACAUGGAAdTdT	956
D-2782	S-3041	GUUCAUGAGUUU GGAGAUCdTdT	1126	A-3041	UAUCUCCAAAC UCAUGAACdTdT	957
D-2783	S-3042	UUCAUGAGUUUG GAGAUACdTdT	1127	A-3042	UUAUCUCCAAA CUCAUGAAdTdT	958
D-2784	S-3043	UGAGUUUGGAGA UAAUACCdTdT	1128	A-3043	UGUAUUAUCUC CAAACUCAdTdT	959
D-2785	S-3044	GAGUUUGGAGAU AAUACACdTdT	1129	A-3044	UUGUAUUAUCU CCAAACUCdTdT	960
D-2786	S-3045	AGGCUGUACCAG UGCAGGCdTdT	1130	A-3045	UCCUGCACUGG UACAGCCUdTdT	961
D-2787	S-3046	GGCUGUACCAGU GCAGGUCdTdT	1131	A-3046	UACCUGCACUG GUACAGCCdTdT	962
D-2788	S-3047	GCAGGUCCUCAC UUUAAUCdTdT	1132	A-3047	UAUUAAAGUGA GGACCUGCdTdT	963
D-2789	S-3048	CAGGUCCUCACU UUAAUCCdTdT	1133	A-3048	UGAUUAAAGUG AGGACCUGdTdT	964
D-2790	S-3049	UCACUUUAAUCC UCUAUCCdTdT	1134	A-3049	UGAUAGAGGAU UAAAGUGAdTdT	965
D-2791	S-3050	CUAUCCAGAAAA CACGGUCdTdT	1135	A-3050	UACCGUGUUUU CUGGAUAGdTdT	966
D-2792	S-3051	UAUCCAGAAAAC ACGGUGCdTdT	1136	A-3051	UCACCGUGUUU UCUGGAUAdTdT	967
D-2793	S-3052	AUCCAGAAAACA CGGUGGCdTdT	1137	A-3052	UCCACCGUGUU UUCUGGAUdTdT	968
D-2794	S-3053	CCAGAAAACACG GUGGGCCdTdT	1138	A-3053	UGCCCACCGUG UUUUCUGGdTdT	969
D-2795	S-3054	GAAAACACGGUG GGCCAACdTdT	1139	A-3054	UUUGGCCCACC GUGUUUUCdTdT	970
D-2796	S-3055	AAAACACGGUGG GCCAAACdTdT	1140	A-3055	UUUUGGCCCAC CGUGUUUUdTdT	971
D-2797	S-3056	CGGUGGGCCAAA GGAUGACdTdT	1141	A-3056	UUCAUCCUUUG GCCCACCGdTdT	972
D-2798	S-3057	AGGAUGAAGAGA GGCAUGCdTdT	1142	A-3057	UCAUGCCUCUC UUCAUCCUdTdT	973
D-2799	S-3058	AUGAAGAGAGGC AUGUUGCdTdT	1143	A-3058	UCAACAUGCCU CUCUUCAUdTdT	974
D-2800	S-3059	GAGAGGCAUGUU GGAGACCdTdT	1144	A-3059	UGUCUCCAACA UGCCUCUCdTdT	975
D-2801	S-3060	AGAGGCAUGUUG GAGACUCdTdT	1145	A-3060	UAGUCUCCAAC AUGCCUCUdTdT	976
D-2802	S-3061	AUGUUGGAGACU UGGGCACdTdT	1146	A-3061	UUGCCCAAGUC UCCAACAUdTdT	977
D-2803	S-3062	GUUGGAGACUUG GGCAAUCdTdT	1147	A-3062	UAUUGCCCAAG UCUCCAACdTdT	978
D-2804	S-3063	GGAGACUUGGGC AAUGUGCdTdT	1148	A-3063	UCACAUUGCCC AAGUCUCCdTdT	979
D-2805	S-3064	GGCAAUGUGACU GCUGACCdTdT	1149	A-3064	UGUCAGCAGUC ACAUUGCCdTdT	980
D-2806	S-3065	CAAUGUGACUGC UGACAACdTdT	1150	A-3065	UUUGUCAGCAG UCACAUUGdTdT	981
D-2807	S-3066	CUGACAAAGAUG GUGUGGCdTdT	1151	A-3066	UCCACACCAUCU UUGUCAGdTdT	982
D-2808	S-3067	UGACAAAGAUGG UGUGGCCdTdT	1152	A-3067	UGCCACACCAUC UUUGUCAdTdT	983
D-2809	S-3068	CUCAGGAGACCA UUGCAUCdTdT	1153	A-3068	UAUGCAAUGGU CUCCUGAGdTdT	984

D-2810	S-3069	UCAGGAGACCAU UGCAUCCdTdT	1154	A-3069	UGAUGCAAUGG UCUCCUGAdTdT	985
D-2811	S-3070	AGACCAUUGCAU CAUUGGCdTdT	1155	A-3070	UCCAAUGAUGC AAUGGUCUdTdT	986
D-2812	S-3071	GACCAUUGCAUC AUUGGCCdTdT	1156	A-3071	UGCCAAUGAUG CAAUGGUCdTdT	987
D-2813	S-3072	AUUGCAUCAUUG GCCGCACdTdT	1157	A-3072	UUGCGGCCAAU GAUGCAAUdTdT	988
D-2814	S-3073	CAUUGGCCGCAC ACUGGUCdTdT	1158	A-3073	UACCAGUGUGC GGCCAAUGdTdT	989
D-2815	S-3074	CGCACACUGGUG GUCCAUCdTdT	1159	A-3074	UAUGGACCACC AGUGUGCGdTdT	990
D-2816	S-3075	CACACUGGUGGU CCAUGACdTdT	1160	A-3075	UUCAUGGACCA CCAGUGUGdTdT	991
D-2817	S-3076	ACACUGGUGGUC CAUGAACdTdT	1161	A-3076	UUUCAUGGACC ACCAGUGUdTdT	992
D-2818	S-3077	UGGUGGUCCAUG AAAAAGCdTdT	1162	A-3077	UCUUUUUCAUG GACCACCAdTdT	993
D-2819	S-3078	UGGUCCAUGAAA AAGCAGCdTdT	1163	A-3078	UCUGCUUUUUC AUGGACCAdTdT	994
D-2820	S-3079	AAAGCAGAUGAC UUGGGCCdTdT	1164	A-3079	UGCCCAAGUCA UCUGCUUUdTdT	995
D-2821	S-3080	GCAGAUGACUUG GGCAAACdTdT	1165	A-3080	UUUUGCCCAAG UCAUCUGCdTdT	996
D-2822	S-3081	AUGACUUGGGCA AAGGUGCdTdT	1166	A-3081	UCACCUUUGCCC AAGUCAUdTdT	997
D-2823	S-3082	UGACUUGGGCAA AGGUGGCdTdT	1167	A-3082	UCCACCUUUGCC CAAGUCAdTdT	998
D-2824	S-3083	GACUUGGGCAAA GGUGGACdTdT	1168	A-3083	UUCCACCUUUG CCCAAGUCdTdT	999
D-2825	S-3084	GUACAAAGACAG GAAACGCdTdT	1169	A-3084	UCGUUUCCUGU CUUUGUACdTdT	1000
D-2826	S-3085	ACAAAGACAGGA AACGCUCdTdT	1170	A-3085	UAGCGUUUCCU GUCUUUGUdTdT	1001
D-2827	S-3086	CAAAGACAGGAA ACGCUGCdTdT	1171	A-3086	UCAGCGUUUCC UGUCUUUGdTdT	1002
D-2828	S-3087	AGGAAACGCUGG AAGUCGCdTdT	1172	A-3087	UCGACUUCCAG CGUUUCCUdTdT	1003
D-2829	S-3088	GUCGUUUGGCUU GUGGUGCdTdT	1173	A-3088	UCACCACAAGCC AAACGACdTdT	1004
D-2830	S-3089	UCGUUUGGCUUG UGGUGUCdTdT	1174	A-3089	UACACCACAAG CCAAACGAdTdT	1005
D-2831	S-3090	CGUUUGGCUUGU GGUGUACdTdT	1175	A-3090	UUACACCACAA GCCAAACGdTdT	1006
D-2832	S-3091	GUUUGGCUUGUG GUGUAACdTdT	1176	A-3091	UUUACACCACA AGCCAAACdTdT	1007
D-2833	S-3092	UUGGCUUGUGGU GUAAUUCdTdT	1177	A-3092	UAAUUACACCA CAAGCCAAdTdT	1008
D-2834	S-3093	GGCUUGUGGUGU AAUUGGCdTdT	1178	A-3093	UCCAAUUACAC CACAAGCCdTdT	1009
D-2835	S-3094	GCUUGUGGUGUA AUUGGGCdTdT	1179	A-3094	UCCCAAUUACA CCACAAGCdTdT	1010
D-2836	S-3095	CUUGUGGUGUAA UUGGGACdTdT	1180	A-3095	UUCCCAAUUAC ACCACAAGdTdT	1011
D-2837	S-3096	UGUGGUGUAAUU GGGAUCCdTdT	1181	A-3096	UGAUCCCAAUU ACACCACAdTdT	1012
D-2838	S-3097	GUGGUGUAAUUG GGAUCGCdTdT	1182	A-3097	UCGAUCCCAAU UACACCACdTdT	1013

D-2839	S-3098	UGGUGUAAUUGG GAUCGCCdTdT	1183	A-3098	UGCGAUCCCAA UUACACCAdTdT	1014
D-2840	S-3099	GUAAUUGGGAUC GCCCAACdTdT	1184	A-3099	UUUGGGCGAUC CCAAUUACdTdT	1015
D-2841	S-3100	UAAUUGGGAUCG CCCAAUCdTdT	1185	A-3100	UAUUGGGCGAU CCCAAUUAdTdT	1016
D-2842	S-3101	AAUUGGGAUCGC CCAAUACdTdT	1186	A-3101	UUAUUGGGCGA UCCCAAUUdTdT	1017
D-2843	S-3102	AUUGGGAUCGCC CAAUAACdTdT	1187	A-3102	UUUAUUGGGCG AUCCCAAUdTdT	1018
D-2844	S-3103	UUGGGAUCGCCC AAUAAACdTdT	1188	A-3103	UUUUAUUGGGC GAUCCCAAdTdT	1019
D-2845	S-3104	UGGGAUCGCCCA AUAAACCdTdT	1189	A-3104	UGUUUAUUGGG CGAUCCCAdTdT	1020
D-2846	S-3105	GGGAUCGCCCAA UAAACACdTdT	1190	A-3105	UUGUUUAUUGG GCGAUCCCdTdT	1021
D-2847	S-3106	AUCGCCCAAUAA ACAUUCCdTdT	1191	A-3106	UGAAUGUUUAU UGGGCGAUdTdT	1022
D-2848	S-3107	CCAAUAAACAUU CCCUUGCdTdT	1192	A-3107	UCAAGGGAAUG UUUAUUGGdTdT	1023
D-2849	S-3108	CAAUAAACAUUC CCUUGGCdTdT	1193	A-3108	UCCAAGGGAAU GUUUAUUGdTdT	1024
D-2850	S-3109	AAUAAACAUUCC CUUGGACdTdT	1194	A-3109	UUCCAAGGGAA UGUUUAUUdTdT	1025
D-2851	S-3110	AUAAACAUUCCC UUGGAUCdTdT	1195	A-3110	UAUCCAAGGGA AUGUUUAUdTdT	1026
D-2852	S-3111	UAAACAUUCCCU UGGAUGCdTdT	1196	A-3111	UCAUCCAAGGG AAUGUUUAdTdT	1027
D-2853	S-3112	AAACAUUCCCUU GGAUGUCdTdT	1197	A-3112	UACAUCCAAGG GAAUGUUUdTdT	1028
D-2854	S-3113	AACAUUCCCUUG GAUGUACdTdT	1198	A-3113	UUACAUCCAAG GGAAUGUUdTdT	1029
D-2855	S-3114	AUUCCCUUGGAU GUAGUCCdTdT	1199	A-3114	UGACUACAUCC AAGGGAAUdTdT	1030
D-2856	S-3115	CUUGGAUGUAGU CUGAGGCdTdT	1200	A-3115	UCCUCAGACUA CAUCCAAGdTdT	1031
D-2857	S-3116	CUGAGGCCCCUU AACUCACdTdT	1201	A-3116	UUGAGUUAAGG GGCCUCAGdTdT	1032
D-2858	S-3117	GAGGCCCCUUAA CUCAUCCdTdT	1202	A-3117	UGAUGAGUUAA GGGGCCUCdTdT	1033
D-2859	S-3118	AGGCCCCUUAAC UCAUCUCdTdT	1203	A-3118	UAGAUGAGUUA AGGGGCCUdTdT	1034
D-2860	S-3119	CCCCUUAACUCA UCUGUUCdTdT	1204	A-3119	UAACAGAUGAG UUAAGGGGdTdT	1035
D-2861	S-3120	CCCUUAACUCAU CUGUUACdTdT	1205	A-3120	UUAACAGAUGA GUUAAGGGdTdT	1036
D-2862	S-3121	CCUUAACUCAUC UGUUAUCdTdT	1206	A-3121	UAUAACAGAUG AGUUAAGGdTdT	1037
D-2863	S-3122	CUUAACUCAUCU GUUAUCCdTdT	1207	A-3122	UGAUAACAGAU GAGUUAAGdTdT	1038
D-2864	S-3123	UUAACUCAUCUG UUAUCCCdTdT	1208	A-3123	UGGAUAACAGA UGAGUUAAdTdT	1039
D-2865	S-3124	UAACUCAUCUGU UAUCCUCdTdT	1209	A-3124	UAGGAUAACAG AUGAGUUAdTdT	1040
D-2866	S-3125	AACUCAUCUGUU AUCCUGCdTdT	1210	A-3125	UCAGGAUAACA GAUGAGUUdTdT	1041
D-2867	S-3126	GUUAUCCUGCUA GCUGUACdTdT	1211	A-3126	UUACAGCUAGC AGGAUAACdTdT	1042

D-2868	S-3127	CUGCUAGCUGUA GAAAUGCdTdT	1212	A-3127	UCAUUUCUACA GCUAGCAGdTdT	1043
D-2869	S-3128	UGCUAGCUGUAG AAAUGUCdTdT	1213	A-3128	UACAUUUCUAC AGCUAGCAdTdT	1044
D-2870	S-3129	GCUGUAGAAAUG UAUCCUCdTdT	1214	A-3129	UAGGAUACAUU UCUACAGCdTdT	1045
D-2871	S-3130	CUGUAGAAAUGU AUCCUGCdTdT	1215	A-3130	UCAGGAUACAU UUCUACAGdTdT	1046
D-2872	S-3131	UGUAGAAAUGUA UCCUGACdTdT	1216	A-3131	UUCAGGAUACA UUUCUACAdTdT	1047
D-2873	S-3132	GUAGAAAUGUAU CCUGAUCdTdT	1217	A-3132	UAUCAGGAUAC AUUUCUACdTdT	1048
D-2874	S-3133	AAAUGUAUCCUG AUAAACCdTdT	1218	A-3133	UGUUUAUCAGG AUACAUUUdTdT	1049
D-2875	S-3134	GUAUCCUGAUAA ACAUUACdTdT	1219	A-3134	UUAAUGUUUAU CAGGAUACdTdT	1050
D-2876	S-3135	UUAAACACUGUA AUCUUACdTdT	1220	A-3135	UUAAGAUUACA GUGUUUAAdTdT	1051
D-2877	S-3136	ACUGUAAUCUUA AAAGUGCdTdT	1221	A-3136	UCACUUUUAAG AUUACAGUdTdT	1052
D-2878	S-3137	CUGUAAUCUUAA AAGUGUCdTdT	1222	A-3137	UACACUUUUAA GAUUACAGdTdT	1053
D-2879	S-3138	UGUAAUCUUAAA AGUGUACdTdT	1223	A-3138	UUACACUUUUA AGAUUACAdTdT	1054
D-2880	S-3139	GUAAUCUUAAAA GUGUAACdTdT	1224	A-3139	UUUACACUUUU AAGAUUACdTdT	1055
D-2881	S-3140	CUUAAAAGUGUA AUUGUGCdTdT	1225	A-3140	UCACAAUUACA CUUUUAAGdTdT	1056
D-2882	S-3141	UACCUGUAGUGA GAAACUCdTdT	1226	A-3141	UAGUUUCUCAC UACAGGUAdTdT	1057
D-2883	S-3142	UUAUGAUCACUU GGAAGACdTdT	1227	A-3142	UUCUUCCAAGU GAUCAUAAdTdT	1058
D-2884	S-3143	AUGAUCACUUGG AAGAUUCdTdT	1228	A-3143	UAAUCUUCCAA GUGAUCAUdTdT	1059
D-2885	S-3144	AUCACUUGGAAG AUUUGUCdTdT	1229	A-3144	UACAAAUCUUC CAAGUGAUdTdT	1060
D-2886	S-3145	UGGAAGAUUUGU AUAGUUCdTdT	1230	A-3145	UAACUAUACAA AUCUUCCAdTdT	1061
D-2887	S-3146	UAUAAAACUCAG UUAAAACdTdT	1231	A-3146	UUUUUAACUGA GUUUUAUAdTdT	1062
D-2888	S-3147	AAACUCAGUUAA AAUGUCCdTdT	1232	A-3147	UGACAUUUUAA CUGAGUUUdTdT	1063
D-2889	S-3148	GUCUGUUUCAAU GACCUGCdTdT	1233	A-3148	UCAGGUCAUUG AAACAGACdTdT	1064
D-2890	S-3149	AUGACCUGUAUU UUGCCACdTdT	1234	A-3149	UUGGCAAAAUA CAGGUCAUdTdT	1065
D-2891	S-3150	ACCUGUAUUUUG CCAGACCdTdT	1235	A-3150	UGUCUGGCAAA AUACAGGUdTdT	1066
D-2892	S-3151	CCUGUAUUUUGC CAGACUCdTdT	1236	A-3151	UAGUCUGGCAA AAUACAGGdTdT	1067
D-2893	S-3152	UAAAUCACAGAU GGGUAUCdTdT	1237	A-3152	UAUACCCAUCU GUGAUUUAdTdT	1068
D-2894	S-3153	AUCACAGAUGGG UAUUAACdTdT	1238	A-3153	UUUAAUACCCA UCUGUGAUdTdT	1069
D-2895	S-3154	UCACAGAUGGGU AUUAAACdTdT	1239	A-3154	UUUUAAUACCC AUCUGUGAdTdT	1070
D-2896	S-3155	ACAGAUGGGUAU UAAACUCdTdT	1240	A-3155	UAGUUUAAUAC CCAUCUGUdTdT	1071

D-2897	S-3156	CAGAUGGGUAUU AAACUUCdTdT	1241	A-3156	UAAGUUUAAUA CCCAUCUGdTdT	1072
D-2898	S-3157	AGAUGGGUAUUA AACUUGCdTdT	1242	A-3157	UCAAGUUUAAU ACCCAUCUdTdT	1073
D-2899	S-3158	AUGGGUAUUAAA CUUGUCCdTdT	1243	A-3158	UGACAAGUUUA AUACCCAUdTdT	1074
D-2900	S-3159	UAAACUUGUCAG AAUUUCCdTdT	1244	A-3159	UGAAAUUCUGA CAAGUUUAdTdT	1075
D-2901	S-3160	UCAUUCAAGCCU GUGAAUCdTdT	1245	A-3160	UAUUCACAGGC UUGAAUGAdTdT	1076
D-2902	S-3161	CAUUCAAGCCUG UGAAUACdTdT	1246	A-3161	UUAUUCACAGG CUUGAAUGdTdT	1077
D-2903	S-3162	AAUAAAAACCCU GUAUGGCdTdT	1247	A-3162	UCCAUACAGGG UUUUUAUUdTdT	1078
D-2904	S-3163	AUAAAAACCCUG UAUGGCCdTdT	1248	A-3163	UGCCAUACAGG GUUUUUAUdTdT	1079
D-2905	S-3164	AACCCUGUAUGG CACUUACdTdT	1249	A-3164	UUAAGUGCCAU ACAGGGUUdTdT	1080
D-2906	S-3165	ACCCUGUAUGGC ACUUAUCdTdT	1250	A-3165	UAUAAGUGCCA UACAGGGUdTdT	1081
D-2907	S-3166	GAGGCUAUUAAA AGAAUCCdTdT	1251	A-3166	UGAUUCUUUUA AUAGCCUCdTdT	1082
D-2908	S-3167	AAAGAAUCCAAA UUCAAACdTdT	1252	A-3167	UUUUGAAUUUG GAUUCUUUdTdT	1083
D-2909	S-3168	GAAUCCAAAUUC AAACUACdTdT	1253	A-3168	UUAGUUUGAAU UUGGAUUCdTdT	1084

[00378] In other embodiments, the siRNA molecules of the present invention targeting SOD1 can be encoded in plasmid vectors, AAV particles, viral genome or other nucleic acid expression vectors for delivery to a cell.

[00379] DNA expression plasmids can be used to stably express the siRNA duplexes or dsRNA of the present invention targeting SOD1 in cells and achieve long-term inhibition of the target gene expression. In one aspect, the sense and antisense strands of a siRNA duplex are typically linked by a short spacer sequence leading to the expression of a stem-loop structure termed short hairpin RNA (shRNA). The hairpin is recognized and cleaved by Dicer, thus generating mature siRNA molecules.

[00380] According to the present invention, AAV particles comprising the nucleic acids encoding the siRNA molecules targeting SOD1 mRNA are produced, the AAV serotypes may be any of the serotypes listed in Table 1. Non-limiting examples of the AAV serotypes include, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ8, AAV-DJ, AAV-PHP.A, and/or AAV-PHP.B, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B-EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B-SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN,

AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B-STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A15/G2A3, AAVG2B4, AAVG2B5, and variants thereof.

[00381] In some embodiments, the siRNA duplexes or encoded dsRNA of the present invention suppress (or degrade) SOD1 mRNA. Accordingly, the siRNA duplexes or encoded dsRNA can be used to substantially inhibit SOD1 gene expression in a cell. In some aspects, the inhibition of SOD1 gene expression refers to an inhibition by at least about 20%, preferably by at least about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%. Accordingly, the protein product of the targeted gene may be inhibited by at least about 20%, preferably by at least about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100%.

[00382] According to the present invention, the siRNA molecules are designed and tested for their ability in reducing SOD1 mRNA levels in cultured cells. Such siRNA molecules may form a duplex such as, but not limited to, include those listed in Table 4. As a non-limiting example, the siRNA duplexes may be siRNA duplex IDs: D-2741 to D-2909.

[00383] In one embodiment, the siRNA molecules comprise a miRNA seed match for SOD1 located in the guide strand. In another embodiment, the siRNA molecules comprise a miRNA seed match for SOD1 located in the passenger strand. In yet another embodiment, the siRNA duplexes or encoded dsRNA targeting SOD1 gene do not comprise a seed match for SOD1 located in the guide or passenger strand.

[00384] In one embodiment, the siRNA duplexes or encoded dsRNA targeting SOD1 gene may have almost no significant full-length off target effects for the guide strand. In another embodiment, the siRNA duplexes or encoded dsRNA targeting SOD1 gene may have almost no significant full-length off target effects for the passenger strand. The siRNA duplexes or encoded dsRNA targeting SOD1 gene may have less than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%,

10%,11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 1-5%, 2-6%, 3-7%, 4-8%, 5-9%, 5-10%, 6-10%, 5-15%, 5-20%, 5-25% 5-30%, 10-20%, 10-30%, 10-40%, 10-50%, 15-30%, 15-40%, 15-45%, 20-40%, 20-50%, 25-50%, 30-40%, 30-50%, 35-50%, 40-50%, 45-50% full-length off target effects for the passenger strand. In yet another embodiment, the siRNA duplexes or encoded dsRNA targeting SOD1 gene may have almost no significant full-length off target effects for the guide strand or the passenger strand. The siRNA duplexes or encoded dsRNA targeting SOD1 gene may have less than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%,11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 1-5%, 2-6%, 3-7%, 4-8%, 5-9%, 5-10%, 6-10%, 5-15%, 5-20%, 5-25% 5-30%, 10-20%, 10-30%, 10-40%, 10-50%, 15-30%, 15-40%, 15-45%, 20-40%, 20-50%, 25-50%, 30-40%, 30-50%, 35-50%, 40-50%, 45-50% full-length off target effects for the guide or passenger strand.

[00385] In one embodiment, the siRNA duplexes or encoded dsRNA targeting SOD1 gene may have high activity *in vitro*. In another embodiment, the siRNA molecules may have low activity *in vitro*. In yet another embodiment, the siRNA duplexes or dsRNA targeting the SOD1 gene may have high guide strand activity and low passenger strand activity *in vitro*.

[00386] In one embodiment, the siRNA molecules targeting SOD1 have a high guide strand activity and low passenger strand activity *in vitro*. The target knock-down (KD) by the guide strand may be at least 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 99.5% or 100%. The target knock-down by the guide strand may be 40-50%, 45-50%, 50-55%, 50-60%, 60-65%, 60-70%, 60-75%, 60-80%, 60-85%, 60-90%, 60-95%, 60-99%, 60-99.5%, 60-100%, 65-70%, 65-75%, 65-80%, 65-85%, 65-90%, 65-95%, 65-99%, 65-99.5%, 65-100%, 70-75%, 70-80%, 70-85%, 70-90%, 70-95%, 70-99%, 70-99.5%, 70-100%, 75-80%, 75-85%, 75-90%, 75-95%, 75-99%, 75-99.5%, 75-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-99.5%, 80-100%, 85-90%, 85-95%, 85-99%, 85-99.5%, 85-100%, 90-95%, 90-99%, 90-99.5%, 90-100%, 95-99%, 95-99.5%, 95-100%, 99-99.5%, 99-100% or 99.5-100%. As a non-limiting example, the target knock-down (KD) by the guide strand is greater than 70%. As a non-limiting example, the target knock-down (KD) by the guide strand is greater than 60%.

[00387] In one embodiment, the siRNA duplex target SOD1 is designed so there is no miRNA seed match for the sense or antise sequence to the non-SOD1 sequence.

[00388] In one embodiment, the IC_{50} of the guide strand in the siRNA duplex targeting SOD1 for the nearest off target is greater than 100 multiplied by the IC_{50} of the guide strand for the on-target gene, SOD1. As a non-limiting example, if the IC_{50} of the guide strand for the nearest off

target is greater than 100 multiplied by the IC_{50} of the guide strand for the target then the siRNA molecules is said to have high guide strand selectivity for inhibiting SOD1 *in vitro*.

[00389] In one embodiment, the 5' processing of the guide strand of the siRNA duplex targeting SOD1 has a correct start (n) at the 5' end at least 75%, 80%, 85%, 90%, 95%, 99% or 100% of the time *in vitro* or *in vivo*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 99% of the time *in vitro*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 99% of the time *in vivo*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 90% of the time *in vitro*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 90% of the time *in vivo*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 85% of the time *in vitro*. As a non-limiting example, the 5' processing of the guide strand is precise and has a correct start (n) at the 5' end at least 85% of the time *in vivo*.

[00390] In one embodiment, the 5' processing of the guide strand of the siRNA duplex targeting SOD1 has a correct start (n) at the 5' end in a range of 75-95%, 75-90%, 75-85%, 75-80%, 80-95%, 80-90%, 80-85%, 85-95%, 85-90%, or 90-95%. As a non-limiting example, the 5' processing of the guide strand of the siRNA duplex targeting SOD1 has a correct start (n) at the 5' end in a range of 75-95%.

[00391] In one embodiment, the 5' processing of the guide strand of the siRNA duplex targeting SOD1 has a correct start (n) at the 5' end for 75%, 75.1%, 75.2%, 75.3%, 75.4%, 75.5%, 75.6%, 75.7%, 75.8%, 75.9%, 76%, 76.1%, 76.2%, 76.3%, 76.4%, 76.5%, 76.6%, 76.7%, 76.8%, 76.9%, 77%, 77.1%, 77.2%, 77.3%, 77.4%, 77.5%, 77.6%, 77.7%, 77.8%, 77.9%, 78%, 78.1%, 78.2%, 78.3%, 78.4%, 78.5%, 78.6%, 78.7%, 78.8%, 78.9%, 79%, 79.1%, 79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8%, 79.9%, 80%, 80.1%, 80.2%, 80.3%, 80.4%, 80.5%, 80.6%, 80.7%, 80.8%, 80.9%, 81%, 81.1%, 81.2%, 81.3%, 81.4%, 81.5%, 81.6%, 81.7%, 81.8%, 81.9%, 82%, 82.1%, 82.2%, 82.3%, 82.4%, 82.5%, 82.6%, 82.7%, 82.8%, 82.9%, 83%, 83.1%, 83.2%, 83.3%, 83.4%, 83.5%, 83.6%, 83.7%, 83.8%, 83.9%, 84%, 84.1%, 84.2%, 84.3%, 84.4%, 84.5%, 84.6%, 84.7%, 84.8%, 84.9%, 85%, 85.1%, 85.2%, 85.3%, 85.4%, 85.5%, 85.6%, 85.7%, 85.8%, 85.9%, 86%, 86.1%, 86.2%, 86.3%, 86.4%, 86.5%, 86.6%, 86.7%, 86.8%, 86.9%, 87%, 87.1%, 87.2%, 87.3%, 87.4%, 87.5%, 87.6%, 87.7%, 87.8%, 87.9%, 88%, 88.1%, 88.2%, 88.3%, 88.4%, 88.5%, 88.6%, 88.7%, 88.8%, 88.9%, 89%, 89.1%, 89.2%, 89.3%, 89.4%, 89.5%, 89.6%, 89.7%, 89.8%, 89.9%, 90%, 90.1%,

90.2%, 90.3%, 90.4%, 90.5%, 90.6%, 90.7%, 90.8%, 90.9%, 91%, 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, 91.6%, 91.7%, 91.8%, 91.9%, 92%, 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, 92.6%, 92.7%, 92.8%, 92.9%, 93%, 93.1%, 93.2%, 93.3%, 93.4%, 93.5%, 93.6%, 93.7%, 93.8%, 93.9%, 94%, 94.1%, 94.2%, 94.3%, 94.4%, 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, or 95% of the constructs expressed. As a non-limiting example, the 5' processing of the guide strand of the siRNA duplex targeting SOD1 has a correct start (n) at the 5' end for 81% of the constructs expressed. As a non-limiting example, the 5' processing of the guide strand of the siRNA duplex targeting SOD1 has a correct start (n) at the 5' end for 90 % of the constructs expressed.

[00392] In one embodiment, a passenger-guide strand duplex for SOD1 is considered effective when the pri- or pre-microRNAs demonstrate, by methods known in the art and described herein, greater than 2-fold guide to passenger strand ratio when processing is measured. As a non-limiting examples, the pri- or pre-microRNAs demonstrate great than 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, or 2 to 5-fold, 2 to 10-fold, 2 to 15-fold, 3 to 5-fold, 3 to 10-fold, 3 to 15-fold, 4 to 5-fold, 4 to 10-fold, 4 to 15-fold, 5 to 10-fold, 5 to 15-fold, 6 to 10-fold, 6 to 15-fold, 7 to 10-fold, 7 to 15-fold, 8 to 10-fold, 8 to 15-fold, 9 to 10-fold, 9 to 15-fold, 10 to 15-fold, 11 to 15-fold, 12 to 15-fold, 13 to 15-fold, or 14 to 15-fold guide to passenger strand ratio when processing is measured.

[00393] In one embodiment, the siRNA molecules may be used to silence wild type or mutant SOD1 by targeting at least one exon on the SOD1 sequence. The exon may be exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, exon 10, exon 11, exon 12, exon 13, exon 14, exon 15, exon 16, exon 17, exon 18, exon 19, exon 20, exon 21, exon 22, exon 23, exon 24, exon 25, exon 26, exon 27, exon 28, exon 29, exon 30, exon 31, exon 32, exon 33, exon 34, exon 35, exon 36, exon 37, exon 38, exon 39, exon 40, exon 41, exon 42, exon 43, exon 44, exon 45, exon 46, exon 47, exon 48, exon 49, exon 50, exon 51, exon 52, exon 53, exon 54, exon 55, exon 56, exon 57, exon 58, exon 59, exon 60, exon 61, exon 62, exon 63, exon 64, exon 65, exon 66, and/or exon 67.

[00394] In one embodiment, the range of guide strands to the total endogenous pool of miRNAs is 0.001-0.6%, 0.005-0.6%, 0.01-0.6%, 0.015-0.6%, 0.02-0.6%, 0.025-0.6%, 0.03-0.6%, 0.035-0.6%, 0.04-0.6%, 0.045-0.6%, 0.05-0.6%, 0.055-0.6%, 0.06-0.6%, 0.065-0.6%, 0.07-0.6%, 0.075-0.6%, 0.08-0.6%, 0.085-0.6%, 0.09-0.6%, 0.095-0.6%, 0.1-0.6%, 0.15-0.6%, 0.2-0.6%, 0.25-0.6%, 0.3-0.6%, 0.35-0.6%, 0.4-0.6%, 0.45-0.6%, 0.5-0.6%, 0.55-0.6%, 0.001-

0.5%, 0.005-0.5%, 0.01-0.5%, 0.015-0.5%, 0.02-0.5%, 0.025-0.5%, 0.03-0.5%, 0.035-0.5%, 0.04-0.5%, 0.045-0.5%, 0.05-0.5%, 0.055-0.5%, 0.06-0.5%, 0.065-0.5%, 0.07-0.5%, 0.075-0.5%, 0.08-0.5%, 0.085-0.5%, 0.09-0.5%, 0.095-0.5%, 0.1-0.5%, 0.15-0.5%, 0.2-0.5%, 0.25-0.5%, 0.3-0.5%, 0.35-0.5%, 0.4-0.5%, 0.45-0.5%, 0.001-0.4%, 0.005-0.4%, 0.01-0.4%, 0.015-0.4%, 0.02-0.4%, 0.025-0.4%, 0.03-0.4%, 0.035-0.4%, 0.04-0.4%, 0.045-0.4%, 0.05-0.4%, 0.055-0.4%, 0.06-0.4%, 0.065-0.4%, 0.07-0.4%, 0.075-0.4%, 0.08-0.4%, 0.085-0.4%, 0.09-0.4%, 0.095-0.4%, 0.1-0.4%, 0.15-0.4%, 0.2-0.4%, 0.25-0.4%, 0.3-0.4%, 0.35-0.4%, 0.001-0.3%, 0.005-0.3%, 0.01-0.3%, 0.015-0.3%, 0.02-0.3%, 0.025-0.3%, 0.03-0.3%, 0.035-0.3%, 0.04-0.3%, 0.045-0.3%, 0.05-0.3%, 0.055-0.3%, 0.06-0.3%, 0.065-0.3%, 0.07-0.3%, 0.075-0.3%, 0.08-0.3%, 0.085-0.3%, 0.09-0.3%, 0.095-0.3%, 0.1-0.3%, 0.15-0.3%, 0.2-0.3%, 0.25-0.3%, 0.001-0.2%, 0.005-0.2%, 0.01-0.2%, 0.015-0.2%, 0.02-0.2%, 0.025-0.2%, 0.03-0.2%, 0.035-0.2%, 0.04-0.2%, 0.045-0.2%, 0.05-0.2%, 0.055-0.2%, 0.06-0.2%, 0.065-0.2%, 0.07-0.2%, 0.075-0.2%, 0.08-0.2%, 0.085-0.2%, 0.09-0.2%, 0.095-0.2%, 0.1-0.2%, 0.15-0.2%, 0.001-0.1%, 0.005-0.1%, 0.01-0.1%, 0.015-0.1%, 0.02-0.1%, 0.025-0.1%, 0.03-0.1%, 0.035-0.1%, 0.04-0.1%, 0.045-0.1%, 0.05-0.1%, 0.055-0.1%, 0.06-0.1%, 0.065-0.1%, 0.07-0.1%, 0.075-0.1%, 0.08-0.1%, 0.085-0.1%, 0.09-0.1%, or 0.095-0.1%. As a non-limiting example, the range is 0.06-0.6%. As a non-limiting example, the range is 0.4-0.5%.

[00395] In one embodiment, the percent of guide strands to the total endogenous pool of miRNAs is 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or 0.6%. As a non-limiting example, the percent is 0.06%. As a non-limiting example, the percent is 0.4%. As a non-limiting example, the percent is 0.5%.

siRNA modification

[00396] In some embodiments, the siRNA molecules of the present invention, when not delivered as a precursor or DNA, may be chemically modified to modulate some features of RNA molecules, such as, but not limited to, increasing the stability of siRNAs *in vivo*. The chemically modified siRNA molecules can be used in human therapeutic applications, and are improved without compromising the RNAi activity of the siRNA molecules. As a non-limiting example, the siRNA molecules modified at both the 3′ and the 5′ end of both the sense strand and the antisense strand.

[00397] In some aspects, the siRNA duplexes of the present invention may contain one or more modified nucleotides such as, but not limited to, sugar modified nucleotides, nucleobase

modifications and/or backbone modifications. In some aspects, the siRNA molecule may contain combined modifications, for example, combined nucleobase and backbone modifications.

[00398] In one embodiment, the modified nucleotide may be a sugar-modified nucleotide. Sugar modified nucleotides include, but are not limited to 2′-fluoro, 2′-amino and 2′-thio modified ribonucleotides, e.g. 2′-fluoro modified ribonucleotides. Modified nucleotides may be modified on the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties may be, or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4′-thioribose, and other sugars, heterocycles, or carbocycles.

[00399] In one embodiment, the modified nucleotide may be a nucleobase-modified nucleotide.

[00400] In one embodiment, the modified nucleotide may be a backbone-modified nucleotide. In some embodiments, the siRNA duplexes of the present invention may further comprise other modifications on the backbone. A normal “backbone”, as used herein, refers to the repeating alternating sugar-phosphate sequences in a DNA or RNA molecule. The deoxyribose/ribose sugars are joined at both the 3'-hydroxyl and 5'-hydroxyl groups to phosphate groups in ester links, also known as "phosphodiester" bonds/linker (PO linkage). The PO backbones may be modified as “phosphorothioate backbone (PS linkage). In some cases, the natural phosphodiester bonds may be replaced by amide bonds but the four atoms between two sugar units are kept. Such amide modifications can facilitate the solid phase synthesis of oligonucleotides and increase the thermodynamic stability of a duplex formed with siRNA complement. See e.g. Mesmaeker et al., *Pure & Appl. Chem.*, 1997, 3, 437-440; the content of which is incorporated herein by reference in its entirety.

[00401] Modified bases refer to nucleotide bases such as, for example, adenine, guanine, cytosine, thymine, uracil, xanthine, inosine, and queuosine that have been modified by the replacement or addition of one or more atoms or groups. Some examples of modifications on the nucleobase moieties include, but are not limited to, alkylated, halogenated, thiolated, aminated, amidated, or acetylated bases, individually or in combination. More specific examples include, for example, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N,N,-dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5-(2-amino)propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine, 7-methylguanosine, 2,2-dimethylguanosine, 5-methylaminoethyluridine, 5-methyloxyuridine,

deazanucleotides such as 7-deaza-adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O- and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4,6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides.

[00402] In one embodiment, the modified nucleotides may be on just the sense strand.

[00403] In another embodiment, the modified nucleotides may be on just the antisense strand.

[00404] In some embodiments, the modified nucleotides may be in both the sense and antisense strands.

[00405] In some embodiments, the chemically modified nucleotide does not affect the ability of the antisense strand to pair with the target mRNA sequence.

[00406] In one embodiment, the AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may encode siRNA molecules which are polycistronic molecules. The siRNA molecules may additionally comprise one or more linkers between regions of the siRNA molecules.

Molecular Scaffold

[00407] In one embodiment, the siRNA molecules may be encoded in a modulatory polynucleotide which also comprises a molecular scaffold. As used herein a “molecular scaffold” is a framework or starting molecule that forms the sequence or structural basis against which to design or make a subsequent molecule.

[00408] In one embodiment, the molecular scaffold comprises at least one 5’ flanking region. As a non-limiting example, the 5’ flanking region may comprise a 5’ flanking sequence which may be of any length and may be derived in whole or in part from wild type microRNA sequence or be a completely artificial sequence.

[00409] In one embodiment, the molecular scaffold comprises at least one 3’ flanking region. As a non-limiting example, the 3’ flanking region may comprise a 3’ flanking sequence which may be of any length and may be derived in whole or in part from wild type microRNA sequence or be a completely artificial sequence.

[00410] In one embodiment, the molecular scaffold comprises at least one loop motif region. As a non-limiting example, the loop motif region may comprise a sequence which may be of any length.

[00411] In one embodiment, the molecular scaffold comprises a 5' flanking region, a loop motif region and/or a 3' flanking region.

[00412] In one embodiment, at least one siRNA, miRNA or other RNAi agent described herein, may be encoded by a modulatory polynucleotide which may also comprise at least one molecular scaffold. The molecular scaffold may comprise a 5' flanking sequence which may be of any length and may be derived in whole or in part from wild type microRNA sequence or be completely artificial. The 3' flanking sequence may mirror the 5' flanking sequence and/or a 3' flanking sequence in size and origin. Either flanking sequence may be absent. The 3' flanking sequence may optionally contain one or more CNNC motifs, where "N" represents any nucleotide.

[00413] Forming the stem of a stem loop structure is a minimum of the modulatory polynucleotide encoding at least one siRNA, miRNA or other RNAi agent described herein. In some embodiments, the siRNA, miRNA or other RNAi agent described herein comprises at least one nucleic acid sequence which is in part complementary or will hybridize to a target sequence. In some embodiments the payload is an siRNA molecule or fragment of an siRNA molecule.

[00414] In some embodiments, the 5' arm of the stem loop structure of the modulatory polynucleotide comprises a nucleic acid sequence encoding a sense sequence. Non-limiting examples of sense sequences, or fragments or variants thereof, which may be encoded by the modulatory polynucleotide are described in Table 3.

[00415] In some embodiments, the 3' arm of the stem loop of the modulatory polynucleotide comprises a nucleic acid sequence encoding an antisense sequence. The antisense sequence, in some instances, comprises a "G" nucleotide at the 5' most end. Non-limiting examples of antisense sequences, or fragments or variants thereof, which may be encoded by the modulatory polynucleotide are described in Table 2.

[00416] In other embodiments, the sense sequence may reside on the 3' arm while the antisense sequence resides on the 5' arm of the stem of the stem loop structure of the modulatory polynucleotide. Non-limiting examples of sense and antisense sequences which may be encoded by the modulatory polynucleotide are described in Tables 2 and 3.

[00417] In one embodiment, the sense and antisense sequences may be completely complementary across a substantial portion of their length. In other embodiments the sense

sequence and antisense sequence may be at least 70, 80, 90, 95 or 99% complementarity across independently at least 50, 60, 70, 80, 85, 90, 95, or 99 % of the length of the strands.

[00418] Neither the identity of the sense sequence nor the homology of the antisense sequence need to be 100% complementarity to the target sequence.

[00419] In one embodiment, separating the sense and antisense sequence of the stem loop structure of the modulatory polynucleotide is a loop sequence (also known as a loop motif, linker or linker motif). The loop sequence may be of any length, between 4-30 nucleotides, between 4-20 nucleotides, between 4-15 nucleotides, between 5-15 nucleotides, between 6-12 nucleotides, 6 nucleotides, 7 nucleotides, 8 nucleotides, 9 nucleotides, 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, and/or 15 nucleotides.

[00420] In some embodiments, the loop sequence comprises a nucleic acid sequence encoding at least one UGUG motif. In some embodiments, the nucleic acid sequence encoding the UGUG motif is located at the 5' terminus of the loop sequence.

[00421] In one embodiment, spacer regions may be present in the modulatory polynucleotide to separate one or more modules (e.g., 5' flanking region, loop motif region, 3' flanking region, sense sequence, antisense sequence) from one another. There may be one or more such spacer regions present.

[00422] In one embodiment, a spacer region of between 8-20, i.e., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides may be present between the sense sequence and a flanking region sequence.

[00423] In one embodiment, the length of the spacer region is 13 nucleotides and is located between the 5' terminus of the sense sequence and the 3' terminus of the flanking sequence. In one embodiment, a spacer is of sufficient length to form approximately one helical turn of the sequence.

[00424] In one embodiment, a spacer region of between 8-20, i.e., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides may be present between the antisense sequence and a flanking sequence.

[00425] In one embodiment, the spacer sequence is between 10-13, i.e., 10, 11, 12 or 13 nucleotides and is located between the 3' terminus of the antisense sequence and the 5' terminus of a flanking sequence. In one embodiment, a spacer is of sufficient length to form approximately one helical turn of the sequence.

[00426] In one embodiment, the molecular scaffold of the modulatory polynucleotide comprises in the 5' to 3' direction, a 5' flanking sequence, a 5' arm, a loop motif, a 3' arm and a

3' flanking sequence. As a non-limiting example, the 5' arm may comprise a nucleic acid sequence encoding a sense sequence and the 3' arm comprises a nucleic acid sequence encoding the antisense sequence. In another non-limiting example, the 5' arm comprises a nucleic acid sequence encoding the antisense sequence and the 3' arm comprises a nucleic acid sequence encoding the sense sequence.

[00427] In one embodiment, the 5' arm, sense and/or antisense sequence, loop motif and/or 3' arm sequence may be altered (e.g., substituting 1 or more nucleotides, adding nucleotides and/or deleting nucleotides). The alteration may cause a beneficial change in the function of the construct (e.g., increase knock-down of the target sequence, reduce degradation of the construct, reduce off target effect, increase efficiency of the payload, and reduce degradation of the payload).

[00428] In one embodiment, the molecular scaffold of the modulatory polynucleotides is aligned in order to have the rate of excision of the guide strand (also referred to herein as the antisense strand) be greater than the rate of excision of the passenger strand (also referred to herein as the sense strand). The rate of excision of the guide or passenger strand may be, independently, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more than 99%. As a non-limiting example, the rate of excision of the guide strand is at least 80%. As another non-limiting example, the rate of excision of the guide strand is at least 90%.

[00429] In one embodiment, the rate of excision of the guide strand is greater than the rate of excision of the passenger strand. In one aspect, the rate of excision of the guide strand may be at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more than 99% greater than the passenger strand.

[00430] In one embodiment, the efficiency of excision of the guide strand is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more than 99%. As a non-limiting example, the efficiency of the excision of the guide strand is greater than 80%.

[00431] In one embodiment, the efficiency of the excision of the guide strand is greater than the excision of the passenger strand from the molecular scaffold. The excision of the guide strand may be 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 times more efficient than the excision of the passenger strand from the molecular scaffold.

[00432] In one embodiment, the molecular scaffold comprises a dual-function targeting modulatory polynucleotide. As used herein, a "dual-function targeting" modulatory

polynucleotide is a polynucleotide where both the guide and passenger strands knock down the same target or the guide and passenger strands knock down different targets.

[00433] In one embodiment, the molecular scaffold of the modulatory polynucleotides described herein may comprise a 5' flanking region, a loop motif region and a 3' flanking region. Non-limiting examples of the sequences for the 5' flanking region, loop motif region (may also be referred to as a linker region) and the 3' flanking region which may be used, or fragments thereof used, in the modulatory polynucleotides described herein are shown in Tables 5 – 7.

Table 5. 5' Flanking Regions for Molecular Scaffold

5' Flanking Region Name	5' Flanking Region Sequence	5' Flanking Region SEQ ID
5F1	GTGCTGGGCGGGGGGCGGCGGGCCCTCCCGC AGAACACCATGCGCTCTTCGGAA	1255
5F2	GAAGCAAAGAAGGGGCAGAGGGAGCCCGTG AGCTGAGTGGGCCAGGGACTGGGAGAAGGAG TGAGGAGGCAGGGCCGGCATGCCTCTGCTGC TGGCCAGA	1256
5F3	GTGCTGGGCGGGGGGCGGCGGGCCCTCCCGC AGAACACCATGCGCTCCACGGAA	1257
5F4	GGGCCCTCCCGCAGAACACCATGCGCTCCAC GGAA	1258
5F5	CTCCCGCAGAACACCATGCGCTCCACGGAA	1259
5F6	GTGCTGGGCGGGGGGCGGCGGGCCCTCCCGC AGAACACCATGCGCTCCACGGAAG	1260
5F7	GTGCTGGGCGGGGGGCGGCGGGCCCTCCCGC AGAACACCATGCGCTCCTCGGAA	1261
5F8	TTTATGCCTCATCCTCTGAGTGCTGAAGGCTT GCTGTAGGCTGTATGCTG	1262
5F9	GTGCTGGGCGGGGGGCGGCGGGCCCTCCCGC AGAACACCATGCGCTCTTCGGGA	1263

Table 6. Loop Motif Regions for Molecular Scaffold

Loop Motif Region Name	Loop Motif Region Sequence	Loop Motif Region SEQ ID
L1	TGTGACCTGG	1264
L2	TGTGATTTGG	1265
L3	GTCTGCACCTGTCACTAG	1266
L4	GTGACCCAAG	1267
L5	GTGGCCACTGAGAAG	1268
L6	GTGACCCAAT	1269
L7	GTGACCCAAC	1270
L8	GTGGCCACTGAGAAA	1271
L9	TATAATTTGG	1272
L10	CCTGACCCAGT	1273

Table 7. 3' Flanking Regions for Molecular Scaffold

3' Flanking Region Name	3' Flanking Region Sequence	3' Flanking Region SEQ ID

3F1	CTGAGGAGCGCCTTGACAGCAGCCATGGGAG GGCCGCCCCCTACCTCAGTGA	1274
3F2	CTGTGGAGCGCCTTGACAGCAGCCATGGGAG GGCCGCCCCCTACCTCAGTGA	1275
3F3	TGGCCGTGTAGTGCTACCCAGCGCTGGCTGCC TCCTCAGCATTGCAATTCCTCTCCCATCTGGG CACCAGTCAGCTACCCTGGTGGGAATCTGGGT AGCC	1276
3F4	CTGAGGAGCGCCTTGACAGCAGCCATGGGAG GGCC	1277
3F5	CTGCGGAGCGCCTTGACAGCAGCCATGGGAG GGCCGCCCCCTACCTCAGTGA	1278
3F6	AGTGTATGATGCCTGTTACTAGCATTCACATG GAACAAATTGCTGCCGTG	1279
3F7	TCCTGAGGAGCGCCTTGACAGCAGCCATGGG AGGGCCGCCCCCTACCTCAGTGA	1280

[00434] In one embodiment, the molecular scaffold may comprise at least one 5' flanking region, fragment or variant thereof listed in Table 5. As a non-limiting example, the 5' flanking region may be 5F1, 5F2, 5F3, 5F4, 5F5, 5F6, 5F7, 5F8, or 5F9.

[00435] In one embodiment, the molecular scaffold may comprise at least one 5F1 flanking region.

[00436] In one embodiment, the molecular scaffold may comprise at least one 5F2 flanking region.

[00437] In one embodiment, the molecular scaffold may comprise at least one 5F3 flanking region.

[00438] In one embodiment, the molecular scaffold may comprise at least one 5F4 flanking region.

[00439] In one embodiment, the molecular scaffold may comprise at least one 5F5 flanking region.

[00440] In one embodiment, the molecular scaffold may comprise at least one 5F6 flanking region.

[00441] In one embodiment, the molecular scaffold may comprise at least one 5F7 flanking region.

[00442] In one embodiment, the molecular scaffold may comprise at least one 5F8 flanking region.

[00443] In one embodiment, the molecular scaffold may comprise at least one 5F9 flanking region.

[00444] In one embodiment, the molecular scaffold may comprise at least one loop motif region, fragment or variant thereof listed in Table 6. As a non-limiting example, the loop motif region may be L1, L2, L3, L4, L5, L6, L7, L8, L9, or L10.

[00445] In one embodiment, the molecular scaffold may comprise at least one L1 loop motif region.

[00446] In one embodiment, the molecular scaffold may comprise at least one L2 loop motif region.

[00447] In one embodiment, the molecular scaffold may comprise at least one L3 loop motif region.

[00448] In one embodiment, the molecular scaffold may comprise at least one L4 loop motif region.

[00449] In one embodiment, the molecular scaffold may comprise at least one L5 loop motif region.

[00450] In one embodiment, the molecular scaffold may comprise at least one L6 loop motif region.

[00451] In one embodiment, the molecular scaffold may comprise at least one L7 loop motif region.

[00452] In one embodiment, the molecular scaffold may comprise at least one L8 loop motif region.

[00453] In one embodiment, the molecular scaffold may comprise at least one L9 loop motif region.

[00454] In one embodiment, the molecular scaffold may comprise at least one L10 loop motif region.

[00455] In one embodiment, the molecular scaffold may comprise at least one 3' flanking region, fragment or variant thereof listed in Table 7. As a non-limiting example, the 3' flanking region may be 3F1, 3F2, 3F3, 3F4, 3F5, 3F6, or 3F7.

[00456] In one embodiment, the molecular scaffold may comprise at least one 3F1 flanking region.

[00457] In one embodiment, the molecular scaffold may comprise at least one 3F2 flanking region.

[00458] In one embodiment, the molecular scaffold may comprise at least one 3F3 flanking region.

[00459] In one embodiment, the molecular scaffold may comprise at least one 3F4 flanking region.

[00460] In one embodiment, the molecular scaffold may comprise at least one 3F5 flanking region.

[00461] In one embodiment, the molecular scaffold may comprise at least one 3F6 flanking region.

[00462] In one embodiment, the molecular scaffold may comprise at least one 3F7 flanking region.

[00463] In one embodiment, the molecular scaffold may comprise at least one 5' flanking region, fragment or variant thereof, and at least one loop motif region, fragment or variant thereof, as described in Tables 5 and 6. As a non-limiting example, the 5' flanking region and the loop motif region may be 5F1 and L1, 5F1 and L2, 5F1 and L3, 5F1 and L4, 5F1 and L5, 5F1 and L6, 5F1 and L7, 5F1 and L8, 5F1 and L9, 5F1 and L10, 5F2 and L1, 5F2 and L2, 5F2 and L3, 5F2 and L4, 5F2 and L5, 5F2 and L6, 5F2 and L7, 5F2 and L8, 5F2 and L9, 5F2 and L10, 5F3 and L1, 5F3 and L2, 5F3 and L3, 5F3 and L4, 5F3 and L5, 5F3 and L6, 5F3 and L7, 5F3 and L8, 5F3 and L9, 5F3 and L10, 5F4 and L1, 5F4 and L2, 5F4 and L3, 5F4 and L4, 5F4 and L5, 5F4 and L6, 5F4 and L7, 5F4 and L8, 5F4 and L9, 5F4 and L10, 5F5 and L1, 5F5 and L2, 5F5 and L3, 5F5 and L4, 5F5 and L5, 5F5 and L6, 5F5 and L7, 5F5 and L8, 5F5 and L9, 5F5 and L10, 5F6 and L1, 5F6 and L2, 5F6 and L3, 5F6 and L4, 5F6 and L5, 5F6 and L6, 5F6 and L7, 5F6 and L8, 5F6 and L9, 5F6 and L10, 5F7 and L1, 5F7 and L2, 5F7 and L3, 5F7 and L4, 5F7 and L5, 5F7 and L6, 5F7 and L7, 5F7 and L8, 5F7 and L9, 5F7 and L10, 5F8 and L1, 5F8 and L2, 5F8 and L3, 5F8 and L4, 5F8 and L5, 5F8 and L6, 5F8 and L7, 5F8 and L8, 5F8 and L9, 5F8 and L10, 5F9 and L1, 5F9 and L2, 5F9 and L3, 5F9 and L4, 5F9 and L5, 5F9 and L6, 5F9 and L7, 5F9 and L8, 5F9 and L9, and 5F9 and L10.

[00464] In one embodiment, the molecular scaffold may comprise at least one 5F2 flanking region and at least one L1 loop motif region.

[00465] In one embodiment, the molecular scaffold may comprise at least one 5F1 flanking region and at least one L4 loop motif region.

[00466] In one embodiment, the molecular scaffold may comprise at least one 5F7 flanking region and at least one L8 loop motif region.

[00467] In one embodiment, the molecular scaffold may comprise at least one 5F3 flanking region and at least one L4 loop motif region.

[00468] In one embodiment, the molecular scaffold may comprise at least one 5F3 flanking region and at least one L5 loop motif region.

[00469] In one embodiment, the molecular scaffold may comprise at least one 5F4 flanking region and at least one L4 loop motif region.

[00470] In one embodiment, the molecular scaffold may comprise at least one 5F3 flanking region and at least one L7 loop motif region.

[00471] In one embodiment, the molecular scaffold may comprise at least one 5F5 flanking region and at least one L4 loop motif region.

[00472] In one embodiment, the molecular scaffold may comprise at least one 5F6 flanking region and at least one L4 loop motif region.

[00473] In one embodiment, the molecular scaffold may comprise at least one 5F3 flanking region and at least one L6 loop motif region.

[00474] In one embodiment, the molecular scaffold may comprise at least one 5F7 flanking region and at least one L4 loop motif region.

[00475] In one embodiment, the molecular scaffold may comprise at least one 5F2 flanking region and at least one L2 loop motif region.

[00476] In one embodiment, the molecular scaffold may comprise at least one 5F1 flanking region and at least one L1 loop motif region.

[00477] In one embodiment, the molecular scaffold may comprise at least one 5F1 flanking region and at least one L2 loop motif region.

[00478] In one embodiment, the molecular scaffold may comprise at least one 3' flanking region, fragment or variant thereof, and at least one motif region, fragment or variant thereof, as described in Tables 6 and 7. As a non-limiting example, the 3' flanking region and the loop motif region may be 3F1 and L1, 3F1 and L2, 3F1 and L3, 3F1 and L4, 3F1 and L5, 3F1 and L6, 3F1 and L7, 3F1 and L8, 3F1 and L9, 3F1 and L10, 3F2 and L1, 3F2 and L2, 3F2 and L3, 3F2 and L4, 3F2 and L5, 3F2 and L6, 3F2 and L7, 3F2 and L8, 3F2 and L9, 3F2 and L10, 3F3 and L1, 3F3 and L2, 3F3 and L3, 3F3 and L4, 3F3 and L5, 3F3 and L6, 3F3 and L7, 3F3 and L8, 3F3 and L9, 3F3 and L10, 3F4 and L1, 3F4 and L2, 3F4 and L3, 3F4 and L4, 3F4 and L5, 3F4 and L6, 3F4 and L7, 3F4 and L8, 3F4 and L9, 3F4 and L10, 3F5 and L1, 3F5 and L2, 3F5 and L3, 3F5 and L4, 3F5 and L5, 3F5 and L6, 3F5 and L7, 3F5 and L8, 3F5 and L9, 3F5 and L10, 3F6 and L1, 3F6 and L2, 3F6 and L3, 3F6 and L4, 3F6 and L5, 3F6 and L6, 3F6 and L7, 3F6 and L8, 3F6 and L9, 3F6 and L10, 3F7 and L1, 3F7 and L2, 3F7 and L3, 3F7 and L4, 3F7 and L5, 3F7 and L6, 3F7 and L7, 3F7 and L8, 3F7 and L9, and 3F7 and L10.

[00479] In one embodiment, the molecular scaffold may comprise at least one L1 loop motif region and at least one 3F2 flanking region.

[00480] In one embodiment, the molecular scaffold may comprise at least one L4 loop motif region and at least one 3F1 flanking region.

[00481] In one embodiment, the molecular scaffold may comprise at least one L8 loop motif region and at least one 3F5 flanking region.

[00482] In one embodiment, the molecular scaffold may comprise at least one L5 loop motif region and at least 3F1 flanking region.

[00483] In one embodiment, the molecular scaffold may comprise at least one L4 loop motif region and at least one 3F4 flanking region.

[00484] In one embodiment, the molecular scaffold may comprise at least one L7 loop motif region and at least one 3F1 flanking region.

[00485] In one embodiment, the molecular scaffold may comprise at least one L6 loop motif region and at least one 3F1 flanking region.

[00486] In one embodiment, the molecular scaffold may comprise at least one L4 loop motif region and at least one 3F5 flanking region.

[00487] In one embodiment, the molecular scaffold may comprise at least one L2 loop motif region and at least one 3F2 flanking region.

[00488] In one embodiment, the molecular scaffold may comprise at least one L1 loop motif region and at least one 3F3 flanking region.

[00489] In one embodiment, the molecular scaffold may comprise at least one L5 loop motif region and at least one 3F4 flanking region.

[00490] In one embodiment, the molecular scaffold may comprise at least one L1 loop motif region and at least one 3F1 flanking region.

[00491] In one embodiment, the molecular scaffold may comprise at least one L2 loop motif region and at least one 3F1 flanking region.

[00492] In one embodiment, the molecular scaffold may comprise at least one 5' flanking region, fragment or variant thereof, and at least one 3' flanking region, fragment or variant thereof, as described in Tables 5 and 7. As a non-limiting example, the flanking regions may be 5F1 and 3F1, 5F1 and 3F2, 5F1 and 3F3, 5F1 and 3F4, 5F1 and 3F5, 5F1 and 3F6, 5F1 and 3F7, 5F2 and 3F1, 5F2 and 3F2, 5F2 and 3F3, 5F2 and 3F4, 5F2 and 3F5, 5F2 and 3F6, 5F2 and 3F7, 5F3 and 3F1, 5F3 and 3F2, 5F3 and 3F3, 5F3 and 3F4, 5F3 and 3F5, 5F3 and 3F6, 5F3 and 3F7, 5F4 and 3F1, 5F4 and 3F2, 5F4 and 3F3, 5F4 and 3F4, 5F4 and 3F5, 5F4 and 3F6, 5F4 and 3F7,

5F5 and 3F1, 5F5 and 3F2, 5F5 and 3F3, 5F5 and 3F4, 5F5 and 3F5, 5F5 and 3F6, 5F5 and 3F7, 5F6 and 3F1, 5F6 and 3F2, 5F6 and 3F3, 5F6 and 3F4, 5F6 and 3F5, 5F6 and 3F6, 5F6 and 3F7, 5F7 and 3F1, 5F7 and 3F2, 5F7 and 3F3, 5F7 and 3F4, 5F7 and 3F5, 5F7 and 3F6, 5F7 and 3F7, 5F8 and 3F1, 5F8 and 3F2, 5F8 and 3F3, 5F8 and 3F4, 5F8 and 3F5, 5F8 and 3F6, and 5F8 and 3F7. 5F9 and 3F1, 5F9 and 3F2, 5F9 and 3F3, 5F9 and 3F4, 5F9 and 3F5, 5F9 and 3F6, and 5F9 and 3F7

[00493] In one embodiment, the molecular scaffold may comprise at least one 5F2 5' flanking region and at least one 3F2 3' flanking region.

[00494] In one embodiment, the molecular scaffold may comprise at least one 5F1 5' flanking region and at least one 3F1 3' flanking region.

[00495] In one embodiment, the molecular scaffold may comprise at least one 5F7 5' flanking region and at least one 3F5 3' flanking region.

[00496] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region and at least one 3F1 3' flanking region.

[00497] In one embodiment, the molecular scaffold may comprise at least one 5F4 5' flanking region and at least one 3F4 3' flanking region.

[00498] In one embodiment, the molecular scaffold may comprise at least one 5F5 5' flanking region and at least one 3F4 3' flanking region.

[00499] In one embodiment, the molecular scaffold may comprise at least one 5F6 5' flanking region and at least one 3F1 3' flanking region.

[00500] In one embodiment, the molecular scaffold may comprise at least one 5F2 5' flanking region and at least one 3F3 3' flanking region.

[00501] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region and at least one 3F4 3' flanking region.

[00502] In one embodiment, the molecular scaffold may comprise at least one 5F1 5' flanking region and at least one 3F2 3' flanking region.

[00503] In one embodiment, the molecular scaffold may comprise at least one 5' flanking region, fragment or variant thereof, at least one loop motif region, fragment or variant thereof, and at least one 3' flanking region as described in Tables 5 – 7. As a non-limiting example, the flanking and loop motif regions may be 5F1, L1 and 3F1; 5F1, L1 and 3F2; 5F1, L1 and 3F3; 5F1, L1 and 3F4; 5F1, L1 and 3F5; 5F1, L1 and 3F6; 5F1, L1 and 3F7; 5F2, L1 and 3F1; 5F2, L1 and 3F2; 5F2, L1 and 3F3; 5F2, L1 and 3F4; 5F2, L1 and 3F5; 5F2, L1 and 3F6; 5F2, L1 and 3F7; 5F3, L1 and 3F1; 5F3, L1 and 3F2; 5F3, L1 and 3F3; 5F3, L1 and 3F4; 5F3, L1 and 3F5;

5F3, L1 and 3F6; 5F3, L1 and 3F7; 5F4, L1 and 3F1; 5F4, L1 and 3F2; 5F4, L1 and 3F3; 5F4, L1 and 3F4; 5F4, L1 and 3F5; 5F4, L1 and 3F6; 5F4, L1 and 3F7; 5F5, L1 and 3F1; 5F5, L1 and 3F2; 5F5, L1 and 3F3; 5F5, L1 and 3F4; 5F5, L1 and 3F5; 5F5, L1 and 3F6; 5F5, L1 and 3F7; 5F6, L1 and 3F1; 5F6, L1 and 3F2; 5F6, L1 and 3F3; 5F6, L1 and 3F4; 5F6, L1 and 3F5; 5F6, L1 and 3F6; 5F6, L1 and 3F7; 5F7, L1 and 3F1; 5F7, L1 and 3F2; 5F7, L1 and 3F3; 5F7, L1 and 3F4; 5F7, L1 and 3F5; 5F7, L1 and 3F6; 5F7, L1 and 3F7; 5F8, L1 and 3F1; 5F8, L1 and 3F2; 5F8, L1 and 3F3; 5F8, L1 and 3F4; 5F8, L1 and 3F5; 5F8, L1 and 3F6; 5F8, L1 and 3F7; 5F9, L1 and 3F1; 5F9, L1 and 3F2; 5F9, L1 and 3F3; 5F9, L1 and 3F4; 5F9, L1 and 3F5; 5F9, L1 and 3F6; 5F9, L1 and 3F7; 5F1, L2 and 3F1; 5F1, L2 and 3F2; 5F1, L2 and 3F3; 5F1, L2 and 3F4; 5F1, L2 and 3F5; 5F1, L2 and 3F6; 5F1, L2 and 3F7; 5F2, L2 and 3F1; 5F2, L2 and 3F2; 5F2, L2 and 3F3; 5F2, L2 and 3F4; 5F2, L2 and 3F5; 5F2, L2 and 3F6; 5F2, L2 and 3F7; 5F3, L2 and 3F1; 5F3, L2 and 3F2; 5F3, L2 and 3F3; 5F3, L2 and 3F4; 5F3, L2 and 3F5; 5F3, L2 and 3F6; 5F3, L2 and 3F7; 5F4, L2 and 3F1; 5F4, L2 and 3F2; 5F4, L2 and 3F3; 5F4, L2 and 3F4; 5F4, L2 and 3F5; 5F4, L2 and 3F6; 5F4, L2 and 3F7; 5F5, L2 and 3F1; 5F5, L2 and 3F2; 5F5, L2 and 3F3; 5F5, L2 and 3F4; 5F5, L2 and 3F5; 5F5, L2 and 3F6; 5F5, L2 and 3F7; 5F6, L2 and 3F1; 5F6, L2 and 3F2; 5F6, L2 and 3F3; 5F6, L2 and 3F4; 5F6, L2 and 3F5; 5F6, L2 and 3F6; 5F6, L2 and 3F7; 5F7, L2 and 3F1; 5F7, L2 and 3F2; 5F7, L2 and 3F3; 5F7, L2 and 3F4; 5F7, L2 and 3F5; 5F7, L2 and 3F6; 5F7, L2 and 3F7; 5F8, L2 and 3F1; 5F8, L2 and 3F2; 5F8, L2 and 3F3; 5F8, L2 and 3F4; 5F8, L2 and 3F5; 5F8, L2 and 3F6; 5F8, L2 and 3F7; 5F9, L2 and 3F1; 5F9, L2 and 3F2; 5F9, L2 and 3F3; 5F9, L2 and 3F4; 5F9, L2 and 3F5; 5F9, L2 and 3F6; 5F9, L2 and 3F7; 5F1, L3 and 3F1; 5F1, L3 and 3F2; 5F1, L3 and 3F3; 5F1, L3 and 3F4; 5F1, L3 and 3F5; 5F1, L3 and 3F6; 5F1, L3 and 3F7; 5F2, L3 and 3F1; 5F2, L3 and 3F2; 5F2, L3 and 3F3; 5F2, L3 and 3F4; 5F2, L3 and 3F5; 5F2, L3 and 3F6; 5F2, L3 and 3F7; 5F3, L3 and 3F1; 5F3, L3 and 3F2; 5F3, L3 and 3F3; 5F3, L3 and 3F4; 5F3, L3 and 3F5; 5F3, L3 and 3F6; 5F3, L3 and 3F7; 5F4, L3 and 3F1; 5F4, L3 and 3F2; 5F4, L3 and 3F3; 5F4, L3 and 3F4; 5F4, L3 and 3F5; 5F4, L3 and 3F6; 5F4, L3 and 3F7; 5F5, L3 and 3F1; 5F5, L3 and 3F2; 5F5, L3 and 3F3; 5F5, L3 and 3F4; 5F5, L3 and 3F5; 5F5, L3 and 3F6; 5F5, L3 and 3F7; 5F6, L3 and 3F1; 5F6, L3 and 3F2; 5F6, L3 and 3F3; 5F6, L3 and 3F4; 5F6, L3 and 3F5; 5F6, L3 and 3F6; 5F6, L3 and 3F7; 5F7, L3 and 3F1; 5F7, L3 and 3F2; 5F7, L3 and 3F3; 5F7, L3 and 3F4; 5F7, L3 and 3F5; 5F7, L3 and 3F6; 5F7, L3 and 3F7; 5F8, L3 and 3F1; 5F8, L3 and 3F2; 5F8, L3 and 3F3; 5F8, L3 and 3F4; 5F8, L3 and 3F5; 5F8, L3 and 3F6; 5F8, L3 and 3F7; 5F9, L3 and 3F1; 5F9, L3 and 3F2; 5F9, L3 and 3F3; 5F9, L3 and 3F4; 5F9, L3 and 3F5; 5F9, L3 and 3F6; 5F9, L3 and 3F7; 5F1, L4 and 3F1; 5F1, L4 and 3F2; 5F1, L4 and 3F3; 5F1, L4 and 3F4; 5F1, L4 and 3F5; 5F1, L4 and 3F6;

5F1, L4 and 3F7; 5F2, L4 and 3F1; 5F2, L4 and 3F2; 5F2, L4 and 3F3; 5F2, L4 and 3F4; 5F2, L4 and 3F5; 5F2, L4 and 3F6; 5F2, L4 and 3F7; 5F3, L4 and 3F1; 5F3, L4 and 3F2; 5F3, L4 and 3F3; 5F3, L4 and 3F4; 5F3, L4 and 3F5; 5F3, L4 and 3F6; 5F3, L4 and 3F7; 5F4, L4 and 3F1; 5F4, L4 and 3F2; 5F4, L4 and 3F3; 5F4, L4 and 3F4; 5F4, L4 and 3F5; 5F4, L4 and 3F6; 5F4, L4 and 3F7; 5F5, L4 and 3F1; 5F5, L4 and 3F2; 5F5, L4 and 3F3; 5F5, L4 and 3F4; 5F5, L4 and 3F5; 5F5, L4 and 3F6; 5F5, L4 and 3F7; 5F6, L4 and 3F1; 5F6, L4 and 3F2; 5F6, L4 and 3F3; 5F6, L4 and 3F4; 5F6, L4 and 3F5; 5F6, L4 and 3F6; 5F6, L4 and 3F7; 5F7, L4 and 3F1; 5F7, L4 and 3F2; 5F7, L4 and 3F3; 5F7, L4 and 3F4; 5F7, L4 and 3F5; 5F7, L4 and 3F6; 5F7, L4 and 3F7; 5F8, L4 and 3F1; 5F8, L4 and 3F2; 5F8, L4 and 3F3; 5F8, L4 and 3F4; 5F8, L4 and 3F5; 5F8, L4 and 3F6; 5F8, L4 and 3F7; 5F9, L4 and 3F1; 5F9, L4 and 3F2; 5F9, L4 and 3F3; 5F9, L4 and 3F4; 5F9, L4 and 3F5; 5F9, L4 and 3F6; 5F9, L4 and 3F7; 5F1, L5 and 3F1; 5F1, L5 and 3F2; 5F1, L5 and 3F3; 5F1, L5 and 3F4; 5F1, L5 and 3F5; 5F1, L5 and 3F6; 5F1, L5 and 3F7; 5F2, L5 and 3F1; 5F2, L5 and 3F2; 5F2, L5 and 3F3; 5F2, L5 and 3F4; 5F2, L5 and 3F5; 5F2, L5 and 3F6; 5F2, L5 and 3F7; 5F3, L5 and 3F1; 5F3, L5 and 3F2; 5F3, L5 and 3F3; 5F3, L5 and 3F4; 5F3, L5 and 3F5; 5F3, L5 and 3F6; 5F3, L5 and 3F7; 5F4, L5 and 3F1; 5F4, L5 and 3F2; 5F4, L5 and 3F3; 5F4, L5 and 3F4; 5F4, L5 and 3F5; 5F4, L5 and 3F6; 5F4, L5 and 3F7; 5F5, L5 and 3F1; 5F5, L5 and 3F2; 5F5, L5 and 3F3; 5F5, L5 and 3F4; 5F5, L5 and 3F5; 5F5, L5 and 3F6; 5F5, L5 and 3F7; 5F6, L5 and 3F1; 5F6, L5 and 3F2; 5F6, L5 and 3F3; 5F6, L5 and 3F4; 5F6, L5 and 3F5; 5F6, L5 and 3F6; 5F6, L5 and 3F7; 5F7, L5 and 3F1; 5F7, L5 and 3F2; 5F7, L5 and 3F3; 5F7, L5 and 3F4; 5F7, L5 and 3F5; 5F7, L5 and 3F6; 5F7, L5 and 3F7; 5F8, L5 and 3F1; 5F8, L5 and 3F2; 5F8, L5 and 3F3; 5F8, L5 and 3F4; 5F8, L5 and 3F5; 5F8, L5 and 3F6; 5F8, L5 and 3F7; 5F9, L5 and 3F1; 5F9, L5 and 3F2; 5F9, L5 and 3F3; 5F9, L5 and 3F4; 5F9, L5 and 3F5; 5F9, L5 and 3F6; 5F9, L5 and 3F7; 5F1, L6 and 3F1; 5F1, L6 and 3F2; 5F1, L6 and 3F3; 5F1, L6 and 3F4; 5F1, L6 and 3F5; 5F1, L6 and 3F6; 5F1, L6 and 3F7; 5F2, L6 and 3F1; 5F2, L6 and 3F2; 5F2, L6 and 3F3; 5F2, L6 and 3F4; 5F2, L6 and 3F5; 5F2, L6 and 3F6; 5F2, L6 and 3F7; 5F3, L6 and 3F1; 5F3, L6 and 3F2; 5F3, L6 and 3F3; 5F3, L6 and 3F4; 5F3, L6 and 3F5; 5F3, L6 and 3F6; 5F3, L6 and 3F7; 5F4, L6 and 3F1; 5F4, L6 and 3F2; 5F4, L6 and 3F3; 5F4, L6 and 3F4; 5F4, L6 and 3F5; 5F4, L6 and 3F6; 5F4, L6 and 3F7; 5F5, L6 and 3F1; 5F5, L6 and 3F2; 5F5, L6 and 3F3; 5F5, L6 and 3F4; 5F5, L6 and 3F5; 5F5, L6 and 3F6; 5F5, L6 and 3F7; 5F6, L6 and 3F1; 5F6, L6 and 3F2; 5F6, L6 and 3F3; 5F6, L6 and 3F4; 5F6, L6 and 3F5; 5F6, L6 and 3F6; 5F6, L6 and 3F7; 5F7, L6 and 3F1; 5F7, L6 and 3F2; 5F7, L6 and 3F3; 5F7, L6 and 3F4; 5F7, L6 and 3F5; 5F7, L6 and 3F6; 5F7, L6 and 3F7; 5F8, L6 and 3F1; 5F8, L6 and 3F2; 5F8, L6 and 3F3; 5F8, L6 and 3F4; 5F8, L6 and 3F5; 5F8, L6 and 3F6; 5F8, L6 and 3F7;

5F9, L6 and 3F1; 5F9, L6 and 3F2; 5F9, L6 and 3F3; 5F9, L6 and 3F4; 5F9, L6 and 3F5; 5F9, L6 and 3F6; 5F9, L6 and 3F7; 5F1, L7 and 3F1; 5F1, L7 and 3F2; 5F1, L7 and 3F3; 5F1, L7 and 3F4; 5F1, L7 and 3F5; 5F1, L7 and 3F6; 5F1, L7 and 3F7; 5F2, L7 and 3F1; 5F2, L7 and 3F2; 5F2, L7 and 3F3; 5F2, L7 and 3F4; 5F2, L7 and 3F5; 5F2, L7 and 3F6; 5F2, L7 and 3F7; 5F3, L7 and 3F1; 5F3, L7 and 3F2; 5F3, L7 and 3F3; 5F3, L7 and 3F4; 5F3, L7 and 3F5; 5F3, L7 and 3F6; 5F3, L7 and 3F7; 5F4, L7 and 3F1; 5F4, L7 and 3F2; 5F4, L7 and 3F3; 5F4, L7 and 3F4; 5F4, L7 and 3F5; 5F4, L7 and 3F6; 5F4, L7 and 3F7; 5F5, L7 and 3F1; 5F5, L7 and 3F2; 5F5, L7 and 3F3; 5F5, L7 and 3F4; 5F5, L7 and 3F5; 5F5, L7 and 3F6; 5F5, L7 and 3F7; 5F6, L7 and 3F1; 5F6, L7 and 3F2; 5F6, L7 and 3F3; 5F6, L7 and 3F4; 5F6, L7 and 3F5; 5F6, L7 and 3F6; 5F6, L7 and 3F7; 5F7, L7 and 3F1; 5F7, L7 and 3F2; 5F7, L7 and 3F3; 5F7, L7 and 3F4; 5F7, L7 and 3F5; 5F7, L7 and 3F6; 5F7, L7 and 3F7; 5F8, L7 and 3F1; 5F8, L7 and 3F2; 5F8, L7 and 3F3; 5F8, L7 and 3F4; 5F8, L7 and 3F5; 5F8, L7 and 3F6; 5F8, L7 and 3F7; ; 5F9, L7 and 3F1; 5F9, L7 and 3F2; 5F9, L7 and 3F3; 5F9, L7 and 3F4; 5F9, L7 and 3F5; 5F9, L7 and 3F6; 5F9, L7 and 3F7; 5F1, L8 and 3F1; 5F1, L8 and 3F2; 5F1, L8 and 3F3; 5F1, L8 and 3F4; 5F1, L8 and 3F5; 5F1, L8 and 3F6; 5F1, L8 and 3F7; 5F2, L8 and 3F1; 5F2, L8 and 3F2; 5F2, L8 and 3F3; 5F2, L8 and 3F4; 5F2, L8 and 3F5; 5F2, L8 and 3F6; 5F2, L8 and 3F7; 5F3, L8 and 3F1; 5F3, L8 and 3F2; 5F3, L8 and 3F3; 5F3, L8 and 3F4; 5F3, L8 and 3F5; 5F3, L8 and 3F6; 5F3, L8 and 3F7; 5F4, L8 and 3F1; 5F4, L8 and 3F2; 5F4, L8 and 3F3; 5F4, L8 and 3F4; 5F4, L8 and 3F5; 5F4, L8 and 3F6; 5F4, L8 and 3F7; 5F5, L8 and 3F1; 5F5, L8 and 3F2; 5F5, L8 and 3F3; 5F5, L8 and 3F4; 5F5, L8 and 3F5; 5F5, L8 and 3F6; 5F5, L8 and 3F7; 5F6, L8 and 3F1; 5F6, L8 and 3F2; 5F6, L8 and 3F3; 5F6, L8 and 3F4; 5F6, L8 and 3F5; 5F6, L8 and 3F6; 5F6, L8 and 3F7; 5F7, L8 and 3F1; 5F7, L8 and 3F2; 5F7, L8 and 3F3; 5F7, L8 and 3F4; 5F7, L8 and 3F5; 5F7, L8 and 3F6; 5F7, L8 and 3F7; 5F8, L8 and 3F1; 5F8, L8 and 3F2; 5F8, L8 and 3F3; 5F8, L8 and 3F4; 5F8, L8 and 3F5; 5F8, L8 and 3F6; 5F8, L8 and 3F7; 5F9, L8 and 3F1; 5F9, L8 and 3F2; 5F9, L8 and 3F3; 5F9, L8 and 3F4; 5F9, L8 and 3F5; 5F9, L8 and 3F6; 5F9, L8 and 3F7; 5F1, L9 and 3F1; 5F1, L9 and 3F2; 5F1, L9 and 3F3; 5F1, L9 and 3F4; 5F1, L9 and 3F5; 5F1, L9 and 3F6; 5F1, L9 and 3F7; 5F2, L9 and 3F1; 5F2, L9 and 3F2; 5F2, L9 and 3F3; 5F2, L9 and 3F4; 5F2, L9 and 3F5; 5F2, L9 and 3F6; 5F2, L9 and 3F7; 5F3, L9 and 3F1; 5F3, L9 and 3F2; 5F3, L9 and 3F3; 5F3, L9 and 3F4; 5F3, L9 and 3F5; 5F3, L9 and 3F6; 5F3, L9 and 3F7; 5F4, L9 and 3F1; 5F4, L9 and 3F2; 5F4, L9 and 3F3; 5F4, L9 and 3F4; 5F4, L9 and 3F5; 5F4, L9 and 3F6; 5F4, L9 and 3F7; 5F5, L9 and 3F1; 5F5, L9 and 3F2; 5F5, L9 and 3F3; 5F5, L9 and 3F4; 5F5, L9 and 3F5; 5F5, L9 and 3F6; 5F5, L9 and 3F7; 5F6, L9 and 3F1; 5F6, L9 and 3F2; 5F6, L9 and 3F3; 5F6, L9 and 3F4; 5F6, L9 and 3F5; 5F6, L9 and 3F6; 5F6, L9 and 3F7; 5F7, L9 and 3F1;

5F7, L9 and 3F2; 5F7, L9 and 3F3; 5F7, L9 and 3F4; 5F7, L9 and 3F5; 5F7, L9 and 3F6; 5F7, L9 and 3F7; 5F8, L9 and 3F1; 5F8, L9 and 3F2; 5F8, L9 and 3F3; 5F8, L9 and 3F4; 5F8, L9 and 3F5; 5F8, L9 and 3F6; 5F8, L9 and 3F7; 5F9, L9 and 3F1; 5F9, L9 and 3F2; 5F9, L9 and 3F3; 5F9, L9 and 3F4; 5F9, L9 and 3F5; 5F9, L9 and 3F6; 5F9, L9 and 3F7; 5F1, L10 and 3F1; 5F1, L10 and 3F2; 5F1, L10 and 3F3; 5F1, L10 and 3F4; 5F1, L10 and 3F5; 5F1, L10 and 3F6; 5F1, L10 and 3F7; 5F2, L10 and 3F1; 5F2, L10 and 3F2; 5F2, L10 and 3F3; 5F2, L10 and 3F4; 5F2, L10 and 3F5; 5F2, L10 and 3F6; 5F2, L10 and 3F7; 5F3, L10 and 3F1; 5F3, L10 and 3F2; 5F3, L10 and 3F3; 5F3, L10 and 3F4; 5F3, L10 and 3F5; 5F3, L10 and 3F6; 5F3, L10 and 3F7; 5F4, L10 and 3F1; 5F4, L10 and 3F2; 5F4, L10 and 3F3; 5F4, L10 and 3F4; 5F4, L10 and 3F5; 5F4, L10 and 3F6; 5F4, L10 and 3F7; 5F5, L10 and 3F1; 5F5, L10 and 3F2; 5F5, L10 and 3F3; 5F5, L10 and 3F4; 5F5, L10 and 3F5; 5F5, L10 and 3F6; 5F5, L10 and 3F7; 5F6, L10 and 3F1; 5F6, L10 and 3F2; 5F6, L10 and 3F3; 5F6, L10 and 3F4; 5F6, L10 and 3F5; 5F6, L10 and 3F6; 5F6, L10 and 3F7; 5F7, L10 and 3F1; 5F7, L10 and 3F2; 5F7, L10 and 3F3; 5F7, L10 and 3F4; 5F7, L10 and 3F5; 5F7, L10 and 3F6; 5F7, L10 and 3F7; 5F8, L10 and 3F1; 5F8, L10 and 3F2; 5F8, L10 and 3F3; 5F8, L10 and 3F4; 5F8, L10 and 3F5; 5F8, L10 and 3F6; 5F8, L10 and 3F7; 5F9, L10 and 3F1; 5F9, L10 and 3F2; 5F9, L10 and 3F3; 5F9, L10 and 3F4; 5F9, L10 and 3F5; 5F9, L10 and 3F6; and 5F9, L10 and 3F7.

[00504] In one embodiment, the molecular scaffold may comprise at least one 5F2 5' flanking region, at least one L1 loop motif region, and at least one 3F2 3' flanking region.

[00505] In one embodiment, the molecular scaffold may comprise at least one 5F1 5' flanking region, at least one L4 loop motif region, and at least one 3F1 3' flanking region.

[00506] In one embodiment, the molecular scaffold may comprise at least one 5F7 5' flanking region, at least one L8 loop motif region, and at least one 3F5 3' flanking region.

[00507] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region, at least one L4 loop motif region, and at least one 3F1 3' flanking region.

[00508] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region, at least one L5 loop motif region, and at least one 3F1 3' flanking region.

[00509] In one embodiment, the molecular scaffold may comprise at least one 5F4 5' flanking region, at least one L4 loop motif region, and at least one 3F4 3' flanking region.

[00510] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region, at least one L7 loop motif region, and at least one 3F1 3' flanking region.

[00511] In one embodiment, the molecular scaffold may comprise at least one 5F5 5' flanking region, at least one L4 loop motif region, and at least one 3F4 3' flanking region.

[00512] In one embodiment, the molecular scaffold may comprise at least one 5F6 5' flanking region, at least one L4 loop motif region, and at least one 3F1 3' flanking region.

[00513] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region, at least one L6 loop motif region, and at least one 3F1 3' flanking region.

[00514] In one embodiment, the molecular scaffold may comprise at least one 5F7 5' flanking region, at least one L4 loop motif region, and at least one 3F5 3' flanking region.

[00515] In one embodiment, the molecular scaffold may comprise at least one 5F2 5' flanking region, at least one L2 loop motif region, and at least one 3F2 3' flanking region.

[00516] In one embodiment, the molecular scaffold may comprise at least one 5F2 5' flanking region, at least one L1 loop motif region, and at least one 3F3 3' flanking region.

[00517] In one embodiment, the molecular scaffold may comprise at least one 5F3 5' flanking region, at least one L5 loop motif region, and at least one 3F4 3' flanking region.

[00518] In one embodiment, the molecular scaffold may comprise at least one 5F1 5' flanking region, at least one L1 loop motif region, and at least one 3F1 3' flanking region.

[00519] In one embodiment, the molecular scaffold may comprise at least one 5F1 5' flanking region, at least one L2 loop motif region, and at least one 3F1 3' flanking region.

[00520] In one embodiment, the molecular scaffold may comprise at least one 5F1 5' flanking region, at least one L1 loop motif region, and at least one 3F2 3' flanking region.

[00521] In one embodiment, the molecular scaffold may comprise at least one 5F2 5' flanking region, at least one L3 loop motif region, and at least one 3F3 3' flanking region.

[00522] In one embodiment, the molecular scaffold may be a natural pri-miRNA scaffold. As a non-limiting example, the molecular scaffold may be a scaffold derived from the human miR155 scaffold.

[00523] In one embodiment, the molecular scaffold may comprise one or more linkers known in the art. The linkers may separate regions or one molecular scaffold from another. As a non-limiting example, the molecular scaffold may be polycistronic.

Modulatory Polynucleotide Comprising Molecular Scaffold and siRNA Molecules Targeting SOD1

[00524] In one embodiment, the modulatory polynucleotide may comprise 5' and 3' flanking regions, loop motif region, and nucleic acid sequences encoding sense sequence and antisense sequence as described in Tables 8 and 9. In Tables 8 and 9, the DNA sequence identifier for the passenger and guide strands are described as well as the 5' and 3' Flanking Regions and the Loop region (also referred to as the linker region). In Tables 8 and 9, the "miR" component of

the name of the sequence does not necessarily correspond to the sequence numbering of miRNA genes (e.g., VOYSOD1miR-102 is the name of the sequence and does not necessarily mean that miR-102 is part of the sequence).

Table 8. SOD1 Modulatory Polynucleotide Sequence Regions (5' to 3')

Modulatory Polynucleotide Construct Name	5' Flanking to 3' Flanking SEQ ID NO	5' Flanking SEQ ID NO	Passenger SEQ ID NO	Loop SEQ ID NO	Guide SEQ ID NO	3' Flanking SEQ ID NO
VOYSOD1miR-101	1281	1262	1331	1268	1332	1279
VOYSOD1miR-102	1282	1257	1331	1268	1332	1274
VOYSOD1miR-103	1283	1257	1333	1268	1332	1274
VOYSOD1miR-104	1284	1257	1334	1268	1332	1274
VOYSOD1miR-105	1285	1257	1335	1268	1332	1274
VOYSOD1miR-106	1286	1257	1336	1268	1332	1274
VOYSOD1miR-107	1287	1257	1337	1268	1332	1274
VOYSOD1miR-108	1288	1257	1339	1268	1332	1274
VOYSOD1miR-109	1289	1257	1331	1264	1332	1274
VOYSOD1miR-110	1290	1257	1331	1272	1332	1274
VOYSOD1miR-111	1291	1257	1338	1273	1332	1274
VOYSOD1miR-112	1292	1257	1331	1268	1332	1275
VOYSOD1miR-113	1293	1257	1333	1268	1332	1275
VOYSOD1miR-114	1294	1257	1336	1268	1332	1275
VOYSOD1miR-115	1295	1257	1338	1273	1332	1275
VOYSOD1miR-116	1296	1257	1334	1268	1332	1275
VOYSOD1miR-117	1297	1257	1340	1268	1341	1274
VOYSOD1miR-118	1298	1257	1342	1268	1343	1274
VOYSOD1miR-119	1299	1257	1344	1268	1345	1274
VOYSOD1miR-127	1300	1255	1331	1265	1332	1276
VOYSOD1miR-102.860	1301	1257	1346	1268	1347	1274
VOYSOD1miR-102.861	1302	1257	1348	1268	1349	1274
VOYSOD1miR-102.866	1303	1257	1350	1268	1345	1274
VOYSOD1miR-102.870	1304	1257	1351	1268	1352	1274
VOYSOD1miR-102.823	1305	1257	1353	1268	1343	1274
VOYSOD1miR-104.860	1306	1257	1354	1268	1347	1274
VOYSOD1miR-104.861	1307	1257	1355	1268	1349	1274
VOYSOD1miR-104.866	1308	1257	1356	1268	1345	1274
VOYSOD1miR-104.870	1309	1257	1357	1268	1352	1274
VOYSOD1miR-104.823	1310	1257	1358	1268	1343	1274
VOYSOD1miR-109.860	1311	1257	1346	1264	1347	1274
VOYSOD1miR-109.861	1312	1257	1348	1264	1349	1274
VOYSOD1miR-109.866	1313	1257	1350	1264	1345	1274
VOYSOD1miR-109.870	1314	1257	1351	1264	1352	1274
VOYSOD1miR-109.823	1315	1257	1353	1264	1343	1274
VOYSOD1miR-114.860	1316	1257	1359	1268	1347	1275
VOYSOD1miR-114.861	1317	1257	1360	1268	1349	1275
VOYSOD1miR-114.866	1318	1257	1361	1268	1345	1275
VOYSOD1miR-114.870	1319	1257	1362	1268	1352	1275
VOYSOD1miR-114.823	1320	1257	1363	1268	1343	1275
VOYSOD1miR-116.860	1321	1257	1354	1268	1347	1275
VOYSOD1miR-116.861	1322	1257	1355	1268	1349	1275
VOYSOD1miR-116.866	1323	1257	1364	1268	1345	1275
VOYSOD1miR-116.870	1324	1257	1357	1268	1352	1275
VOYSOD1miR-116.823	1325	1257	1358	1268	1343	1275
VOYSOD1miR-127.860	1326	1255	1365	1265	1347	1276
VOYSOD1miR-127.861	1327	1255	1348	1265	1349	1276

VOYSOD1miR-127.866	1328	1255	1350	1265	1345	1276
VOYSOD1miR-127.870	1329	1255	1351	1265	1352	1276
VOYSOD1miR-127.823	1330	1255	1366	1265	1343	1276

Table 9. SOD1 Modulatory Polynucleotide Sequence Region (5' to 3')

Name	5' Flanking to 3' Flanking SEQ ID NO	5' Flanking SEQ ID NO	Passenger SEQ ID NO	Loop SEQ ID NO	Guide SEQ ID NO	3' Flanking SEQ ID NO
VOYSOD1miR-120	1367	1263	1368	1264	1369	1280

AAV Particles Comprising Modulatory Polynucleotides

[00525] In one embodiment, the AAV particle comprises a viral genome with a payload region comprising a modulatory polynucleotide sequences. In such an embodiment, a viral genome encoding more than one polypeptide may be replicated and packaged into a viral particle. A target cell transduced with a viral particle comprising a modulatory polynucleotide may express the encoded sense and/or antisense sequences in a single cell.

[00526] In some embodiments, the AAV particles are useful in the field of medicine for the treatment, prophylaxis, palliation or amelioration of neurological diseases and/or disorders.

[00527] In one embodiment, the AAV particles comprising modulatory polynucleotide sequence which comprises a nucleic acid sequence encoding at least one siRNA molecule may be introduced into mammalian cells.

[00528] Where the AAV particle payload region comprises a modulatory polynucleotide, the modulatory polynucleotide may comprise sense and/or antisense sequences to knock down a target gene. The AAV viral genomes encoding modulatory polynucleotides described herein may be useful in the fields of human disease, viruses, infections veterinary applications and a variety of *in vivo* and *in vitro* settings.

[00529] In one embodiment, the AAV particle viral genome may comprise at least one inverted terminal repeat (ITR) region. The ITR region(s) may, independently, have a length such as, but not limited to, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, and 175 nucleotides. The length of the ITR region for the viral genome may be

75-80, 75-85, 75-100, 80-85, 80-90, 80-105, 85-90, 85-95, 85-110, 90-95, 90-100, 90-115, 95-100, 95-105, 95-120, 100-105, 100-110, 100-125, 105-110, 105-115, 105-130, 110-115, 110-120, 110-135, 115-120, 115-125, 115-140, 120-125, 120-130, 120-145, 125-130, 125-135, 125-150, 130-135, 130-140, 130-155, 135-140, 135-145, 135-160, 140-145, 140-150, 140-165, 145-150, 145-155, 145-170, 150-155, 150-160, 150-175, 155-160, 155-165, 160-165, 160-170, 165-170, 165-175, and 170-175 nucleotides. As a non-limiting example, the viral genome comprises an ITR that is about 105 nucleotides in length. As a non-limiting example, the viral genome comprises an ITR that is about 141 nucleotides in length. As a non-limiting example, the viral genome comprises an ITR that is about 130 nucleotides in length.

[00530] In one embodiment, the AAV particle viral genome may comprises two inverted terminal repeat (ITR) regions. Each of the ITR regions may independently have a length such as, but not limited to, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, and 175 nucleotides. The length of the ITR regions for the viral genome may be 75-80, 75-85, 75-100, 80-85, 80-90, 80-105, 85-90, 85-95, 85-110, 90-95, 90-100, 90-115, 95-100, 95-105, 95-120, 100-105, 100-110, 100-125, 105-110, 105-115, 105-130, 110-115, 110-120, 110-135, 115-120, 115-125, 115-140, 120-125, 120-130, 120-145, 125-130, 125-135, 125-150, 130-135, 130-140, 130-155, 135-140, 135-145, 135-160, 140-145, 140-150, 140-165, 145-150, 145-155, 145-170, 150-155, 150-160, 150-175, 155-160, 155-165, 160-165, 160-170, 165-170, 165-175, and 170-175 nucleotides. As a non-limiting example, the viral genome comprises an ITR that is about 105 nucleotides in length and 141 nucleotides in length. As a non-limiting example, the viral genome comprises an ITR that is about 105 nucleotides in length and 130 nucleotides in length. As a non-limiting example, the viral genome comprises an ITR that is about 130 nucleotides in length and 141 nucleotides in length.

[00531] In one embodiment, the AAV particle viral genome may comprise at least one sequence region as described in Tables 10-17. The regions may be located before or after any of the other sequence regions described herein.

[00532] In one embodiment, the AAV particle viral genome comprises at least one inverted terminal repeat (ITR) sequence region. Non-limiting examples of ITR sequence regions are described in Table 10.

Table 10. Inverted Terminal Repeat (ITR) Sequence Regions

Sequence Region Name	SEQ ID NO
ITR1	1370
ITR2	1371
ITR3	1372
ITR4	1373

[00533] In one embodiment, the AAV particle viral genome comprises two ITR sequence regions. In one embodiment, the ITR sequence regions are the ITR1 sequence region and the ITR3 sequence region. In one embodiment, the ITR sequence regions are the ITR1 sequence region and the ITR4 sequence region. In one embodiment, the ITR sequence regions are the ITR2 sequence region and the ITR3 sequence region. In one embodiment, the ITR sequence regions are the ITR2 sequence region and the ITR4 sequence region.

[00534] In one embodiment, the AAV particle viral genome may comprise at least one multiple cloning site (MCS) sequence region. The MCS region(s) may, independently, have a length such as, but not limited to, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150 nucleotides. The length of the MCS region for the viral genome may be 2-10, 5-10, 5-15, 10-20, 10-30, 10-40, 15-20, 15-25, 20-30, 20-40, 20-50, 25-30, 25-35, 30-40, 30-50, 30-60, 35-40, 35-45, 40-50, 40-60, 40-70, 45-50, 45-55, 50-60, 50-70, 50-80, 55-60, 55-65, 60-70, 60-80, 60-90, 65-70, 65-75, 70-80, 70-90, 70-100, 75-80, 75-85, 80-90, 80-100, 80-110, 85-90, 85-95, 90-100, 90-110, 90-120, 95-100, 95-105, 100-110, 100-120, 100-130, 105-110, 105-115, 110-120, 110-130, 110-140, 115-120, 115-125, 120-130, 120-140, 120-150, 125-130, 125-135, 130-140, 130-150, 135-140, 135-145, 140-150, and 145-150 nucleotides. As a non-limiting example, the viral genome comprises a MCS region that is about 5 nucleotides in length. As a non-limiting example, the viral genome comprises a MCS region that is about 10 nucleotides in length. As a non-limiting example, the viral genome comprises a MCS region that is about 14 nucleotides in length. As a non-limiting example, the viral genome comprises a MCS region that is about 18 nucleotides in length. As a non-limiting example, the viral genome comprises a MCS region that is about 73 nucleotides in length. As a non-limiting example, the viral genome comprises a MCS region that is about 121 nucleotides in length.

[00535] In one embodiment, the AAV particle viral genome comprises at least one multiple cloning site (MCS) sequence regions. Non-limiting examples of MCS sequence regions are described in Table 11.

Table 11. Multiple Cloning Site (MCS) Sequence Regions

Sequence Region Name	SEQ ID NO or Sequence
MCS1	1374
MCS2	1375
MCS3	1376
MCS4	1377
MCS5	TCGAG
MCS6	1378

[00536] In one embodiment, the AAV particle viral genome comprises one MCS sequence region. In one embodiment, the MCS sequence region is the MCS1 sequence region. In one embodiment, the MCS sequence region is the MCS2 sequence region. In one embodiment, the MCS sequence region is the MCS3 sequence region. In one embodiment, the MCS sequence region is the MCS4 sequence region. In one embodiment, the MCS sequence region is the MCS5 sequence region. In one embodiment, the MCS sequence region is the MCS6 sequence region.

[00537] In one embodiment, the AAV particle viral genome comprises two MCS sequence regions. In one embodiment, the two MCS sequence regions are the MCS1 sequence region and the MCS2 sequence region. In one embodiment, the two MCS sequence regions are the MCS1 sequence region and the MCS3 sequence region. In one embodiment, the two MCS sequence regions are the MCS1 sequence region and the MCS4 sequence region. In one embodiment, the two MCS sequence regions are the MCS1 sequence region and the MCS5 sequence region. In one embodiment, the two MCS sequence regions are the MCS1 sequence region and the MCS6 sequence region. In one embodiment, the two MCS sequence regions are the MCS2 sequence region and the MCS3 sequence region. In one embodiment, the two MCS sequence regions are the MCS2 sequence region and the MCS4 sequence region. In one embodiment, the two MCS sequence regions are the MCS2 sequence region and the MCS5 sequence region. In one embodiment, the two MCS sequence regions are the MCS2 sequence region and the MCS6 sequence region. In one embodiment, the two MCS sequence regions are the MCS3 sequence region and the MCS4 sequence region. In one embodiment, the two MCS sequence regions are the MCS3 sequence region and the MCS5 sequence region. In one embodiment, the two MCS sequence regions are the MCS3 sequence region and the MCS6 sequence region. In one embodiment, the two MCS sequence regions are the MCS4 sequence region and the MCS5 sequence region. In one embodiment, the two MCS sequence regions are the MCS4 sequence

region and the MCS6 sequence region. In one embodiment, the two MCS sequence regions are the MCS5 sequence region and the MCS6 sequence region.

[00538] In one embodiment, the AAV particle viral genome comprises two or more MCS sequence regions.

[00539] In one embodiment, the AAV particle viral genome comprises three MCS sequence regions. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS2 sequence region, and the MCS3 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS2 sequence region, and the MCS4 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS2 sequence region, and the MCS5 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS2 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS3 sequence region, and the MCS4 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS3 sequence region, and the MCS5 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS3 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS4 sequence region, and the MCS5 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS4 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS1 sequence region, the MCS5 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS2 sequence region, the MCS3 sequence region, and the MCS4 sequence region. In one embodiment, the three MCS sequence regions are the MCS2 sequence region, the MCS3 sequence region, and the MCS5 sequence region. In one embodiment, the three MCS sequence regions are the MCS2 sequence region, the MCS3 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS2 sequence region, the MCS4 sequence region, and the MCS5 sequence region. In one embodiment, the three MCS sequence regions are the MCS2 sequence region, the MCS4 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS2 sequence region, the MCS5 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS3 sequence region, the MCS4 sequence region, and the MCS5 sequence region. In one embodiment, the three MCS sequence regions are the MCS3 sequence region, the MCS4 sequence region, and the

MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS3 sequence region, the MCS5 sequence region, and the MCS6 sequence region. In one embodiment, the three MCS sequence regions are the MCS4 sequence region, the MCS5 sequence region, and the MCS6 sequence region.

[00540] In one embodiment, the AAV particle viral genome may comprise at least one multiple filler sequence region. The filler region(s) may, independently, have a length such as, but not limited to, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572,

573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157,

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3138, 3139, 3140, 3141, 3142, 3143, 3144, 3145, 3146, 3147, 3148, 3149, 3150, 3151, 3152, 3153, 3154, 3155, 3156, 3157, 3158, 3159, 3160, 3161, 3162, 3163, 3164, 3165, 3166, 3167, 3168, 3169, 3170, 3171, 3172, 3173, 3174, 3175, 3176, 3177, 3178, 3179, 3180, 3181, 3182, 3183, 3184, 3185, 3186, 3187, 3188, 3189, 3190, 3191, 3192, 3193, 3194, 3195, 3196, 3197, 3198, 3199, 3200, 3201, 3202, 3203, 3204, 3205, 3206, 3207, 3208, 3209, 3210, 3211, 3212, 3213, 3214, 3215, 3216, 3217, 3218, 3219, 3220, 3221, 3222, 3223, 3224, 3225, 3226, 3227, 3228, 3229, 3230, 3231, 3232, 3233, 3234, 3235, 3236, 3237, 3238, 3239, 3240, 3241, 3242, 3243, 3244, 3245, 3246, 3247, 3248, 3249, and 3250 nucleotides. The length of any filler region for the viral genome may be 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, 1150-1200, 1200-1250, 1250-1300, 1300-1350, 1350-1400, 1400-1450, 1450-1500, 1500-1550, 1550-1600, 1600-1650, 1650-1700, 1700-1750, 1750-1800, 1800-1850, 1850-1900, 1900-1950, 1950-2000, 2000-2050, 2050-2100, 2100-2150, 2150-2200, 2200-2250, 2250-2300, 2300-2350, 2350-2400, 2400-2450, 2450-2500, 2500-2550, 2550-2600, 2600-2650, 2650-2700, 2700-2750, 2750-2800, 2800-2850, 2850-2900, 2900-2950, 2950-3000, 3000-3050, 3050-3100, 3100-3150, 3150-3200, and 3200-3250 nucleotides. As a non-limiting example, the viral genome comprises a filler region that is about 55 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 56 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 97 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 103 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 105 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 357 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 363 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 712 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 714 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1203 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1209 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1512 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1519 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 2395 nucleotides in length. As a non-limiting example, the viral genome comprises a filler

region that is about 2403 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 2405 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 3013 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 3021 nucleotides in length.

[00541] In one embodiment, the AAV particle viral genome may comprise at least one multiple filler sequence region. The filler region(s) may, independently, have a length such as, but not limited to, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572,

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1653, 1654, 1655, 1656, 1657, 1658, 1659, 1660, 1661, 1662, 1663, 1664, 1665, 1666, 1667, 1668, 1669, 1670, 1671, 1672, 1673, 1674, 1675, 1676, 1677, 1678, 1679, 1680, 1681, 1682, 1683, 1684, 1685, 1686, 1687, 1688, 1689, 1690, 1691, 1692, 1693, 1694, 1695, 1696, 1697, 1698, 1699, 1700, 1701, 1702, 1703, 1704, 1705, 1706, 1707, 1708, 1709, 1710, 1711, 1712, 1713, 1714, 1715, 1716, 1717, 1718, 1719, 1720, 1721, 1722, 1723, 1724, 1725, 1726, 1727, 1728, 1729, 1730, 1731, 1732, 1733, 1734, 1735, 1736, 1737, 1738, 1739, 1740, 1741, 1742, 1743, 1744, 1745, 1746, 1747, 1748, 1749, 1750, 1751, 1752, 1753, 1754, 1755, 1756, 1757, 1758, 1759, 1760, 1761, 1762, 1763, 1764, 1765, 1766, 1767, 1768, 1769, 1770, 1771, 1772, 1773, 1774, 1775, 1776, 1777, 1778, 1779, 1780, 1781, 1782, 1783, 1784, 1785, 1786, 1787, 1788, 1789, 1790, 1791, 1792, 1793, 1794, 1795, 1796, 1797, 1798, 1799, 1800, 1801, 1802, 1803, 1804, 1805, 1806, 1807, 1808, 1809, 1810, 1811, 1812, 1813, 1814, 1815, 1816, 1817, 1818, 1819, 1820, 1821, 1822, 1823, 1824, 1825, 1826, 1827, 1828, 1829, 1830, 1831, 1832, 1833, 1834, 1835, 1836, 1837, 1838, 1839, 1840, 1841, 1842, 1843, 1844, 1845, 1846, 1847, 1848, 1849, 1850, 1851, 1852, 1853, 1854, 1855, 1856, 1857, 1858, 1859, 1860, 1861, 1862, 1863, 1864, 1865, 1866, 1867, 1868, 1869, 1870, 1871, 1872, 1873, 1874, 1875, 1876, 1877, 1878, 1879, 1880, 1881, 1882, 1883, 1884, 1885, 1886, 1887, 1888, 1889, 1890, 1891, 1892, 1893, 1894, 1895, 1896, 1897, 1898, 1899, 1900, 1901, 1902, 1903, 1904, 1905, 1906, 1907, 1908, 1909, 1910, 1911, 1912, 1913, 1914, 1915, 1916, 1917, 1918, 1919, 1920, 1921, 1922, 1923, 1924, 1925, 1926, 1927, 1928, 1929, 1930, 1931, 1932, 1933, 1934, 1935, 1936, 1937, 1938, 1939, 1940, 1941, 1942, 1943, 1944, 1945, 1946, 1947, 1948, 1949, 1950, 1951, 1952, 1953, 1954, 1955, 1956, 1957, 1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147,

2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642,

2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 2679, 2680, 2681, 2682, 2683, 2684, 2685, 2686, 2687, 2688, 2689, 2690, 2691, 2692, 2693, 2694, 2695, 2696, 2697, 2698, 2699, 2700, 2701, 2702, 2703, 2704, 2705, 2706, 2707, 2708, 2709, 2710, 2711, 2712, 2713, 2714, 2715, 2716, 2717, 2718, 2719, 2720, 2721, 2722, 2723, 2724, 2725, 2726, 2727, 2728, 2729, 2730, 2731, 2732, 2733, 2734, 2735, 2736, 2737, 2738, 2739, 2740, 2741, 2742, 2743, 2744, 2745, 2746, 2747, 2748, 2749, 2750, 2751, 2752, 2753, 2754, 2755, 2756, 2757, 2758, 2759, 2760, 2761, 2762, 2763, 2764, 2765, 2766, 2767, 2768, 2769, 2770, 2771, 2772, 2773, 2774, 2775, 2776, 2777, 2778, 2779, 2780, 2781, 2782, 2783, 2784, 2785, 2786, 2787, 2788, 2789, 2790, 2791, 2792, 2793, 2794, 2795, 2796, 2797, 2798, 2799, 2800, 2801, 2802, 2803, 2804, 2805, 2806, 2807, 2808, 2809, 2810, 2811, 2812, 2813, 2814, 2815, 2816, 2817, 2818, 2819, 2820, 2821, 2822, 2823, 2824, 2825, 2826, 2827, 2828, 2829, 2830, 2831, 2832, 2833, 2834, 2835, 2836, 2837, 2838, 2839, 2840, 2841, 2842, 2843, 2844, 2845, 2846, 2847, 2848, 2849, 2850, 2851, 2852, 2853, 2854, 2855, 2856, 2857, 2858, 2859, 2860, 2861, 2862, 2863, 2864, 2865, 2866, 2867, 2868, 2869, 2870, 2871, 2872, 2873, 2874, 2875, 2876, 2877, 2878, 2879, 2880, 2881, 2882, 2883, 2884, 2885, 2886, 2887, 2888, 2889, 2890, 2891, 2892, 2893, 2894, 2895, 2896, 2897, 2898, 2899, 2900, 2901, 2902, 2903, 2904, 2905, 2906, 2907, 2908, 2909, 2910, 2911, 2912, 2913, 2914, 2915, 2916, 2917, 2918, 2919, 2920, 2921, 2922, 2923, 2924, 2925, 2926, 2927, 2928, 2929, 2930, 2931, 2932, 2933, 2934, 2935, 2936, 2937, 2938, 2939, 2940, 2941, 2942, 2943, 2944, 2945, 2946, 2947, 2948, 2949, 2950, 2951, 2952, 2953, 2954, 2955, 2956, 2957, 2958, 2959, 2960, 2961, 2962, 2963, 2964, 2965, 2966, 2967, 2968, 2969, 2970, 2971, 2972, 2973, 2974, 2975, 2976, 2977, 2978, 2979, 2980, 2981, 2982, 2983, 2984, 2985, 2986, 2987, 2988, 2989, 2990, 2991, 2992, 2993, 2994, 2995, 2996, 2997, 2998, 2999, 3000, 3001, 3002, 3003, 3004, 3005, 3006, 3007, 3008, 3009, 3010, 3011, 3012, 3013, 3014, 3015, 3016, 3017, 3018, 3019, 3020, 3021, 3022, 3023, 3024, 3025, 3026, 3027, 3028, 3029, 3030, 3031, 3032, 3033, 3034, 3035, 3036, 3037, 3038, 3039, 3040, 3041, 3042, 3043, 3044, 3045, 3046, 3047, 3048, 3049, 3050, 3051, 3052, 3053, 3054, 3055, 3056, 3057, 3058, 3059, 3060, 3061, 3062, 3063, 3064, 3065, 3066, 3067, 3068, 3069, 3070, 3071, 3072, 3073, 3074, 3075, 3076, 3077, 3078, 3079, 3080, 3081, 3082, 3083, 3084, 3085, 3086, 3087, 3088, 3089, 3090, 3091, 3092, 3093, 3094, 3095, 3096, 3097, 3098, 3099, 3100, 3101, 3102, 3103, 3104, 3105, 3106, 3107, 3108, 3109, 3110, 3111, 3112, 3113, 3114, 3115, 3116, 3117, 3118, 3119, 3120, 3121, 3122, 3123, 3124, 3125, 3126, 3127, 3128, 3129, 3130, 3131, 3132, 3133, 3134, 3135, 3136, 3137,

3138, 3139, 3140, 3141, 3142, 3143, 3144, 3145, 3146, 3147, 3148, 3149, 3150, 3151, 3152, 3153, 3154, 3155, 3156, 3157, 3158, 3159, 3160, 3161, 3162, 3163, 3164, 3165, 3166, 3167, 3168, 3169, 3170, 3171, 3172, 3173, 3174, 3175, 3176, 3177, 3178, 3179, 3180, 3181, 3182, 3183, 3184, 3185, 3186, 3187, 3188, 3189, 3190, 3191, 3192, 3193, 3194, 3195, 3196, 3197, 3198, 3199, 3200, 3201, 3202, 3203, 3204, 3205, 3206, 3207, 3208, 3209, 3210, 3211, 3212, 3213, 3214, 3215, 3216, 3217, 3218, 3219, 3220, 3221, 3222, 3223, 3224, 3225, 3226, 3227, 3228, 3229, 3230, 3231, 3232, 3233, 3234, 3235, 3236, 3237, 3238, 3239, 3240, 3241, 3242, 3243, 3244, 3245, 3246, 3247, 3248, 3249, and 3250 nucleotides. The length of any filler region for the viral genome may be 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, 1150-1200, 1200-1250, 1250-1300, 1300-1350, 1350-1400, 1400-1450, 1450-1500, 1500-1550, 1550-1600, 1600-1650, 1650-1700, 1700-1750, 1750-1800, 1800-1850, 1850-1900, 1900-1950, 1950-2000, 2000-2050, 2050-2100, 2100-2150, 2150-2200, 2200-2250, 2250-2300, 2300-2350, 2350-2400, 2400-2450, 2450-2500, 2500-2550, 2550-2600, 2600-2650, 2650-2700, 2700-2750, 2750-2800, 2800-2850, 2850-2900, 2900-2950, 2950-3000, 3000-3050, 3050-3100, 3100-3150, 3150-3200, and 3200-3250 nucleotides. As a non-limiting example, the viral genome comprises a filler region that is about 55 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 56 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 97 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 103 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 105 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 357 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 363 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 712 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 714 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1203 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1209 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1512 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 1519 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 2395 nucleotides in length. As a non-limiting example, the viral genome comprises a filler

region that is about 2403 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 2405 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 3013 nucleotides in length. As a non-limiting example, the viral genome comprises a filler region that is about 3021 nucleotides in length.

[00542] In one embodiment, the AAV particle viral genome comprises at least one filler sequence regions. Non-limiting examples of filler sequence regions are described in Table 12.

Table 12. Filler Sequence Regions

Sequence Region Name	SEQ ID NO
FILL1	1379
FILL2	1380
FILL3	1381
FILL4	1382
FILL5	1383
FILL6	1384
FILL7	1385
FILL8	1386
FILL9	1387
FILL10	1388
FILL11	1389
FILL12	1390
FILL13	1391
FILL14	1392
FILL15	1393
FILL16	1394
FILL17	1395
FILL18	1396

[00543] In one embodiment, the AAV particle viral genome comprises one filler sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region. In one embodiment, the filler sequence

region is the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL18 sequence region.

[00544] In one embodiment, the AAV particle viral genome comprises two filler sequence regions. In one embodiment, the two filler sequence regions are the FILL1 sequence region, and the FILL2 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL3 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL4 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, and the FILL3 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL4 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the

FILL3 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL3 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL4 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL10 sequence region. In one embodiment, the

filler sequence region is the FILL5 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL5 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL6 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL15 sequence region. In one

embodiment, the filler sequence region is the FILL7 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL7 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL8 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the

FILL16 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler

sequence region is the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL17 sequence region, and the FILL18 sequence region.

[00545] In one embodiment, the AAV particle viral genome comprises three filler sequence regions. In one embodiment, the two filler sequence regions are the FILL1 sequence region, the FILL2 sequence region, and the FILL3 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL4 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL2 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL4 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence

region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL3 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL4 sequence

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region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL8 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL8 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL8 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL8 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL8 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL8 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL9 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence

region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL10 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL11 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL12 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL12 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL12 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL12 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL12 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL12 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL13 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL13 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL13 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL13 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL13 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL14 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL14 sequence

region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL1 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL4 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL5 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL6 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL7 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL8 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL9 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL10 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL11 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL2 sequence region, the FILL3 sequence

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region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL14 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL9 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL12 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL11 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL12 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL12 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL12 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL12 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL12 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL12 sequence

region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL13 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL13 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL13 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL13 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL13 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL14 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL14 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL10 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL12 sequence region, and the FILL13 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL12 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL12 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL12 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL12 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL12 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL13 sequence

region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL13 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL13 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL13 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL13 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL14 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL14 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL11 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL13 sequence region, and the FILL14 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL13 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL13 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL13 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL13 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL14 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL14 sequence

region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL12 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL14 sequence region, and the FILL15 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL14 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL14 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL14 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL13 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, the FILL15 sequence region, and the FILL16 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, the FILL15 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, the FILL15 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, the FILL16 sequence

region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL14 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region, the FILL16 sequence region, and the FILL17 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region, the FILL16 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL15 sequence region, the FILL17 sequence region, and the FILL18 sequence region. In one embodiment, the filler sequence region is the FILL16 sequence region, the FILL17 sequence region, and the FILL18 sequence region.

[00546] In one embodiment, the AAV particle viral genome may comprise at least one enhancer sequence region. The enhancer sequence region(s) may, independently, have a length such as, but not limited to, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, and 400 nucleotides. The length of the enhancer region for the viral genome may be 300-310, 300-325, 305-315, 310-320, 315-325, 320-330, 325-335, 325-350, 330-340, 335-345, 340-350, 345-355, 350-360, 350-375, 355-365, 360-370, 365-375, 370-380, 375-385, 375-400, 380-390, 385-395, and 390-400 nucleotides. As a non-limiting example, the viral genome comprises an enhancer region that is about 303 nucleotides in length. As a non-limiting example, the viral genome comprises an enhancer region that is about 382 nucleotides in length.

[00547] In one embodiment, the AAV particle viral genome comprises at least one enhancer sequence region. Non-limiting examples of enhancer sequence regions are described in Table 13.

Table 13. Enhancer Sequence Regions

Sequence Region Name	SEQ ID NO
Enhancer1	1397
Enhancer2	1398

[00548] In one embodiment, the AAV particle viral genome comprises one enhancer sequence region. In one embodiment, the enhancer sequence regions is the Enhancer1 sequence region. In one embodiment, the enhancer sequence regions is the Enhancer2 sequence region.

[00549] In one embodiment, the AAV particle viral genome comprises two enhancer sequence regions. In one embodiment, the enhancer sequence regions are the Enhancer1 sequence region and the Enhancer 2 sequence region.

[00550] In one embodiment, the AAV particle viral genome may comprise at least one promoter sequence region. The promoter sequence region(s) may, independently, have a length such as, but not limited to, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, and 600 nucleotides. The length of the promoter region for the viral

genome may be 4-10, 10-20, 10-50, 20-30, 30-40, 40-50, 50-60, 50-100, 60-70, 70-80, 80-90, 90-100, 100-110, 100-150, 110-120, 120-130, 130-140, 140-150, 150-160, 150-200, 160-170, 170-180, 180-190, 190-200, 200-210, 200-250, 210-220, 220-230, 230-240, 240-250, 250-260, 250-300, 260-270, 270-280, 280-290, 290-300, 300-310, 300-350, 310-320, 320-330, 330-340, 340-350, 350-360, 350-400, 360-370, 370-380, 380-390, 390-400, 400-410, 400-450, 410-420, 420-430, 430-440, 440-450, 450-460, 450-500, 460-470, 470-480, 480-490, 490-500, 500-510, 500-550, 510-520, 520-530, 530-540, 540-550, 550-560, 550-600, 560-570, 570-580, 580-590, and 590-600 nucleotides. As a non-limiting example, the viral genome comprises a promoter region that is about 4 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 17 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 204 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 219 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 260 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 303 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 382 nucleotides in length. As a non-limiting example, the viral genome comprises a promoter region that is about 588 nucleotides in length.

[00551] In one embodiment, the AAV particle viral genome comprises at least one promoter sequence region. Non-limiting examples of promoter sequence regions are described in Table 14.

Table 14. Promoter Sequence Regions

Sequence Region Name	SEQ ID NO or Sequence
Promoter1	1399
Promoter2	1400
Promoter3	GTTG
Promoter4	1401
Promoter5	1402
Promoter6	1403

[00552] In one embodiment, the AAV particle viral genome comprises one promoter sequence region. In one embodiment, the promoter sequence region is Promoter1. In one embodiment, the promoter sequence region is Promoter2. In one embodiment, the promoter sequence region is Promoter3. In one embodiment, the promoter sequence region is Promoter4. In one embodiment, the promoter sequence region is Promoter5. In one embodiment, the promoter sequence region is Promoter6.

[00553] In one embodiment, the AAV particle viral genome comprises two promoter sequence regions. In one embodiment, the promoter sequence region is Promoter1 sequence region, and the Promoter2 sequence region. In one embodiment, the promoter sequence region is

Promoter1 sequence region, and the Promoter3 sequence region. In one embodiment, the promoter sequence region is Promoter1 sequence region, and the Promoter4 sequence region. In one embodiment, the promoter sequence region is Promoter1 sequence region, and the Promoter5 sequence region. In one embodiment, the promoter sequence region is Promoter1 sequence region, and the Promoter6 sequence region. In one embodiment, the promoter sequence region is Promoter2 sequence region, and the Promoter3 sequence region. In one embodiment, the promoter sequence region is Promoter2 sequence region, and the Promoter4 sequence region. In one embodiment, the promoter sequence region is Promoter2 sequence region, and the Promoter5 sequence region. In one embodiment, the promoter sequence region is Promoter2 sequence region, and the Promoter6 sequence region. In one embodiment, the promoter sequence region is Promoter3 sequence region, and the Promoter4 sequence region. In one embodiment, the promoter sequence region is Promoter3 sequence region, and the Promoter5 sequence region. In one embodiment, the promoter sequence region is Promoter3 sequence region, and the Promoter6 sequence region. In one embodiment, the promoter sequence region is Promoter4 sequence region, and the Promoter5 sequence region. In one embodiment, the promoter sequence region is Promoter4 sequence region, and the Promoter6 sequence region. In one embodiment, the promoter sequence region is Promoter5 sequence region, and the Promoter6 sequence region.

[00554] In one embodiment, the AAV particle viral genome may comprise at least one exon sequence region. The exon region(s) may, independently, have a length such as, but not limited to, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150 nucleotides. The length of the exon region for the viral genome may be 2-10, 5-10, 5-15, 10-20, 10-30, 10-40, 15-20, 15-25, 20-30, 20-40, 20-50, 25-30, 25-35, 30-40, 30-50, 30-60, 35-40, 35-45, 40-50, 40-60, 40-70, 45-50, 45-55, 50-60, 50-70, 50-80, 55-60, 55-65, 60-70, 60-80, 60-90, 65-70, 65-75, 70-80, 70-90, 70-100, 75-80, 75-85, 80-90, 80-100, 80-110, 85-90, 85-95, 90-100, 90-110, 90-120, 95-100, 95-105, 100-110, 100-120, 100-130, 105-110, 105-115, 110-120, 110-130, 110-140, 115-120, 115-125, 120-130, 120-140, 120-150, 125-130, 125-135, 130-140, 130-150, 135-140, 135-145, 140-150, and 145-150 nucleotides. As a non-limiting example, the viral genome comprises an exon region that is about

53 nucleotides in length. As a non-limiting example, the viral genome comprises an exon region that is about 134 nucleotides in length.

[00555] In one embodiment, the AAV particle viral genome comprises at least one Exon sequence region. Non-limiting examples of Exon sequence regions are described in Table 15.

Table 15. Exon Sequence Regions

Sequence Region Name	SEQ ID NO
Exon1	1404
Exon2	1405

[00556] In one embodiment, the AAV particle viral genome comprises one Exon sequence region. In one embodiment, the Exon sequence regions is the Exon1 sequence region. In one embodiment, the Exon sequence regions is the Exon2 sequence region.

[00557] In one embodiment, the AAV particle viral genome comprises two Exon sequence regions. In one embodiment, the Exon sequence regions are the Exon1 sequence region and the Exon 2 sequence region.

[00558] In one embodiment, the AAV particle viral genome may comprise at least one intron sequence region. The intron region(s) may, independently, have a length such as, but not limited to, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, and 350 nucleotides. The length of the intron region for the viral genome may be 25-35, 25-50, 35-45, 45-55, 50-75, 55-65, 65-75, 75-85, 75-100, 85-95, 95-105, 100-125, 105-115, 115-125, 125-135, 125-150, 135-145, 145-155, 150-175, 155-165, 165-175, 175-185, 175-200,

185-195, 195-205, 200-225, 205-215, 215-225, 225-235, 225-250, 235-245, 245-255, 250-275, 255-265, 265-275, 275-285, 275-300, 285-295, 295-305, 300-325, 305-315, 315-325, 325-335, 325-350, and 335-345 nucleotides. As a non-limiting example, the viral genome comprises an intron region that is about 32 nucleotides in length. As a non-limiting example, the viral genome comprises an intron region that is about 172 nucleotides in length. As a non-limiting example, the viral genome comprises an intron region that is about 201 nucleotides in length. As a non-limiting example, the viral genome comprises an intron region that is about 347 nucleotides in length.

[00559] In one embodiment, the AAV particle viral genome comprises at least one intron sequence region. Non-limiting examples of intron sequence regions are described in Table 16.

Table 16. Intron Sequence Regions

Sequence Region Name	SEQ ID NO
Intron1	1406
Intron2	1407
Intron3	1408
Intron4	1409

[00560] In one embodiment, the AAV particle viral genome comprises one intron sequence region. In one embodiment, the intron sequence regions is the Intron1 sequence region. In one embodiment, the intron sequence regions is the Intron2 sequence region. In one embodiment, the intron sequence regions is the Intron3 sequence region. In one embodiment, the intron sequence regions is the Intron4 sequence region.

[00561] In one embodiment, the AAV particle viral genome comprises two intron sequence regions. In one embodiment, the intron sequence regions are the Intron1 sequence region and the Intron2 sequence region. In one embodiment, the intron sequence regions are the Intron1 sequence region and the Intron3 sequence region. In one embodiment, the intron sequence regions are the Intron1 sequence region and the Intron4 sequence region. In one embodiment, the intron sequence regions are the Intron2 sequence region and the Intron3 sequence region. In one embodiment, the intron sequence regions are the Intron2 sequence region and the Intron4 sequence region. In one embodiment, the intron sequence regions are the Intron3 sequence region and the Intron4 sequence region.

[00562] In one embodiment, the AAV particle viral genome comprises three intron sequence regions. In one embodiment, the intron sequence regions are the Intron1 sequence region, the Intron2 sequence region, and the Intron3 sequence region. In one embodiment, the intron sequence regions are the Intron1 sequence region, the Intron2 sequence region, and the Intron4 sequence region. In one embodiment, the intron sequence regions are the Intron1 sequence

region, the Intron3 sequence region, and the Intron4 sequence region. In one embodiment, the intron sequence regions are the Intron2 sequence region, the Intron3 sequence region, and the Intron4 sequence region.

[00563] In one embodiment, the AAV particle viral genome may comprise at least one polyadenylation signal sequence region. The polyadenylation signal region sequence region(s) may, independently, have a length such as, but not limited to, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550,

551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, and 600 nucleotides. The length of the polyadenylation signal sequence region for the viral genome may be 4-10, 10-20, 10-50, 20-30, 30-40, 40-50, 50-60, 50-100, 60-70, 70-80, 80-90, 90-100, 100-110, 100-150, 110-120, 120-130, 130-140, 140-150, 150-160, 150-200, 160-170, 170-180, 180-190, 190-200, 200-210, 200-250, 210-220, 220-230, 230-240, 240-250, 250-260, 250-300, 260-270, 270-280, 280-290, 290-300, 300-310, 300-350, 310-320, 320-330, 330-340, 340-350, 350-360, 350-400, 360-370, 370-380, 380-390, 390-400, 400-410, 400-450, 410-420, 420-430, 430-440, 440-450, 450-460, 450-500, 460-470, 470-480, 480-490, 490-500, 500-510, 500-550, 510-520, 520-530, 530-540, 540-550, 550-560, 550-600, 560-570, 570-580, 580-590, and 590-600 nucleotides. As a non-limiting example, the viral genome comprises a polyadenylation signal sequence region that is about 127 nucleotides in length. As a non-limiting example, the viral genome comprises a polyadenylation signal sequence region that is about 225 nucleotides in length. As a non-limiting example, the viral genome comprises a polyadenylation signal sequence region that is about 476 nucleotides in length. As a non-limiting example, the viral genome comprises a polyadenylation signal sequence region that is about 477 nucleotides in length.

[00564] In one embodiment, the AAV particle viral genome comprises at least one polyadenylation (polyA) signal sequence region. Non-limiting examples of polyA signal sequence regions are described in Table 17.

Table 17. PolyA Signal Sequence Regions

Sequence Region Name	SEQ ID NO
PolyA1	1410
PolyA2	1411
PolyA3	1412
PolyA4	1413

[00565] In one embodiment, the AAV particle viral genome comprises one polyA signal sequence region. In one embodiment, the polyA signal sequence regions is the PolyA1 sequence region. In one embodiment, the polyA signal sequence regions is the PolyA2 sequence region. In one embodiment, the polyA signal sequence regions is the PolyA3 sequence region. In one embodiment, the polyA signal sequence regions is the PolyA4 sequence region.

[00566] In one embodiment, the AAV particle viral genome comprises more than one polyA signal sequence region.

[00567] Non-limiting examples of ITR to ITR sequences of AAV particles comprising a viral genome with a payload region comprising a modulatory polynucleotide sequence are described in Table 18.

Table 18. ITR to ITR Sequences of AAV Particles comprising Modulatory Polynucleotides

ITR to ITR Construct Name	ITR to ITR SEQ ID NO	Modulatory Polynucleotide SEQ ID NO
VOYSOD1	1414	1326
VOYSOD2	1415	1326
VOYSOD3	1416	1317
VOYSOD4	1417	1317
VOYSOD5	2240	1326
VOYSOD6	2241	1326

[00568] In one embodiment, the AAV particle comprises a viral genome which comprises a sequence which has a percent identity to any of SEQ ID NOs: 1414-1417, 2240, and 2241. The viral genome may have 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identity to any of SEQ ID NOs: 1414-1417, 2240, and 2241. The viral genome may have 1-10%, 10-20%, 30-40%, 50-60%, 50-70%, 50-80%, 50-90%, 50-99%, 50-100%, 60-70%, 60-80%, 60-90%, 60-99%, 60-100%, 70-80%, 70-90%, 70-99%, 70-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-100%, 90-95%, 90-99%, or 90-100% to any of SEQ ID NOs: 1414-1417, 2240, and 2241. As a non-limiting example, the viral genome comprises a sequence which as 80% identity to any of SEQ ID NO: 1414-1417, 2240, and 2241. As another non-limiting example, the viral genome comprises a sequence which as 85% identity to any of SEQ ID NO: 1414-1417, 2240, and 2241. As another non-limiting example, the viral genome comprises a sequence which as 90% identity to any of SEQ ID NO: 1414-1417, 2240, and 2241. As another non-limiting example, the viral genome comprises a sequence which as 95% identity to any of SEQ ID NO: 1414-1417, 2240, and 2241. As another non-limiting example, the viral genome comprises a sequence which as 99% identity to any of SEQ ID NO: 1414-1417, 2240, and 2241.

[00569] In one embodiment, the AAV particle viral genome comprises at least one inverted terminal repeat (ITR) sequence region, at least one multiple cloning site (MCS) sequence region, at least one exon sequence region, at least one intron sequence region, at least one modulatory polynucleotide region, and at least one polyadenylation signal sequence region.

[00570] In one embodiment, the AAV particle viral genome comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, two multiple cloning site (MCS) sequence regions, an exon sequence region, two intron sequence regions, a modulatory polynucleotide region, and a polyadenylation signal sequence region.

[00571] In one embodiment, the AAV particle viral genome comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, two MCS sequence regions, an exon sequence regions (human beta globin (hbglobin) exon 3 or fragments thereof), two intron sequence regions (ie1 intron 1 and hbglobin intron 2 or fragments thereof), a modulatory polynucleotide region, and a rabbit betaglobin polyadenylation signal sequence region. A non-limiting example of an ITR to ITR sequence for use in the AAV particles of the present invention having all of the sequence modules above are described in Table 19. In Table 19, the sequence identifier or sequence of the sequence region (Region SEQ ID NO) and the length of the sequence region (Region length) are described as well as the name and sequence identifier of the ITR to ITR sequence (e.g., VOYSOD1 (SEQ ID NO: 1414)).

Table 19. Sequence Regions in ITR to ITR Sequences

Sequence Regions	VOYSOD1 (SEQ ID NO: 1414)		VOYSOD3 (SEQ ID NO: 1416)	
	Region SEQ ID NO	Region length	Region SEQ ID NO	Region length
5' ITR	1371	105	1371	105
MCS	TCGAG	5	TCGAG	5
Ie1 intron1	1407	32	1407	32
hbglobin intron2	1408	347	1408	347
hbglobin exon3	1405	53	1405	53
Modulatory Polynucleotide	1328	260	1317	158
MCS	TCGAG	5	TCGAG	5
Rabbit betaglobin polyA	1410	127	1410	127
3' ITR	1373	130	1373	130

[00572] In one embodiment, the AAV particle viral genome comprises SEQ ID NO: 1414 (VOYSOD1) which comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, two multiple cloning site (MCS) sequence regions, an exon sequence region, two intron sequence regions (ie1 intron 1 and hbglobin intron 2), a modulatory polynucleotide region, and a rabbit globin polyadenylation signal sequence region.

[00573] In one embodiment, the AAV particle viral genome comprises SEQ ID NO: 1416 (VOYSOD3) which comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, two multiple cloning site (MCS) sequence regions, an exon sequence region, two intron sequence regions (ie1 intron 1 and hbglobin intron 2), a modulatory polynucleotide region, and a rabbit globin polyadenylation signal sequence region.

[00574] In one embodiment, the AAV particle viral genome comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, a multiple cloning site (MCS) sequence region, two exon sequence regions, two intron sequence regions, a modulatory polynucleotide region, and a polyadenylation signal sequence region.

[00575] In one embodiment, the AAV particle viral genome comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, a MCS sequence region, two exon sequence regions (ie1 exon 1, human beta globin (hbglobin) exon 3 or fragments thereof), two intron sequence regions (ie1 intron 1 and hbglobin intron 2 or fragments thereof), a modulatory polynucleotide region, and a rabbit betaglobin polyadenylation signal sequence region. A non-limiting example of an ITR to ITR sequence for use in the AAV particles of the present invention having all of the sequence modules above are described in Table 20. In Table 20, the sequence identifier or sequence of the sequence region (Region SEQ ID NO) and the length of the sequence region (Region length) are described as well as the name and sequence identifier of the ITR to ITR sequence (e.g., VOYSOD2 (SEQ ID NO: 1415)).

Table 20. Sequence Regions in ITR to ITR Sequences

Sequence Regions	VOYSOD2 (SEQ ID NO: 1415)		VOYSOD4 (SEQ ID NO: 1417)	
	Region SEQ ID NO	Region length	Region SEQ ID NO	Region length
5' ITR	1371	105	1371	105
Ie1 exon1	1404	134	1404	134
Ie1 intron1	1407	32	1407	32
hbglobin intron2	1408	347	1408	347
hbglobin exon3	1405	53	1405	53
Modulatory Polynucleotide	1328	260	1317	158
MCS	TCGAG	5	TCGAG	5
Rabbit betaglobin polyA	1410	127	1410	127
3' ITR	1373	130	1373	130

[00576] In one embodiment, the AAV particle viral genome comprises SEQ ID NO: 1415 (VOYSOD2) which comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR sequence region, a multiple cloning site (MCS) sequence region, two exon sequence regions, two intron sequence regions (ie1 intron 1 and hbglobin intron 2), a modulatory polynucleotide region, and a rabbit globin polyadenylation signal sequence region.

[00577] In one embodiment, the AAV particle viral genome comprises SEQ ID NO: 1416 (VOYSOD4) which comprises a 5' inverted terminal repeat (ITR) sequence region and a 3' ITR

sequence region, a multiple cloning site (MCS) sequence region, two exon sequence regions, two intron sequence regions (ie1 intron 1 and hbglobin intron 2), a modulatory polynucleotide region, and a rabbit globin polyadenylation signal sequence region.

[00578] AAV particles may be modified to enhance the efficiency of delivery. Such modified AAV particles comprising the nucleic acid sequence encoding the siRNA molecules of the present invention can be packaged efficiently and can be used to successfully infect the target cells at high frequency and with minimal toxicity.

[00579] In some embodiments, the AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be a human serotype AAV particle. Such human AAV particle may be derived from any known serotype, e.g., from any one of serotypes AAV1-AAV11. As non-limiting examples, AAV particles may be vectors comprising an AAV1-derived genome in an AAV1-derived capsid; vectors comprising an AAV2-derived genome in an AAV2-derived capsid; vectors comprising an AAV4-derived genome in an AAV4 derived capsid; vectors comprising an AAV6-derived genome in an AAV6 derived capsid or vectors comprising an AAV9-derived genome in an AAV9 derived capsid.

[00580] In other embodiments, the AAV particle comprising a nucleic acid sequence for encoding siRNA molecules of the present invention may be a pseudotyped hybrid or chimeric AAV particle which contains sequences and/or components originating from at least two different AAV serotypes. Pseudotyped AAV particles may be vectors comprising an AAV genome derived from one AAV serotype and a capsid protein derived at least in part from a different AAV serotype. As non-limiting examples, such pseudotyped AAV particles may be vectors comprising an AAV2-derived genome in an AAV1-derived capsid; or vectors comprising an AAV2-derived genome in an AAV6-derived capsid; or vectors comprising an AAV2-derived genome in an AAV4-derived capsid; or an AAV2-derived genome in an AAV9-derived capsid. In like fashion, the present invention contemplates any hybrid or chimeric AAV particle.

[00581] In other embodiments, AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be used to deliver siRNA molecules to the central nervous system (e.g., U.S. Pat. No. 6,180,613; the contents of which is herein incorporated by reference in its entirety).

[00582] In some aspects, the AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may further comprise a modified capsid including peptides from non-viral origin. In other aspects, the AAV particle may contain a CNS specific

chimeric capsid to facilitate the delivery of encoded siRNA duplexes into the brain and the spinal cord. For example, an alignment of cap nucleotide sequences from AAV variants exhibiting CNS tropism may be constructed to identify variable region (VR) sequence and structure.

Viral production

[00583] The present disclosure provides a method for the generation of parvoviral particles, *e.g.* AAV particles, by viral genome replication in a viral replication cell comprising contacting the viral replication cell with an AAV polynucleotide or AAV genome.

[00584] The present disclosure provides a method for producing an AAV particle having enhanced (increased, improved) transduction efficiency comprising the steps of: 1) co-transfecting competent bacterial cells with a bacmid vector and either a viral construct vector and/or AAV payload construct vector, 2) isolating the resultant viral construct expression vector and AAV payload construct expression vector and separately transfecting viral replication cells, 3) isolating and purifying resultant payload and viral construct particles comprising viral construct expression vector or AAV payload construct expression vector, 4) co-infecting a viral replication cell with both the AAV payload and viral construct particles comprising viral construct expression vector or AAV payload construct expression vector, 5) harvesting and purifying the viral particle comprising a parvoviral genome.

[00585] In one embodiment, the present invention provides a method for producing an AAV particle comprising the steps of 1) simultaneously co-transfecting mammalian cells, such as, but not limited to HEK293 cells, with a payload region, a construct expressing rep and cap genes and a helper construct, 2) harvesting and purifying the AAV particle comprising a viral genome.

Cells

[00586] The present disclosure provides a cell comprising an AAV polynucleotide and/or AAV genome.

[00587] Viral production disclosed herein describes processes and methods for producing AAV particles that contact a target cell to deliver a payload construct, *e.g.* a recombinant viral construct, which comprises a polynucleotide sequence encoding a payload molecule.

[00588] In one embodiment, the AAV particles may be produced in a viral replication cell that comprises an insect cell.

[00589] Growing conditions for insect cells in culture, and production of heterologous products in insect cells in culture are well-known in the art, *see* U.S. Pat. No. 6,204,059, the contents of which are herein incorporated by reference in their entirety.

[00590] Any insect cell which allows for replication of parvovirus and which can be maintained in culture can be used in accordance with the present invention. Cell lines may be used from *Spodoptera frugiperda,* including, but not limited to the Sf9 or Sf21 cell lines, *Drosophila* cell lines, or mosquito cell lines, such as *Aedes albopictus* derived cell lines. Use of insect cells for expression of heterologous proteins is well documented, as are methods of introducing nucleic acids, such as vectors, *e.g.*, insect-cell compatible vectors, into such cells and methods of maintaining such cells in culture. *See*, for example, Methods in Molecular Biology, ed. Richard, Humana Press, NJ (1995); O'Reilly *et al.*, Baculovirus Expression Vectors, A Laboratory Manual, Oxford Univ. Press (1994); Samulski *et al.*, *J. Vir.*63:3822-8 (1989); Kajigaya *et al.*, *Proc. Nat'l. Acad. Sci. USA* 88: 4646-50 (1991); Ruffing *et al.*, J. *Vir.* 66:6922-30 (1992); Kimbauer *et al.*, *Vir.*219:37-44 (1996); Zhao *et al.*, Vir.272:382-93 (2000); and Samulski *et al.*, U.S. Pat. No. 6,204,059, the contents of each of which is herein incorporated by reference in its entirety.

[00591] The viral replication cell may be selected from any biological organism, including prokaryotic (*e.g.*, bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells. Viral replication cells may comprise mammalian cells such as A549, WEH1, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO. W138, HeLa, HEK293, Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals. Viral replication cells comprise cells derived from mammalian species including, but not limited to, human, monkey, mouse, rat, rabbit, and hamster or cell type, including but not limited to fibroblast, hepatocyte, tumor cell, cell line transformed cell, etc.

Small scale production of AAV Particles

[00592] Viral production disclosed herein describes processes and methods for producing AAV particles that contact a target cell to deliver a payload, *e.g.* a recombinant viral construct, which comprises a polynucleotide sequence encoding a payload.

[00593] In one embodiment, the AAV particles may be produced in a viral replication cell that comprises a mammalian cell.

[00594] Viral replication cells commonly used for production of recombinant AAV particles include, but are not limited to 293 cells, COS cells, HeLa cells, KB cells, and other mammalian cell lines as described in U.S. Pat. Nos. 6,156,303, 5,387,484, 5,741,683, 5,691,176, and 5,688,676; U.S. patent application 2002/0081721, and International Patent Applications WO

00/47757, WO 00/24916, and WO 96/17947, the contents of each of which are herein incorporated by reference in their entireties.

[00595] In one embodiment, AAV particles are produced in mammalian-cells wherein all three VP proteins are expressed at a stoichiometry approaching 1:1:10 (VP1:VP2:VP3). The regulatory mechanisms that allow this controlled level of expression include the production of two mRNAs, one for VP1, and the other for VP2 and VP3, produced by differential splicing.

[00596] In another embodiment, AAV particles are produced in mammalian cells using a triple transfection method wherein a payload construct, parvoviral Rep and parvoviral Cap and a helper construct are comprised within three different constructs. The triple transfection method of the three components of AAV particle production may be utilized to produce small lots of virus for assays including transduction efficiency, target tissue (tropism) evaluation, and stability.

Baculovirus

[00597] Particle production disclosed herein describes processes and methods for producing AAV particles that contact a target cell to deliver a payload construct which comprises a polynucleotide sequence encoding a payload.

[00598] Briefly, the viral construct vector and the AAV payload construct vector are each incorporated by a transposon donor/acceptor system into a bacmid, also known as a baculovirus plasmid, by standard molecular biology techniques known and performed by a person skilled in the art. Transfection of separate viral replication cell populations produces two baculoviruses, one that comprises the viral construct expression vector, and another that comprises the AAV payload construct expression vector. The two baculoviruses may be used to infect a single viral replication cell population for production of AAV particles.

[00599] Baculovirus expression vectors for producing viral particles in insect cells, including but not limited to *Spodoptera frugiperda* (Sf9) cells, provide high titers of viral particle product. Recombinant baculovirus encoding the viral construct expression vector and AAV payload construct expression vector initiates a productive infection of viral replicating cells. Infectious baculovirus particles released from the primary infection secondarily infect additional cells in the culture, exponentially infecting the entire cell culture population in a number of infection cycles that is a function of the initial multiplicity of infection, *see* Urabe, M. *et al.*, J Virol. 2006 Feb; 80 (4):1874-85, the contents of which are herein incorporated by reference in their entirety.

[00600] Production of AAV particles with baculovirus in an insect cell system may address known baculovirus genetic and physical instability. In one embodiment, the production system

addresses baculovirus instability over multiple passages by utilizing a titerless infected-cells preservation and scale-up system. Small scale seed cultures of viral producing cells are transfected with viral expression constructs encoding the structural, non-structural, components of the viral particle. Baculovirus-infected viral producing cells are harvested into aliquots that may be cryopreserved in liquid nitrogen; the aliquots retain viability and infectivity for infection of large scale viral producing cell culture Wasilko DJ *et al.*, Protein Expr Purif. 2009 Jun; 65(2):122-32, the contents of which are herein incorporated by reference in their entirety.

[00601] A genetically stable baculovirus may be used to produce source of the one or more of the components for producing AAV particles in invertebrate cells. In one embodiment, defective baculovirus expression vectors may be maintained episomally in insect cells. In such an embodiment the bacmid vector is engineered with replication control elements, including but not limited to promoters, enhancers, and/or cell-cycle regulated replication elements.

[00602] In one embodiment, baculoviruses may be engineered with a (non-) selectable marker for recombination into the chitinase/cathepsin locus. The chia/v-cath locus is non-essential for propagating baculovirus in tissue culture, and the V-cath (EC 3.4.22.50) is a cysteine endoprotease that is most active on Arg-Arg dipeptide containing substrates. The Arg-Arg dipeptide is present in densovirus and parvovirus capsid structural proteins but infrequently occurs in dependovirus VP1.

[00603] In one embodiment, stable viral replication cells permissive for baculovirus infection are engineered with at least one stable integrated copy of any of the elements necessary for AAV replication and viral particle production including, but not limited to, the entire AAV genome, Rep and Cap genes, Rep genes, Cap genes, each Rep protein as a separate transcription cassette, each VP protein as a separate transcription cassette, the AAP (assembly activation protein), or at least one of the baculovirus helper genes with native or non-native promoters.

Large-scale production

[00604] In some embodiments, AAV particle production may be modified to increase the scale of production. Large scale viral production methods according to the present disclosure may include any of those taught in US Patent Nos. 5,756,283, 6,258,595, 6,261,551, 6,270,996, 6,281,010, 6,365,394, 6,475,769, 6,482,634, 6,485,966, 6,943,019, 6,953,690, 7,022,519, 7,238,526, 7,291,498 and 7,491,508 or International Publication Nos. WO1996039530, WO1998010088, WO1999014354, WO1999015685, WO1999047691, WO2000055342, WO2000075353 and WO2001023597, the contents of each of which are herein incorporated by reference in their entirety. Methods of increasing viral particle production scale typically

comprise increasing the number of viral replication cells. In some embodiments, viral replication cells comprise adherent cells. To increase the scale of viral particle production by adherent viral replication cells, larger cell culture surfaces are required. In some cases, large-scale production methods comprise the use of roller bottles to increase cell culture surfaces. Other cell culture substrates with increased surface areas are known in the art. Examples of additional adherent cell culture products with increased surface areas include, but are not limited to CELLSTACK®, CELLCUBE® (Corning Corp., Corning, NY) and NUNC™ CELL FACTORY™ (Thermo Scientific, Waltham, MA.) In some cases, large-scale adherent cell surfaces may comprise from about 1,000 cm^2 to about 100,000 cm^2. In some cases, large-scale adherent cell cultures may comprise from about 10^7 to about 10^9 cells, from about 10^8 to about 10^{10} cells, from about 10^9 to about 10^{12} cells or at least 10^{12} cells. In some cases, large-scale adherent cultures may produce from about 10^9 to about 10^{12}, from about 10^{10} to about 10^{13}, from about 10^{11} to about 10^{14}, from about 10^{12} to about 10^{15} or at least 10^{15} viral particles.

[00605] In some embodiments, large-scale viral production methods of the present disclosure may comprise the use of suspension cell cultures. Suspension cell culture allows for significantly increased numbers of cells. Typically, the number of adherent cells that can be grown on about 10-50 cm^2 of surface area can be grown in about 1 cm^3 volume in suspension.

[00606] Transfection of replication cells in large-scale culture formats may be carried out according to any methods known in the art. For large-scale adherent cell cultures, transfection methods may include, but are not limited to the use of inorganic compounds (*e.g.* calcium phosphate), organic compounds [*e.g.* polyethyleneimine (PEI)] or the use of non-chemical methods (*e.g.* electroporation.) With cells grown in suspension, transfection methods may include, but are not limited to the use of calcium phosphate and the use of PEI. In some cases, transfection of large scale suspension cultures may be carried out according to the section entitled "Transfection Procedure" described in Feng, L. *et al.*, 2008. Biotechnol Appl. Biochem. 50:121-32, the contents of which are herein incorporated by reference in their entirety. According to such embodiments, PEI-DNA complexes may be formed for introduction of plasmids to be transfected. In some cases, cells being transfected with PEI-DNA complexes may be 'shocked' prior to transfection. This comprises lowering cell culture temperatures to 4°C for a period of about 1 hour. In some cases, cell cultures may be shocked for a period of from about 10 minutes to about 5 hours. In some cases, cell cultures may be shocked at a temperature of from about 0°C to about 20°C.

[00607] In some cases, transfections may include one or more vectors for expression of an RNA effector molecule to reduce expression of nucleic acids from one or more AAV payload construct. Such methods may enhance the production of viral particles by reducing cellular resources wasted on expressing payload constructs. In some cases, such methods may be carried according to those taught in US Publication No. US2014/0099666, the contents of which are herein incorporated by reference in their entirety.

Bioreactors

[00608] In some embodiments, cell culture bioreactors may be used for large scale viral production. In some cases, bioreactors comprise stirred tank reactors. Such reactors generally comprise a vessel, typically cylindrical in shape, with a stirrer (*e.g.* impeller.) In some embodiments, such bioreactor vessels may be placed within a water jacket to control vessel temperature and/or to minimize effects from ambient temperature changes. Bioreactor vessel volume may range in size from about 500 ml to about 2 L, from about 1 L to about 5 L, from about 2.5 L to about 20 L, from about 10 L to about 50 L, from about 25 L to about 100 L, from about 75 L to about 500 L, from about 250 L to about 2,000 L, from about 1,000 L to about 10,000 L, from about 5,000 L to about 50,000 L or at least 50,000 L. Vessel bottoms may be rounded or flat. In some cases, animal cell cultures may be maintained in bioreactors with rounded vessel bottoms.

[00609] In some cases, bioreactor vessels may be warmed through the use of a thermocirculator. Thermocirculators pump heated water around water jackets. In some cases, heated water may be pumped through pipes (*e.g.* coiled pipes) that are present within bioreactor vessels. In some cases, warm air may be circulated around bioreactors, including, but not limited to air space directly above culture medium. Additionally, pH and CO_2 levels may be maintained to optimize cell viability.

[00610] In some cases, bioreactors may comprise hollow-fiber reactors. Hollow-fiber bioreactors may support the culture of both anchorage dependent and anchorage independent cells. Further bioreactors may include, but are not limited to packed-bed or fixed-bed bioreactors. Such bioreactors may comprise vessels with glass beads for adherent cell attachment. Further packed-bed reactors may comprise ceramic beads.

[00611] In some cases, viral particles are produced through the use of a disposable bioreactor. In some embodiments, such bioreactors may include WAVE™ disposable bioreactors.

[00612] In some embodiments, AAV particle production in animal cell bioreactor cultures may be carried out according to the methods taught in US Patent Nos. 5,064764, 6,194,191,

6,566,118, 8,137,948 or US Patent Application No. US2011/0229971, the contents of each of which are herein incorporated by reference in their entirety.

Cell Lysis

[00613] Cells of the invention, including, but not limited to viral production cells, may be subjected to cell lysis according to any methods known in the art. Cell lysis may be carried out to obtain one or more agents (*e.g.* viral particles) present within any cells of the invention. In some embodiments, cell lysis may be carried out according to any of the methods listed in US Patent Nos. 7,326,555, 7,579,181, 7,048,920, 6,410,300, 6,436,394, 7,732,129, 7,510,875, 7,445,930, 6,726,907, 6,194,191, 7,125,706, 6,995,006, 6,676,935, 7,968,333, 5,756,283, 6,258,595, 6,261,551, 6,270,996, 6,281,010, 6,365,394, 6,475,769, 6,482,634, 6,485,966, 6,943,019, 6,953,690, 7,022,519, 7,238,526, 7,291,498 and 7,491,508 or International Publication Nos. WO1996039530, WO1998010088, WO1999014354, WO1999015685, WO1999047691, WO2000055342, WO2000075353 and WO2001023597, the contents of each of which are herein incorporated by reference in their entirety. Cell lysis methods may be chemical or mechanical. Chemical cell lysis typically comprises contacting one or more cells with one or more lysis agent. Mechanical lysis typically comprises subjecting one or more cells to one or more lysis condition and/or one or more lysis force.

[00614] In some embodiments, chemical lysis may be used to lyse cells. As used herein, the term "lysis agent" refers to any agent that may aid in the disruption of a cell. In some cases, lysis agents are introduced in solutions, termed lysis solutions or lysis buffers. As used herein, the term "lysis solution" refers to a solution (typically aqueous) comprising one or more lysis agent. In addition to lysis agents, lysis solutions may include one or more buffering agents, solubilizing agents, surfactants, preservatives, cryoprotectants, enzymes, enzyme inhibitors and/or chelators. Lysis buffers are lysis solutions comprising one or more buffering agent. Additional components of lysis solutions may include one or more solubilizing agent. As used herein, the term "solubilizing agent" refers to a compound that enhances the solubility of one or more components of a solution and/or the solubility of one or more entities to which solutions are applied. In some cases, solubilizing agents enhance protein solubility. In some cases, solubilizing agents are selected based on their ability to enhance protein solubility while maintaining protein conformation and/or activity.

[00615] Exemplary lysis agents may include any of those described in US Patent Nos. 8,685,734, 7,901,921, 7,732,129, 7,223,585, 7,125,706, 8,236,495, 8,110,351, 7,419,956, 7,300,797, 6,699,706 and 6,143,567, the contents of each of which are herein incorporated by

reference in their entirety. In some cases, lysis agents may be selected from lysis salts, amphoteric agents, cationic agents, ionic detergents and non-ionic detergents. Lysis salts may include, but are not limited to sodium chloride (NaCl) and potassium chloride (KCl.) Further lysis salts may include any of those described in US Patent Nos. 8,614,101, 7,326,555, 7,579,181, 7,048,920, 6,410,300, 6,436,394, 7,732,129, 7,510,875, 7,445,930, 6,726,907, 6,194,191, 7,125,706, 6,995,006, 6,676,935 and 7,968,333, the contents of each of which are herein incorporated by reference in their entirety. Concentrations of salts may be increased or decreased to obtain an effective concentration for rupture of cell membranes. Amphoteric agents, as referred to herein, are compounds capable of reacting as an acid or a base. Amphoteric agents may include, but are not limited to lysophosphatidylcholine, 3-((3-Cholamidopropyl) dimethylammonium)-1-propanesulfonate (CHAPS), ZWITTERGENT® and the like. Cationic agents may include, but are not limited to cetyltrimethylammonium bromide (C (16) TAB) and Benzalkonium chloride. Lysis agents comprising detergents may include ionic detergents or non-ionic detergents. Detergents may function to break apart or dissolve cell structures including, but not limited to cell membranes, cell walls, lipids, carbohydrates, lipoproteins and glycoproteins. Exemplary ionic detergents include any of those taught in US Patent Nos. 7,625,570 and 6,593,123 or US Publication No. US2014/0087361, the contents of each of which are herein incorporated by reference in their entirety. Some ionic detergents may include, but are not limited to sodium dodecyl sulfate (SDS), cholate and deoxycholate. In some cases, ionic detergents may be included in lysis solutions as a solubilizing agent. Non-ionic detergents may include, but are not limited to octylglucoside, digitonin, lubrol, C12E8, TWEEN®-20, TWEEN®-80, Triton X-100 and Noniodet P-40. Non-ionic detergents are typically weaker lysis agents, but may be included as solubilizing agents for solubilizing cellular and/or viral proteins. Further lysis agents may include enzymes and urea. In some cases, one or more lysis agents may be combined in a lysis solution in order to enhance one or more of cell lysis and protein solubility. In some cases, enzyme inhibitors may be included in lysis solutions in order to prevent proteolysis that may be triggered by cell membrane disruption.

[00616] In some embodiments, mechanical cell lysis is carried out. Mechanical cell lysis methods may include the use of one or more lysis condition and/or one or more lysis force. As used herein, the term “lysis condition” refers to a state or circumstance that promotes cellular disruption. Lysis conditions may comprise certain temperatures, pressures, osmotic purity, salinity and the like. In some cases, lysis conditions comprise increased or decreased temperatures. According to some embodiments, lysis conditions comprise changes in

temperature to promote cellular disruption. Cell lysis carried out according to such embodiments may include freeze-thaw lysis. As used herein, the term "freeze-thaw lysis" refers to cellular lysis in which a cell solution is subjected to one or more freeze-thaw cycle. According to freeze-thaw lysis methods, cells in solution are frozen to induce a mechanical disruption of cellular membranes caused by the formation and expansion of ice crystals. Cell solutions used according freeze-thaw lysis methods, may further comprise one or more lysis agents, solubilizing agents, buffering agents, cryoprotectants, surfactants, preservatives, enzymes, enzyme inhibitors and/or chelators. Once cell solutions subjected to freezing are thawed, such components may enhance the recovery of desired cellular products. In some cases, one or more cryoprotectants are included in cell solutions undergoing freeze-thaw lysis. As used herein, the term "cryoprotectant" refers to an agent used to protect one or more substance from damage due to freezing. Cryoprotectants may include any of those taught in US Publication No. US2013/0323302 or US Patent Nos. 6,503,888, 6,180,613, 7,888,096, 7,091,030, the contents of each of which are herein incorporated by reference in their entirety. In some cases, cryoprotectants may include, but are not limited to dimethyl sulfoxide, 1,2-propanediol, 2,3-butanediol, formamide, glycerol, ethylene glycol, 1,3-propanediol and n-dimethyl formamide, polyvinylpyrrolidone, hydroxyethyl starch, agarose, dextrans, inositol, glucose, hydroxyethylstarch, lactose, sorbitol, methyl glucose, sucrose and urea. In some embodiments, freeze-thaw lysis may be carried out according to any of the methods described in US Patent No. 7,704,721, the contents of which are herein incorporated by reference in their entirety.

[00617] As used herein, the term "lysis force" refers to a physical activity used to disrupt a cell. Lysis forces may include, but are not limited to mechanical forces, sonic forces, gravitational forces, optical forces, electrical forces and the like. Cell lysis carried out by mechanical force is referred to herein as "mechanical lysis." Mechanical forces that may be used according to mechanical lysis may include high shear fluid forces. According to such methods of mechanical lysis, a microfluidizer may be used. Microfluidizers typically comprise an inlet reservoir where cell solutions may be applied. Cell solutions may then be pumped into an interaction chamber via a pump (*e.g.* high-pressure pump) at high speed and/or pressure to produce shear fluid forces. Resulting lysates may then be collected in one or more output reservoir. Pump speed and/or pressure may be adjusted to modulate cell lysis and enhance recovery of products (*e.g.* viral particles.) Other mechanical lysis methods may include physical disruption of cells by scraping.

[00618] Cell lysis methods may be selected based on the cell culture format of cells to be lysed. For example, with adherent cell cultures, some chemical and mechanical lysis methods may be used. Such mechanical lysis methods may include freeze-thaw lysis or scraping. In another example, chemical lysis of adherent cell cultures may be carried out through incubation with lysis solutions comprising surfactant, such as Triton-X-100. In some cases, cell lysates generated from adherent cell cultures may be treated with one more nuclease to lower the viscosity of the lysates caused by liberated DNA.

[00619] In one embodiment, a method for harvesting AAV particles without lysis may be used for efficient and scalable AAV particle production. In a non-limiting example, AAV particles may be produced by culturing an AAV particle lacking a heparin binding site, thereby allowing the AAV particle to pass into the supernatant, in a cell culture, collecting supernatant from the culture; and isolating the AAV particle from the supernatant, as described in US Patent Application 20090275107, the contents of which are incorporated herein by reference in their entirety.

Clarification

[00620] Cell lysates comprising viral particles may be subjected to clarification. Clarification refers to initial steps taken in purification of viral particles from cell lysates. Clarification serves to prepare lysates for further purification by removing larger, insoluble debris. Clarification steps may include, but are not limited to centrifugation and filtration. During clarification, centrifugation may be carried out at low speeds to remove larger debris only. Similarly, filtration may be carried out using filters with larger pore sizes so that only larger debris is removed. In some cases, tangential flow filtration may be used during clarification. Objectives of viral clarification include high throughput processing of cell lysates and to optimize ultimate viral recovery. Advantages of including a clarification step include scalability for processing of larger volumes of lysate. In some embodiments, clarification may be carried out according to any of the methods presented in US Patent Nos. 8,524,446, 5,756,283, 6,258,595, 6,261,551, 6,270,996, 6,281,010, 6,365,394, 6,475,769, 6,482,634, 6,485,966, 6,943,019, 6,953,690, 7,022,519, 7,238,526, 7,291,498, 7,491,508, US Publication Nos. US2013/0045186, US2011/0263027, US2011/0151434, US2003/0138772, and International Publication Nos. WO2002012455, WO1996039530, WO1998010088, WO1999014354, WO1999015685, WO1999047691, WO2000055342, WO2000075353 and WO2001023597, the contents of each of which are herein incorporated by reference in their entirety.

[00621] Methods of cell lysate clarification by filtration are well understood in the art and may be carried out according to a variety of available methods including, but not limited to passive filtration and flow filtration. Filters used may comprise a variety of materials and pore sizes. For example, cell lysate filters may comprise pore sizes of from about 1 μM to about 5 μM, from about 0.5 μM to about 2 μM, from about 0.1 μM to about 1 μM, from about 0.05 μM to about 0.05 μM and from about 0.001 μM to about 0.1 μM. Exemplary pore sizes for cell lysate filters may include, but are not limited to, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.95, 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1, 0.05, 0.22, 0.21, 0.20, 0.19, 0.18, 0.17, 0.16, 0.15, 0.14, 0.13, 0.12, 0.11, 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.02, 0.019, 0.018, 0.017, 0.016, 0.015, 0.014, 0.013, 0.012, 0.011, 0.01, 0.009, 0.008, 0.007, 0.006, 0.005, 0.004, 0.003, 0.002, 0.001 and 0.001 μM. In one embodiment, clarification may comprise filtration through a filter with 2.0 μM pore size to remove large debris, followed by passage through a filter with 0.45 μM pore size to remove intact cells.

[00622] Filter materials may be composed of a variety of materials. Such materials may include, but are not limited to polymeric materials and metal materials (e.g. sintered metal and pored aluminum.) Exemplary materials may include, but are not limited to nylon, cellulose materials (e.g. cellulose acetate), polyvinylidene fluoride (PVDF), polyethersulfone, polyamide, polysulfone, polypropylene, and polyethylene terephthalate. In some cases, filters useful for clarification of cell lysates may include, but are not limited to ULTIPLEAT PROFILE™ filters (Pall Corporation, Port Washington, NY), SUPOR™ membrane filters (Pall Corporation, Port Washington, NY)

[00623] In some cases, flow filtration may be carried out to increase filtration speed and/or effectiveness. In some cases, flow filtration may comprise vacuum filtration. According to such methods, a vacuum is created on the side of the filter opposite that of cell lysate to be filtered. In some cases, cell lysates may be passed through filters by centrifugal forces. In some cases, a pump is used to force cell lysate through clarification filters. Flow rate of cell lysate through one or more filters may be modulated by adjusting one of channel size and/or fluid pressure.

[00624] According to some embodiments, cell lysates may be clarified by centrifugation. Centrifugation may be used to pellet insoluble particles in the lysate. During clarification, centrifugation strength [expressed in terms of gravitational units (g), which represents multiples of standard gravitational force] may be lower than in subsequent purification steps. In some cases, centrifugation may be carried out on cell lysates at from about 200 g to about 800 g, from

about 500 g to about 1500 g, from about 1000 g to about 5000 g, from about 1200 g to about 10000 g or from about 8000 g to about 15000 g. In some embodiments, cell lysate centrifugation is carried out at 8000 g for 15 minutes. In some cases, density gradient centrifugation may be carried out in order to partition particulates in the cell lysate by sedimentation rate. Gradients used according to methods of the present disclosure may include, but are not limited to cesium chloride gradients and iodixanol step gradients.

Purification: Chromatography

[00625] In some cases, AAV particles may be purified from clarified cell lysates by one or more methods of chromatography. Chromatography refers to any number of methods known in the art for separating out one or more elements from a mixture. Such methods may include, but are not limited to ion exchange chromatography (*e.g.* cation exchange chromatography and anion exchange chromatography), immunoaffinity chromatography and size-exclusion chromatography. In some embodiments, methods of viral chromatography may include any of those taught in US Patent Nos. 5,756,283, 6,258,595, 6,261,551, 6,270,996, 6,281,010, 6,365,394, 6,475,769, 6,482,634, 6,485,966, 6,943,019, 6,953,690, 7,022,519, 7,238,526, 7,291,498 and 7,491,508 or International Publication Nos. WO1996039530, WO1998010088, WO1999014354, WO1999015685, WO1999047691, WO2000055342, WO2000075353 and WO2001023597, the contents of each of which are herein incorporated by reference in their entirety.

[00626] In some embodiments, ion exchange chromatography may be used to isolate viral particles. Ion exchange chromatography is used to bind viral particles based on charge-charge interactions between capsid proteins and charged sites present on a stationary phase, typically a column through which viral preparations (*e.g.* clarified lysates) are passed. After application of viral preparations, bound viral particles may then be eluted by applying an elution solution to disrupt the charge-charge interactions. Elution solutions may be optimized by adjusting salt concentration and/or pH to enhance recovery of bound viral particles. Depending on the charge of viral capsids being isolated, cation or anion exchange chromatography methods may be selected. Methods of ion exchange chromatography may include, but are not limited to any of those taught in US Patent Nos. 7,419,817, 6,143,548, 7,094,604, 6,593,123, 7,015,026 and 8,137,948, the contents of each of which are herein incorporated by reference in their entirety.

[00627] In some embodiments, immunoaffinity chromatography may be used. Immunoaffinity chromatography is a form of chromatography that utilizes one or more immune compounds (*e.g.* antibodies or antibody-related structures) to retain viral particles. Immune

compounds may bind specifically to one or more structures on viral particle surfaces, including, but not limited to one or more viral coat protein. In some cases, immune compounds may be specific for a particular viral variant. In some cases, immune compounds may bind to multiple viral variants. In some embodiments, immune compounds may include recombinant single-chain antibodies. Such recombinant single chain antibodies may include those described in Smith, R.H. *et al.*, 2009. Mol. Ther. 17(11):1888-96, the contents of which are herein incorporated by reference in their entirety. Such immune compounds are capable of binding to several AAV capsid variants, including, but not limited to AAV1, AAV2, AAV6 and AAV8.

[00628] In some embodiments, size-exclusion chromatography (SEC) may be used. SEC may comprise the use of a gel to separate particles according to size. In viral particle purification, SEC filtration is sometimes referred to as "polishing." In some cases, SEC may be carried out to generate a final product that is near-homogenous. Such final products may in some cases be used in pre-clinical studies and/or clinical studies (Kotin, R.M. 2011. Human Molecular Genetics. 20(1):R2-R6, the contents of which are herein incorporated by reference in their entirety.) In some cases, SEC may be carried out according to any of the methods taught in US Patent Nos. 6,143,548, 7,015,026, 8,476,418, 6,410,300, 8,476,418, 7,419,817, 7,094,604, 6,593,123, and 8,137,948, the contents of each of which are herein incorporated by reference in their entirety.

[00629] In one embodiment, the compositions comprising at least one AAV particle may be isolated or purified using the methods described in US Patent No. US 6146874, the contents of which are herein incorporated by reference in its entirety.

[00630] In one embodiment, the compositions comprising at least one AAV particle may be isolated or purified using the methods described in US Patent No. US 6660514, the contents of which are herein incorporated by reference in its entirety.

[00631] In one embodiment, the compositions comprising at least one AAV particle may be isolated or purified using the methods described in US Patent No. US 8283151, the contents of which are herein incorporated by reference in its entirety.

[00632] In one embodiment, the compositions comprising at least one AAV particle may be isolated or purified using the methods described in US Patent No. US 8524446, the contents of which are herein incorporated by reference in its entirety.

II. FORMULATION AND DELIVERY

Pharmaceutical compositions and formulation

[00633] In addition to the pharmaceutical compositions (AAV particles comprising a modulatory polynucleotide sequence encoding the siRNA molecules), provided herein are

pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, *e.g.,* to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00634] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers either to the synthetic siRNA duplexes, the modulatory polynucleotide encoding the siRNA duplex, or the AAV particle comprising a modulatory polynucleotide encoding the siRNA duplex described herein.

[00635] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00636] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

[00637] The AAV particles comprising the modulatory polynucleotide sequence encoding the siRNA molecules of the present invention can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection or transduction; (3) permit the sustained or delayed release; or (4) alter the biodistribution (e.g., target the AAV particle to specific tissues or cell types such as brain and neurons).

[00638] Formulations of the present invention can include, without limitation, saline, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with AAV particles (e.g., for transplantation into a subject),

nanoparticle mimics and combinations thereof. Further, the AAV particles of the present invention may be formulated using self-assembled nucleic acid nanoparticles.

[00639] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00640] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00641] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[00642] In some embodiments, a pharmaceutically acceptable excipient may be at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use for humans and for veterinary use. In some embodiments, an excipient may be approved by United States Food and Drug Administration. In some embodiments, an excipient may be of pharmaceutical grade. In some embodiments, an excipient may meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00643] Excipients, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams &

Wilkins, Baltimore, MD, 2006; incorporated herein by reference in its entirety). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00644] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

[00645] In some embodiments, the formulations may comprise at least one inactive ingredient. As used herein, the term "inactive ingredient" refers to one or more inactive agents included in formulations. In some embodiments, all, none or some of the inactive ingredients which may be used in the formulations of the present invention may be approved by the US Food and Drug Administration (FDA).

[00646] Formulations of vectors comprising the nucleic acid sequence for the siRNA molecules of the present invention may include cations or anions. In one embodiment, the formulations include metal cations such as, but not limited to, Zn2+, Ca2+, Cu2+, Mg+ and combinations thereof.

[00647] As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g., by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, acetic acid, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzene sulfonic acid, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts,

and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, *Pharmaceutical Salts: Properties, Selection, and Use*, P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., *Journal of Pharmaceutical Science*, 66, 1-19 (1977); the content of each of which is incorporated herein by reference in their entirety.

[00648] The term "pharmaceutically acceptable solvate," as used herein, means a compound of the invention wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N,N'*-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate."

[00649] According to the present invention, the AAV particle comprising the modulatory polynucleotide sequence encoding for the siRNA molecules may be formulated for CNS delivery. Agents that cross the brain blood barrier may be used. For example, some cell penetrating peptides that can target siRNA molecules to the brain blood barrier endothelium may be used to formulate the siRNA duplexes targeting the gene of interest.

Inactive Ingredients

[00650] In some embodiments, formulations may comprise at least one excipient which is an inactive ingredient. As used herein, the term "inactive ingredient" refers to one or more inactive agents included in formulations. In some embodiments, all, none or some of the inactive ingredients which may be used in the formulations of the present disclosure may be approved by the US Food and Drug Administration (FDA).

[00651] Formulations of AAV particles described herein may include cations or anions. In one embodiment, the formulations include metal cations such as, but not limited to, Zn2+, Ca2+, Cu2+, Mg+ and combinations thereof. As a non-limiting example, formulations may include polymers and compositions described herein complexed with a metal cation (*See e.g.*, U.S. Pat. Nos. 6,265,389 and 6,555,525, each of which is herein incorporated by reference in its entirety).

Delivery

[00652] In one embodiment, the AAV particles described herein may be administered or delivered using the methods for the delivery of AAV virions described in European Patent Application No. EP1857552, the contents of which are herein incorporated by reference in its entirety.

[00653] In one embodiment, the AAV particles described herein may be administered or delivered using the methods for delivering proteins using AAV particles described in European Patent Application No. EP2678433, the contents of which are herein incorporated by reference in its entirety.

[00654] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering DNA molecules using AAV particles described in US Patent No. US 5,858,351, the contents of which are herein incorporated by reference in its entirety.

[00655] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering DNA to the bloodstream described in US Patent No. US 6,211,163, the contents of which are herein incorporated by reference in its entirety.

[00656] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering AAV virions described in US Patent No. US 6,325,998, the contents of which are herein incorporated by reference in its entirety.

[00657] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering a payload to the central nervous system described in US Patent No. US 7,588,757, the contents of which are herein incorporated by reference in its entirety.

[00658] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering a payload described in US Patent No. US 8283151, the contents of which are herein incorporated by reference in its entirety.

[00659] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering a payload using a glutamic acid decarboxylase (GAD) delivery vector described in International Patent Publication No. WO2001089583, the contents of which are herein incorporated by reference in its entirety.

[00660] In one embodiment, the AAV particle described herein may be administered or delivered using the methods for delivering a payload to neural cells described in International Patent Publication No. WO2012057363, the contents of which are herein incorporated by reference in its entirety.

Delivery to Cells

[00661] The present disclosure provides a method of delivering to a cell or tissue any of the above-described AAV polynucleotides or AAV genomes, comprising contacting the cell or tissue with said AAV polynucleotide or AAV genomes or contacting the cell or tissue with a particle comprising said AAV polynucleotide or AAV genome, or contacting the cell or tissue with any of the described compositions, including pharmaceutical compositions. The method of delivering the AAV polynucleotide or AAV genome to a cell or tissue can be accomplished *in vitro, ex vivo*, or *in vivo*.

Introduction into cells- Synthetic dsRNA

[00662] To ensure the chemical and biological stability of siRNA molecules (e.g., siRNA duplexes and dsRNA), it is important to deliver siRNA molecules inside the target cells. In some embodiments, the cells may include, but are not limited to, cells of mammalian origin, cells of human origins, embryonic stem cells, induced pluripotent stem cells, neural stem cells, and neural progenitor cells.

[00663] Nucleic acids, including siRNA, carry a net negative charge on the sugar-phosphate backbone under normal physiological conditions. In order to enter the cell, a siRNA molecule must come into contact with a lipid bilayer of the cell membrane, whose head groups are also negatively charged.

[00664] The siRNA duplexes can be complexed with a carrier that allows them to traverse cell membranes such as package particles to facilitate cellular uptake of the siRNA. The package particles may include, but are not limited to, liposomes, nanoparticles, cationic lipids, polyethylenimine derivatives, dendrimers, carbon nanotubes and the combination of carbon-made nanoparticles with dendrimers. Lipids may be cationic lipids and/or neutral lipids. In

addition to well established lipophilic complexes between siRNA molecules and cationic carriers, siRNA molecules can be conjugated to a hydrophobic moiety, such as cholesterol (e.g., U.S. Patent Publication No. 20110110937; the content of which is herein incorporated by reference in its entirety). This delivery method holds a potential of improving *in vitro* cellular uptake and *in vivo* pharmacological properties of siRNA molecules. The siRNA molecules of the present invention may also be conjugated to certain cationic cell-penetrating peptides (CPPs), such as MPG, transportan or penetratin covalently or non-covalently (e.g., U.S. Patent Publication No. 20110086425; the content of which is herein incorporated by reference in its entirety).

Introduction into cells- AAV particles

[00665] The siRNA molecules (e.g., siRNA duplexes) of the present invention may be introduced into cells using any of a variety of approaches such as, but not limited to, AAV particles. These AAV particles are engineered and optimized to facilitate the entry of siRNA molecule into cells that are not readily amendable to transfection. Also, some synthetic AAV particles possess an ability to integrate the shRNA into the cell genome, thereby leading to stable siRNA expression and long-term knockdown of a target gene. In this manner, AAV particles are engineered as vehicles for specific delivery while lacking the deleterious replication and/or integration features found in wild-type virus.

[00666] In some embodiments, the siRNA molecules of the present invention are introduced into a cell by contacting the cell with an AAV particle comprising a modulatory polynucleotide sequence encoding a siRNA molecule, and a lipophilic carrier. In other embodiments, the siRNA molecule is introduced into a cell by transfecting or infecting the cell with an AAV particle comprising a nucleic acid sequence capable of producing the siRNA molecule when transcribed in the cell. In some embodiments, the siRNA molecule is introduced into a cell by injecting into the cell an AAV particle comprising a nucleic acid sequence capable of producing the siRNA molecule when transcribed in the cell.

[00667] In some embodiments, prior to transfection, an AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be transfected into cells.

[00668] In other embodiments, the AAV particles comprising the nucleic acid sequence encoding the siRNA molecules of the present invention may be delivered into cells by electroporation (e.g. U.S. Patent Publication No. 20050014264; the content of which is herein incorporated by reference in its entirety).

[00669] Other methods for introducing AAV particles comprising the nucleic acid sequence encoding the siRNA molecules described herein may include photochemical internalization as described in U. S. Patent publication No. 20120264807; the content of which is herein incorporated by reference in its entirety.

[00670] In some embodiments, the formulations described herein may contain at least one AAV particle comprising the nucleic acid sequence encoding the siRNA molecules described herein. In one embodiment, the siRNA molecules may target the gene of interest at one target site. In another embodiment, the formulation comprises a plurality of AAV particles, each AAV particle comprising a nucleic acid sequence encoding a siRNA molecule targeting the gene of interest at a different target site. The gene of interest may be targeted at 2, 3, 4, 5 or more than 5 sites.

[00671] In one embodiment, the AAV particles from any relevant species, such as, but not limited to, human, dog, mouse, rat or monkey may be introduced into cells.

[00672] In one embodiment, the AAV particles may be introduced into cells which are relevant to the disease to be treated. As a non-limiting example, the disease is ALS and the target cells are neurons and astrocytes. As another non-limiting example, the disease is ALS and the target cells are medium spiny neurons, cortical neurons and astrocytes.

[00673] In one embodiment, the AAV particles may be introduced into cells which have a high level of endogenous expression of the target sequence.

[00674] In another embodiment, the AAV particles may be introduced into cells which have a low level of endogenous expression of the target sequence.

[00675] In one embodiment, the cells may be those which have a high efficiency of AAV transduction.

Delivery to Subjects

[00676] The present disclosure additionally provides a method of delivering to a subject, including a mammalian subject, any of the above-described AAV polynucleotides or AAV genomes comprising administering to the subject said AAV polynucleotide or AAV genome, or administering to the subject a particle comprising said AAV polynucleotide or AAV genome, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[00677] The pharmaceutical compositions of AAV particles described herein may be characterized by one or more of bioavailability, therapeutic window and/or volume of distribution.

III. ADMINISTRATION AND DOSING

Administration

[00678] The AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to, within the parenchyma of an organ such as, but not limited to, a brain (e.g., intraparenchymal), corpus striatum (intrastriatal), enteral (into the intestine), gastroenteral, epidural, oral (by way of the mouth), transdermal, peridural, intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), subpial (under the pia), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intravenous bolus, intravenous drip, intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraganglionic (into the ganglion), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection (into a pathologic cavity) intracavitary (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), transvaginal, insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), in ear drops, auricular (in or by way of the ear), buccal (directed toward the cheek), conjunctival, cutaneous, dental (to a tooth or teeth), electro-osmosis, endocervical, endosinusial, endotracheal, extracorporeal, hemodialysis, infiltration, interstitial, intra-abdominal, intra-amniotic, intra-articular, intrabiliary, intrabronchial, intrabursal, intracartilaginous (within a cartilage), intracaudal (within the cauda equine), intracisternal (within the cisterna magna cerebellomedularis), intracorneal (within the cornea), dental intracornal, intracoronary (within the coronary arteries), intracorporus cavernosum (within the dilatable spaces of the corporus cavernosa of the penis), intradiscal (within a disc), intraductal (within a duct of a gland), intraduodenal (within the duodenum), intradural (within or beneath the dura), intraepidermal (to the epidermis), intraesophageal (to the esophagus), intragastric (within the stomach), intragingival (within the gingivae), intraileal (within the distal portion of the small intestine), intralesional (within or introduced directly to a localized lesion), intraluminal (within a lumen of a tube), intralymphatic (within the lymph), intramedullary (within the marrow cavity of a bone), intrameningeal (within the meninges), intraocular (within the eye), intraovarian (within the ovary), intrapericardial (within the pericardium), intrapleural (within the pleura), intraprostatic (within the prostate gland), intrapulmonary (within the lungs or

its bronchi), intrasinal (within the nasal or periorbital sinuses), intraspinal (within the vertebral column), intrasynovial (within the synovial cavity of a joint), intratendinous (within a tendon), intratesticular (within the testicle), intrathecal (within the cerebrospinal fluid at any level of the cerebrospinal axis), intrathoracic (within the thorax), intratubular (within the tubules of an organ), intratumor (within a tumor), intratympanic (within the aurus media), intravascular (within a vessel or vessels), intraventricular (within a ventricle), iontophoresis (by means of electric current where ions of soluble salts migrate into the tissues of the body), irrigation (to bathe or flush open wounds or body cavities), laryngeal (directly upon the larynx), nasogastric (through the nose and into the stomach), occlusive dressing technique (topical route administration which is then covered by a dressing which occludes the area), ophthalmic (to the external eye), oropharyngeal (directly to the mouth and pharynx), parenteral, percutaneous, periarticular, peridural, perineural, periodontal, rectal, respiratory (within the respiratory tract by inhaling orally or nasally for local or systemic effect), retrobulbar (behind the pons or behind the eyeball), soft tissue, subarachnoid, subconjunctival, submucosal, topical, transplacental (through or across the placenta), transtracheal (through the wall of the trachea), transtympanic (across or through the tympanic cavity), ureteral (to the ureter), urethral (to the urethra), vaginal, caudal block, diagnostic, nerve block, biliary perfusion, cardiac perfusion, photopheresis or spinal.

[00679] In specific embodiments, compositions of AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered in a way which facilitates the vectors or siRNA molecule to enter the central nervous system and penetrate into medium spiny and/or cortical neurons and/or astrocytes.

[00680] In some embodiments, the AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered by intramuscular injection.

[00681] In one embodiment, the AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered via intraparenchymal injection.

[00682] In one embodiment, the AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered via intraparenchymal injection and intrathecal injection.

[00683] In one embodiment, the AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered via intrastriatal injection.

[00684] In one embodiment, the AAV particles comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered via intrastriatal injection and another route of administration described herein.

[00685] In some embodiments, AAV particles that express siRNA duplexes of the present invention may be administered to a subject by peripheral injections (e.g., intravenous) and/or intranasal delivery. It was disclosed in the art that the peripheral administration of AAV particles for siRNA duplexes can be transported to the central nervous system, for example, to the neurons (e.g., U. S. Patent Publication Nos. 20100240739; and 20100130594; the content of each of which is incorporated herein by reference in their entirety).

[00686] In other embodiments, compositions comprising at least one AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered to a subject by intracranial delivery (See, e.g., U. S. Pat. No. 8,119,611; the content of which is incorporated herein by reference in its entirety).

[00687] The AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered in any suitable form, either as a liquid solution or suspension, as a solid form suitable for liquid solution or suspension in a liquid solution. The siRNA duplexes may be formulated with any appropriate and pharmaceutically acceptable excipient.

[00688] The AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be administered in a "therapeutically effective" amount, i.e., an amount that is sufficient to alleviate and/or prevent at least one symptom associated with the disease, or provide improvement in the condition of the subject.

[00689] In one embodiment, the AAV particle may be administered to the CNS in a therapeutically effective amount to improve function and/or survival for a subject with ALS. As a non-limiting example, the vector may be administered by direct infusion into the striatum.

[00690] In one embodiment, the AAV particle may be administered to a subject (e.g., to the CNS of a subject via intrathecal administration) in a therapeutically effective amount for the siRNA duplexes or dsRNA to target the medium spiny neurons, cortical neurons and/or astrocytes. As a non-limiting example, the siRNA duplexes or dsRNA may target SOD1 and reduce the expression of SOD1 protein or mRNA. As another non-limiting example, the siRNA duplexes or dsRNA target SOD1 and can suppress SOD1 and reduce SOD1 mediated toxicity. The reduction of SOD1 protein and/or mRNA as well as SOD1 mediated toxicity may be accomplished with almost no enhanced inflammation.

[00691] In one embodiment, the AAV particle may be administered to a subject (e.g., to the CNS of a subject) in a therapeutically effective amount to slow the functional decline of a subject (e.g., determined using a known evaluation method). As a non-limiting example, the vector may be administered via intraparenchymal injection.

[00692] In one embodiment, the AAV particle may be administered to the cisterna magna in a therapeutically effective amount to transduce medium spiny neurons, cortical neurons and/or astrocytes. As a non-limiting example, the vector may be administered intrathecally.

[00693] In one embodiment, the AAV particle may be administered using intrathecal infusion in a therapeutically effective amount to transduce medium spiny neurons, cortical neurons and/or astrocytes. As a non-limiting example, the vector may be administered intrathecally.

[00694] In one embodiment, the AAV particle may be administered to the cisterna magna in a therapeutically effective amount to transduce medium spiny neurons, cortical neurons and/or astrocytes. As a non-limiting example, the vector may be administered by intraparenchymal injection.

[00695] In one embodiment, the AAV particle comprising a modulatory polynucleotide may be formulated. As a non-limiting example the baricity and/or osmolality of the formulation may be optimized to ensure optimal drug distribution in the central nervous system or a region or component of the central nervous system.

[00696] In one embodiment, the AAV particle comprising a modulatory polynucleotide may be delivered to a subject via a single route administration.

[00697] In one embodiment, the AAV particle comprising a modulatory polynucleotide may be delivered to a subject via a multi-site route of administration. A subject may be administered the AAV particle comprising a modulatory polynucleotide at 2, 3, 4, 5 or more than 5 sites.

[00698] In one embodiment, a subject may be administered the AAV particle comprising a modulatory polynucleotide described herein using a bolus injection.

[00699] In some embodiments, the efficacy of aministration of the AAV particle comprising a modulatory polynucleotide using a bolus injection may be measured by monitoring the gene transfer to the spinal cord, brain stem, or motor cortex. The biodistribution and cellular tropism may be monitored by any methods known in the art, such as, but not limited to, immunostaining, and the vector genome levels may be measured by digital PCR.

[00700] In one embodiment, a subject may be administered the AAV particle comprising a modulatory polynucleotide described herein using sustained delivery over a period of minutes,

hours or days. The infusion rate may be changed depending on the subject, distribution, formulation or another delivery parameter.

[00701] In one embodiment, the AAV particle described herein is administered via putamen and caudate infusion. As a non-limiting example, the dual infusion provides a broad striatal distribution as well as a frontal and temporal cortical distribution.

[00702] In one embodiment, the AAV particle is AAV-DJ8 which is administered via unilateral putamen infusion. As a non-limiting example, the distribution of the administered AAV-DJ8 is similar to the distribution of AAV1 delivered via unilateral putamen infusion.

[00703] In one embodiment, the AAV particle described herein is administered via intrathecal (IT) infusion at C1. The infusion may be for 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more than 15 hours.

[00704] In one embodiment, the selection of subjects for administration of the AAV particle described herein and/or the effectiveness of the dose, route of administration and/or volume of administration may be evaluated using imaging of the perivascular spaces (PVS) which are also known as Virchow-Robin spaces. PVS surround the arterioles and venules as they perforate brain parenchyma and are filled with cerebrospinal fluid (CSF)/interstitial fluid. PVS are common in the midbrain, basal ganglia, and centrum semiovale. While not wishing to be bound by theory, PVS may play a role in the normal clearance of metabolites and have been associated with worse cognition and several disease states including Parkinson's disease. PVS are usually are normal in size but they can increase in size in a number of disease states. Potter et al. (Cerebrovasc Dis. 2015 Jan; 39(4): 224–231; the contents of which are herein incorporated by reference in its entirety) developed a grading method where they studied a full range of PVS and rated basal ganglia, centrum semiovale and midbrain PVS. They used the frequency and range of PVS used by Mac and Lullich et al. (J Neurol Neurosurg Psychiatry. 2004 Nov;75(11):1519-23; the contents of which are herein incorporated by reference in its entirety) and Potter et al. gave 5 ratings to basal ganglia and centrum semiovale PVS: 0 (none), 1 (1-10), 2 (11-20), 3 (21-40) and 4 (>40) and 2 ratings to midbrain PVS: 0 (non visible) or 1 (visible). The user guide for the rating system by Potter et al. can be found at: www.sbirc.ed.ac.uk/documents/epvs-rating-scale-user-guide.pdf.

Dosing

[00705] The pharmaceutical compositions of the present invention may be administered to a subject using any amount effective for reducing, preventing and/or treating a disease and/or disorder. The exact amount required will vary from subject to subject, depending on the species,

age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like.

[00706] The compositions of the present invention are typically formulated in unit dosage form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutic effectiveness for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the siRNA duplexes employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[00707] As a non-limiting example, the dose may be determined based on the total volume of CSF of a subject. For example cynomolgus monkeys have a total estimated cerebrospinal fluid (CSF) volume of approximately 6 to 12 mL and humans have a total estimated CSF of approximately 120 to 150 mL, a factor of at least 10 to 12-fold. Therefore, a factor of 10x to 12x may be used to determine a human dose based on the dose to a cynomolgus monkey. In one embodiment, the factor is 10x. In another embodiment the factor is 11x. In yet another embodiment, the factor is 12x. In yet another embodiment, the factor may be, but is not limited to, 10x, 10.1x, 10.2x, 10.3x, 10.4x, 10.5x, 10.6x, 10.7x, 10.8x, 10.9x, 11x, 11.1x, 11.2x, 11.3x, 11.4x, 11.5x, 11.6x, 11.7x, 11.8x, 11.9x, 12x, 12.1x, 12.2x, 12.3x, 12.4x, and 12.5x.

[00708] In one embodiment, the age and sex of a subject may be used to determine the dose of the compositions of the present invention. As a non-limiting example, a subject who is older may receive a larger dose (e.g., 5-10%, 10-20%, 15-30% ,20-50%, 25-50% or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% more) of the composition as compared to a younger subject. As another non-limiting example, a subject who is younger may receive a larger dose (e.g., 5-10%, 10-20%, 15-30%, 20-50%, 25-50% or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% more) of the composition as compared to an older subject. As yet another non-limiting example, a subject who is female may receive a larger dose (e.g., 5-10%, 10-20%, 15-30% ,20-50%, 25-50% or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% more) of the composition as compared to a male subject. As yet another non-

limiting example, a subject who is male may receive a larger dose (e.g., 5-10%, 10-20%, 15-30% ,20-50%, 25-50% or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more than 90% more) of the composition as compared to a female subject

[00709] In some specific embodiments, the doses of AAV particles for delivering siRNA duplexes of the present invention may be adapted depending on the disease condition, the subject and the treatment strategy.

[00710] In one embodiment, delivery of the compositions in accordance with the present invention to cells comprises a rate of delivery defined by [VG/hour = mL/hour * VG/mL] wherein VG is viral genomes, VG/mL is composition concentration, and mL/hour is rate of prolonged delivery.

[00711] In one embodiment, delivery of compositions in accordance with the present invention to cells may comprise a total concentration per subject between about $1x10^6$ VG and about $1x10^{16}$ VG. In some embodiments, delivery may comprise a composition concentration of about $1x10^6$, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$, $8x10^6$, $9x10^6$, $1x10^7$, $2x10^7$, $3x10^7$, $4x10^7$, $5x10^7$, $6x10^7$, $7x10^7$, $8x10^7$, $9x10^7$, $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, $5x10^{10}$, $6x10^{10}$, $7x10^{10}$, $8x10^{10}$, $9x10^{10}$, $1x10^{11}$, $1.1x10^{11}$, $1.2x10^{11}$, $1.3x10^{11}$, $1.4x10^{11}$, $1.5x10^{11}$, $1.6x10^{11}$, $1.7x10^{11}$, $1.8x10^{11}$, $1.9x10^{11}$, $2x10^{11}$, $2.1x10^{11}$, $2.2x10^{11}$, $2.3x10^{11}$, $2.4x10^{11}$, $2.5x10^{11}$, $2.6x10^{11}$, $2.7x10^{11}$, $2.8x10^{11}$, $2.9x10^{11}$, $3x10^{11}$, $4x10^{11}$, $5x10^{11}$, $6x10^{11}$, $7x10^{11}$, $7.1x10^{11}$, $7.2x10^{11}$, $7.3x10^{11}$, $7.4x10^{11}$, $7.5x10^{11}$, $7.6x10^{11}$, $7.7x10^{11}$, $7.8x10^{11}$, $7.9x10^{11}$, $8x10^{11}$, $9x10^{11}$, $1x10^{12}$, $1.1\ x10^{12}$, $1.2x10^{12}$, $1.3x10^{12}$, $1.4x10^{12}$, $1.5x10^{12}$, $1.6x10^{12}$, $1.7x10^{12}$, $1.8x10^{12}$, $1.9x10^{12}$, $2x10^{12}$, $2.1x10^{12}$, $2.2x10^{12}$, $2.3x10^{12}$, $2.4x10^{12}$, $2.5x10^{12}$, $2.6x10^{12}$, $2.7x10^{12}$, $2.8x10^{12}$, $2.9x10^{12}$, $3x10^{12}$, $3.1x10^{12}$, $3.2x10^{12}$, $3.3x10^{12}$, $3.4x10^{12}$, $3.5x10^{12}$, $3.6x10^{12}$, $3.7x10^{12}$, $3.8x10^{12}$, $3.9x10^{12}$, $4x10^{12}$, $4.1x10^{12}$, $4.2x10^{12}$, $4.3x10^{12}$, $4.4x10^{12}$, $4.5x10^{12}$,$4.6x10^{12}$, $4.7x10^{12}$, $4.8x10^{12}$, $4.9x10^{12}$, $5x10^{12}$, $6x10^{12}$, $6.1x10^{12}$, $6.2x10^{12}$, $6.3x10^{12}$, $6.4x10^{12}$, $6.5x10^{12}$, $6.6x10^{12}$, $6.7x10^{12}$, $6.8x10^{12}$, $6.9x10^{12}$, $7x10^{12}$, $8x10^{12}$, $8.1x10^{12}$, $8.2x10^{12}$, $8.3x10^{12}$, $8.4x10^{12}$, $8.5x10^{12}$, $8.6x10^{12}$, $8.7x10^{12}$, $8.8\ x10^{12}$, $8.9x10^{12}$, $9x10^{12}$, $1x10^{13}$, $1.1x10^{13}$, $1.2x10^{13}$, $1.3x10^{13}$, $1.4x10^{13}$, $1.5x10^{13}$, $1.6x10^{13}$, $1.7x10^{13}$, $1.8x10^{13}$, $1.9x10^{13}$, $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$, $6x10^{13}$, $6.7x10^{13}$, $7x10^{13}$, $8x10^{13}$, $9x10^{13}$, $1x10^{14}$, $2x10^{14}$, $3x10^{14}$, $4x10^{14}$, $5x10^{14}$, $6x10^{14}$, $7x10^{14}$, $8x10^{14}$, $9x10^{14}$, $1x10^{15}$, $2x10^{15}$, $3x10^{15}$, $4x10^{15}$, $5x10^{15}$, $6x10^{15}$, $7x10^{15}$, $8x10^{15}$, $9x10^{15}$, or $1x10^{16}$ VG/subject.

[00712] In one embodiment, delivery of compositions in accordance with the present invention to cells may comprise a total concentration per subject between about $1x10^6$ VG/kg

and about $1x10^{16}$ VG/kg. In some embodiments, delivery may comprise a composition concentration of about $1x10^{6}$, $2x10^{6}$, $3x10^{6}$, $4x10^{6}$, $5x10^{6}$, $6x10^{6}$, $7x10^{6}$, $8x10^{6}$, $9x10^{6}$, $1x10^{7}$, $2x10^{7}$, $3x10^{7}$, $4x10^{7}$, $5x10^{7}$, $6x10^{7}$, $7x10^{7}$, $8x10^{7}$, $9x10^{7}$, $1x10^{8}$, $2x10^{8}$, $3x10^{8}$, $4x10^{8}$, $5x10^{8}$, $6x10^{8}$, $7x10^{8}$, $8x10^{8}$, $9x10^{8}$, $1x10^{9}$, $2x10^{9}$, $3x10^{9}$, $4x10^{9}$, $5x10^{9}$, $6x10^{9}$, $7x10^{9}$, $8x10^{9}$, $9x10^{9}$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, $5x10^{10}$, $6x10^{10}$, $7x10^{10}$, $8x10^{10}$, $9x10^{10}$, $1x10^{11}$, $1.1x10^{11}$, $1.2x10^{11}$, $1.3x10^{11}$, $1.4x10^{11}$, $1.5x10^{11}$, $1.6x10^{11}$, $1.7x10^{11}$, $1.8x10^{11}$, $1.9x10^{11}$, $2x10^{11}$, $2.1x10^{11}$, $2.2x10^{11}$, $2.3x10^{11}$, $2.4x10^{11}$, $2.5x10^{11}$, $2.6x10^{11}$, $2.7x10^{11}$, $2.8x10^{11}$, $2.9x10^{11}$, $3x10^{11}$, $4x10^{11}$, $5x10^{11}$, $6x10^{11}$, $7x10^{11}$, $7.1x10^{11}$, $7.2x10^{11}$, $7.3x10^{11}$, $7.4x10^{11}$, $7.5x10^{11}$, $7.6x10^{11}$, $7.7x10^{11}$, $7.8x10^{11}$, $7.9x10^{11}$, $8x10^{11}$, $9x10^{11}$, $1x10^{12}$, $1.1\ x10^{12}$, $1.2x10^{12}$, $1.3x10^{12}$, $1.4x10^{12}$, $1.5x10^{12}$, $1.6x10^{12}$, $1.7x10^{12}$, $1.8x10^{12}$, $1.9x10^{12}$, $2x10^{12}$, $2.1x10^{12}$, $2.2x10^{12}$, $2.3x10^{12}$, $2.4x10^{12}$, $2.5x10^{12}$, $2.6x10^{12}$, $2.7x10^{12}$, $2.8x10^{12}$, $2.9x10^{12}$, $3x10^{12}$, $3.1x10^{12}$, $3.2x10^{12}$, $3.3x10^{12}$, $3.4x10^{12}$, $3.5x10^{12}$, $3.6x10^{12}$, $3.7x10^{12}$, $3.8x10^{12}$, $3.9x10^{12}$, $4x10^{12}$, $4.1x10^{12}$, $4.2x10^{12}$, $4.3x10^{12}$, $4.4x10^{12}$, $4.5x10^{12}$,$4.6x10^{12}$, $4.7x10^{12}$, $4.8x10^{12}$, $4.9x10^{12}$, $5x10^{12}$, $6x10^{12}$, $6.1x10^{12}$, $6.2x10^{12}$, $6.3x10^{12}$, $6.4x10^{12}$, $6.5x10^{12}$, $6.6x10^{12}$, $6.7x10^{12}$, $6.8x10^{12}$, $6.9x10^{12}$, $7x10^{12}$, $8x10^{12}$, $8.1x10^{12}$, $8.2x10^{12}$, $8.3x10^{12}$, $8.4x10^{12}$, $8.5x10^{12}$, $8.6x10^{12}$, $8.7x10^{12}$, $8.8\ x10^{12}$, $8.9x10^{12}$, $9x10^{12}$, $1x10^{13}$, $1.1x10^{13}$, $1.2x10^{13}$, $1.3x10^{13}$, $1.4x10^{13}$, $1.5x10^{13}$, $1.6x10^{13}$, $1.7x10^{13}$, $1.8x10^{13}$, $1.9x10^{13}$, $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$, $6x10^{13}$, $6.7x10^{13}$, $7x10^{13}$, $8x10^{13}$, $9x10^{13}$, $1x10^{14}$, $2x10^{14}$, $3x10^{14}$, $4x10^{14}$, $5x10^{14}$, $6x10^{14}$, $7x10^{14}$, $8x10^{14}$, $9x10^{14}$, $1x10^{15}$, $2x10^{15}$, $3x10^{15}$, $4x10^{15}$, $5x10^{15}$, $6x10^{15}$, $7x10^{15}$, $8x10^{15}$, $9x10^{15}$, or $1x10^{16}$ VG/kg.

[00713] In one embodiment, about 10^{5} to 10^{6} viral genome (unit) may be administered per dose.

[00714] In one embodiment, delivery of the compositions in accordance with the present invention to cells may comprise a total concentration between about $1x10^{6}$ VG/mL and about $1x10^{16}$ VG/mL. In some embodiments, delivery may comprise a composition concentration of about $1x10^{6}$, $2x10^{6}$, $3x10^{6}$, $4x10^{6}$, $5x10^{6}$, $6x10^{6}$, $7x10^{6}$, $8x10^{6}$, $9x10^{6}$, $1x10^{7}$, $2x10^{7}$, $3x10^{7}$, $4x10^{7}$, $5x10^{7}$, $6x10^{7}$, $7x10^{7}$, $8x10^{7}$, $9x10^{7}$, $1x10^{8}$, $2x10^{8}$, $3x10^{8}$, $4x10^{8}$, $5x10^{8}$, $6x10^{8}$, $7x10^{8}$, $8x10^{8}$, $9x10^{8}$, $1x10^{9}$, $2x10^{9}$, $3x10^{9}$, $4x10^{9}$, $5x10^{9}$, $6x10^{9}$, $7x10^{9}$, $8x10^{9}$, $9x10^{9}$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, $5x10^{10}$, $6x10^{10}$, $7x10^{10}$, $8x10^{10}$, $9x10^{10}$, $1x10^{11}$, $1.1x10^{11}$, $1.2x10^{11}$, $1.3x10^{11}$, $1.4x10^{11}$, $1.5x10^{11}$, $1.6x10^{11}$, $1.7x10^{11}$, $1.8x10^{11}$, $1.9x10^{11}$, $2x10^{11}$, $3x10^{11}$, $4x10^{11}$, $5x10^{11}$, $6x10^{11}$, $7x10^{11}$, $8x10^{11}$, $9x10^{11}$, $1x10^{12}$, $1.1x10^{12}$, $1.2x10^{12}$, $1.3x10^{12}$, $1.4x10^{12}$, $1.5x10^{12}$, $1.6x10^{12}$, $1.7x10^{12}$, $1.8x10^{12}$, $1.9x10^{12}$, $2x10^{12}$, $2.1x10^{12}$, $2.2x10^{12}$, $2.3x10^{12}$, $2.4x10^{12}$, $2.5x10^{12}$, $2.6x10^{12}$, $2.7x10^{12}$, $2.8x10^{12}$, $2.9x10^{12}$, $3x10^{12}$, $3.1x10^{12}$, $3.2x10^{12}$, $3.3x10^{12}$, $3.4x10^{12}$, $3.5x10^{12}$, $3.6x10^{12}$, $3.7x10^{12}$, $3.8x10^{12}$, $3.9x10^{12}$, $4x10^{12}$, $4.1x10^{12}$, $4.2x10^{12}$, $4.3x10^{12}$, $4.4x10^{12}$, $4.5x10^{12}$,

$4.6x10^{12}$, $4.7x10^{12}$, $4.8x10^{12}$, $4.9x10^{12}$, $5x10^{12}$, $6x10^{12}$, $6.1x10^{12}$, $6.2x10^{12}$, $6.3x10^{12}$, $6.4x10^{12}$, $6.5x10^{12}$, $6.6x10^{12}$, $6.7x10^{12}$, $6.8x10^{12}$, $6.9x10^{12}$, $7x10^{12}$, $8x10^{12}$, $9x10^{12}$, $1x10^{13}$, $1.1x10^{13}$, $1.2x10^{13}$, $1.3x10^{13}$, $1.4x10^{13}$, $1.5x10^{13}$, $1.6x10^{13}$, $1.7x10^{13}$, $1.8x10^{13}$, $1.9x10^{13}$, $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$, $6x10^{13}$, $6.7x10^{13}$, $7x10^{13}$, $8x10^{13}$, $9x10^{13}$, $1x10^{14}$, $2x10^{14}$, $3x10^{14}$, $4x10^{14}$, $5x10^{14}$, $6x10^{14}$, $7x10^{14}$, $8x10^{14}$, $9x10^{14}$, $1x10^{15}$, $2x10^{15}$, $3x10^{15}$, $4x10^{15}$, $5x10^{15}$, $6x10^{15}$, $7x10^{15}$, $8x10^{15}$, $9x10^{15}$, or $1x10^{16}$ VG/mL.

[00715] In certain embodiments, the desired siRNA duplex dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses, e.g., two or more administrations of the single unit dose. As used herein, a "single unit dose" is a dose of any modulatory polynucleotide therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a "total daily dose" is an amount given or prescribed in a 24 hour period. It may be administered as a single unit dose. In one embodiment, the AAV particles comprising the modulatory polynucleotides of the present invention are administered to a subject in split doses. They may be formulated in buffer only or in a formulation described herein.

[00716] In one embodiment, the dose, concentration and/or volume of the composition described herein may be adjusted depending on the contribution of the caudate or putamen to cortical and subcortical distribution after administration. The administration may be intracerebroventricular, intraputamenal, intrathalmic, intraparenchymal, subpial, and/or intrathecal administration.

[00717] In one embodiment, the dose, concentration and/or volume of the composition described herein may be adjusted depending on the cortical and neuraxial distribution following administration by intracerebroventricular, intraputamenal, intrathalmic, intraparenchymal, subpial, and/or intrathecal delivery.

IV. METHODS AND USES OF THE COMPOSITIONS OF THE INVENTION

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS)

[00718] Amyotrophic lateral sclerosis (ALS), an adult-onset neurodegenerative disorder, is a progressive and fatal disease characterized by the selective death of motor neurons in the motor cortex, brainstem and spinal cord. The incidence of ALS is about 1.9 per 100,000. Patients

diagnosed with ALS develop a progressive muscle phenotype characterized by spasticity, hyperreflexia or hyporeflexia, fasciculations, muscle atrophy and paralysis. These motor impairments are caused by the denervation of muscles due to the loss of motor neurons. The major pathological features of ALS include degeneration of the corticospinal tracts and extensive loss of lower motor neurons (LMNs) or anterior horn cells (Ghatak et al., *J Neuropathol Exp Neurol*., 1986, 45, 385-395), degeneration and loss of Betz cells and other pyramidal cells in the primary motor cortex (Udaka et al., *Acta Neuropathol*, 1986, 70, 289-295; Maekawa et al., *Brain*, 2004, 127, 1237-1251) and reactive gliosis in the motor cortex and spinal cord (Kawamata et al., *Am J Pathol*., 1992, 140,691-707; and Schiffer et al., *J Neurol Sci.*, 1996, 139, 27-33). ALS is usually fatal within 3 to 5 years after the diagnosis due to respiratory defects and/or inflammation (Rowland LP and Shneibder NA, *N Engl. J. Med*., 2001, 344, 1688-1700).

[00719] A cellular hallmark of ALS is the presence of proteinaceous, ubiquitinated, cytoplasmic inclusions in degenerating motor neurons and surrounding cells (e.g., astrocytes). Ubiquitinated inclusions (i.e., Lewy body-like inclusions or Skein-like inclusions) are the most common and specific type of inclusion in ALS and are found in LMNs of the spinal cord and brainstem, and in corticospinal upper motor neurons (UMNs) (Matsumoto et al., *J Neurol Sci*., 1993, 115, 208-213; and Sasak and Maruyama, *Acta Neuropathol*., 1994, 87, 578-585). A few proteins have been identified to be components of the inclusions, including ubiquitin, Cu/Zn superoxide dismutase 1 (SOD1), peripherin and Dorfin. Neurofilamentous inclusions are often found in hyaline conglomerate inclusions (HCIs) and axonal 'spheroids' in spinal cord motor neurons in ALS. Other types and less specific inclusions include Bunina bodies (cystatin C-containing inclusions) and Crescent shaped inclusions (SCIs) in upper layers of the cortex. Other neuropathological features seen in ALS include fragmentation of the Golgi apparatus, mitochondrial vacuolization and ultrastructural abnormalities of synaptic terminals (Fujita et al., *Acta Neuropathol*. 2002, 103, 243-247).

[00720] In addition, in frontotemporal dementia ALS (FTD-ALS) cortical atrophy (including the frontal and temporal lobes) is also observed, which may cause cognitive impairment in FTD-ALS patients.

[00721] ALS is a complex and multifactorial disease and multiple mechanisms hypothesized as responsible for ALS pathogenesis include, but are not limited to, dysfunction of protein degradation, glutamate excitotoxicity, mitochondrial dysfunction, apoptosis, oxidative stress, inflammation, protein misfolding and aggregation, aberrant RNA metabolism, and altered gene expression.

[00722] About 10%-15% of ALS cases have family history of the disease, and these patients are referred to as familial ALS (fALS) or inherited patients, commonly with a Mendelian dominant mode of inheritance and high penetrance. The remainder (approximately 85%-95%) is classified as sporadic ALS (sALS), as they are not associated with a documented family history, but instead are thought to be due to other risk factors including, but not limited to environmental factors, genetic polymorphisms, somatic mutations, and possibly gene-environmental interactions. In most cases, familial (or inherited) ALS is inherited as autosomal dominant disease, but pedigrees with autosomal recessive and X-linked inheritance and incomplete penetrance exist. Sporadic and familial forms are clinically indistinguishable suggesting a common pathogenesis. The precise cause of the selective death of motor neurons in ALS remains elusive. Progress in understanding the genetic factors in fALS may shed light on both forms of the disease.

[00723] Recently, an exploration into genetic causes of ALS has discovered mutations in more than 10 different genes that are known to cause fALS. The most common ones are found in the genes encoding Cu/Zn superoxide dismutase 1 (SOD1; ~ 20%) (Rosen DR et al., Nature, 1993, 362, 59-62), fused in sarcoma/translated in liposarcoma (FUS/TLS; 1-5%) and TDP-43 (TARDBP; 1-5%). Recently, a hexanucleotide repeat expansion (GGGGCC)n in the C9orF72 gene was identified as the most frequent cause of fALS (~ 40%) in the Western population (reviewed by Renton et al., Nat. Neurosci., 2014, 17, 17-23). Other genes mutated in ALS include alsin (ALS2), senataxin (SETX), vesicle-associated membrane protein (VAPB), and angiogenin (ANG). fALS genes control different cellular mechanisms, suggesting that the pathogenesis of ALS is complicated and may be related to several different processes finally leading to motor neuron degeneration.

[00724] Subjects with ALS and an identified mutation in a known ALS-causative gene may be referred to as having "genetically defined ALS." Non-limiting examples of ALS-causative genes include SOD1, C9orf72, TARDBP (also termed TDP-43), FUS/TLS, OPTN, UBQLN2, PFN1, DCTN1, and TBK1.

[00725] SOD1 is one of the three human superoxide dismutases identified and characterized in mammals: copper-zinc superoxide dismutase (Cu/ZnSOD or SOD1), manganese superoxide dismutase (MnSOD or SOD2), and extracellular superoxide dismutase (ECSOD or SOD3). SOD1 is a 32 kDa homodimer of a 153-residue polypeptide with one copper- and one zinc-binding site per subunit, which is encoded by the SOD1 gene (GeneBank access No.: NM_000454.4; SEQ ID NO: 1254) on human chromosome 21. SOD1 catalyzes the reaction of

superoxide anion (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) at a bound copper ion. The intracellular concentration of SOD1 is high (ranging from 10 to 100 μM), accounting for 1% of the total protein content in the central nervous system (CNS). The protein is localized not only in the cytoplasm but also in the nucleus, lysosomes, peroxisomes, and mitochondrial intermembrane spaces in eukaryotic cells (Lindenau J et al., *Glia*, 2000, 29, 25–34).

[00726] Mutations in the SOD1 gene are carried by 15–20% of fALS patients and by 1-2% of all ALS cases. Currently, at least 170 different mutations distributed throughout the 153-amino acid SOD1 polypeptide have been found to cause ALS, and an updated list can be found at the ALS online Genetic Database (ALSOD) (Wroe R et al., *Amyotroph Lateral Scler.*, 2008, 9, 249-250). Table 21 lists some examples of mutations in SOD1 in ALS. These mutations are predominantly single amino acid substitutions (i.e. missense mutations) although deletions, insertions, and C-terminal truncations also occur. Different SOD1 mutations display different geographic distribution patterns. For instance, 40-50% of all Americans with ALS caused by SOD1 gene mutations have a particular mutation Ala4Val (or A4V). The A4V mutation is typically associated with more severe signs and symptoms and the survival period is typically 2-3 years. The I113T mutation is by far the most common mutation in the United Kingdom. The most prevalent mutation in Europe is D90A substitute and the survival period is usually greater than 10 years.

Table 21. Examples of SOD1 mutations in ALS

Location	Mutations
Exon1 (220bp)	Q22L; E21K,G; F20C;N19S; G16A,S; V14M,S; G12R; G10G,V,R; L8Q,V; V7E; C6G,F; V5L; A4T,V,S
Exon2 (97bp)	T54R; E49K; H48R,Q; V47F,A; H46R; F45C; H43R; G41S,D; G37R; V29,insA
Exon3 (70bp)	D76Y,V; G72S,C; L67R; P66A; N65S; S59I,S
Exon4 (118bp)	D124G,V; V118L,InsAAAAC; L117V; T116T; R115G; G114A; I113T,F; I112M,T; G108V; L106V,F; S106L,delTCACTC; I104F; D101G,Y,H,N; E100G,K; I99V; V97L,M; D96N,V; A95T,V; G93S,V,A, C,R,D; D90V,A; A89T,V; T88delACTGCTGAC; V87A,M; N86I,S,D,K; G85R,S; L84V,F; H80R
Exon5 (461bp)	I151T,S; I149T; V148I,G; G147D,R; C146R, stop; A145T,G; L144F,S; G141E,stop; A140A,G; N139D,K,H,N; G138E; T137R; S134N; E133V,delGAA,insTT; E132insTT; G127R,InsTGGG; L126S,delITT,stop; D126,delTT

[00727] To investigate the mechanism of neuronal death associated with SOD1 gene defects, several rodent models of SOD1-linked ALS were developed in the art, which express the human SOD1 gene with different mutations, including missense mutations, small deletions or insertions. Non-limiting examples of ALS mouse models include $SOD1^{G93A}$, $SOD1^{A4V}$, $SOD1^{G37R}$, $SOD1^{G85R}$, $SOD1^{D90A}$, $SOD1^{L84V}$, $SOD1^{I113T}$, $SOD1^{H36R/H48Q}$, $SOD1^{G127X}$, $SOD1^{L126X}$ and $SOD1^{L126delTT}$. There are two transgenic rat models carrying two different human SOD1 mutations: $SOD1^{H46R}$ and $SOD1^{G93R}$. These rodent ALS models can develop muscle weakness similar to human ALS patients and other pathogenic features that reflect several characteristics of the human disease, in particular, the selective death of spinal motor neurons, aggregation of protein inclusions in motor neurons and microglial activation. It is well known in the art that the transgenic rodents are good models of human SOD1-associated ALS disease and provide models for studying disease pathogenesis and developing disease treatment.

[00728] Studies in animal and cellular models showed that SOD1 pathogenic variants cause ALS by gain of function. That is to say, the superoxide dismutase enzyme gains new but harmful properties when altered by SOD1 mutations. For example, some SOD1 mutated variants in ALS increase oxidative stress (e.g., increased accumulation of toxic superoxide radicals) by disrupting the redox cycle. Other studies also indicate that some SOD1 mutated variants in ALS might acquire toxic properties that are independent of its normal physiological function (such as abnormal aggregation of misfolded SOD1 variants. In the aberrant redox chemistry model, mutant SOD1 is unstable and through aberrant chemistry interacts with nonconventional substrates causing overproduction of reactive oxygen species (ROS). In the protein toxicity model, unstable, misfolded SOD1 aggregates into cytoplasmic inclusion bodies, sequestering proteins crucial for cellular processes. These two hypotheses are not mutually exclusive. It has been shown that oxidation of selected histidine residues that bind metals in the active site mediates SOD1 aggregation.

[00729] The aggregated mutant SOD1 protein may also induce mitochondrial dysfunction (Vehvilainen P et al., *Front Cell Neurosci*., 2014, 8, 126), impairment of axonal transport, aberrant RNA metabolism, glial cell pathology and glutamate excitotoxicity. In some sporadic ALS cases, misfolded wild-type SOD1 protein is found in diseased motor neurons which forms a "toxic conformation" that is similar to that which is seen with familial ALS-linked SOD1 variants (Rotunno MS and Bosco DA, *Front Cell Neurosci*., 2013, 16, 7, 253). Such evidence suggests that ALS is a protein folding diseases analogous to other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.

[00730] Currently, no curative treatments are available for patients suffering from ALS. The only FDA approved drug Riluzole, an inhibitor of glutamate release, has a moderate effect on ALS, only extending survival by 2-3 months if it is taken for 18 months. Unfortunately, patients taking riluzole do not experience any slowing in disease progression or improvement in muscle function. Therefore, riluzole does not present a cure, or even an effective treatment. Researchers continue to search for better therapeutic agents.

[00731] Therapeutic approaches that may prevent or ameliorate SOD1 aggregation have been tested previously. For example, arimoclomol, a hydroxylamine derivative, is a drug that targets heat shock proteins, which are cellular defense mechanisms against these aggregates. Studies demonstrated that treatment with arimoclomol improved muscle function in SOD1 mouse models. Other drugs that target one or more cellular defects in ALS may include AMPA antagonists such as talampanel, beta-lactam antibiotics, which may reduce glutamate-induced excitotoxicity to motor neurons; Bromocriptine that may inhibit oxidative induced motor neuron death (e.g. U.S. Patent publication No. 20110105517; the content of which is incorporated herein by reference in its entirety); 1,3-diphenylurea derivative or multikinase inhibitor which may reduce SOD1 gene expression (e.g., U.S. Patent Publication No.20130225642; the content of which is incorporated herein by reference in its entirety); dopamine agonist pramipexole and its anantiomer dexpramipexole, which may ameliorate the oxidative response in mitochondria; nimesulide, which inhibits cyclooxygenase enzyme (e.g., U.S. Patent Publication No. 20060041022; the content of which is incorporated herein by reference in its entirety); drugs that act as free radical scavengers (e.g. U.S. Pat. No.: 6,933,310 and PCT Patent Publication No.: WO2006075434; the content of each of which is incorporated herein by reference in their entirety).

[00732] Another approach to inhibit abnormal SOD1 protein aggregation is to silence/inhibit SOD1 gene expression in ALS. It has been reported that small interfering RNAs for specific gene silencing of the mutated allele are therapeutically beneficial for the treatment of fALS (e.g., Ralgh GS et al., *Nat. Medicine*, 2005, 11(4), 429-433; and Raoul C et al., *Nat. Medicine*, 2005, 11(4), 423-428; and Maxwell MM et al., *PNAS*, 2004, 101(9), 3178-3183; and Ding H et al., *Chinese Medical J.*, 2011, 124(1), 106-110; and Scharz DS et al., *Plos Genet.*, 2006, 2(9), e140; the content of each of which is incorporated herein by reference in their entirety).

[00733] Many other RNA therapeutic agents that target the SOD1 gene and modulate SOD1 expression in ALS are taught in the art. Such RNA based agents include antisense oligonucleotides and double stranded small interfering RNAs. See, e.g., Wang H et al., *J Biol.*

Chem., 2008, 283(23), 15845-15852); U.S. Pat. Nos. 7,498,316; 7,632,938; 7,678,895; 7,951,784; 7,977,314; 8,183,219; 8,309,533 and 8, 586, 554; and U.S. Patent publication Nos. 2006/0229268 and 2011/0263680; the content of each of which is herein incorporated by reference in their entirety.

[00734] The present invention provides AAV particles comprising modulatory polynucleotides comprising sequences encoding siRNA molecules targeting the SOD1 gene and methods for their design and manufacture. The AAV particles comprising the nucleic acid sequence encoding the siRNA molecules of the present invention may increase the delivery of active agents into motor neurons. The siRNA duplexes or encoding dsRNA targeting the SOD1 gene may be able to inhibit SOD1 gene expression (e.g., mRNA level) significantly inside cells; therefore, ameliorating SOD1 expression induced stress inside the cells such as aggregation of protein and formation of inclusions, increased free radicals, mitochondrial dysfunction and RNA metabolism.

[00735] Such siRNA mediated SOD1 expression inhibition may be used for treating ALS. According to the present invention, methods for treating and/or ameliorating ALS in a patient comprises administering to the patient an effective amount of AAV particle comprising a nucleic acid sequence encoding the siRNA molecules of the present invention into cells. The administration of the AAV particle comprising such a nucleic acid sequence will encode the siRNA molecules which cause the inhibition/silence of SOD1 gene expression.

[00736] In one embodiment, the AAV particle comprising the modulatory polynucleotide, reduce the expression of mutant SOD1 in a subject. The reduction of mutant SOD1 can also reduce the formation of toxic aggregates which can cause mechanisms of toxicity such as, but not limited to, oxidative stress, mitochondrial dysfunction, impaired axonal transport, aberrant RNA metabolism, glial cell pathology and/or glutamate excitotoxicity.

[00737] In one embodiment, the vector, e.g., AAV particles, reduces the amount of SOD1 in a subject in need thereof and thus provides a therapeutic benefit as described herein.

Methods of treatment of ALS

[00738] Provided in the present invention are methods for introducing the AAV particles comprising modulatory polynucleotides comprising sequences comprising a nucleic acid sequence encoding the siRNA molecules of the present invention into cells, the method comprising introducing into said cells any of the vectors in an amount sufficient for degradation of target SOD1 mRNA to occur, thereby activating target-specific RNAi in the cells. In some

aspects, the cells may be stem cells, neurons such as motor neurons, muscle cells and glial cells such as astrocytes.

[00739] Disclosed in the present invention are methods for treating ALS associated with abnormal SOD1 function in a subject in need of treatment. The method optionally comprises administering to the subject a therapeutically effective amount of a composition comprising at least AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention. As a non-limiting example, the siRNA molecules can silence SOD1 gene expression, inhibit SOD1 protein production, and reduce one or more symptoms of ALS in the subject such that ALS is therapeutically treated.

[00740] In some embodiments, the composition comprising the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention is administered to the central nervous system of the subject. In other embodiments, the composition comprising the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention is administered to the muscles of the subject

[00741] In particular, the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be delivered into specific types of targeted cells, including motor neurons; glial cells including oligodendrocyte, astrocyte and microglia; and/or other cells surrounding neurons such as T cells. Studies in human ALS patients and animal SOD1 ALS models implicate glial cells as playing an early role in the dysfunction and death of motor neurons. Normal SOD1 in the surrounding, protective glial cells can prevent the motor neurons from dying even though mutant SOD1 is present in motor neurons (e.g., reviewed by Philips and Rothstein, *Exp. Neurol.*, 2014, May 22. pii: S0014-4886(14)00157-5; the content of which is incorporated herein by reference in its entirety).

[00742] In some specific embodiments, the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be used as a therapy for ALS.

[00743] In some embodiments, the present composition is administered as a solo therapeutics or combination therapeutics for the treatment of ALS.

[00744] The AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules targeting the SOD1 gene may be used in combination with one or more other therapeutic agents. By "in combination with," it is not intended to imply

that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent.

[00745] Therapeutic agents that may be used in combination with the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention can be small molecule compounds which are antioxidants, anti-inflammatory agents, anti-apoptosis agents, calcium regulators, antiglutamatergic agents, structural protein inhibitors, and compounds involved in metal ion regulation.

[00746] Compounds tested for treating ALS which may be used in combination with the vectors described herein include, but are not limited to, antiglutamatergic agents: Riluzole, Topiramate, Talampanel, Lamotrigine, Dextromethorphan, Gabapentin and AMPA antagonist; Anti-apoptosis agents: Minocycline, Sodium phenylbutyrate and Arimoclomol; Anti-inflammatory agent: ganglioside, Celecoxib, Cyclosporine, Azathioprine, Cyclophosphamide, Plasmaphoresis, Glatiramer acetate and thalidomide; Ceftriaxone (Berry et al., *Plos One*, 2013, 8(4)); Beat-lactam antibiotics; Pramipexole (a dopamine agonist) (Wang et al., *Amyotrophic Lateral Scler.*, 2008, 9(1), 50-58); Nimesulide in U.S. Patent Publication No. 20060074991; Diazoxide disclosed in U.S. Patent Publication No. 20130143873); pyrazolone derivatives disclosed in US Patent Publication No. 20080161378; free radical scavengers that inhibit oxidative stress-induced cell death, such as bromocriptine (US. Patent Publication No. 20110105517); phenyl carbamate compounds discussed in PCT Patent Publication No. 2013100571; neuroprotective compounds disclosed in US Pat. Nos. 6,933,310 and 8,399,514 and US Patent Publication Nos. 20110237907 and 20140038927; and glycopeptides taught in U.S. Patent Publication No. 20070185012; the content of each of which is incorporated herein by reference in their entirety.

[00747] Therapeutic agents that may be used in combination therapy with the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention may be hormones or variants that can protect neuronal loss, such as adrenocorticotropic hormone (ACTH) or fragments thereof (e.g., U.S. Patent Publication No. 20130259875); Estrogen (e.g., U.S. Pat. Nos. 6,334,998 and 6,592,845); the content of each of which is incorporated herein by reference in their entirety.

[00748] Neurotrophic factors may be used in combination therapy with the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention for treating ALS. Generally, a neurotrophic factor is defined as a substance that promotes survival, growth, differentiation, proliferation and /or maturation of a neuron, or stimulates increased activity of a neuron. In some embodiments, the present methods further comprise delivery of one or more trophic factors into the subject in need of treatment. Trophic factors may include, but are not limited to, IGF-I, GDNF, BDNF, CTNF, VEGF, Colivelin, Xaliproden, Thyrotrophin-releasing hormone and ADNF, and variants thereof.

[00749] In one aspect, the vector, e.g., AAV particle, encoding the nucleic acid sequence for the at least one siRNA duplex targeting the SOD1 gene may be co-administered with AAV particles expressing neurotrophic factors such as AAV-IGF-I (Vincent et al., *Neuromolecular medicine*, 2004, 6, 79-85; the content of which is incorporated herein by reference in its entirety) and AAV-GDNF (Wang et al., *J Neurosci*., 2002, 22, 6920-6928; the content of which is incorporated herein by reference in its entirety).

[00750] In some embodiments, the composition of the present invention for treating ALS is administered to the subject in need intravenously, intramuscularly, subcutaneously, intraperitoneally, intrathecally and/or intraventricularly, allowing the siRNA molecules or vectors comprising the siRNA molecules to pass through one or both the blood-brain barrier and the blood spinal cord barrier. In some aspects, the method includes administering (e.g., intraventricularly administering and/or intrathecally administering) directly to the central nervous system (CNS) of a subject (using, e.g., an infusion pump and/or a delivery scaffold) a therapeutically effective amount of a composition comprising AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention. The vectors may be used to silence or suppress SOD1 gene expression, and/or reducing one or more symptoms of ALS in the subject such that ALS is therapeutically treated.

[00751] In certain aspects, the symptoms of ALS include, but are not limited to, motor neuron degeneration, muscle weakness, muscle atrophy, the stiffness of muscle, difficulty in breathing, slurred speech, fasciculation development, frontotemporal dementia and/or premature death are improved in the subject treated. In other aspects, the composition of the present invention is applied to one or both of the brain and the spinal cord. In other aspects, one or both of muscle coordination and muscle function are improved. In other aspects, the survival of the subject is prolonged.

[00752] In one embodiment, administration of the AAV particles comprising modulatory polynucleotides comprising a nucleic acid sequence encoding the siRNA molecules of the present invention, to a subject may lower mutant SOD1 in the CNS of a subject. In another embodiment, administration of the AAV particles, to a subject may lower wild-type SOD1 in the CNS of a subject. In yet another embodiment, administration of the AAV particles, to a subject may lower both mutant SOD1 and wild-type SOD1 in the CNS of a subject. The mutant and/or wild-type SOD1 may be lowered by about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100% in the CNS, a region of the CNS, or a specific cell of the CNS of a subject. As a non-limiting example, the AAV particles may lower the expression of wild-type SOD1 by at least 50% in the motor neurons (e.g., ventral horn motor neurons) and/or astrocytes. As another non-limiting example, the AAV particles may lower the expression of mutant SOD1 by at least 50% in the motor neurons (e.g., ventral horn motor neurons) and/or astrocytes. As yet another non-limiting example, the AAV particles may lower the expression of wild-type SOD1 and mutant SOD1 by at least 50% in the motor neurons (e.g., ventral horn motor neurons) and/or astrocytes.

[00753] In one embodiment, administration of the AAV particles, to a subject will reduce the expression of mutant and/or wild-type SOD1 in the spinal cord and the reduction of expression of the mutant and/or wild-type SOD1 will reduce the effects of ALS in a subject.

[00754] In one embodiment, the AAV particles may be administered to a subject who is in the early stages of ALS. Early stage symptoms include, but are not limited to, muscles which are weak and soft or stiff, tight and spastic, cramping and twitching (fasciculations) of muscles, loss of muscle bulk (atrophy), fatigue, poor balance, slurred words, weak grip, and/or tripping when walking. The symptoms may be limited to a single body region or a mild symptom may affect more than one region. As a non-limiting example, administration of the AAV particles may reduce the severity and/or occurrence of the symptoms of ALS.

[00755] In one embodiment, the AAV particles may be administered to a subject who is in the middle stages of ALS. The middle stage of ALS includes, but is not limited to, more widespread muscle symptoms as compared to the early stage, some muscles are paralyzed while others are weakened or unaffected, continued muscle twitchings (fasciculations), unused muscles may

cause contractures where the joints become rigid, painful and sometimes deformed, weakness in swallowing muscles may cause choking and greater difficulty eating and managing saliva, weakness in breathing muscles can cause respiratory insufficiency which can be prominent when lying down, and/or a subject may have bouts of uncontrolled and inappropriate laughing or crying (pseudobulbar affect). As a non-limiting example, administration of the AAV particles may reduce the severity and/or occurrence of the symptoms of ALS.

[00756] In one embodiment, the AAV particles may be administered to a subject who is in the late stages of ALS. The late stage of ALS includes, but is not limited to, voluntary muscles which are mostly paralyzed, the muscles that help move air in and out of the lungs are severely compromised, mobility is extremely limited, poor respiration may cause fatigue, fuzzy thinking, headaches and susceptibility to infection or diseases (e.g., pneumonia), speech is difficult and eating or drinking by mouth may not be possible.

[00757] In one embodiment, the AAV particles may be used to treat a subject with ALS who has a C9orf72 mutation.

[00758] In one embodiment, the AAV particles may be used to treat a subject with ALS who has TDP-43 mutations.

[00759] In one embodiment, the AAV particles may be used to treat a subject with ALS who has FUS mutations.

[00760] In one embodiment, the AAV particle of the present invention comprises an AAVrh10 capsid and a self-complementary AAV viral genome comprising an H1 promoter, a stuffer sequence originating from a pLKO.1 lentiviral vector and a SOD1 targeting payload.

[00761] In one embodiment, the AAV particle of the present invention comprises an AAV2 capsid and a self-complementary AAV viral genome.

[00762] In one embodiment, the AAV particle of the present invention comprises an AAV2 capsid and a self-complementary AAV viral genome comprising an H1 promoter, a stuffer sequence originating from a pLKO.1 lentiviral vector and a SOD1 targeting payload.

V. DEFINITIONS

[00763] Unless stated otherwise, the following terms and phrases have the meanings described below. The definitions are not meant to be limiting in nature and serve to provide a clearer understanding of certain aspects of the present invention.

[00764] As used herein, the term "nucleic acid", "polynucleotide" and 'oligonucleotide" refer to any nucleic acid polymers composed of either polydeoxyribonucleotides (containing 2-deoxy-

D-ribose), or polyribonucleotides (containing D-ribose), or any other type of polynucleotide which is an N glycoside of a purine or pyrimidine base, or modified purine or pyrimidine bases. There is no intended distinction in length between the term "nucleic acid", "polynucleotide" and "oligonucleotide", and these terms will be used interchangeably. These terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single stranded RNA.

[00765] As used herein, the term "RNA" or "RNA molecule" or "ribonucleic acid molecule" refers to a polymer of ribonucleotides; the term "DNA" or "DNA molecule" or "deoxyribonucleic acid molecule" refers to a polymer of deoxyribonucleotides. DNA and RNA can be synthesized naturally, e.g., by DNA replication and transcription of DNA, respectively; or be chemically synthesized. DNA and RNA can be single-stranded (i.e., ssRNA or ssDNA, respectively) or multi-stranded (e.g., double stranded, i.e., dsRNA and dsDNA, respectively). The term "mRNA" or "messenger RNA", as used herein, refers to a single stranded RNA that encodes the amino acid sequence of one or more polypeptide chains.

[00766] As used herein, the term "RNA interfering" or "RNAi" refers to a sequence specific regulatory mechanism mediated by RNA molecules which results in the inhibition or interfering or "silencing" of the expression of a corresponding protein-coding gene. RNAi has been observed in many types of organisms, including plants, animals and fungi. RNAi occurs in cells naturally to remove foreign RNAs (e.g., viral RNAs). Natural RNAi proceeds via fragments cleaved from free dsRNA which direct the degradative mechanism to other similar RNA sequences. RNAi is controlled by the RNA-induced silencing complex (RISC) and is initiated by short/small dsRNA molecules in cell cytoplasm, where they interact with the catalytic RISC component argonaute. The dsRNA molecules can be introduced into cells exogenously. Exogenous dsRNA initiates RNAi by activating the ribonuclease protein Dicer, which binds and cleaves dsRNAs to produce double-stranded fragments of 21-25 base pairs with a few unpaired overhang bases on each end. These short double stranded fragments are called small interfering RNAs (siRNAs).

[00767] As used herein, the terms "short interfering RNA," "small interfering RNA" or "siRNA" refer to an RNA molecule (or RNA analog) comprising between about 5-60 nucleotides (or nucleotide analogs) which is capable of directing or mediating RNAi. Preferably, a siRNA molecule comprises between about 15-30 nucleotides or nucleotide analogs, such as between about 16-25 nucleotides (or nucleotide analogs), between about 18-23 nucleotides (or nucleotide analogs), between about 19-22 nucleotides (or nucleotide analogs) (e.g., 19, 20, 21 or

22 nucleotides or nucleotide analogs), between about 19-25 nucleotides (or nucleotide analogs), and between about 19-24 nucleotides (or nucleotide analogs). The term "short" siRNA refers to a siRNA comprising 5-23 nucleotides, preferably 21 nucleotides (or nucleotide analogs), for example, 19, 20, 21 or 22 nucleotides. The term "long" siRNA refers to a siRNA comprising 24-60 nucleotides, preferably about 24-25 nucleotides, for example, 23, 24, 25 or 26 nucleotides. Short siRNAs may, in some instances, include fewer than 19 nucleotides, e.g., 16, 17 or 18 nucleotides, or as few as 5 nucleotides, provided that the shorter siRNA retains the ability to mediate RNAi. Likewise, long siRNAs may, in some instances, include more than 26 nucleotides, e.g., 27, 28, 29, 30, 35, 40, 45, 50, 55, or even 60 nucleotides, provided that the longer siRNA retains the ability to mediate RNAi or translational repression absent further processing, e.g., enzymatic processing, to a short siRNA. siRNAs can be single stranded RNA molecules (ss-siRNAs) or double stranded RNA molecules (ds-siRNAs) comprising a sense strand and an antisense strand which hybridized to form a duplex structure called siRNA duplex.

[00768] As used herein, the term "the antisense strand" or "the first strand" or "the guide strand" of a siRNA molecule refers to a strand that is substantially complementary to a section of about 10-50 nucleotides, e.g., about 15-30, 16-25, 18-23 or 19-22 nucleotides of the mRNA of the gene targeted for silencing. The antisense strand or first strand has sequence sufficiently complementary to the desired target mRNA sequence to direct target-specific silencing, e.g., complementarity sufficient to trigger the destruction of the desired target mRNA by the RNAi machinery or process.

[00769] As used herein, the term "the sense strand" or "the second strand" or "the passenger strand" of a siRNA molecule refers to a strand that is complementary to the antisense strand or first strand. The antisense and sense strands of a siRNA molecule are hybridized to form a duplex structure. As used herein, a "siRNA duplex" includes a siRNA strand having sufficient complementarity to a section of about 10-50 nucleotides of the mRNA of the gene targeted for silencing and a siRNA strand having sufficient complementarity to form a duplex with the other siRNA strand.

[00770] As used herein, the term "complementary" refers to the ability of polynucleotides to form base pairs with one another. Base pairs are typically formed by hydrogen bonds between nucleotide units in antiparallel polynucleotide strands. Complementary polynucleotide strands can form base pair in the Watson-Crick manner (e.g., A to T, A to U, C to G), or in any other manner that allows for the formation of duplexes. As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be

complementary to adenosine. However, when a U is denoted in the context of the present invention, the ability to substitute a T is implied, unless otherwise stated. Perfect complementarity or 100% complementarity refers to the situation in which each nucleotide unit of one polynucleotide strand can form hydrogen bond with a nucleotide unit of a second polynucleotide strand. Less than perfect complementarity refers to the situation in which some, but not all, nucleotide units of two strands can form hydrogen bond with each other. For example, for two 20-mers, if only two base pairs on each strand can form hydrogen bond with each other, the polynucleotide strands exhibit 10% complementarity. In the same example, if 18 base pairs on each strand can form hydrogen bonds with each other, the polynucleotide strands exhibit 90% complementarity.

[00771] As used herein, the term "substantially complementary" means that the siRNA has a sequence (e.g., in the antisense strand) which is sufficient to bind the desired target mRNA, and to trigger the RNA silencing of the target mRNA.

[00772] As used herein, "targeting" means the process of design and selection of nucleic acid sequence that will hybridize to a target nucleic acid and induce a desired effect.

[00773] The term "gene expression" refers to the process by which a nucleic acid sequence undergoes successful transcription and in most instances translation to produce a protein or peptide. For clarity, when reference is made to measurement of "gene expression", this should be understood to mean that measurements may be of the nucleic acid product of transcription, e.g., RNA or mRNA or of the amino acid product of translation, e.g., polypeptides or peptides. Methods of measuring the amount or levels of RNA, mRNA, polypeptides and peptides are well known in the art.

[00774] As used herein, the term "mutation" refers to any changing of the structure of a gene, resulting in a variant (also called "mutant") form that may be transmitted to subsequent generations. Mutations in a gene may be caused by the alternation of single base in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes.

[00775] As used herein, the term "vector" means any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule such as the siRNA molecule of the invention. A "viral genome" or "vector genome" or "viral vector" refers to a sequence which comprises one or more polynucleotide regions encoding or comprising a molecule of interest, e.g., a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide or a modulatory nucleic acid such as small interfering RNA (siRNA). Viral genomes are commonly used to deliver genetic materials into cells. Viral genomes are often modified for specific

applications. Types of viral genome sequence include retroviral viral genome sequences, lentiviral viral genome sequences, adenoviral viral genome sequences and adeno-associated viral genome sequences.

[00776] The term "adeno-associated virus" or "AAV" as used herein refers to any vector which comprises or derives from components of an adeno-associated vector and is suitable to infect mammalian cells, preferably human cells. The term AAV vector typically designates an AAV type viral particle or virion comprising a payload. The AAV vector may be derived from various serotypes, including combinations of serotypes (i.e., "pseudotyped" AAV) or from various genomes (e.g., single stranded or self-complementary). In addition, the AAV vector may be replication defective and/or targeted.

[00777] As used herein, the phrase "inhibit expression of a gene" means to cause a reduction in the amount of an expression product of the gene. The expression product can be a RNA molecule transcribed from the gene (*e.g.*, an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[00778] As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, in a Petri dish, *etc.*, rather than within an organism (*e.g.*, animal, plant, or microbe).

[00779] As used herein, the term "*in vivo*" refers to events that occur within an organism (*e.g.*, animal, plant, or microbe or cell or tissue thereof).

[00780] As used herein, the term "modified" refers to a changed state or structure of a molecule of the invention. Molecules may be modified in many ways including chemically, structurally, and functionally.

[00781] As used herein, the term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or polypeptides or other molecules of the present invention may be chemical or enzymatic.

[00782] As used herein, the term "transfection" refers to methods to introduce exogenous nucleic acids into a cell. Methods of transfection include, but are not limited to, chemical methods, physical treatments and cationic lipids or mixtures. The list of agents that can be transfected into a cell is large and includes, but is not limited to, siRNA, sense and/or anti-sense sequences, DNA encoding one or more genes and organized into an expression plasmid, proteins, protein fragments, and more.

[00783] As used herein, "off target" refers to any unintended effect on any one or more target, gene, or cellular transcript.

[00784] As used herein, the phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00785] As used herein, the term "effective amount" of an agent is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats ALS, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of ALS, as compared to the response obtained without administration of the agent.

[00786] As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[00787] As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the invention may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates such as chimpanzees and other apes and monkey species, and humans) and/or plants.

[00788] As used herein, the term "preventing" or "prevention" refers to delaying or forestalling the onset, development or progression of a condition or disease for a period of time, including weeks, months, or years.

[00789] The term "treatment" or "treating," as used herein, refers to the application of one or more specific procedures used for the cure or amelioration of a disease. In certain embodiments, the specific procedure is the administration of one or more pharmaceutical agents. In the context of the present invention, the specific procedure is the administration of one or more siRNA molecules.

[00790] As used herein, the term "amelioration" or “ameliorating” refers to a lessening of severity of at least one indicator of a condition or disease. For example, in the context of neurodegeneration disorder, amelioration includes the reduction of neuron loss.

[00791] As used herein, the term "administering" refers to providing a pharmaceutical agent or composition to a subject.

[00792] As used herein, the term “neurodegeneration” refers to a pathologic state which results in neural cell death. A large number of neurological disorders share neurodegeneration as a common pathological state. For example, Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS) all cause chronic neurodegeneration, which is characterized by a slow, progressive neural cell death over a period of several years, whereas acute neurodegeneration is characterized by a sudden onset of neural cell death as a result of ischemia, such as stroke, or trauma, such as traumatic brain injury, or as a result of axonal transection by demyelination or trauma caused, for example, by spinal cord injury or multiple sclerosis. In some neurological disorders, mainly one type of neuronal cell is degenerative, for example, medium spiny neuron degeneration in early ALS.

VI. EQUIVALENTS AND SCOPE

[00793] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00794] In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[00795] It is also noted that the term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term “comprising” is used herein, the term “consisting of” is thus also encompassed and disclosed.

[00796] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00797] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (e.g., any antibiotic, therapeutic or active ingredient; any method of production; any method of use; etc.) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[00798] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

[00799] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

VII. EXAMPLES

Example 1. SOD1 siRNA design, synthesis and analysis

SOD1 siRNA design

[00800] siRNA design is carried out to identify siRNAs targeting human SOD1 gene. The design uses the SOD1 transcripts for human (Genebank access NO. NM_ 000454.4 (SEQ ID NO: 1254).

[00801] The siRNA duplexes are designed to have 100% identity to the human SOD1 transcript for positions 2-18 of the antisense strand, and partial or 100% identity to the non-human primate SOD1 transcript for positions 2-18 of the antisense strand. In all siRNA duplexes, position 1 of the antisense strand is engineered to have a U and position 19 of the sense strand is engineered to have a C, in order to unpair the duplex at this position.

SOD1 siRNA sequence selection and synthesis

[00802] Based on predicted selectivity of the antisense strand for human, cynomolgus and rhesus SOD1 genes, and lack of match of the seed sequence at positions 2-7 of the antisense strand to human sequences in miRBase20.0, sense human SOD1 derived oligonucleotides are synthesized and formed into duplexes. Examples of SOD1 derived oligonucleotides and duplexes can be found in Table 3 of International Patent Application No. PCT/US2015/060562, the contents of which is herein incorporated by reference in its entirety. The siRNA duplexes are then tested for *in vitro* inhibitory activity on endogenous SOD1 gene expression (SOD1 mRNA levels). The oligoribonucleotides are synthesized as described in Example 1, SOD1 siRNA synthesis, of International Patent Application No. PCT/US2015/060562, the contents of which is herein incorporated by reference in its entirety.

In Vitro Screening of SOD1 siRNAs

[00803] Human SOD1 targeting siRNAs are assayed for inhibition of endogenous SOD1 expression in HeLa cells, using the bDNA (branched DNA) assay to quantify SOD1 mRNA. Results from two dose assays are used to select a subset of SOD1 dsRNA duplexes for dose response experiments in 4 types of cultured cells to calculate IC_{50}'s.

Cell Culture, Transfection and Assays

[00804] HeLa cells are obtained from ATCC (ATCC in Partnership with LGC Standards, Wesel, Germany) and cultured in HAM's F-12 Medium (Biochrom GmbH, Berlin, Germany) supplemented to contain 10% fetal calf serum (Ultra-low IgG from GIBCO/Life Technologies) and 1% Pen/Strep (Biochrom GmbH, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator.

[00805] For transfection with siRNA, HeLa cells are seeded at a density of 19,000 – 20,000 cells/well in 96-well plates. Transfection of siRNA is carried out with Lipofectamine 2000 (Invitrogen/Life Technologies) according to the manufacturer's instructions. For the two-dose screen, SOD1 siRNA concentrations of 1 nM or 0.1 nM are used. Dose response experiments are done with SOD1 siRNA concentrations of 10, 2.5, 0.6, 0.16, 0.039, 0.0098, 0.0024, 0.0006, 0.00015, and 0.000038 nM. Control wells are transfected with luciferase siRNA, Aha-1 siRNA, PLGF siRNA, or a control mix of unrelated siRNAs.

[00806] After a 24-hour incubation with siRNA, media is removed and cells are lysed and prepped for analysis by QuantiGene 2.0 and then bDNA data analysis as described in Example 2 of International Patent Application No. PCT/US2015/060562, the contents of which is herein incorporated by reference in its entirety.

Example 2. In vitro screen of selected SOD1 siRNAs against endogenous SOD1 mRNA expression in SH-SY5Y cells, U87 cells and primary human astrocytes

[00807] SH-SY5Y cells are obtained from ATCC (ATCC in Partnership with LGC Standards, Wesel, Germany) and cultured in Dulbecco's MEM (Biochrom GmbH, Berlin, Germany) supplemented to contain 15% FCS (Ultra-low IgG from GIBCO/Life Technologies), 1% L-Glutamine (Biochrom GmbH, Berlin, Germany) and 1% Pen/Strep (Biochrom GmbH, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator.

[00808] U87MG cells are obtained from ATCC (ATCC in Partnership with LGC Standards, Wesel, Germany) and cultured in ATCC-formulated Eagle's Minimum Essential Medium (ATCC in Partnership with LGC Standards, Wesel, Germany) supplemented to contain 10% FCS (Ultra-low IgG from GIBCO/Life Technologies) and 1% Pen/Strep (Biochrom GmbH, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator.

[00809] Primary human astrocytes are obtained from LONZA (Lonza Sales Ltd, Basel, Switzerland) and cultured in ABM Basal Medium (Lonza Sales Ltd, Basel, Switzerland) supplemented with AGM SingleQuot Kit (Lonza Sales Ltd, Basel, Switzerland) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator.

[00810] Transfection of SH-SY5Y cells, U87MG cells and primary human astrocytes with selected siRNAs, and quantitation of SOD1 and GAPDH mRNA levels with bDNA are performed in a similar manner to that described for HeLa cells, except that the transfection reagents are Lipofectamine2000 (Invitrogen/Life Technologies) for SH-SY5Y cells, RNAiMAX (Invitrogen/Life Technologies) for U87 cells, and Lipofectamine2000 (Invitrogen/Life Technologies) for primary human astrocytes.

Example 3. siRNA targeting SOD1

[00811] The passenger-guide strand duplexes of the SOD1 siRNA found to be efficacious are engineered into expression vectors and transfected into cells of the central nervous system or neuronal cell lines. Even though overhang utilized in the siRNA knockdown study is a canonical dTdT for siRNA, the overhang in the constructs may comprise any dinucleotide overhang.

[00812] The cells used may be primary cells or derived from induced pluripotent stem cells (iPS cells).

[00813] SOD1 knockdown is then measured and deep sequencing performed to determine the exact passenger and guide strand processed from each construct administered in the expression vector.

[00814] A guide to passenger strand ratio is calculated to determine the efficiency of knockdown, e.g., of RNA Induced Silencing Complex (RISC) processing.

[00815] The N-terminus is sequenced to determine the cleavage site and to determine the percent homogeneous cleavage of the target. It is expected that cleavage will be higher than 90 percent.

[00816] HeLa cells are co-transfected in a parallel study to analyze in vitro knockdown of SOD1. A luciferase construct is used as a control to determine off-target effects.

[00817] Deep sequencing is again performed.

Example 4. SOD1 siRNA constructs in AAV-miRNA vectors

[00818] The passenger-guide strand duplexes of the SOD1 siRNA are engineered into AAV-miRNA expression vectors. The construct from ITR to ITR, recited 5' to 3', comprises a mutant ITR, a promoter (either a CMV (which includes an SV40 intron or a beta-globin intron), a U6, H1 or the CBA promoter (which includes a CMVie enhancer, a CB promoter and an SV40 intron or a beta-globin intron)), the passenger and guide strand (with a loop between the passenger and guide strand, a 5' flanking region before the passenger strand and a 3' flanking region after the guide strand, a rabbit globin polyA and wild type ITR. *In vitro* and *in vivo* studies are performed to test the efficacy of the AAV-miRNA expression vectors.

[00819] Exemplary ITR to ITR sequences are shown in Table 22. These sequences comprise either a CBA promoter with a beta-globin intron or a CMV promoter with a beta globin (β-Globin) intron as well as a modulatory polynucleotide including a 5' flanking region, passenger strand, loop, guide strand and a 3' flanking region.

Table 22. ITR to ITR Sequences

Construct Name	ITR to ITR Promoter and Intron	ITR to ITR SEQ ID NO	Modulatory Polynucleotide Name Description	Modulatory Polynucleotide SEQ ID
VOYSOD1	CBA promoter; β-Globin intron	1414	VOYmiR-127.860	1326
VOYSOD2	CMV promoter; β-Globin intron	1415	VOYmiR-127.860	1326
VOYSOD3	CBA promoter; β-Globin intron	1416	VOYmiR-114.861	1327
VOYSOD4	CMV promoter; β-Globin intron	1417	VOYmiR-114.861	1327

Example 5. Activity of constructs

HeLa Cells

[00820] SOD1 siRNA constructs and a control are transfected in HeLa cells at a MOI of 1e4 vg/cell, 1e3 vg/cell, or 1e2 vg/cell to test the activity of the constructs. After 48-72 hours the endogenous mRNA expression is evaluated using methods known in the art.

HEK293 Cells

[00821] SOD1 siRNA constructs and a control are transfected into HEK293 cells at a MOI of 1e4 vg/cell, 1e3 vg/cell, or 1e2 vg/cell to test the activity of the constructs. After 24 -48 hours the endogenous mRNA expression is evaluated using methods known in the art.

Human Motor Neuron Progenitors (HMNPs)

[00822] SOD1 siRNA constructs and a control are transfected into human motor neuron progenitor (HMNP) cells at a MOI of 1e4 vg/cell, 1e3 vg/cell, or 1e2 vg/cell to test the activity of the constructs. After 48 hours the endogenous mRNA expression is evaluated using methods known in the art.

U251MG

[00823] SOD1 siRNA constructs and a control are transfected into the human astrocyte cell line U251MG at a MOI of 1e4 vg/cell, 1e3 vg/cell, or 1e2 vg/cell to test the activity of the constructs. After 48-60 hours the endogenous mRNA expression is evaluated using methods known in the art.

Human Astrocyte (HA)

[00824] SOD1 siRNA constructs and a control are transfected into primary human astrocyte cells at a MOI of 1e4 vg/cell, 1e3 vg/cell, or 1e2 vg/cell to test the activity of the constructs. After 48-60 hours the endogenous mRNA expression is evaluated using methods known in the art.

Example 6. SOD1 Knock-Down *in vivo*

[00825] Self-complementary pri-miRNAs are packaged in AAV and are administered by a 10 minute intrastriatal infusion. Female or male Tg(SOD1)3Cje/J mice (Jackson Laboratory, Bar Harbor, ME), which express human SOD1, and of approximately 20-30 g body weight, receive unilateral injections of 5 uL test article which is targeted to the striatum (anteroposterior +0.5 mm, mediolateral + 2 mm, relative to bregma; dorsoventral 3.8 mm, relative to skull surface). Test articles are injected (5 animals per test article) at 0.5 uL/min. using pre-filled, pump-regulated Hamilton micro-syringes (1701 model, 10 μl) with 33 gauge needles. At 1, 2, 3, 4 or 6 weeks following the injection, animals are sacrificed, brains are removed, and ipsilateral striata encompassing the infusion site from a 1 mm coronal slab, as well as striatal tissue from the adjacent 1 mm coronal slabs are dissected and flash frozen. Mouse tissue samples are lysed, and

human SOD1 protein levels, and SOD1 and mouse GAPDH (mGAPDH) mRNA levels are quantified. SOD1 protein levels are quantified by ELISA (eBioscience (Affymetrix, San Diego, CA)), and total protein levels are quantified by BCA analysis (ThermoFisher Scientific, Waltham, MA). For each tissue sample, the level of SOD1 protein normalized to total protein is calculated as an average of 2 determinations. These normalized SOD1 protein levels are further normalized to the vehicle group, then averaged to obtain a group (treatment) average. SOD1 and mGAPDH mRNA levels are quantified by qRT-PCR. For each tissue sample, the ratio of SOD1/mGAPDH (normalized SOD1 mRNA level) is calculated as an average of 3 determinations. These ratios are then averaged to obtain a group (treatment) average. These group averages are further normalized to the vehicle group.

[00826] In non-human primates, test articles ($1 \times 10^{13} - 3 \times 10^{13}$vg of pri-miRNA packaged in AAV) or vehicle are administered by intrathecal lumbar bolus. Female cynomolgus monkeys (Macaca fascicularis, CR Research Model Houston, Houston, TX) of approximately 2.5-8.5 kg body weight, receive implanted single intrathecal catheters with the tip of the catheter located at the lumbar spine. Test articles are administered (4 animals per test article) three 1 mL bolus injections (1 mL/minute), at approximately 60 minute intervals. At 4 to 6 weeks following the administration, animals are sacrificed, and selected tissues harvested for bioanalytical and histological evaluation. SOD1 protein and mRNA levels are assessed for suppression after treatment with pri-miRNA packaged in AAV-DJ with a CBA promoter, relative to the vehicle group.

Example 7. SOD1 Knock-Down *in vivo*

hSOD1 Transgenic Mice

[00827] Self-complementary pri-miRNAs targeting SOD1 as described herein were packaged in AAV-DJ. Female or male Tg(SOD1)3Cje/J mice (Jackson Laboratory, Bar Harbor, ME) (n=11-13/group), which express human SOD1 received a single unilateral injection of 5 uL vector comprising self-complementary pri-miRNA targeting SOD1 (VOYmir102.860, VOYmir104.861, VOYmir109.861, VOYmir109.866, VOYmir116.860, VOYmir116.866, VOYmir127.860, VOYmir127.866, VOYmir109.860, VOYmir114.861, VOYmir114.860, or VOYmir102.861; Table 23 and Table 24) packaged in AAV-DJ, or vehicle which was targeted to the striatum. Test articles were injected at 0.5 uL/min with 3.1 to 6.4 x 10^{12} vg/ml vector concentrations (total doses were 1.5 to 3.2 x 10^{10} vg) using pre-filled, pump-regulated Hamilton micro-syringes (1701 model, 10 µl) with 33-gauge needles. At 22 (n=6) or 29 (n=5 to 7) days following the injection, animals were sacrificed, brains were removed, and ipsilateral striata

encompassing the infusion site from a 1 mm coronal slab, as well as striatal tissue from the adjacent 1 mm coronal slabs were dissected and flash frozen. The striatum tissue samples from adjacent 1 mm coronal slabs were pooled for each hemisphere and used for hSOD1 mRNA quantification. The striatum tissue samples from the infusion site were collected and used for hSOD1 protein quantification. SOD1 and mGAPDH mRNA levels were quantified by qRT-PCR. Total RNA was extracted from striatal tissue samples using the RNeasy Mini Kit according to the manufacturer's protocol (QIAGEN). Complementary DNA synthesis was performed by reverse transcription using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). All TaqMan assays and master mixes were ordered from Life Technologies and used according to the manufacturer's recommendations. RT-qPCR was performed using the LightCycler 480II (Roche) or the CFX384 real-time system (BIO-RAD) and data were analyzed with the ΔΔCT method. hSOD1 mRNA levels were normalized to mGAPDH mRNA levels, and then further normalized to the vehicle control group. These group averages were calculated to obtain a group (treatment) average. SOD1 protein levels were quantified by ELISA (eBioscience (Affymetrix, San Diego, CA)), and total protein levels were quantified by BCA analysis (ThermoFisher Scientific, Waltham, MA). The SOD1 protein levels were normalized to the vehicle group, then averaged to obtain a group (treatment) average. Along with the dose, the results for days 22 and 29 are shown below in Table 23 (qRT-PCR mRNA) and Table 24 (ELISA protein).

Table 23. Modulatory Polynucleotide, Dose, and qRT-PCR mRNA Results

Modulatory Polynucleotide Name	Dose	SOD1 mRNA normalized to GAPDH (% of Vehicle ± SD)	
		Day 22	Day 29
VOYmir102.860	1.9E10	92±25	66±13
VOYmir104.861	2.3E10	94±20	66±8
VOYmir109.861	2.1E10	74±3	65±7
VOYmir109.866	2.4E10	93±10	87±16
VOYmir116.860	2.5E10	68±10	62±13
VOYmir116.866	2.8E10	83±6	82±9
VOYmir127.860	2.9E10	47±5	54±32
VOYmir127.866	3.2E10	98±20	64±8
VOYmir109.860	1.7E10	64±14	56±8
VOYmir114.861	3.2E10	58±6	64±5
VOYmir114.860	1.5E10	78±6	55±14
VOYmir102.861	1.9E10	77±13	68±15
N/A (Vehicle only)	0	100±19	100±9

Table 24. Modulatory Polynucleotide, Dose, and ELISA Protein Results

Modulatory Polynucleotide Name	Dose	SOD1 mRNA normalized to Vehicle (% of Vehicle ± SD)

		Day 22	Day 29
VOYmir102.860	1.9E10	90.3±5.2	80.8±3.6
VOYmir104.861	2.3E10	88.8±9.4	73.1±5
VOYmir109.861	2.1E10	103.7±16.3	70.7±2.5
VOYmir109.866	2.4E10	129.8±17.6	75.8±6.9
VOYmir116.860	2.5E10	103.4±9.8	77.2±5.9
VOYmir116.866	2.8E10	94.0±6.1	83.0±4.2
VOYmir127.860	2.9E10	75.3±8.6	75.9±14.4
VOYmir127.866	3.2E10	87.8±8.2	76.9±5.9
VOYmir109.860	1.7E10	89.2±5.3	80.2±3.7
VOYmir114.861	3.2E10	86.1±4.4	78.0±3.6
VOYmir114.860	1.5E10	81.4±6.6	88.2±5.5
VOYmir102.861	1.9E10	87.5±2.8	92.4±5.2
N/A (Vehicle only)	0	100±11.7	100±7.8

[00828] VOYmiR127.860 and VOYmiR114.861 packaged in AAV-DJ resulted in the greatest SOD1 mRNA suppression of 53% ($p<0.0001$) and 42% ($p<0.001$) reduction at 22 days, respectively, and 46% ($p<0.0001$) and 36% ($p<0.001$) reduction at 29 days respectfully, relative to the vehicle group. VOYmiR127.860 and VOYmiR114.861 packaged in AAV-DJ also resulted in protein suppression of 25% ($p<0.001$) and 14% reduction at 22 days, respectively, and 24% ($p<0.0001$) and 22% ($p<0.0001$) reduction at 29 days, respectively, relative to the vehicle group.

[00829] Additionally, deep sequencing was performed for VOYmiR127.860 and VOYmiR114.861 packaged in AAV-DJ, from mouse striatum samples (n=2-3) 4 weeks after a single unilateral injection of 5 uL of vector at 0.5ul/min, for a total dose of 2.9E10 vg for VOYmiR127.860 or 3.2E10 vg for VOYmiR114.861. In brief, total RNA from striatum was isolated using mirVana miRNA Isolation kit (Cat# AM1560, ThermoFisher Scientific, Waltham, MA). Small RNA sequencing libraries were prepared using Illumina TruSeq Small RNA library Prep Kit (Cat# RS-200-0012, Illumina, San Diego, CA), starting from 1 µg RNA, according to the manufacturer's protocol. Libraries were pooled and sequenced on the Illumina HiSeq2500. The results showed high guide: passenger ratios (99 for VOYmiR127.860 and 11.5 for VOYmiR114.861) demonstrating that the primary miRNA is processed primarily to the guide strand (the strand targeting SOD1 mRNA for RNAi -mediated reduction) with relatively low levels of the passenger strand. Low levels of guide and passenger strands relative to the total endogenous pool of miRNAs (0.3% for both VOYmiR127.860 and VOYmiR114.861) was also measured.

[00830] In an additional study, transgenic mice expressing human wild-type SOD1 (C57BL/6-Tg(SOD1)3Cje/J) (n=4-5 per group) were treated with self-complementary pri-miRNA targeting SOD1 (VOYmiR127.860 or VOYmiR114.861) packaged in AAVrh10, or vehicle by a single

unilateral intrastriatal infusion of 5 ul of vector (1.6×10^{12} vg/ml) at 0.5 ul/min, for a total dose of 8×10^{9} vg). Three weeks after dosing, striatal tissues from the site of administration were evaluated for SOD1 mRNA suppression by the same RT-qPCR method as described above. Treatment with VOYmiR127.860 packaged in AAVrh10 resulted in the suppression of SOD1 mRNA by 36 ±17.5 % (average ± SD) ($p<0.01$). Treatment with VOYmiR11.861 packaged in AAVrh10 resulted in the suppression of SOD1 mRNA by 30 ±9.7 % (average ± SD) ($p<0.01$).

Non-Human Primates

[00831] In non-human primates, self-complementary pri-miRNA targeting SOD1 (VOYmiR127.860) packaged in AAVrh10 at 1.1×10^{13}vg/ml, or vehicle was administered by intrathecal lumbar infusion. Four female cynomolgus monkeys (Macaca fascicularis, CR Research Model Houston, Houston, TX) of approximately 2.5-8.5 kg body weight, received implanted single intrathecal catheters with the tip of the catheter located at the lumbar spine. Test articles were administered two 2.5 mL IT lumbar (L1) injections (over 20 minutes in the Trendelenburg position) 6 hours apart. The total dose administered was 5.4×10^{13} vg per animal. Tolerability as assessed by body weight, clinical signs, clinical pathology, cerebral spinal fluid (CSF) chemistry, and CSF total cell counts was evaluated, as well as neutralizing antibodies in serum and CSF before and after dosing, as described below. At four weeks following the administration, animals were sacrificed, and selected tissues were harvested for evaluation of SOD1 mRNA by RT-qPCR, co-detection of vector genomes (Vg) and cynomolgus SOD1 mRNA by duplex RNAscope in situ hybridization assay, vector genome levels by droplet digital PCR, precision and efficiency of pri-miRNA processing by deep sequencing, and histopathology, as described below.

[00832] All animals survived until the scheduled termination. There were no significant test article-related effects of VOYmiR127.860 packaged in AAVrh10 on body weight, clinical signs, clinical pathology (comprising serum chemistry, hematology, and coagulation on Day 15 and Day 29), cerebral spinal fluid (CSF) chemistry, or CSF total cell counts on Day 29.

[00833] Serum and CSF samples were evaluated for neutralizing antibodies at approximately one week prior to dosing, approximately Day 15 (serum only) and approximately Day 29 at the time of necropsy, using a functional in vitro assay that quantifies inhibition of AAVrh10.GFP (green fluorescent protein) infection using flow cytometric analysis. Nonhuman primates were pre-screened for absent or minimal circulating neutralizing antibodies in serum prior to enrollment into the study. Neutralizing antibodies were evaluated again in both serum and CSF samples at approximately 1 week prior to dosing, confirming the absence or minimal circulating

neutralizing antibodies in all but three animals. On Day 15 and Day 29 post-dosing, there were significant neutralizing antibodies present in both serum and CSF of all animals.

[00834] SOD1 mRNA levels were assessed for suppression after treatment with pri-miRNA VOYmiR127.860 packaged in AAVrh10, relative to the vehicle group. RT-qPCR on laser captured lumbar and sacral motor neurons was evaluated (approximately 500 motor neurons per pool for the lumbar samples and 250 motor neurons per pool for the sacral samples). For laser captured motor neuron samples, SOD1 mRNA, levels of a motor neuron specific gene (choline acetyltransferase (ChAT)), and two reference genes (alanyl-tRNA synthetase (AARS) and Beta-actin (ACTB)) were determined by qRT-PCR. hSOD1 mRNA levels were normalized to the geometric mean of the two reference gene mRNA levels, and then expressed relative to the vehicle control group. Intrathecal infusion of pri-miRNA VOYmiR127.860 packaged in AAVrh10, resulted in 33 +/- 17.8% (average ± SD, $p<0.05$) and 78 +/- 12.7% (average ± SD, $p<0.0001$) suppression of SOD1 mRNA in laser captured motor neurons from the lumbar and sacral spinal cord, respectively, relative to the vehicle group.

[00835] In addition, a duplex RNAscope in situ hybridization (ISH) assay was used for co-detection of vector genomes (Vg) and cynomolgus SOD1 mRNA in motor neurons in the non-human primate lumbar spinal cord after treatment with pri-miRNA VOYmiR127.860 packaged in AAVrh10, relative to the vehicle group. RNA ISH for vg and cynnomolgus SOD1 was performed on an automation platform using the RNAscope 2.5 LS Duplex Reagent Kit (Advanced Cell Diagnostics, Inc., Newark, CA) according to the manufacturer's instructions. Briefly, 5 μm formalin fixed, paraffin embedded (FFPE) tissue sections were pretreated with heat and protease prior to hybridization with the target oligo probes. Preamplifier, amplifier and HRP/AP-labels oligo was then hybridized sequentially, followed by chromogenic precipitate development. Each sample was quality controlled for RNA integrity with a RNAscope probe specific to PPIB/POLR2A RNA and for background with a probe specific to bacterial dapB RNA. Specific RNA staining signal was identified as green (C1) and red (C2), punctate dots. Samples were counterstained with Mayer's Hematoxylin. Visual scoring was performed by a qualified scientist to assign a single score to a sample based on the average number of dots per cell throughout the entire sample. SOD1 expression levels in the Vg+ lumbar motor neurons were scored and there was a 53 +/- 11.2% (average ± SD, $p=0.0002$) reduction of SOD1 signal observed in Vg+ lumbar motor neurons from animals treated with pri-miRNA VOYmiR127.860 packaged in AAVrh10, as compared to vehicle control lumbar motor neurons. In contrast, there was a 10 +/- 13.4% (average ± SD, $p = 0.17$) reduction of SOD1 signal observed in Vg- lumbar

motor neurons from animals treated with pri-miRNA VOYmiR127.860 packaged in AAVrh10, as compared to vehicle control lumbar motor neurons.

[00836] Shown in Table 25 and Table 26 are the levels of the vector genome in different tissues. Vector genome was determined by Droplet Digital PCR (ddPCR). Briefly, DNA was purified from LCM samples with DNA Clean and Concentrator Kit (Zymoresearch) and whole cell DNA was prepared from tissue by DNeasy Blood and Tissue Kit (Qiagen, catalog #69506) under the conditions recommended by the manufacturer. This DNA was used as template in ddPCR reactions utilizing QX200 Droplet Digital PCR System (Bio-Rad). Briefly, reactions comprised of template, 2x Supermix (BioRad, catalog#1863023), HindIII restriction endonuclease (New England Biolabs) and oligonucleotide probesets with homology specific to the vector (FAM labelled) and non-human primate host RNaseP gene (VIC labelled, [ThermoFisher Scientific, # 4403328]) were set up. Reactions were incorporated into droplets with Bio-Rad Automatic Droplet Generator with conditions and reagents recommended by the manufacturer. Droplets underwent 40 cycles of thermocycling to endpoint. After PCR was complete, samples were read on QX200 Droplet Reader (Bio-Rad). Data were analyzed by QuantaSoft software to yield copies/reaction of each target. Vector genome distribution was reported as copies of vector genome per diploid cell (VG/DC) by normalizing the copies of vector to half the copies of host. Included in the tables are the averaged group data with and without the neutralizing antibody-positive animals. The presence of neutralizing antibodies did not diminish the VG/DC levels in the CNS tissues sampled, whereas the VG/DC levels in peripheral tissues were lower than in animals that were negative for neutralizing antibodies. In the laser captured motor neurons (approximately 75 motor neurons per pool), significant vector genomes averaging 13.64 VG/DC were measured. In spinal cord white matter sections (depleted of ventral horn gray matter and pia), average (all 4 animals) levels of 35.4 vg/dc were observed. In the liver, the vg levels averaged 120.36 +/- 121.01 VG/DC (all 4 animals) whereas the sero-negative animals averaged 224.75 +/- 4.23 VG/DC. Vector genome levels in heart, lung, kidney, and ovary were markedly lower than those observed in liver or spinal cord white matter, with the levels in ovary and kidney at or approaching the limit of detection for the assay.

Table 25. Biodistribution by Vector Genome Determination in Nonhuman Primate Peripheral Tissues

		Average Vector genomes/cell (VG/DC)				
Construct	**Number of animals**	**Liver**	**Heart**	**Lung**	**Ovary**	**Kidney**
Vehicle	4	1.95+/-0.01	1.97+/-0.01	1.99+/-0.00	1.98+/-0.02	1.97+/-0.01
VOYmiR127.860	4	120.36+/-121.01	3.30+/-1.90	2.69+/-0.82	2.03+/-0.07	2.19+/-0.19

	2 (without neutralizing antibody-positive animals)	224.75+/-4.23	4.60+/-2.02	3.29+/-0.74	2.09+/-0.02	2.24+/-0.25

Table 26. Biodistribution by Vector Genome Determination in Nonhuman Primate Spinal Cord Samples

		Average Vector genomes/cell (VG/DC)		
Construct	**Number of animals**	**Pia Mater**	**White Matter**	**Laser Captured Motor Neurons**
Vehicle	4	1.96+/-0.01	1.97+/-0.07	1.58+/-0.63
VOYmiR127.860	4	201.84+/-107.93	35.40+/-8.59	13.64+/-11.67
	2 (without neutralizing antibody-positive animals)	141.82+/-85.84	31.86+/-4.14	6.59

[00837] Precision and efficiency of pri-miRNA processing was evaluated by deep sequencing of ventral horn punches from the lumbar spinal cord, using the same methods as described above. These results showed that treatment with VOYmiR127.860 packaged in AAVrh10 resulted in a high guide to passenger ratio (>30.5), a high percentage of miRNAs with precise processing at the 5' end (90.7 +/- 4.5%), and a low level of guide and passenger strands relative to the total endogenous pool of miRNAs (0.00096 +/- 0.00054 %).

[00838] Histology was conducted on animals euthanized on Day 29. No animals in any treatment group had gross lesions at necropsy. Microscopic histopathology was conducted on spinal cord and liver, and no histopathological findings related to VOYmiR127.860 packaged in AAVrh10 were detected.

Example 8. SOD1 Knock-Down Mammalian Studies

Pharmacology and Efficacy

[00839] To study the efficacy and pharmacology of the VOYmiR127.860, studies will be conducted in SOD1-G93A mouse models of ALS.

[00840] Mice will be administered by IT administration of at least 2 dosage levels (low and high vg). Alternatively, IV dosing will be used as a surrogate route of administration.

[00841] For the pharmacology study, three groups of 10 mice/group, approximately 40 to 50 days of age and balanced for gender, will receive vehicle or VOYmiR127.860 packaged in AAVrh10 at either high or low dose levels. All animals will be euthanized after four weeks and spinal cord tissue samples will be evaluated for SOD1 suppression by RT-qPCR. Body weights and cage-side observation will also be collected.

[00842] After the pharmacology study, an efficacy study of VOYmiR127.860 packaged in AAVrh10 will be conducted in SOD1-G93A mice. Two groups of approximately 32 mice/group,

approximately 40 to 50 days of age and balanced for gender will receive vehicle or VOYmiR127-860 packaged in AAVrh10 at a high or low dose level. Endpoints will include body weight, neurological monitoring, and survival. Spinal cord tissue samples will also be evaluated for SOD1 suppression by RT-qPCR.

Pharmacology, Toxicity, and Biodistribution Study in NHPs

[00843] In order to evaluate the safety and distribution of VOYmiR127.860 packaged in AAVrh10, a study in NHP with a one-time IT infusion, will be conducted. Three time points - 4, 13 and 26 weeks - will be evaluated. The 4-week survival period after a one-time dose is intended to provide sufficient time for SOD1 suppression to be attained and to provide information on the short-term effects of VOYmiR127.860 packaged in AAVrh10. The 13-week and 26-week time points is intended to provide information on the long-term effects of VOYmiR127.860 packaged in AAVrh10.

[00844] Prior to enrollment, animals will be screened for the presence of neutralizing antibodies to the AAVrh10 capsid, as well as for abnormal serum chemistry profiles. Three dose levels will be tested. A control of vehicle only will also be tested.

[00845] Vector genomes will be quantified in all of the dose groups at 4 weeks. Tissues that have no detectable vector genomes at four weeks will not be evaluated at the 13-week or 26-week time points. Tissues that have detectable vector genomes at four weeks will be evaluated at the 13-week time point. All tissues and organs will be archived for possible vg quantification.

[00846] Primary outcome measures will include a PCR analysis of vector genomes within different CNS regions and major peripheral organs, including heart and lungs, a histopathological assessment of target and non-target tissues that have significant vector genome levels, and clinical pathology including serum chemistry, hematology, and coagulation. Safety pharmacology (central nervous, cardiovascular, and respiratory systems) and local tolerance will also be evaluated.

[00847] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

[00848] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification,

including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

CLAIMS

We claim:

1. An adeno-associated viral (AAV) vector comprising a first nucleic acid sequence comprising two inverted terminal repeats (ITRs), a promoter, an intron and a second nucleic acid sequence, wherein said second nucleic acid sequence when expressed inhibits or suppresses expression of SOD1 in a cell, wherein said second nucleic acid sequence comprises a sense strand sequence and an antisense strand sequence, wherein the sense strand sequence comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of sequences listed in Table 3 and the antisense strand sequence comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of sequences listed in Table 2 and wherein said sense strand sequence and antisense strand sequence share a region of complementarity of at least four nucleotides in length.

2. The AAV vector of claim 1, wherein the promoter is selected from the group consisting of CMV, CBA and H1.

3. The AAV vector of claim 2, wherein the intron is beta-globin.

4. The AAV vector of claim 3, wherein the promoter is CMV.

5. The AAV vector of claim 3, wherein the promoter is CBA.

6. The AAV vector of claim 3, wherein the promoter is H1.

7. The AAV vector of claim 1, wherein the AAV vector comprises a capsid serotype selected from the group consisting AAV1, AAV2, AAV2G9, AAV3, AAV3a, AAV3b, AAV3-3, AAV4, AAV4-4, AAV5, AAV6, AAV6.1, AAV6.2, AAV6.1.2, AAV7, AAV7.2, AAV8, AAV9, AAV9.11, AAV9.13, AAV9.16, AAV9.24, AAV9.45, AAV9.47, AAV9.61, AAV9.68, AAV9.84, AAV9.9, AAV10, AAV11, AAV12, AAV16.3, AAV24.1, AAV27.3, AAV42.12, AAV42-1b, AAV42-2, AAV42-3a, AAV42-3b, AAV42-4, AAV42-5a, AAV42-5b, AAV42-6b, AAV42-8, AAV42-10, AAV42-11, AAV42-12, AAV42-13, AAV42-15,

AAV42-aa, AAV43-1, AAV43-12, AAV43-20, AAV43-21, AAV43-23, AAV43-25, AAV43-5, AAV44.1, AAV44.2, AAV44.5, AAV223.1, AAV223.2, AAV223.4, AAV223.5, AAV223.6, AAV223.7, AAV1-7/rh.48, AAV1-8/rh.49, AAV2-15/rh.62, AAV2-3/rh.61, AAV2-4/rh.50, AAV2-5/rh.51, AAV3.1/hu.6, AAV3.1/hu.9, AAV3-9/rh.52, AAV3-11/rh.53, AAV4-8/r11.64, AAV4-9/rh.54, AAV4-19/rh.55, AAV5-3/rh.57, AAV5-22/rh.58, AAV7.3/hu.7, AAV16.8/hu.10, AAV16.12/hu.11, AAV29.3/bb.1, AAV29.5/bb.2, AAV106.1/hu.37, AAV114.3/hu.40, AAV127.2/hu.41, AAV127.5/hu.42, AAV128.3/hu.44, AAV130.4/hu.48, AAV145.1/hu.53, AAV145.5/hu.54, AAV145.6/hu.55, AAV161.10/hu.60, AAV161.6/hu.61, AAV33.12/hu.17, AAV33.4/hu.15, AAV33.8/hu.16, AAV52/hu.19, AAV52.1/hu.20, AAV58.2/hu.25, AAVA3.3, AAVA3.4, AAVA3.5, AAVA3.7, AAVC1, AAVC2, AAVC5, AAV-DJ, AAV-DJ8, AAVF3, AAVF5, AAVH2, AAVrh.72, AAVhu.8, AAVrh.68, AAVrh.70, AAVpi.1, AAVpi.3, AAVpi.2, AAVrh.60, AAVrh.44, AAVrh.65, AAVrh.55, AAVrh.47, AAVrh.69, AAVrh.45, AAVrh.59, AAVhu.12, AAVH6, AAVLK03, AAVH-1/hu.1, AAVH-5/hu.3, AAVLG-10/rh.40, AAVLG-4/rh.38, AAVLG-9/hu.39, AAVN721-8/rh.43, AAVCh.5, AAVCh.5R1, AAVcy.2, AAVcy.3, AAVcy.4, AAVcy.5, AAVCy.5R1, AAVCy.5R2, AAVCy.5R3, AAVCy.5R4, AAVcy.6, AAVhu.1, AAVhu.2, AAVhu.3, AAVhu.4, AAVhu.5, AAVhu.6, AAVhu.7, AAVhu.9, AAVhu.10, AAVhu.11, AAVhu.13, AAVhu.15, AAVhu.16, AAVhu.17, AAVhu.18, AAVhu.20, AAVhu.21, AAVhu.22, AAVhu.23.2, AAVhu.24, AAVhu.25, AAVhu.27, AAVhu.28, AAVhu.29, AAVhu.29R, AAVhu.31, AAVhu.32, AAVhu.34, AAVhu.35, AAVhu.37, AAVhu.39, AAVhu.40, AAVhu.41, AAVhu.42, AAVhu.43, AAVhu.44, AAVhu.44R1, AAVhu.44R2, AAVhu.44R3, AAVhu.45, AAVhu.46, AAVhu.47, AAVhu.48, AAVhu.48R1, AAVhu.48R2, AAVhu.48R3, AAVhu.49, AAVhu.51, AAVhu.52, AAVhu.54, AAVhu.55, AAVhu.56, AAVhu.57, AAVhu.58, AAVhu.60, AAVhu.61, AAVhu.63, AAVhu.64, AAVhu.66, AAVhu.67, AAVhu.14/9, AAVhu.t 19, AAVrh.2, AAVrh.2R, AAVrh.8, AAVrh.8R, AAVrh.10, AAVrh.12, AAVrh.13, AAVrh.13R, AAVrh.14, AAVrh.17, AAVrh.18, AAVrh.19, AAVrh.20, AAVrh.21, AAVrh.22, AAVrh.23, AAVrh.24, AAVrh.25, AAVrh.31, AAVrh.32, AAVrh.33, AAVrh.34, AAVrh.35, AAVrh.36, AAVrh.37, AAVrh.37R2, AAVrh.38, AAVrh.39, AAVrh.40, AAVrh.46, AAVrh.48, AAVrh.48.1, AAVrh.48.1.2, AAVrh.48.2, AAVrh.49, AAVrh.51, AAVrh.52, AAVrh.53, AAVrh.54, AAVrh.56, AAVrh.57, AAVrh.58, AAVrh.61, AAVrh.64, AAVrh.64R1, AAVrh.64R2, AAVrh.67, AAVrh.73, AAVrh.74, AAVrh8R, AAVrh8R A586R mutant, AAVrh8R R533A mutant, AAAV, BAAV, caprine AAV, bovine AAV, AAVhE1.1,

AAVhEr1.5, AAVhER1.14, AAVhEr1.8, AAVhEr1.16, AAVhEr1.18, AAVhEr1.35, AAVhEr1.7, AAVhEr1.36, AAVhEr2.29, AAVhEr2.4, AAVhEr2.16, AAVhEr2.30, AAVhEr2.31, AAVhEr2.36, AAVhER1.23, AAVhEr3.1, AAV2.5T , AAV-PAEC, AAV-LK01, AAV-LK02, AAV-LK03, AAV-LK04, AAV-LK05, AAV-LK06, AAV-LK07, AAV-LK08, AAV-LK09, AAV-LK10, AAV-LK11, AAV-LK12, AAV-LK13, AAV-LK14, AAV-LK15, AAV-LK16, AAV-LK17, AAV-LK18, AAV-LK19, AAV-PAEC2, AAV-PAEC4, AAV-PAEC6, AAV-PAEC7, AAV-PAEC8, AAV-PAEC11, AAV-PAEC12, AAV-2-pre-miRNA-101 , AAV-8h, AAV-8b, AAV-h, AAV-b, AAV SM 10-2 , AAV Shuffle 100-1 , AAV Shuffle 100-3, AAV Shuffle 100-7, AAV Shuffle 10-2, AAV Shuffle 10-6, AAV Shuffle 10-8, AAV Shuffle 100-2, AAV SM 10-1, AAV SM 10-8 , AAV SM 100-3, AAV SM 100-10, BNP61 AAV, BNP62 AAV, BNP63 AAV, AAVrh.50, AAVrh.43, AAVrh.62, AAVrh.48, AAVhu.19, AAVhu.11, AAVhu.53, AAV4-8/rh.64, AAVLG-9/hu.39, AAV54.5/hu.23, AAV54.2/hu.22, AAV54.7/hu.24, AAV54.1/hu.21, AAV54.4R/hu.27, AAV46.2/hu.28, AAV46.6/hu.29, AAV128.1/hu.43, true type AAV (ttAAV), UPENN AAV 10, Japanese AAV 10 serotypes, AAV CBr-7.1, AAV CBr-7.10, AAV CBr-7.2, AAV CBr-7.3, AAV CBr-7.4, AAV CBr-7.5, AAV CBr-7.7, AAV CBr-7.8, AAV CBr-B7.3, AAV CBr-B7.4, AAV CBr-E1, AAV CBr-E2, AAV CBr-E3, AAV CBr-E4, AAV CBr-E5, AAV CBr-e5, AAV CBr-E6, AAV CBr-E7, AAV CBr-E8, AAV CHt-1, AAV CHt-2, AAV CHt-3, AAV CHt-6.1, AAV CHt-6.10, AAV CHt-6.5, AAV CHt-6.6, AAV CHt-6.7, AAV CHt-6.8, AAV CHt-P1, AAV CHt-P2, AAV CHt-P5, AAV CHt-P6, AAV CHt-P8, AAV CHt-P9, AAV CKd-1, AAV CKd-10, AAV CKd-2, AAV CKd-3, AAV CKd-4, AAV CKd-6, AAV CKd-7, AAV CKd-8, AAV CKd-B1, AAV CKd-B2, AAV CKd-B3, AAV CKd-B4, AAV CKd-B5, AAV CKd-B6, AAV CKd-B7, AAV CKd-B8, AAV CKd-H1, AAV CKd-H2, AAV CKd-H3, AAV CKd-H4, AAV CKd-H5, AAV CKd-H6, AAV CKd-N3, AAV CKd-N4, AAV CKd-N9, AAV CLg-F1, AAV CLg-F2, AAV CLg-F3, AAV CLg-F4, AAV CLg-F5, AAV CLg-F6, AAV CLg-F7, AAV CLg-F8, AAV CLv-1, AAV CLv1-1, AAV Clv1-10, AAV CLv1-2, AAV CLv-12, AAV CLv1-3, AAV CLv-13, AAV CLv1-4, AAV Clv1-7, AAV Clv1-8, AAV Clv1-9, AAV CLv-2, AAV CLv-3, AAV CLv-4, AAV CLv-6, AAV CLv-8, AAV CLv-D1, AAV CLv-D2, AAV CLv-D3, AAV CLv-D4, AAV CLv-D5, AAV CLv-D6, AAV CLv-D7, AAV CLv-D8, AAV CLv-E1, AAV CLv-K1, AAV CLv-K3, AAV CLv-K6, AAV CLv-L4, AAV CLv-L5, AAV CLv-L6, AAV CLv-M1, AAV CLv-M11, AAV CLv-M2, AAV CLv-M5, AAV CLv-M6, AAV CLv-M7, AAV CLv-M8, AAV CLv-M9, AAV CLv-R1, AAV CLv-R2, AAV CLv-R3, AAV CLv-R4, AAV CLv-R5, AAV CLv-

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8. A method for inhibiting the expression of SOD1 gene in a cell comprising administering to the cell a composition comprising an AAV vector of any one of claims 1-7.

9. The method of claim 8, wherein the cell is a mammalian cell.

10. The method of claim 9, wherein the mammalian cell is a motor neuron.

11. The method of claim 9, wherein the mammalian cell is an astrocyte.

12. A method for treating and/or ameliorating amyotrophic lateral sclerosis (ALS) in a subject in need of treatment, the method comprising administering to the subject a therapeutically effective amount of a composition comprising an AAV vector of any one of claims 1-7.

13. The method of claim 12, wherein the expression of SOD1 is inhibited or suppressed.

14. The method of claim 13, wherein the SOD1 is wild type SOD1, mutated SOD1 with at least one mutation or both wild type SOD1 and mutated SOD1 with at least one mutation.

15. The method of claim 12, wherein the expression of SOD1 is inhibited or suppressed by about 20% to about 100%.

16. The method of claim 12, wherein the ALS is familial ALS with an identified SOD1 gene mutation.

17. The method of claim 12, wherein the ALS is sporadic ALS.

18. A method for inhibiting the expression of SOD1 gene in a cell wherein SOD1 gene embraces a mutation that causes a gain of function effect inside the cell, comprising administering the cell a composition comprising an AAV vector of any one of claims 1-7.

19. The method of claim 18, wherein the cell is a mammalian cell.

20. The method of claim 19, wherein the mammalian cell is a motor neuron.

21. The method of claim 19, wherein the mammalian cell is an astrocyte.

FIG. 1

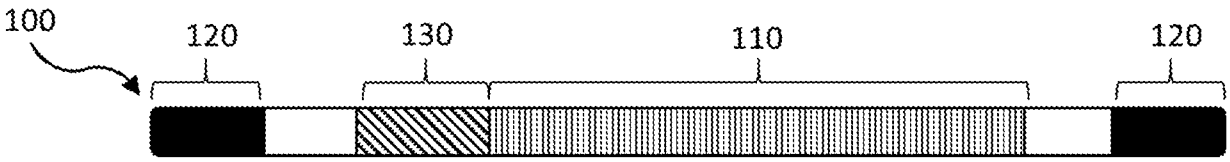


FIG. 2

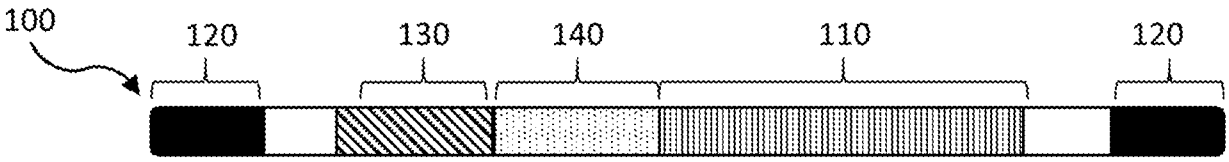


FIG. 3

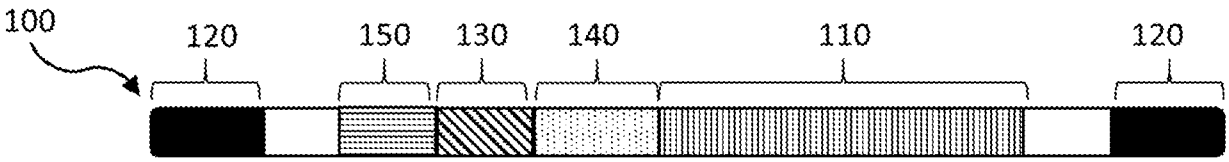


FIG. 4

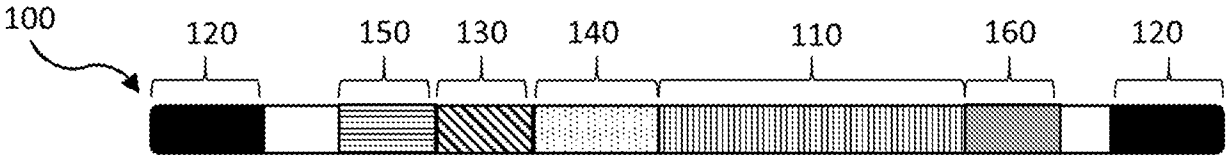


FIG. 5

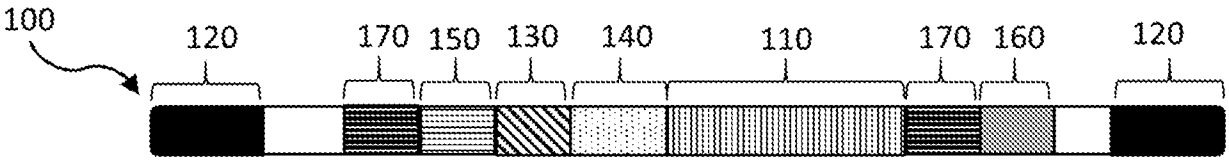


FIG. 6

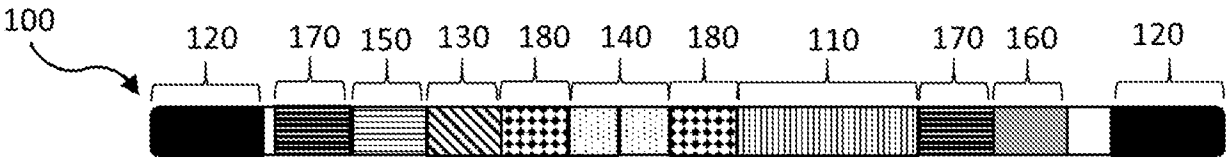


FIG. 7

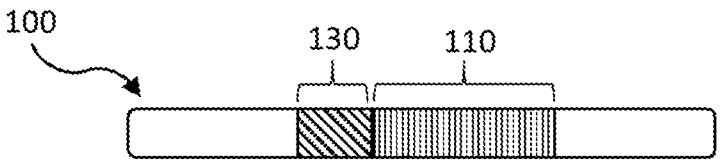


FIG. 8

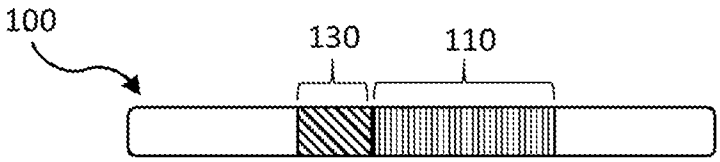
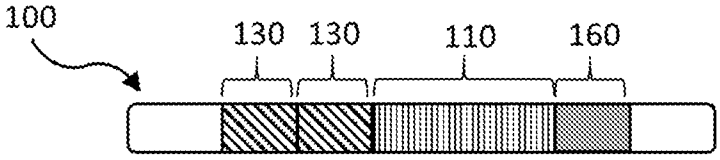


FIG. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/031089

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/713; A61K 48/00; A61P 25/28; C12N 15/11; C12N 15/113; C12N 15/86 (2018.01)

CPC - A61K 48/00; C12N 15/111; C12N 15/113; C12N 15/1137; C12N 15/86; C12N 2310/14; C12N 2750/14143; C12Y 115/01001 (2018.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/93.2; 435/6; 435/375; 435/455; 435/320.1; 514/44A; 536/23.1; 536/24.5 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2016/077687 A1 (VOYAGER THERAPEUTICS, INC.) 19 May 2016 (19.05.2016) entire document	1-5, 7-21 ---------- 6
Y	US 2013/0171726 A1 (BENITEC, INC) 04 July 2013 (04.07.2013) entire document	6
A	US 2017/0037410 A1 (IONIS PHARMACEUTICALS, INC.) 09 February 2017 (09.02.2017) entire document	1-21
A	US 2008/0113375 A1 (KHVOROVA et al) 15 May 2008 (15.05.2008) entire document	1-21
A	US 2014/0349390 A1 (VERITAS BIO, LLC) 27 November 2014 (27.11.2014) entire document	1-21
A	WO 2016/077689 A1 (VOYAGER THERAPEUTICS, INC.) 19 May 2016 (19.05.2016) entire document	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 2018	13 SEP 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/031089

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21 to the extent that they read on a sense nucleic acid sequence of SEQ ID NO:1085 and an antisense nucleic acid sequence of SEQ ID NO:916.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/031089

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-21 are drawn to sense nucleic acid sequences and antisense nucleic acid sequences for inhibiting expression of SOD1, and compositions and methods comprising the same.

The first invention of Group I+ is restricted to a sense nucleic acid sequence and an antisense nucleic acid sequence, and compositions and methods comprising the same, wherein the sense nucleic acid sequence is selected to be SEQ ID NO: 1085; and the antisense nucleic acid sequence is selected to be SEQ ID NO:916. It is believed that claims 1-21 read on this first named invention and thus these claims will be searched without fee to the extent that they read on a sense nucleic acid sequence of SEQ ID NO:1085 and an antisense nucleic acid sequence of SEQ ID NO:916.

Applicant is invited to elect additional sense nucleic acid sequences and antisense nucleic acid sequences, each with specified SEQ ID NO, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a sense nucleic acid sequence and an antisense nucleic acid sequence, and compositions and methods comprising the same, wherein the sense nucleic acid sequence is selected to be SEQ ID NO: 1086; and the antisense nucleic acid sequence is selected to be SEQ ID NO:917. Additional sense nucleic acid sequences and antisense nucleic acid sequences will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for inhibiting expression of a SOD1 gene, requiring the selection of alternatives for the sense nucleic acid sequence and the antisense nucleic acid sequence, where "the sense strand sequence comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of sequences listed in Table 3 and the antisense strand sequence comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of sequences listed in Table 2".

Additionally, even if Groups I+ were considered to share the technical features of an adeno-associated viral (AAV) vector comprising a first nucleic acid sequence comprising two inverted terminal repeats (ITRs), a promoter, an intron and a second nucleic acid sequence, wherein said second nucleic acid sequence when expressed inhibits or suppresses expression of SOD1 in a cell, wherein said second nucleic acid sequence comprises a sense strand sequence and an antisense strand sequence, wherein the sense strand sequence comprises at least 15 contiguous nucleotides and the antisense strand sequence comprises at least 15 contiguous nucleotides and wherein said sense strand sequence and antisense strand sequence share a region of complementarity of at least four nucleotides in length; these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2016/077689 A1 to Voyager Therapeutics, Inc. discloses an adeno-associated viral (AAV) vector (such modulatory polynucleotides may be encoded by or contained within plasmids or vectors or recombinant adeno-associated viruses (AAV) and may comprise artificial microRNAs, Para. [0009]) comprising a first nucleic acid sequence comprising two inverted terminal repeats (ITRs) (may be located between the 5' end of the flip ITR and the 3' end of the flop ITR in an expression vector, Para. [0051]), a promoter (modulatory polynucleotide may be located downstream of a promoter such as, but not limited to, CMV, U6, CBA or a CBA, Para. [0048]), an intron (promoter with a SV40 intron in an expression vector, Para. [0041]) and a second nucleic acid sequence, wherein said second nucleic acid sequence when expressed inhibits or suppresses expression of SOD1 in a cell (the modulatory polynucleotides of the invention may target any gene known in the art. As a non-limiting example, the gene may be SOD1, Para. [00139]; the first payload region sequence may be a guide strand of a siRNA construct and the second payload region sequence may be a passenger strand of an siRNA construct. The passenger and guide sequences may be substantially complementary to each other, Para. [0075]), wherein said second nucleic acid sequence comprises a sense strand sequence and an antisense strand sequence, wherein the sense strand sequence comprises at least 15 contiguous nucleotides (The 19mers, along with the 5' most position of the sense strand are shown in Table 4 along with the antisense strand which is the reverse complement of the sense strand, Para. [00331]) and the antisense strand sequence comprises at least 15 contiguous nucleotides (3' arm of the stem loop comprises a guide strand. This strand is also known as the antisense strand in that it reflects homology to a target. The guide strand may be between 15-30 nucleotides in length, 21-25 nucleotides or 22 nucleotides in length, Para. [0058]) and wherein said sense strand sequence and antisense strand sequence share a region of complementarity of at least four nucleotides in length (The passenger and guide strands may be completely complementary across a substantial portion of their length, Para. [0061]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/031089

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 916-925 and 1085-1094 were searched.