



(43) International Publication Date
4 December 2014 (04.12.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/194132 A1

(51) International Patent Classification:

A61K 48/00 (2006.01) C12N 7/00 (2006.01)
A61K 35/76 (2006.01)

(21) International Application Number:

PCT/US2014/040083

(22) International Filing Date:

29 May 2014 (29.05.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/829,735 31 May 2013 (31.05.2013) US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, 12th Floor, Oakland, California 94607 (US).

(72) Inventors: SCHAFFER, David V.; 19 Rima Court, Danville, California 94526 (US). KOTTERMAN, Melissa A.; 1635 Scenic Avenue, Apartment 1, Berkeley, California 94709 (US). HWANG, Bum-Yeol; 1070 Jackson Street, Apartment 707, Albany, California 94706 (US). KOERBER, James T.; 171 Texas Street, San Francisco, California 94107 (US).

(74) Agent: BORDEN, Paula A.; Bozicevic, Field & Francis LLP, 1900 University Avenue, Suite 200, East Palo Alto, California 94303 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: ADENO-ASSOCIATED VIRUS VARIANTS AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides infectious recombinant adeno-associated virus (rAAV) virions that comprise a variant capsid protein and a heterologous nucleic acid. The present disclosure further provides the variant adeno-associated virus (AAV) capsid proteins (and/or a nucleic acid encoding the variant AAV capsid proteins), which confer to an infectious rAAV virion an increased resistance to human AAV neutralizing antibodies. The present disclosure further provides host cells comprising an infectious rAAV virion and/or a nucleic acid encoding a subject variant AAV capsid protein. The present disclosure further provides methods of delivering a heterologous nucleic acid to a target cell where the target cell is contacted with a subject infectious rAAV virion. The present disclosure further provides methods of delivering a gene product to an individual, the methods generally involving administering an effective amount of a subject rAAV virion to an individual in need thereof.



WO 2014/194132 A1

ADENO-ASSOCIATED VIRUS VARIANTS AND METHODS OF USE THEREOF**CROSS-REFERENCE**

- [0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/829,735, filed May 31, 2013, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

- [0002] This invention was made with government support under Grant No. HL081527 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

- [0003] A Sequence Listing is provided herewith as a text file, "BERK-216WO_ST25.txt" created on May 28, 2014 and having a size of 169 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

- [0004] Gene delivery vectors based on adeno-associated viruses (AAV) have demonstrated promise in both preclinical disease models and recently in human clinical trials for several disease targets. Vectors based on AAV are extremely safe because wild-type AAV is nonpathogenic and has no etiologic association with any known diseases. In addition, AAV offers the capability for highly efficient gene delivery and sustained transgene expression in numerous tissues, including liver, muscle, lung, retina, and brain.
- [0005] AAV is a single stranded DNA virus that contains two open reading frames, rep and cap. The first gene encodes four proteins necessary for genome replication (Rep78, Rep68, Rep52, and Rep40), and the second expresses three structural proteins (VP1-3) that assemble to form the viral capsid. As its name implies, AAV is dependent upon the presence of a helper virus, such as an adenovirus or herpesvirus, for active replication. In the absence of a helper it establishes a latent state in which its genome is maintained episomally or integrated into the host chromosome. Multiple homologous primate AAV

serotypes and numerous nonhuman primate types have been identified. AAV2 is the best characterized as a gene delivery vehicle.

- [0006] As of 2010, there were 75 ongoing clinical trials that used AAV as the gene delivery vehicle. However, the high prevalence of anti-capsid neutralizing antibodies, due to widespread exposure to numerous AAV variants and serotypes within the human population, decrease the efficacy of AAV gene therapy. This pre-existing immunity, as well as the subsequent development of immunity due to vector administration, can impede the broader implementation of AAV gene therapy. For example, to date, AAV has been most successful in clinical studies involving delivery to immune privileged regions.
- [0007] Recent analysis indicated that the prevalence of anti-AAV IgG antibodies in humans was highest for AAV2 (72%) and AAV1 (67%), but AAV9 (47%), AAV6 (46%), AAV5 (40%), and AAV8 (38%) antibodies were also present in a large portion of the population studied. Several studies found that humoral immunity to the AAV capsid during gene therapy could be prevented by lowering the amount of rAAV particles delivered. Unfortunately, administration of low vector doses leads to low transduction and thus low therapeutic gene expression.
- [0008] There is a need in the art for the development of novel AAV variants that are resistant to neutralization by anti-AAV antibodies.

Literature

- [0009] Asuri et al., Mol Ther. 2012 Feb;20(2):329-38; Bainbridge et al., N Engl J Med. 2008 May 22;358(21):2231-9; Excoffon et al., Proc Natl Acad Sci U S A. 2009 Mar 10;106(10):3865-70; Grimm et al., J Virol. 2008 Jun;82(12):5887-911; Jang et al., Mol Ther. 2011 Apr;19(4):667-75; Klimczak et al., PLoS One. 2009 Oct 14;4(10):e7467; Koerber et al.; Mol Ther. 2008 Oct;16(10):1703-9; Koerber et al.; Mol Ther. 2009 Dec;17(12):2088-95; Maguire et al., N Engl J Med. 2008 May 22;358(21):2240-8; Maguire et al., Lancet. 2009 Nov 7;374(9701):1597-605; Maheshri et al., Nat Biotechnol. 2006 Feb;24(2):198-204; Perabo et al., J Gene Med. 2006 Feb;8(2):155-62; Yang et al., Proc Natl Acad Sci U S A. 2009 Mar 10;106(10):3946-51; WO2012145601; U.S. Patent Publication No. US20050053922

SUMMARY

- [0010] The present disclosure provides infectious recombinant adeno-associated virus (rAAV) virions that comprise a variant capsid protein and a heterologous nucleic acid. The present disclosure further provides the variant adeno-associated virus (AAV) capsid proteins (and/or a nucleic acid encoding the variant AAV capsid proteins), which confer to an infectious rAAV virion an increased resistance to human AAV neutralizing antibodies. The present disclosure further provides host cells comprising an infectious rAAV virion and/or a nucleic acid encoding a subject variant AAV capsid protein. The present disclosure further provides libraries of the above virions, capsid proteins, nucleic acids, and/or host cells; where the variant AAV capsid protein of at least one member of the library comprises an amino acid sequence having at least one amino acid substitution relative to the amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33.
- [0011] The present disclosure further provides methods of delivering a heterologous nucleic acid to a target cell where the target cell is contacted with a subject infectious rAAV virion. The present disclosure further provides methods of delivering a gene product to an individual, the methods generally involving administering an effective amount of a subject rAAV virion to an individual in need thereof. Also provided herein are compositions and kits for practicing the subject methods.

FEATURES

- [0012] Features of the present disclosure include an infectious recombinant adeno-associated virus (rAAV) virion comprising (a) a variant adeno-associated virus (AAV) capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33; and (b) a heterologous nucleic acid. In some cases, the variant AAV capsid protein comprises an amino acid sequence having at least about 95% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33. In some cases, the variant AAV capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33.
- [0013] Features of the present disclosure include an infectious recombinant adeno-associated virus (rAAV) virion comprising (a) a variant adeno-associated virus (AAV) capsid protein that comprises an amino acid sequence having at least about 95% amino acid

sequence identity to the amino acid sequence set forth in SEQ ID NO: 10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to SEQ ID NO: 2; and (b) a heterologous nucleic acid. In some cases, the variant AAV capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 10. In some cases, the rAAV exhibits increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by AAV2 (wild type AAV serotype 2). In some cases, the rAAV exhibits at least about 1.5-fold (e.g., at least about 3-fold, at least about 5-fold, at least about 10-fold, at least about 30-fold, etc.) greater resistance to human AAV neutralizing antibodies than the resistance exhibited by AAV2. In some cases, the rAAV exhibits increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction of mammalian cells exhibited by wild type AAV serotype 2 (AAV2). In some cases, the mammalian cells are liver cells, pancreatic cells, skeletal muscle cells, heart muscle cells, fibroblasts, retinal cells, synovial joint cells, lung cells, T cells, neurons, glial cells, stem cells (e.g., hematopoietic stem cells, hematopoietic progenitor cells, neural stem cells, neural progenitor cells, neural crest stem cells, embryonic stem cells, induced pluripotent stem cells (iPS cells), mesenchymal stem cells, mesodermal stem cells, liver stem cells, pancreatic stem cells, pancreatic progenitor cells, muscle stem cells, retinal stem cells, and the like), endothelial cells, or cancer cells. In some cases, the heterologous nucleic acid comprises an RNA interfering agent. In some cases, the heterologous nucleic acid comprises a nucleotide sequence encoding a polypeptide.

[0014] Features of the present disclosure include an isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33. In some cases, the encoded variant AAV capsid protein comprises an amino acid sequence having at least about 95% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33. In some cases, the encoded variant AAV capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33.

[0015] Features of the present disclosure include an isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein

that comprises an amino acid sequence having at least about 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to SEQ ID NO: 2.

- [0016] In some cases, the encoded variant AAV capsid protein (encoded by an isolated nucleic acid) confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by AAV2 (wild type AAV serotype 2). In some cases, increased resistance is at least about 1.5-fold (e.g., at least about 3-fold, at least about 5-fold, at least about 10-fold, at least about 30-fold, etc.) greater than the resistance exhibited by AAV2. In some cases, the encoded variant AAV capsid protein (encoded by an isolated nucleic acid) confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by AAV2.
- [0017] Features of the present disclosure include an isolated host cell comprising a subject nucleic acid as described above. In some cases, the host cell is stably transfected with the nucleic acid. In some cases, the host cell further comprises a nucleic acid comprising a nucleotide sequence encoding an AAV rep protein. In some cases, the host cell further comprises a recombinant AAV vector.
- [0018] Features of the present disclosure include a method of delivering a heterologous nucleic acid to a target cell, comprising contacting the target cell with a subject virion (described above). In some cases, the target cell is a liver cell, a pancreatic cell, a skeletal muscle cell, a heart muscle cell, a fibroblast, a retinal cell, a synovial joint cell, a lung cell, a T cell, a neuron, a glial cell, a stem cell (e.g., a hematopoietic stem cell, a hematopoietic progenitor cell, a neural stem cell, a neural progenitor cell, a neural crest stem cell, an embryonic stem cell, an induced pluripotent stem cell (iPS cell), a mesenchymal stem cell, a mesodermal stem cell, a liver stem cell, a pancreatic stem cell, a pancreatic progenitor cell, a muscle stem cell, or a retinal stem cell, and the like), an endothelial cell, or a cancer cell. In some cases, the target cell is in vitro. In some cases, the target cell is in vivo.
- [0019] Features of the present disclosure include a method of delivering a gene product to an individual in need thereof, the method comprising administering to the individual an

effective amount of a subject infectious recombinant adeno-associated virus (rAAV) virion (described above). In some cases, the heterologous nucleic acid of the rAAV virion comprises an RNA interfering agent. In some cases, the heterologous nucleic acid of the rAAV virion comprises a nucleotide sequence encoding a polypeptide. In some cases, the administering step comprises the indirect delivery of the infectious rAAV virion. In some cases, the administering step comprises the direct delivery of the infectious rAAV virion.

[0020] Features of the present disclosure include a variant adeno-associated virus (AAV) capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33. In some cases, the AAV capsid protein comprises an amino acid sequence having at least about 95% amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33. In some cases, the AAV capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs: 11-13 and 26-33.

[0021] Features of the present disclosure include a variant adeno-associated virus (AAV) capsid protein that comprises an amino acid sequence having at least about 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to SEQ ID NO: 2. In some cases, the variant AAV capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 10. In some cases, the variant AAV capsid protein confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by AAV2. In some cases, the increased resistance is at least about 1.5-fold (e.g., at least about 3-fold, at least about 5-fold, at least about 10-fold, at least about 30-fold, etc.) greater than the resistance exhibited by AAV2. In some cases, the variant AAV capsid protein confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by AAV2.

[0022] Features of the present disclosure include a library comprising at least one of: (i) two or more infectious rAAV virions, each comprising a variant adeno-associated virus (AAV) capsid protein and a heterologous nucleic acid; (ii) two or more isolated nucleic acids, each comprising a nucleotide sequence that encodes a variant AAV capsid protein; (iii)

two or more host cells, each comprising a nucleic acid that comprises a nucleotide sequence that encodes a variant AAV capsid protein; and (iv) two or more variant AAV capsid proteins; wherein the variant AAV capsid protein of at least one member of the library comprises an amino acid sequence having at least one amino acid substitution relative to the amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33.

[0023] Features of the present disclosure include a method of generating and identifying a modified infectious rAAV virion that exhibits an altered property of infection relative to a starter (parent) virion comprising a starter capsid protein, the method comprising: (a) generating variant adeno-associated virus (AAV) capsid proteins from the starter capsid protein, wherein the starter capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33, and wherein each variant AAV capsid protein comprises at least one amino acid substitution relative to the starter capsid protein; (b) generating variant AAV virions, each comprising a variant capsid AAV protein generated in step (a); and (c) assaying variant AAV virions generated in step (b) for the altered property of infection to identify the modified infectious rAAV virion. In some cases, the generation of the library of variant AAV capsid proteins comprises a method of mutagenesis selected from the group consisting of: polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, loop-swapping mutagenesis, fragment shuffling mutagenesis, and a combination thereof. In some cases, the altered property of infection is an increased resistance to human neutralizing AAV antibodies compared to the resistance exhibited by the starter virion. In some cases, the altered property of infection is an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by the starter virion. In some cases, the modified infectious rAAV virion comprises a modified AAV capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to the starter capsid protein.

[0024] Features of the present disclosure include a method of generating a variant AAV capsid protein from a starter capsid protein, the method comprising: subjecting a nucleic acid that comprises a nucleotide sequence encoding the starter capsid protein to a type of mutagenesis selected from the group consisting of: polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, loop-swapping mutagenesis, fragment shuffling mutagenesis, and a combination thereof;

wherein the starter capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0025] **Figures 1A-B** depict directed Evolution of AAV for Enhanced Antibody Evasion.
- [0026] **Figures 2A-B** depict the neutralization profiles of antibody evading variants using human IVIG.
- [0027] **Figures 3A-C** depict the neutralization profiles of antibody evading variants using human sera acquired from individuals that were excluded from hemophilia B clinical trials due to the presence of high neutralizing antibody titers against AAV.
- [0028] **Figures 4A-B** depict the amino acid sequences of loop-swap/shuffle and saturation mutagenesis clones.
- [0029] **Figure 5** demonstrates the *in vitro* tropism of AAV variants.
- [0030] **Figures 6A-B** show *in vivo* localization and neutralization of novel AAV variants.
- [0031] **Figures 7A-D** demonstrate the generation of human antibody evaders.
- [0032] **Figures 8A-I** depict the capsid protein sequence of Shuffle 100-1 (SEQ ID NO: 11) aligned with the wild type capsid protein sequences of AAV1-9 (SEQ ID NOs: 1-9).
- [0033] **Figures 9A-I** depict the capsid protein sequence of Shuffle 100-3 (SEQ ID NO: 12) aligned with the wild type capsid protein sequences of AAV1-9 (SEQ ID NOs: 1-9).
- [0034] **Figures 10A-I** depict the capsid protein sequence of Shuffle 100-7 (SEQ ID NO: 13) aligned with the wild type capsid protein sequences of AAV1-9 (SEQ ID NOs: 1-9).
- [0035] **Figure 11** shows the neutralizing antibody titers of library clones and parent serotypes in immunized mouse sera.

DEFINITIONS

- [0036] Adeno-associated virus is a nonpathogenic parvovirus composed of a 4.7 kb single-stranded DNA genome within a non-enveloped, icosahedral capsid. "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The genome contains three open reading frames (ORF) flanked by inverted terminal repeats (ITR) that function as the viral origin of replication and packaging signal. The *rep* ORF encodes four nonstructural proteins that play roles in viral replication, transcriptional regulation, site-specific integration, and virion assembly.

The *cap* ORF encodes three structural proteins (VP1-3) that assemble to form a 60-mer viral capsid. Finally, an ORF present as an alternate reading frame within the *cap* gene produces the assembly-activating protein (AAP), a viral protein that localizes AAV capsid proteins to the nucleolus and functions in the capsid assembly process.

- [0037] There are several naturally occurring serotypes and over 100 variants of AAV, each of which differs in amino acid sequence, particularly within the hypervariable regions of the capsid proteins, and thus in their gene delivery properties. No AAV has been associated with any human disease, making recombinant AAV attractive for clinical applications.
- [0038] The term “AAV” as used herein covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The term “AAV” includes AAV type 1 (AAV-1 or AAV1), AAV type 2 (AAV-2 or AAV2), AAV type 3 (AAV-3 or AAV3), AAV type 4 (AAV-4 or AAV4), AAV type 5 (AAV-5 or AAV5), AAV type 6 (AAV-6 or AAV6), AAV type 7 (AAV-7 or AAV7), AAV type 8 (AAV-8 or AAV8), AAV type 9 (AAV-9 or AAV9), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. “Primate AAV” refers to AAV that infect primates, “non-primate AAV” refers to AAV that infect non-primate mammals, “bovine AAV” refers to AAV that infect bovine mammals, etc.
- [0039] The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077.1 (AAV-1), AF063497.1 (AAV-1), NC_001401.2 (AAV-2), AF043303.1 (AAV-2), J01901.1 (AAV-2), U48704.1 (AAV-3), NC_001729.1 (AAV-3), NC_001829.1 (AAV-4), U89790.1 (AAV-4), NC_006152.1 (AAV-5), AF085716.1 (AAV-5), AF028704.1 (AAV-6), NC_006260.1 (AAV-7), AF513851.1 (AAV-7), AF513852.1 (AAV-8) NC_006261.1 (AAV-8), and AY530579.1 (AAV-9); the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Srivistava et al. (1983) *J. Virology* 45:555; Chiorini et al. (1998) *J. Virology* 71:6823; Chiorini et al. (1999) *J. Virology* 73:1309; Bantel-Schaal et al. (1999) *J. Virology* 73:939; Xiao et al. (1999) *J. Virology* 73:3994; Muramatsu et al. (1996) *Virology* 221:208; Shade et al., (1986) *J. Virol.* 58:921; Gao et al. (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; Moris et al. (2004)

Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Pat. No. 6,156,303.

- [0040] The sequences of naturally existing *cap* (capsid) proteins associated with AAV serotypes are known in the art and include: AAV1 (SEQ ID NO: 1), AAV2 (SEQ ID NO: 2), AAV3 (SEQ ID NO: 3), AAV4 (SEQ ID NO: 4), AAV5 (SEQ ID NO: 5), AAV6 (SEQ ID NO: 6), AAV7 (SEQ ID NO: 7), AAV8 (SEQ ID NO: 8), and AAV9 (SEQ ID NO: 9). The term “variant AAV capsid protein” is an AAV capsid protein comprising an amino acid sequence that includes at least one substitution (including deletion, insertion, etc.) relative to one of the naturally existing AAV capsid protein sequences set forth in SEQ ID NOs:1-9.
- [0041] An “AAV virion” or “AAV viral particle” refers to a viral particle composed of at least one AAV capsid protein and an encapsidated AAV polynucleotide.
- [0042] “Recombinant,” as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.
- [0043] If an AAV virion comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome, e.g., a transgene to be delivered to a target cell, an RNAi agent or CRISPR agent to be delivered to a target cell, etc.), it is typically referred to as a “recombinant AAV (rAAV) virion” or an “rAAV viral particle.” In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs).
- [0044] The term “rAAV vector” encompasses rAAV virions (i.e., rAAV viral particles) (e.g., an infectious rAAV virion), which by definition include an rAAV polynucleotide; and also encompasses polynucleotides encoding rAAV (e.g., a single stranded polynucleotide encoding rAAV (ss-rAAV); a double stranded polynucleotide encoding rAAV (ds-rAAV), e.g., plasmids encoding rAAV; and the like).
- [0045] “Packaging” refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

- [0046] AAV "rep" and "cap" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV "packaging genes."
- [0047] A "helper virus" for AAV refers to a virus that allows AAV (e.g. wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpesviruses and poxviruses such as vaccinia. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.
- [0048] "Helper virus function(s)" refers to function(s) encoded in a helper virus genome which allow AAV replication and packaging (in conjunction with other requirements for replication and packaging described herein). As described herein, "helper virus function" may be provided in a number of ways, including by providing helper virus or providing, for example, polynucleotide sequences encoding the requisite function(s) to a producer cell in trans. For example, a plasmid or other expression vector comprising nucleotide sequences encoding one or more adenoviral proteins is transfected into a producer cell along with an rAAV vector.
- [0049] An "infectious" virus or viral particle is one that comprises a competently assembled viral capsid and is capable of delivering a polynucleotide component into a cell for which the viral species is tropic. The term does not necessarily imply any replication capacity of the virus. Assays for counting infectious viral particles are described elsewhere in this disclosure and in the art. Viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Methods of determining the ratio of infectious viral particle to total viral particle are known in the art. See, e.g., Grainger et al. (2005) *Mol. Ther.* 11:S337 (describing a TCID50 infectious titer assay); and Zolotukhin et al. (1999) *Gene Ther.* 6:973. See also the Examples.
- [0050] The term "tropism" as used herein refers to the preferential targeting of specific host species or specific cell types within a host species by a virus (e.g., an AAV). For

example, a virus that can infect cells of the heart, lung, liver, and muscle has a broader (i.e., increased) tropism relative to a virus that can infect only lung and muscle cells. Tropism can also include the dependence of a virus on particular types of cell surface molecules of the host. For example, some viruses can infect only cells with surface glycosaminoglycans, while other viruses can infect only cells with sialic acid (such dependencies can be tested using various cells lines deficient in particular classes of molecules as potential host cells for viral infection). In some cases, the tropism of a virus describes the virus's relative preferences. For example, a first virus may be able to infect all cell types but is much more successful in infecting those cells with surface glycosaminoglycans. A second virus can be considered to have a similar (or identical) tropism as the first virus if the second virus also prefers the same characteristics (e.g., the second virus is also more successful in infecting those cells with surface glycosaminoglycans), even if the absolute transduction efficiencies are not similar. For example, the second virus might be more efficient than the first virus at infecting every given cell type tested, but if the relative preferences are similar (or identical), the second virus can still be considered to have a similar (or identical) tropism as the first virus. In some embodiments, the tropism of a virion comprising a subject variant AAV capsid protein is not altered relative to a naturally occurring virion. In some embodiments, the tropism of a virion comprising a subject variant AAV capsid protein is expanded (i.e., broadened) relative to a naturally occurring virion. In some embodiments, the tropism of a virion comprising a subject variant AAV capsid protein is reduced relative to a naturally occurring virion.

[0051] A "replication-competent" virus (e.g. a replication-competent AAV) refers to a phenotypically wild-type virus that is infectious, and is also capable of being replicated in an infected cell (i.e. in the presence of a helper virus or helper virus functions). In the case of AAV, replication competence generally requires the presence of functional AAV packaging genes. In general, rAAV vectors as described herein are replication-incompetent in mammalian cells (especially in human cells) by virtue of the lack of one or more AAV packaging genes. Typically, such rAAV vectors lack any AAV packaging gene sequences in order to minimize the possibility that replication competent AAV are generated by recombination between AAV packaging genes and an incoming rAAV vector. In many embodiments, rAAV vector preparations as described herein are those

which contain few if any replication competent AAV (rcAAV, also referred to as RCA) (e.g., less than about 1 rcAAV per 10^2 rAAV particles, less than about 1 rcAAV per 10^4 rAAV particles, less than about 1 rcAAV per 10^8 rAAV particles, less than about 1 rcAAV per 10^{12} rAAV particles, or no rcAAV).

- [0052] The term "polynucleotide" refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment herein that comprises a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.
- [0053] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in *Methods in Enzymology*, vol. 266: *Computer Methods for Macromolecular Sequence Analysis* (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443-453 (1970)
- [0054] A "gene" refers to a polynucleotide that performs a function of some kind in the cell. For example, a gene can contain an open reading frame that is capable of encoding a

particular protein after being transcribed and translated. On the other hand a gene can encode a functional RNA product that is not translated (e.g., an aptamer, an interfering RNA, a ribosomal RNA (rRNA), a transfer RNA (tRNA), etc.).

- [0055] A “gene expression product” or “gene product” is a molecule resulting from expression of a particular gene, as defined above. Gene expression products include, e.g., a polypeptide, an aptamer, an interfering RNA, a messenger RNA (mRNA), an rRNA, a tRNA, a non-coding RNA (ncRNA), and the like.
- [0056] An “RNA interfering agent” or “RNAi agent” encompasses any agent (or a polynucleotide encoding such an agent) that can be used to change the expression of a gene (as defined above). Examples of RNAi agents known to one of ordinary skill in the art include, but are not limited to, (i) siRNA agents; (ii) antisense RNA; (iii) CRISPR agents; (iv) Zinc finger nuclease agents, and (v) Transcription activator-like effector nuclease (TALEN) agents.
- [0057] (i) an siRNA agent (“small interfering” or “short interfering RNA” (or siRNA)) is an RNA duplex of nucleotides that is targeted to a gene interest (a “target gene”). An “RNA duplex” refers to the structure formed by the complementary pairing between two regions of a RNA molecule, forming a region of double stranded RNA (dsRNA). siRNA is “targeted” to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In some embodiments, the length of the duplex of siRNAs is less than 30 nucleotides. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 nucleotides in length. In some embodiments, the length of the duplex is 19-25 nucleotides in length. The RNA duplex portion of the siRNA can be part of a hairpin structure. siRNA agents that contain a hairpin can also be referred to as “shRNA (short hairpin RNA) agents.” In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12 or 13 nucleotides in length. The hairpin structure can also contain 3' or 5' overhang portions. In some embodiments, the overhang is a 3' or a 5' overhang 0, 1, 2, 3, 4 or 5 nucleotides in length. In general, the level of expression product (e.g., mRNA, polypeptide, etc.) of a target gene is reduced by an siRNA agent (e.g., an siRNA, an shRNA, etc.) that contains specific double stranded nucleotide sequences that are complementary to at least a 19-25

nucleotide long segment (e.g., a 20-21 nucleotide sequence) of the target gene transcript, including the 5' untranslated (UT) region, the ORF, or the 3' UT region. In some embodiments, short interfering RNAs are about 19-25 nt in length. See, e.g., PCT applications WO0/44895, WO99/32619, WO01/75164, WO01/92513, WO01/29058, WO01/89304, WO02/16620, and WO02/29858; and U.S. Patent Publication No. 20040023390 for descriptions of siRNA technology. The siRNA and/or shRNA can be encoded by a nucleic acid sequence, and the nucleic acid sequence can also include a promoter. The nucleic acid sequence can also include a polyadenylation signal. In some embodiments, the polyadenylation signal is a synthetic minimal polyadenylation signal.

[0058] (ii) antisense RNA is RNA that is complementary to a gene expression product. For example, an antisense RNA targeted to a specific mRNA is an RNA-based agent (or can be a modified RNA) that is complementary to the mRNA, where hybridization of the antisense RNA to the mRNA alters the expression of the mRNA (e.g., via altering the stability of the RNA, altering the translation of the RNA, etc.). Also included in “antisense RNA” are nucleic acids encoding an antisense RNA.

[0059] (iii) CRISPR agents. CRISPR (Clustered regularly interspaced short palindromic repeats)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. The Cas 9 protein (or functional equivalent and/or variant thereof, i.e., Cas9-like protein) naturally contains DNA endonuclease activity that depends on association of the protein with two naturally occurring or synthetic RNA molecules called crRNA and tracrRNA (also called guide RNAs). In some cases, the two molecules are covalently linked to form a single molecule (also called a single guide RNA (“sgRNA”)). Thus, the Cas9 or Cas9-like protein associates with a DNA-targeting RNA (which term encompasses both the two-molecule guide RNA configuration and the single-molecule guide RNA configuration), which activates the Cas9 or Cas9-like protein and guides the protein to a target nucleic acid sequence. If the Cas9 or Cas9-like protein retains its natural enzymatic function, it will cleave target DNA to create a double-strand break, which can lead to genome alteration (i.e., editing: deletion, insertion (when a donor polynucleotide is present), replacement, etc.), thereby altering gene expression. Some variants of Cas9 (which variants are encompassed by the term Cas9-like) have been altered such that they have a decreased DNA cleaving activity (in

some cases, they cleave a single strand instead of both strands of the target DNA, while in other cases, they have severely reduced to no DNA cleavage activity). Cas9-like proteins with decreased DNA-cleavage activity (even no DNA-cleaving activity) can still be guided to a target DNA and can block RNA polymerase activity. Thus enzymatically inactive Cas9-like proteins can be targeted to a specific location in a target DNA by a DNA-targeting RNA in order to block transcription of the target DNA. Detailed information regarding CRISPR agents can be found, for example in (a) Jinek et al., *Science*. 2012 Aug 17;337(6096):816-21: “A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity”; (b) Qi et al., *Cell*. 2013 Feb 28;152(5):1173-83: “Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression”, and (c) US patent application number 13/842,859 and PCT application number PCT/US13/32589; all of which are hereby incorporated by reference in their entirety. Thus, the term “CRISPR agent” as used herein encompasses any agent (or nucleic acid encoding such an agent), comprising naturally occurring and/or synthetic sequences, that can be used in the Cas9-based system (e.g., a Cas9 or Cas9-like protein; any component of a DNA-targeting RNA, e.g., a crRNA-like RNA, a tracrRNA-like RNA, a single guide RNA, etc.; a donor polynucleotide; and the like).

[0060] (iv) Zinc finger nuclease (ZFN) agents. Zinc-finger nucleases (ZFNs) are artificial DNA endonucleases generated by fusing a zinc finger DNA binding domain to a DNA cleavage domain. ZFNs can be engineered to target desired DNA sequences and this enables zinc-finger nucleases to cleave unique target sequences. When introduced into a cell, ZFNs can be used to edit target DNA in the cell (e.g., the cell’s genome) by inducing double strand breaks. For more information on the use of ZFNs, see, for example: Asuri et al., *Mol Ther*. 2012 Feb;20(2):329-38; Bibikova et al. *Science*. 2003 May 2;300(5620):764; Wood et al. *Science*. 2011 Jul 15;333(6040):307; Ochiai et al. *Genes Cells*. 2010 Aug;15(8):875-85; Takasu et. al., *Insect Biochem Mol Biol*. 2010 Oct;40(10):759-65; Ekker et al, *Zebrafish* 2008 Summer;5(2):121-3; Young et al, *Proc Natl Acad Sci U S A*. 2011 Apr 26;108(17):7052-7; Goldberg et al, *Cell*. 2010 Mar 5;140(5):678-91; Geurts et al, *Science*. 2009 Jul 24;325(5939):433; Flisikowska et al, *PLoS One*. 2011;6(6):e21045. doi: 10.1371/journal.pone.0021045. Epub 2011 Jun 13; Hauschild et al, *Proc Natl Acad Sci U S A*. 2011 Jul 19;108(29):12013-7; and Yu et al, *Cell Res*. 2011 Nov;21(11):1638-40; all of which are herein incorporated by reference

for their teachings related to ZFNs. The term "ZFN agent" encompasses a zinc finger nuclease and/or a polynucleotide comprising a nucleotide sequence encoding a zinc finger nuclease.

[0061] (v) Transcription activator-like effector nuclease (TALEN) agents. Transcription activator-like effector nucleases (TALENs) are artificial DNA endonucleases generated by fusing a TAL (Transcription activator-like) effector DNA binding domain to a DNA cleavage domain. TALENS can be quickly engineered to bind practically any desired DNA sequence and when introduced into a cell, TALENs can be used to edit target DNA in the cell (e.g., the cell's genome) by inducing double strand breaks. For more information on the use of TALENs, see, for example: Hockemeyer et al. *Nat Biotechnol.* 2011 Jul 7;29(8):731-4; Wood et al. *Science.* 2011 Jul 15;333(6040):307; Tesson et al. *Nat Biotechnol.* 2011 Aug 5;29(8):695-6; and Huang et. al., *Nat Biotechnol.* 2011 Aug 5;29(8):699-700; all of which are herein incorporated by reference for their teachings related to TALENs. The term "TALEN agent" encompasses a TALEN and/or a polynucleotide comprising a nucleotide sequence encoding a TALEN.

[0062] A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter.

[0063] "Operatively linked" or "operably linked" refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[0064] An "expression vector" is a vector comprising a region which encodes a polypeptide of interest, and is used for effecting the expression of the protein in an intended target cell.

An expression vector also comprises control elements operatively linked to the encoding region to facilitate expression of the protein in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an "expression cassette," a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0065] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter. Thus, for example, an rAAV that includes a heterologous nucleic acid encoding a heterologous gene product is an rAAV that includes a nucleic acid not normally included in a naturally-occurring, wild-type AAV, and the encoded heterologous gene product is a gene product not normally encoded by a naturally-occurring, wild-type AAV.

[0066] The terms "genetic alteration" and "genetic modification" (and grammatical variants thereof), are used interchangeably herein to refer to a process wherein a genetic element (e.g., a polynucleotide) is introduced into a cell other than by mitosis or meiosis. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alteration may be effected, for example, by transfecting a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, or contacting with a polynucleotide-liposome complex. Genetic alteration may also be effected, for example, by transduction or infection with a DNA or RNA virus or viral vector. Generally, the genetic element is introduced into a chromosome or mini-chromosome in the cell; but any alteration that changes the phenotype and/or genotype of the cell and its progeny is included in this term.

[0067] A cell has been "genetically modified" or "transformed" or "transfected" by exogenous DNA (e.g. via a recombinant virus), when such DNA has been introduced inside the cell. The presence of the exogenous DNA results in permanent or transient genetic change. The transforming DNA may or may not be integrated (covalently linked) into the

genome of the cell. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

[0068] A cell is said to be "stably" altered, transduced, genetically modified, or transformed with a genetic sequence if the sequence is available to perform its function during extended culture of the cell in vitro and/or for an extended period of time in vivo. Generally, such a cell is "heritably" altered (genetically modified) in that a genetic alteration is introduced which is also inheritable by progeny of the altered cell.

[0069] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, phosphorylation, or conjugation with a labeling component. Polypeptides such as anti-angiogenic polypeptides, neuroprotective polypeptides, and the like, when discussed in the context of delivering a gene product to a mammalian subject, and compositions therefor, refer to the respective intact polypeptide, or any fragment or genetically engineered derivative thereof, which retains the desired biochemical function of the intact protein. Similarly, references to nucleic acids encoding anti-angiogenic polypeptides, nucleic acids encoding neuroprotective polypeptides, and other such nucleic acids for use in delivery of a gene product to a mammalian subject (which may be referred to as "transgenes" to be delivered to a recipient cell), include polynucleotides encoding the intact polypeptide or any fragment or genetically engineered derivative possessing the desired biochemical function.

[0070] An "isolated" plasmid, nucleic acid, vector, virus, virion, host cell, protein, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially prepared from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this disclosure are increasingly more isolated. An isolated plasmid, nucleic acid, vector, virus, host cell, or other substance is in some embodiments purified, e.g., from about

80% to about 90% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least about 99%, or more, pure.

- [0071] As used herein, the terms “treatment,” “treating,” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment,” as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease (and/or symptoms caused by the disease) from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease (and/or symptoms caused by the disease), i.e., arresting its development; and (c) relieving the disease (and/or symptoms caused by the disease), i.e., causing regression of the disease (and/or symptoms caused by the disease).
- [0072] The terms “individual,” “host,” “subject,” and “patient” are used interchangeably herein, and refer to a mammal, including, but not limited to, humans; non-human primates, including simians; mammalian sport animals (e.g., horses); mammalian farm animals (e.g., sheep, goats, etc.); mammalian pets (dogs, cats, etc.); and rodents (e.g., mice, rats, etc.).
- [0073] In some embodiments, the individual is a human who has previously been naturally exposed to AAV and as a result harbors anti-AAV antibodies (i.e., AAV neutralizing antibodies). In some embodiments, the individual is a human who has previously been administered an AAV vector (and as a result may harbor anti-AAV antibodies) and needs re-administration of vector for treatment of a different condition or for further treatment of the same condition. Based on positive results in clinical trials involving AAV gene delivery to, for example, liver, muscle, and retina - all tissues affected by neutralizing antibodies against this vehicle - there are many such therapeutic applications/disease targets.
- [0074] The term “effective amount” as used herein is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this disclosure, an effective amount of a compound (e.g., an infectious rAAV virion) is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, prevent, slow or delay the progression of (and/or symptoms associated

with) a particular disease state (e.g., cancer). Accordingly, an effective amount of an infectious rAAV virion is an amount of the infectious rAAV virion that is able to evade the neutralizing activity of an individual's anti-AAV antibodies, thus effectively delivering the heterologous nucleic acid to a target cell (or target cells) of the individual.

- [0075] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.
- [0076] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.
- [0077] 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. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.
- [0078] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an infectious recombinant adeno-associated virus (rAAV) virion" includes a plurality of such virions and reference to "the infectious recombinant adeno-associated virus (rAAV) virion" includes reference to one or more such virions and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such,

this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0079] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0080] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0081] The present disclosure provides infectious recombinant adeno-associated virus (rAAV) virions that comprise a variant capsid protein and a heterologous nucleic acid. The present disclosure further provides the variant adeno-associated virus (AAV) capsid proteins (and/or a nucleic acid encoding the variant AAV capsid proteins), which confer to an infectious rAAV virion an increased resistance to human AAV neutralizing antibodies. The present disclosure further provides host cells comprising an infectious rAAV virion and/or a nucleic acid encoding a subject variant AAV capsid protein. The present disclosure further provides libraries of the above virions, capsid proteins, nucleic acids, and/or host cells; where the variant AAV capsid protein of at least one member of the library comprises an amino acid sequence having at least one amino acid substitution relative to the amino acid sequence set forth in one of SEQ ID NOs:10-13 and 26-33.

[0082] The present disclosure further provides methods of delivering a heterologous nucleic acid to a target cell where the target cell is contacted with a subject infectious rAAV virion. The present disclosure further provides methods of delivering a gene product to an individual, the methods generally involving administering an effective amount of a subject rAAV virion to an individual in need thereof. Also provided herein are compositions and kits for practicing the subject methods. In many embodiments, a subject infectious rAAV virion, a subject nucleic acid, a subject variant AAV capsid protein, a subject host cell, etc., is isolated.

VARIANT AAV CAPSID POLYPEPTIDES

[0083] A subject variant AAV capsid polypeptide (or the variant AAV capsid protein encoded by a subject nucleic acid) confers to an infectious rAAV virion comprising the variant AAV capsid polypeptide an increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein. In some embodiments, the increased resistance is at least about 1.5-fold (e.g., at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 17-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, etc.) greater than the resistance exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein.

[0084] A subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) can be said to confer to an infectious rAAV virion an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein. In some embodiments, the increased transduction is at least about 1.5-fold (e.g., at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 17-fold, at least about 20-fold, at least about 25-fold, at least about

30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, etc.) greater than the transduction exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein.

[0085] In some embodiments, a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) exhibits decreased binding to a neutralizing antibody that binds a wild-type AAV capsid protein. For example, a subject variant AAV capsid protein can exhibit at least about 1.5-fold (e.g., at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 17-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, etc.) reduced binding (e.g., reduced affinity) to a neutralizing antibody that binds a wild-type capsid AAV protein, compared to the binding affinity of the antibody to wild-type AAV capsid protein.

[0086] In some embodiments, an anti-AAV neutralizing antibody binds to a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) with an affinity of less than about 10^{-7} M, less than about 5×10^{-6} M, less than about 10^{-6} M, less than about 5×10^{-5} M, less than about 10^{-5} M, less than about 10^{-4} M, or lower.

[0087] The term “variant capsid protein” does not encompass wild type AAV capsid proteins. A “variant AAV capsid protein” does not comprise an amino acid sequence present in a naturally occurring AAV capsid protein. For example, a subject variant capsid protein does not comprise an amino acid sequence having 100% sequence identity to any of the sequences set forth in SEQ ID NOs:1-9. In other words, a subject variant capsid protein does not comprise an amino acid sequence as set forth in any of SEQ ID NOs:1-9. A variant capsid protein can differ in amino acid sequence from a “starter” or “parental” AAV capsid protein, which parental AAV capsid protein may be a wild-type AAV capsid protein or non-wild-type AAV capsid protein.

[0088] In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at

least about 90% (e.g., at least about 92%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:10-13 and 26-33.

[0089] In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 90% (e.g., at least about 92%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to the amino acid sequence set forth in one of SEQ ID NOs:10-13 and 26-33.

[0090] In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to the AAV capsid protein of AAV2 (e.g., SEQ ID NO: 2), or the corresponding positions in another AAV parental serotype.

[0091] In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to the AAV capsid protein of AAV2 (e.g., SEQ ID NO: 2), or the corresponding positions in another AAV parental serotype.

[0092] In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:31, and includes the amino acid substitutions N312K, N449D, N551S, and I698V relative to the AAV capsid protein

of AAV2 (e.g., SEQ ID NO:2), or the corresponding positions in another AAV parental serotype.

- [0093]** In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:31, and includes the amino acid substitutions N312K, N449D, N551S, and I698V relative to the AAV capsid protein of AAV2 (e.g., SEQ ID NO:2), or the corresponding positions in another AAV parental serotype.
- [0094]** In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:32, and includes the amino acid substitutions D180N, N312K, Q385R, N449D, N551S, I698V, and S721T relative to the AAV capsid protein of AAV2 (e.g., SEQ ID NO:2), or the corresponding positions in another AAV parental serotype.
- [0095]** In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:32, and includes the amino acid substitutions D180N, N312K, Q385R, N449D, N551S, I698V, and S721T relative to the AAV capsid protein of AAV2 (e.g., SEQ ID NO:2), or the corresponding positions in another AAV parental serotype.
- [0096]** In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:33, and includes the amino acid substitutions N312K, N449D, T450A, N551S, and I698V relative to the AAV

capsid protein of AAV2 (e.g., SEQ ID NO:2), or the corresponding positions in another AAV parental serotype.

[0097] In some embodiments a subject variant AAV capsid protein (or the variant AAV capsid protein encoded by a subject nucleic acid) comprises an amino acid sequence having at least about 95% (e.g., at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100%) amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:33, and includes the amino acid substitutions N312K, N449D, T450A, N551S, and I698V relative to the AAV capsid protein of AAV2 (e.g., SEQ ID NO:2), or the corresponding positions in another AAV parental serotype.

[0098] Exemplary variant AAV capsid proteins include, but are not limited to (see Figures 8-10 for selected exemplary sequence alignments):

[0099] SM 10-2 (amino acid sequence) (SEQ ID NO: 10):

[00100] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EF
QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPD
SSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPPAAPSGLGTNTMATGS
GAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYK
QISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFPRKRL
KFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFP
ADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYLSRTDTPSGTTTQSRLQFSQAGASDIRNQSR
NWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNPAMASHK
DDEEKFFPQSGVLIFGKQGSEKTSVDIEKVMITDEEEIRTTPVATEQYGSVSTNL
QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMG
GFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS
KRWNPEVQYTSNYNKS VNVDFTVDTNGVYSEPRPIGTRYLTRNQ;

[00101] SM10-2 (nucleotide sequence) (SEQ ID NO: 22):

[00102] atggctgccgatgggtatctccagattggctcgaggacactctctgaaggaataagacagtgggtgaagctcaa
acctggccccaccaccacaaagcccgagagcggcagacaggggtctgtgcttctgggtacaaagtacctc
ggaccttcaacggactcgacaaggagagccgggtcaacgaggcagacgccgcggccctcgagcacgacaaagcctatg
accggcagctcgacagcggagacaaccgtacctcaagtacaaccacgccgacgcggagtctcaggaacgcctaaagaag

atacgtcttttgggggcaacctcggacgagcagcttccaggcgaaaaagagggttctgaacctctgggcctggtgaggaac
 ctgttaagacggctccgggaaaaagaggccggtagagcactctctgtggagccagactcctcctcgggaaccggaaagg
 cgggccagcagcctgcaagaaaaagattgaatttggtcagactggagacgcagactcagtaacctgacccccagcctctcgg
 acagccaccagcagccccctctggtctgggaactaatac gatggctacaggcagtgggcgaccaatggcagacaataacga
 gggcgccgacggagtgggtaattcctcgggaaattggcattgcgattccacatggatggcgacagagtcaccaccagc
 acccgaacctgggcctgcccactacaacaaccacctctacaacaaatttcagccaatcaggagcctcgaacgacaatca
 ctactttggctacagcacccttgggggtatttgaactcaacagattccactgccactttaccacgtgactggcaagactcat
 caacaacaactggggattccgaccaagagactcaagttcaagctcttaacattcaagtaaagggtcacgcagaatgacg
 gtacgacgacgattgccaataaccttaccagcacggttcaggtgttactgactcggagtaccagctcccgtagctcctcggctc
 ggcgcatcaaggatgcctcccggcgttcccagcagacgttctcatggtgccacagtatggatactcaccctgaacaacggga
 gtcaggcagtaggacgctcttcttactgcctggagtacttctctcagatgctgcgtaccgtaacaactttaccttcagcta
 cacttttgaggacgttcttccacagcagctacgctcacagccagagtctggaccgtctcatgaatcctctcatcgaccagtacc
 tgtattacttgagcagaacagacactccaagtgggaaccaccacgcagtcaggcttcagtttctcaggccggagcgagtgaca
 ttcggaaccagtctaggaactggcttctggaccctgttaccgccagcagcgagtatcaaagacatctcgggataacaacaaca
 gtgaatactcgtggactggagctaccaagtaccacctcaatggcagagactctctggtgaatccggggcccgccatggcaagc
 cacaaggacgatgaagaaaagttttcctcagagcgggggttctcatctttgggaagcaaggctcagagaaaacaagtgtggac
 attgaaaaaggtcatgattacagacgaagaggaaatcaggacaaccaatcccgtggctacggagcagtatggttctgtatctacc
 aacctccagagaggcaacagacaagcagctaccgcagatgtcaacacacaaggcgttcttcaggcatggtctggcaggac
 agagatgtgtaccttcaggggcccctctgggcaaagattccacacacggacggacattttacccctctcccctcatgggtgga
 ttcggacttaaacacctctccacagattctcatcaagaacaccccgtacctgcgaatcttcgaccaccttcagtgcggcaa
 agtttgcctctctcatcacacagtactccacgggacaggtcagcgtggagatcagtgaggagctgcagaaggaaaacagcaaa
 cgctggaatcccgaagttcagtagacttccaactacaacaagtctgttaatgtggactttactgtggacactaatggcgtgtattca
 gagcctcgccccattggcaccagatacctgactcgtaatcagtaa

[00103] Shuffle 100-1 (amino acid sequence) (SEQ ID NO: 11):

[00104] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
 GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADA
 FQQRLQGDTSGGNLGRAVFQAKKRVLEPLGLVEQAGETAPGKKRPLIESPQQP
 DSSTGIGKKGKQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASGG
 GAPMADNNEGADGVGNASGNWHCDSTWLGDREVITSTRTWALPTYNNHLYK
 QISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFRPKRL
 NFKLFNIQVKEVTTNDGVTTIANNLSTVQVFSDSDYQLPYVLGSAHEGCLPPFP
 ADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF

HSSYAHSQSLDRLMNPLIDQYLYLNRQTQNSGSAQNKDLLFSRGSPAGMSVQ
 PKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH
 KDDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVA
 VNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPL
 MGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKE
 NSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL;

[00105] Shuffle 100-1 (nucleotide sequence) (SEQ ID NO: 23):

[00106] atggctgctgatggttatctccagattggctcgaggacactctctgaaggaataagacagtgggtggaagctcaaa
 cctggcccaccaccaccaaagcccgcagagcggcataaggacgacagcaggggtcttgtgcttctgggtacaagtacctcg
 gaccttcaacggactcgacaagggagagccggtcaacgaggcagacgcagcggccctcgagcacgacaaggcctacga
 ccagcagctcaaggccggtgacaaccctacctcaagtacaaccacgccgacgcggagttccagcagcggcttcaggcgga
 cacatcgtttgggggaacctcggcagagcagcttccaggccaaaaagagggttctgaacctcttggtctggtgagcaagc
 ggggtgagacggctcctggaaagaagagaccgttgattgaatccccagcagcccgactcctccacgggtatcgcaaaaaa
 ggcaagcagccggctaaaaagagactcaatttggtcagactggcgactcagagtcagtcctccgaccacaacctctcggag
 aacctccagcaacccccgctgctgtgggacctactacaatggcttcagggtggtggcgaccaatggcagacaataacgaagg
 cgccgacggagtgggtaatgcctcaggaaattggcattgcgattccacatggtggtggcgacagagtcaccaccagcacc
 cgcacctgggccttggccacctacaataaacacctctacaagcaaatctccagtgttcaacggggggccagcaacgacaacca
 ctacttcggctacagcacccttgggggtattttgacttcaacagattccactgccacttttaccacgtgactggcagcgactca
 tcaacaacaattggggattccggcccaagagactcaacttcaactcttcaacatccaagtcaaggaggtcacgacgaatgatg
 gcgtcacaaccatcgctaataaccttaccagcacgggtcaagtcttctggactcagactatcagctcccgtagctgctcgggtc
 gggtcacgaggggtgctctcccgccgttccagcagcgttctcatggtgccacagtatggatactcaccctgaacaacggga
 gtcaggcagtaggacgctcttcttactgcctggagtgacttctctctcagatgctgcgtaccggaaacaactttacctcagcta
 cacttttgaggacgttcttccacagcagctacgctcacagccagagctctggaccgtctcatgaatcctctcatcgaccagtacc
 tgtattacctgaacagaactcagaatcagtcgggaagtgcacaaaacaaggacttgctgtttagccgggggtctccagctggca
 tgtctgttcagcccaaaaactggctacctggaccctgttatcggcagcagcgcgtttctaaaacaaaaacagacaacaaca
 gcaactttacctggactggtgcttcaaaaataaccttaatgggcgtgaatctataatcaacctggcactgctatggcctcacaca
 aagacgacaaagacaagttcttcccatgagcgggtgcatgattttggaaaggagagcggcggagcttcaaacactgcattgg
 acaatgtcatgatcacagacgaagaggaaatcaaagccactaacccccgtggccaccgaaagatttgggactgtggcagtcatt
 ctccagagcagcagcacagacctgcgaccggagatgtgcatgttatgggagccttacctggatggtgtggcaagacagag
 acgtatactgcaggggtccatttgggcaaaaattctcacacagatggacacttccacctctctcttatggcggttga
 ctcaagaacccgctcctcagatcctcatcaaaaacacgcctgttctcgcaatcctccggcgagttttagctacaaagtgtg
 ttcatcatcaccataactccacaggacaagtgagtggtgaaattgaatgggagctgcagaaagaaaacagcaagcgtgga

atcccgaaagtcagtagacatccaattatgcaaaatctgccaacgttgattttactgtggacaacaatggactttatactgagcctc
gccccattggcacccgttacctcacccgtcccctgtaa;

[00107] Shuffle 100-3 (amino acid sequence) (SEQ ID NO: 12):

[00108] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAE
FQQRLLQGDTSFGGNLGRAVFQAKKRVLEPLGLVEQAGETAPGKKRPLIESPQQP
DSSTGIGKKGKQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASGG
GAPMADNNEGADGVGNASGNWHCDSTWLGDREVTTSTRTWALPTYNNHLYK
QISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFRPKRL
NFKLFNIQVKEVTTNDGVTTIANNLSTTVQVFSDSDYQLPYVLGSAHEGCLPPFP
ADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYLNRQTQNSGSAQNKDLLFSRGSPTGMSVQP
KNWLPGPCYRQQRVSKTKTDNNSNFTWTGASKYNLNGRESIINPGTAMASHK
DDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAV
NLQSSSTD PATGDVHAMGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPL
MGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKE
NSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL;

[00109] Shuffle 100-3 (nucleotide sequence) (SEQ ID NO: 24):

[00110] atggctgctgatggttatcttcagattggctcgaggacactctctgaaggaataagacagtggtggaagctcaaa
cctggcccaccaccacaaagcccgcagagcggcataaggacgacagcaggggtcttgtgcttctgggtacaagtacctcg
gaccttcaacggactcgacaagggagagccggtcaacgaggcagacgcagcggccctcgagcacgacaaggcctacga
ccagcagctcaagggcgtgacaaccctacctcaagtacaaccacgccgacgcggagttccagcagcggctcaggcgga
cacatcgtttgggggcaacctcggcagagcagcttccaggccaaaaagagggttctgaacctcttggtctggtgagcaagc
gggtgagacggctcctggaaagaagagaccgttgattgaatccccccagcagcccgactcctccacgggtatcggaacaaa
ggcaagcagccggctaaaaagagactcaatttggtcagactggcgactcagagtcagtcacccgaccacaacctctcggag
aacctccagcaacccccgctgctgtgggacactactacaatggcttcagggtggtggcgaccaatggcagacaataacgaagg
cgccgacggagtggttaatgcctcaggaaattggcattgcgattccacatggctggcgacagagtcaccaccagcacc
cgcacctgggccttggccacctacaataaacacctctacaagcaaatctccagtgttcaacggggggccagcaacgacaacca
ctactcggctacagcacccccctgggggtattttgacttcaacagattccactgccactttaccacgtgactggcagcgactca
tcaacaacaattggggattccggcccaagagactcaacttcaactcttcaacatccaagtcaaggaggtcacgacgaatgatg
gcgtcacaaccatcgctaataaccttaccagcacgggtcaagtcttctcgactcagactatcagctcccgtagctgctcgggtc
gggtcacgaggggtgctccccgccgttccagcagacgttctcatggtgccacagtatggatactcacctgaacaacggga

gtcaggcagtaggacgctcttactgtcctggagtacttctctcagatgctgcgtaccggaacaactttaccttcagcta
 cacttttgaggacgttcttccacagcagctacgctcacagccagagtctggaccgtctcatgaatcctctcatcaccagtacc
 tgtattacctgaacagaactcagaatcagtcgggaagtgcacaaaacaaggacttgctgtttagccgggggtctccaactggca
 tgtctgttcagcccaaaaactggctacctggaccctgtatcggcagcagcgcgtttctaaaacaaaacagacaacaaca
 gcaactttacctggactggtgcttcaaaatataaccttaatgggcgtgaatctataatcaacctggcactgctatggcctcacaca
 aagacgacaaagacaagtcttcccatgagcgggtgcatgattttggaaaggagagcgcgggagcttcaaacactgcattgg
 acaatgcatgatcacagacgaagaggaaatcaaagccactaacccgtggccactgaaagatttgggactgtggcagtcaat
 ctccagagcagcagcacagaccctgcgaccggagatgtgcatgccatgggagccttacctggaatggtgtggcaagacaga
 gacgtatactgcaggggtcctatttgggccaaaattcctcacacggatggacactttcacccgtctcctctcatgggcggccttgg
 actcaagaacccgctcctcagatcctcataaaaacacgcctgttctgcgaatcctccggcggagttttagctacaaagtgg
 cttcattcatcaccagtattccacaggacaagtgagcgtggagattgaatgggagctgcagaaagaaaacgacaaacgctgg
 aatcccgaagtgcagtatacatctaactatgcaaaatctgccaacgttgatttactgtggacaacaatggactttatactgagcct
 cgccccattggcaccgttacctcacccgtccctgttaa;

[00111] Shuffle 100-7 (amino acid sequence) (SEQ ID NO: 13):

[00112] MAADGYLPDWLEDNLSEGIREWALKPGAPKPKANQQKQDDGRGLVL
 PGYKYLGPFNGLDKGEPVNAADAALEHDKAYDQQLKAGDNPYLRYNHADA
 EFQERLQEDTSFGGNLGRAVFQAKKRVLLEPLGLVEEGAKTAPGKKRPVEQSPQE
 PDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASSG
 GGAPMADNNEGADGVGNASGNWHCDSTWLGDREVITTSTRTWALPTYNNHLY
 KQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFRPK
 RLSFKLFNIQVKEVTTNDGVTTIANNLSTVQVFSDSEYQLPYVLGSAHQGCLPP
 FPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEEVP
 FHSSYAHSQSLDRLMNPLIDQYLYLNRNQNSGSAQNKDLLFSRGSPAGMSV
 QPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMAS
 HKDDEDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTV
 AVNFQSSSTD PATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSP
 LMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQK
 ENSKRWNPEVQYTSNYAKSANIDFTVDNNGLYTEPRPIGTRYLTRPQ;

[00113] Shuffle 100-7 (nucleotide sequence) (SEQ ID NO: 25):

[00114] atggctgccgatgggtatctccagattggctcgaggacaacctctctgagggcattcgcgagtgtggggcgctgaa
 acctggagccccgaagcccaagccaaccagcaaaagcaggacgacggccggggtctggtgcttctggctacaagtacct
 cggacccttcaacggactcgacaagggggagcccgtaacgcggcgatgcagcggccctcgagcacgacaaggcctac

gaccagcagctcaaagcgggtgacaatccgtacctgcggtataaccacgccgacgccgagtttcaggagcgtctgcaagaa
gatacgtcttttgggggcaacctcgggcgagcagcttccaggccaagaagcgggtctcgaacctctcggctctggttgagga
aggcgctaagacggctcctggaaagaaacgtccggtagagcaatcgccacaagagccagactcctcctcgggcatcggcaa
gacaggccagcagcccgtaaaaagagactcaattttggtcagactggcgactcagagtcagtcctccgacccacaacctctc
ggagaacctccagcaacccccgtgctgtgggacctactacaatggcttcaggcgggtggcgaccaatggcagacaataacg
aaggcgccgacggagtggttaatgcctcaggaaattggcattgcgattccacatggctgggcgacagagtcaccaccag
caccgaacatgggccttggccacctataacaaccacctctacaagcaaatctccagtgttcgacggggggccagcaacgac
aaccactacttcggctacagcaccacctgggggtattttgactttaacagattccactgccacttttaccacgtgactggcagcg
actcatcaacaacaactggggattccggccaagagactcagcttcaagctcttcaacatccagggtcaaggaggtcacgacga
atgatggcgtcacaaccatcgtataaaccttaccagcacgggttcaagtcttctcggactcggagtaccagcttccgtacgtctc
ggctctgcgcaccagggtgcctccctccgttccggcgagcgtgttcattgattccgcaatcaggctacgtgacgtcaacaat
ggcagccaagccgtgggacgttcattcttttactgcttgaatatttccctctcagatgtgagaacgggcaacaactttaccttc
agctacacctttgaggaagtgcctttccacagcagctacgcgcacagccagagcctggaccggctgatgaatcctctcatcga
caatactgtattacctgaacagaactcaaaatcagtcgggaagtgcacaaaacaggacttgcgttttagccgtgggtctccag
ctggcatgtctgttcagcccaaaactggctacctggaccctgttatcggcagcagcgcgtttctaaaacaaaaacagacaaca
acaacagcaattttacctggactggtgcttcaaaatataacctcaatgggcgtgaatccatcatcaacctggcactgctatggcc
tcacataaagacgacgaagacaagttcttcccatgagcgggtgcatgatttttgaaaagagagcggcggagcttcaaacact
gcattggacaatgtcatgattacagacgaagaggaaattaaagccactaacctgtggccaccgaaagatttgggaccgtggc
agtcaatttccagagcagcagcacagacctgcgaccggagatgtgcatgctatgggagcattacctggcatggtgtggcaag
atagagacgtgtacctgcagggtcccatttgggccaaaattctcacacagatggacactttcaccgtctcctcttatgggcgg
ctttgactcaagaacccgcctctcagatcctcatcaaaaacacgcctgttctcgcgaatcctccggcggagttttcagctacaa
agtttgcttcattcatcaccactccacaggacaagtgagcgtggagattgaatgggagctgcagaaagaaaacagcaaa
cgctggaatcccgaagtgcagtatacatctaactatgcaaatctgccaacattgatttactgtggacaacaatggactttatact
gagcctcgccccattggcacccgttacctcacccgtccccagtaa;

[00115] Shuffle 10-2 (amino acid sequence) (SEQ ID NO: 26):

[00116] MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVL
PGYKYLGPFNGLDKGEPVNAADAALEHDKAYDQQLKAGDNPYLRYNHADA
EFQERLQEDTSFGGNLGRAVFQAKKRVLLEPLGLVEEAAKTAPGKKRPVEQSPQE
PDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPDQPLGEPPAAPSGVGSMTMASG
GGAPMADNNEGADGVGNASGNWHCDSTWLGDREVTTSTRTWALPTYNNHLY
KQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFRPK
RLNFKLFNIQVKEVTTNDGVTTIANNNLTSTVQVFSDSEYQLPYVLGSAHQGCLPP

FPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEEVP
 FHSSYAHSQSLDRLMNPLIDQYLYYLNRTQNQSGSAQNKDLLFSRGSPAGMSV
 QPKNWLPGPCYRQQCVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMAS
 HKDDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTV
 AVNLQSSSTD PATGDVHVMGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPS
 PLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ
 KENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL;

[00117] Shuffle 10-2 (nucleotide sequence) (SEQ ID NO: 34):

[00118] atggctgccgatgggtatctccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggactgaa
 acctggagccccgaaacccaaagccaaccagcaaaagcaggacgacggccgggtctggtgcttctggctacaagtacct
 cggacccttcaacggactcgacaagggggagcccgtaacgcggcggtatgcagcgccctcgagcacgacaaggcctac
 gaccagcagctcaaaagcgggtgacaatccgtacctcggtataaccacgccgacgccgagtttcaggagcgtctgcaagaag
 atacgtcttttgggggcaacctcgggcgagcagcttccaggccaaaagagggttctcgaacctctcggtctggttgaggag
 cggctaagacggctcctggaaagaaacgtccggtagagcagtcgccacaagagccagactcctcctcgggcattggcaaga
 caggccagcagcccgtaaaaagagactcaattttggtcagactggcgactcagagtcagtcctccgaccacaacctctcgg
 agaacctcccgagccccctcaggtgtgggatctcttacaatggcttcaggtgtggcgacccaatggcagacaataacgaag
 gcggcgacggagtggtgaatgcctcaggaaattggcattgcgattccacatggctgggcgacagagtcaccaccagcac
 ccgcacctgggacctgcccacctacaataaccacctctacaagcaaatctccagtgttcaacggggggccagcaacgacaacc
 actacttcggctacagcacccttgggggtattttgacttcaacagattccactgccacttttaccacgtgactggcaagactc
 atcaacaacaattggggattccggcccaagagactcaacttcaagctcttcaacatccaagtcaaggaggtcacgacgaatgat
 ggcgtcacgaccatcgctaataaccttaccagcacgggtcaagtcttctcgactcggagtaccagtgccgtacgtcctcggct
 ctgcgcaccagggtgcctccctccgttcccggcgacgtgttcattgattccgcagtcaggtacctaacgtcaacaatggca
 gccaggcagtgggacgggtacatcttttactgcctggaatatttccatcgagatgctgagaacgggcaacaactttacctcag
 ctacacctttgaggaagtgcctttccacagcagctacgcgcacagccagagcctggaccggctgatgaatcctctcagacca
 gtacctgtattacctgaacagaactcaaaatcagtcgggaagtgcacaaaacaaggacttgcgttttagccgtgggtctcagct
 ggcatgtctgttcagccccaaaactggctacctggacctgttaccggcagcagtcggtttctaaaacaaaaacagacaacaac
 aacagcaactttacctggactggtgcttcaaaatataaccttaattggcggtgaatctataatcaacctggcactgctatggcctca
 cacaagacgacaagacaagttcttccatgagcgggtgtcatgatttttgaaaggagagcgccggagcttcaaacactgca
 ttggacaatgtcatgatcacagacgaagaggaaatcaaaagccactaaccccggtggccaccgaaagatttgggactgtggcagt
 caatctccagagcagcagcacagacctgcgaccggagatgtgcatgttatgggagccttacctggaatggtgtggcaagac
 agagacgtataacctgcagggtcctatttggccaaaatcctcacacagatggacactttacccgtctcctcttatggcggtctt
 ggactcaagaacccgctcctcagatcctcatcaaaaacacgcctgttctcgcgaatcctccggcgagttttcagctacaaagt

ttgcttcattcatcacccaatactccacaggacaagtgagcgtggagattgaatgggagctgcagaaagaaaacagcaagcgc
 tggaatcccgaagtgcagtacacatccaattatgcaaaatctgccaacgttgatttactgtggacaacaatggactttatactga
 gcctcgccccattggcacccgttacctcacccgtccctgttaa;

[00119] Shuffle 10-6 (amino acid sequence) (SEQ ID NO: 27):

[00120] MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKVNQQKQDNARGLVL
 PGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADA
 EFQERLQEDTSFGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQE
 PDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASG
 GGAPMADNNEGADGVGNASGNWHCDSTWLGDREVTTSTRTWALPTYNNHLY
 KQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFPRK
 RLNFKLFNIQVKEVTTNDGVTTIANNLSTVQVFSDSEYQLPYVLGSAHQGCLPP
 FPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDV
 PFHSSYAHSQLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGSPTGMSV
 QPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMAS
 HKDDEDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTV
 AVNLQSSSTD PATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPS
 PLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ
 KENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL;

[00121] Shuffle 10-6 (nucleotide sequence) (SEQ ID NO: 35):

[00122] atggctgccgatgggtatctccagattggctcgaggacaacctctctgagggcattcgcaatggtgggacttgaaa
 cctggagccccgaaaccaaagtaaccagcaaaagcaggacaacgctcggggctctgtgcttccgggttacaataacctcg
 gaccttcaacggactcgacaagggggagcccgtaacgcggcgagcgcagcggccctcgagcacgacaaggcctacga
 ccagcagctcaaagcgggtgacaatccgtacctcggtataaccacgccgacgccgagtttcaggagcgtctgcaagaagat
 acgtcttttgggggcaaccttggacgagcagtcctccaggccaagaagagggttctcgaaccttttggtctggttgaggaagg
 gctaagacggctcctggaaagaaacgtccggtagcagcgcgcacaagagccagactcctcctcgggcattggcaagaca
 ggccagcagccccgtaaaaagagactcaattttggtcagactggcgactcagagtcagtcctccgaccacaacctctcggag
 aacctccagcaacccccgctgctgtgggacctactacaatggcttcaggcgggtggcgaccaatggcagacaataacgaagg
 cgccgacggagtggttaatgcctcaggaaattggcattgcgattccacatggctgggcgacagagtcaccaccagcacc
 cgacctgggccttggccacctacaataaccacctctacaagcaaatctccagtgttcaacggggggccagcaacgacaacca
 ctacttgggtacagcacccccctgggggtattttgacttcaacagattccactgccacttttaccacgtgactggcaaagactcat
 caacaacaattggggattccggcccaagagactcaactcaagctcttcaacatccaagtaaggaggtcacgacgaatgatg
 gcgtcacgaccatcgtaataaccttaccagcacgggtcaagtcttctcgactcggagtaggtgcccgtacgtcctcggctc

tgcgcaccagggctgcctccctccgttccccggcggacgtgttcatgattccgaatacggctacctgacgctcaacaatggcag
ccaggcagtgaggacgggtcatccttttactgcctggaatatttcccatcgcagatgctgagaacgggcaataactttaccttcagct
acacttttgaggacgttctttccacagcagctacgctcacagccagagcctggaccggctgatgaatcctctcatcgaccagta
cctgtattacctgaacagaactcagaatcagtcgggaagtgcceaaaacaaggacttgctgtttagccgtgggtctccaactggc
atgtctgttcagcccaaaaactggctacctggaccctgttatcggcagcagcgcgtttctaaaacaaaaacagacaacaacaac
agcaactttacctggactgggtgcttcaaaatataaccttaatgggcgtgaatctataatcaacctggcactgctatggcctcacac
aaagacgacgaagacaagttctttccatgagcgggtgcatgattttggaaaggagagcggcggagcttcaaacactgcattg
gacaatgcatgatcacagacgaagaggaaatcaaagccactaaccctgtggccactgaaagatttgggactgtggcagtc
tctccagagcagcagcacagaccctgcgaccggagatgtgcatgccatgggagccttacctggaatggtgtggcaagacaga
gacgtatactgcagggtcctatttgggcaaaaattcctcacacggatggacactttcaccgtctcctctcatggcggcgtttgg
acttaagcaccgcctcctcagatcctcatcaaaaacacgcctgttctgcgaatcctccggcagagtttctggctacaaagtgg
cttcattcatcaccagttattccacaggacaagtgagcgtggagattgaatgggagctgcagaaagaaaacagcaaacgctgg
aatcccgaagtgcagtatacatctaactatgcaaaatctgccaacgttgatttactgtggacaacaatggactttatactgagcct
cgccccattggcacccgttacctcaccgtccctgtaa;

[00123] Shuffle 10-8 (amino acid sequence) (SEQ ID NO: 28):

[00124] MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKVNQQKQDNARGLVL
PGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADA
EFQERLQEDTSFGGNLGRAVFQAKKRVLFPFGLVEEGAKTAPGKKRPVEQSPQE
PDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASG
GGAPMADNNEGADGVGNASGNWHCDSTWLGDREVITTSTRTWALPTYNNHLY
KQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPK
RLNFKLFNQVKETTDVTTIANNLSTVQVFSDSEYQLPYVLGSAHQGCLPPFPAD
VFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTSYTFEDVPFHSS
YAHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGSPTGMSVQPKN
WLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDD
EDKFFPMSGVMIFGKESAGASNTALDNVMITDEEATNPVATERFGTVAVNLQSS
PATDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHP
PPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENS KRWNPEV
QYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRP;

[00125] Shuffle 10-8 (nucleotide sequence) (SEQ ID NO: 36):

[00126] atggctgccgatgggtatcttccagattggctcgaggacaacctctctgagggcattcgcgaatgggtgggacttga
cctggagccccgaaaccaaagtcaaccagcaaaagcaggacaacgctcggggcttctgtgcttccgggttacaataacctg

gacccttcaacggactcgacaagggggagcccgtcaacgcggcgagcgcagcggccctcgagcacgacaaggcctacga
ccagcagctcaaaagcgggtgacaatccgtacctcgggtataaccacgccgacgccgagttcaggagcgtctgcaagaagat
acgtcttttgggggcaaccttggacgagcagcttccaggccaagaagggttctgaaccttttggctgtggtgaggaaggt
gctaagacggctcctgaaaagaaacgtccggtagagcagtcgccacaagagccagactcctcctcgggcattggcaagaca
ggccagcagcccgtaaaaagagactcaatttggtcagactggcgactcagagtcagtcctccgacccacaacctctcggag
aacctccagcaacccccgctgctgtgggacactactacaatggcttcaggcgggtggcgaccaatggcagacaataacgaagg
cgccgacggagtggtgaatgcctcaggaaattggcattgcgattccacatggctgggcgacagagtcaccaccagcacc
cgaacatgggccttggccacctataacaaccacctctacaagcaaattcctcagtgcttcaacggggggccagcaacgacaacca
ctactcggctacagcaccctcgggggtatttggatttcaacagattccactgccacttttaccacgtgactggcagcgactcat
caataacaattggggattccggcccaagagactcaacttcaaactcttcaacntccaagtcaaggagggnnacgacgaangatg
ncgtcacaaccatcgctaataaccttaccagcacgggtcaagtcttctcggactcggagtaccagcttccgtacgtctcggctct
gcgcaccaggggtgcctccctccgttcccggcggacgtgttcatgattccgcaatacggctacctgacgtcaacaatggcag
ccaggcagtgggacgggtcatccttttactgcctggaatatttccatcgcagatgctgagaacgggcaataactttacctncagct
acacttttgaggacgttctttccacagcagctacgctcacagccagagcctggaccggctgatgaatcctctcatcgaccagta
cctgtattacctgaacagaactcagaatcagtcgggaagtgcacaaaacaaggacttgctgtttagccgtgggtctccaactggc
atgtctgttcagcccaaaaactggctacctggaccctgttatcggcagcagcgcgttttaaaacaaaaacagacaacaacaac
agcaactttacctggactgggtgcttcaaaatataaccttaatggcggtgaatctataatcaacctggcactgctatggcctcacac
aaagacgacgaagacaagttcttccatgagcgggtgcatgattttggaaaggagagcggcggagcttcaaacactgcattg
gacaatgcatgatcacagacgaagagannnnnaagccactaaccctggccactgaaagatttgggactgtggcagtc
tctccaagcagcacannnacctgcgaccgnagatgtgcatgccatgggagccttacctggaatggtgtggcaagacagag
acgtatactgcagggctcctatttggccaaaattcctcacacggatggacactttaccctcctcctcatggcgggctttgga
cttaagcaccgcctcctcagatcctcatcaaaaacacgcctgttctcgcgaatcctccggcagagtttcggctacaaagtgtgc
ttcattcatcaccagttattccacaggacaagtgagcgtggagattgaatgggagctgcagaaagaaaacagcaaacgctgga
atcccgaagtgcagtatacatctaactatgcaaaatctgccaacgttgatttactgtggacaacaatggactttatactgagcctc
gccccattggcaccggtacctcaccgtccccngtaa;

[00127] Shuffle 100-2 (amino acid sequence) (SEQ ID NO: 29):

[00128] MASDGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVL
PGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLRAGDNPYLRYNHADA
EFQERLQEDTSFGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQE
PDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASG
GGAPMADNNEGADGVGNASGNWHCDSTWLGDREVITSTRTWALPTYNNHLY
KQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFRPK

RLNFKLFNIQVKEVTTNDGVTTIANNLSTVQVFSSEYQLPYVLGSAHQGCLPP
 FPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDV
 PFHSSYAHSQSLDRLMNPLIDQYLYLNRQTQNSGSAQNKDLLFSRGSPAGMSV
 QPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMAS
 HKDDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTV
 AVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHP
 PLMGGFGLKHPPPQILIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ
 KENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL;

[00129] Shuffle 100-2 (nucleotide sequence) (SEQ ID NO: 37):

[00130] atggcttcgatggttatcttcagattggctcgaggacaacctctctgaggcatccgcgagtggtgggacttgaac
 cctggagccccgaaaccaaagccaaccagcaaaagcaggacgacggccgggtctggtgcttctggtacaagtaccc
 ggaccttaacggactcgacaagggggagcccgtaacgcggcgatgcagcggccctcgagcacgacaaggcctacg
 accagcagctcagagcgggtgacaatccgtacctgcggtataaccacggcagccgagtttcaggagcgtctgcaagaag
 atacgtcttttgggggcaacctcgggcgagcagcttccaggccaagaagagggttctgaaccttttggtctggtgaggaag
 gtgctaagacggctcctggaagaacgtccggtagagcagtcgccacaagagccagactcctcctcgggcattggcaaga
 caggccagcagcccgtaaaaagagactcaattttgtcagactggcgactcagagtcagtcctccgaccacaacctctcgg
 agaacctccagcaacccccgtgctgtgggacctactacaatggcttcaggcgggtggcgaccaatggcagacaataacgaa
 ggcgccgacggagtggtaatgcctcaggaaattggcattgcgattccacatggctgggcgacagagtcaccaccagca
 cccgaacatgggccttggccacctataacaaccacctctacaagcaaatctccagtgtcttaacgggggccagcaacgacaac
 cactacttcggctacagcaccctcgggggtattttgattcaacagattccactgccatttctaccacgtgactggcagcgact
 catcaacaacattggggattccggcccaagagactcaacttcaaatcttcaacatccaagtcaaggaggtcacgacgaatga
 tggcgtcacaaccatcgctaataaccttaccagcacgggtcaagtctctcggactcggagtaccagcttcgtacgtcctcggct
 ctgcgcaccagggtgcctccctccgttccggcgacgtgttcattgattccgcagtagcgttacctaacgtcaacaatggca
 gccaggcagtgaggacgggtacatccttttactgcctggaattttcccatcgagatgctgagaacgggcaataactttaccttcagc
 tacaccttgaggacgtgcctttccacagcagctacgcgcacagccagagcctggaccggctgatgaatcctctcatcgacca
 gtacctgtattacctgaacagaactcagaatcagtcgggaagtgcacaaaacaaggacttgctgttttagccgggggtctccagc
 tggcatgtctgttcagccaaaactggctacctggaccctgttaccggcagcagcgcgtttctaaaacaaaacagacaacaa
 caacagcaactttacctggactggtgcttcaaaatataaccttaattggcggtgaatctataatcaaccttggcactgctatggcctc
 acacaaagacgacaaagacaagttcttcccatgagcgggtgtcatgattttgaaaggagagcggcgaggcttcaaacactgc
 attggacaatgtcatgatcacagacgaagaggaaatcaagccactaaccccgaggccaccgaaagatttgggactgtggca
 gtcaatctccagagcagcagcagaccctgcgaccggagatgtgcatgttatgggagccttacctggaatggtgtggcaaga
 cagagacgtatactcgagggtccatttgggcaaaattctcacacagatggacactttacccgtctcctcttatggcgggc

tttgacttaagcaccgcctctcagatcctcatcaaaaacacgcctgtcctgcgaatcctccggcagagtttccggctacaaa
gtttgcttcattcatcaccagctattctactggccaagtcagcgtggagattgaatgggagctgcagaaagaaaacagcaaacg
ctggaatcccgaagtgcagtatacatctaactatgcaaaatctgccaacgttgattcactgtggacaacaatggactttatactga
gcctcgtccattggcaccggttacctcaccggtccctgtaa;

[00131] SM 10-1 (amino acid sequence) (SEQ ID NO: 30):

[00132] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLRYNHADAEF
QERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEP
DSSSGIGKTGQPPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMASGG
GAPMADNNEGADGVGNASGNWHCDSTWLGDREVTTSTRTWALPTYNNHLYK
QISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFRPKRL
SFKLFNIQVKEVTTNDGVTIANNTSTVQVFSDSEYQLPYVLGSAHQGCLPPFP
ADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEEVPF
HSSYAHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGSPAGMSVQ
PKNWLPGPCYRQQRVSKTKTDNNSNFTWTGASKYNLNGRESIINPGTAMASH
KDDDEDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVA
VNFQSSSTDPATGDVHAMGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSP
MGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKE
NSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL;

[00133] SM 10-1 (nucleotide sequence) (SEQ ID NO: 38):

[00134] atggctgccgatggttatctccagattggctcgaggacactctctgaaggaataagacagtggtggaagctcaa
acctggccccaccaccacaaagcccgagagcggcagacagcaggggtctgtgcttctgggtacaaagtacctc
ggaccttcaacggactcgacaaggagagccgggtcaacgaggcagacgccgcggccctcgagcacgacaaggcctacg
accagcagctcaaagcgggtgacaatccgtacctgcgggtataaccacgccgacgccgagtttcaggagcgtctgcaagaaga
tacgtcttttgggggcaacctcgggcgagcagctctccaggccaagaagcgggtctcgaacctctcggctctggttaggaag
gcgctaagacggctcctggaaagaaacgtccggtagagcagtcgccacaagagccagactcctcctcgggcacggaaga
caggccagcagcccgtaaaaagagactcaatttgggtcagactggcgactcagagtcagtcctccgaccacaacctctcgg
agaacctccagcaacccccgctgctgtgggacctactacaatggcttcaggcgggtggcgaccaatggcagacaataacgaa
ggcgccgacggagtgggtaatgcctcaggaaattggcattgcgattccacatggctgggcgacagatcatcaccaccagca
cccgaacatgggccttggccacctataacaaccacctctacaagcaaatctccagtgttcgacggggggccagcaacgacaa
ccactacttcggctacagcaccctcgggggtatttgaacttaacagattccactgccactttcaccacgtgactggcagcgac
tcatcaacaataactggggattccggccaagagactcagcttaagctcttcaacatccagggtcaaggaggtcacgacgaatg

atggcggtcacaaccategctaataacettaccagcacgggtcaagtcttctcggactcggagtaccagcttcgtacgtcctcgg
ctctgcgcaccagggctgcctccctccgttcccgccggacgtgttcattgattccgcaataggctacgtgacgtcaacaatgg
cagccaagccgtgggacgttcattctttactgcctggaatatttccctctcagatgctgagaacgggcaacaactttaccttcag
ctacacctttgaggaagtgcctttccacagcagctacggcgacagccagagcctggaccggctgatgaatcctctcatcgcata
atacctgtattacctgaacagaactcaaaatcagtcgggaagtgcacaaaacaaggacttgctgtttagccgtgggtctccagct
ggcatgtctgttcagcccaaaaactggctacctggaccctgttatggcagcagcgcgtttctaaaacaaaaacagacaacaac
aacagcaattttacctggactgggtgcttcaaaatataacctcaatggcggtgaatccatcatcaaccctggcactgctatggctc
acacaaagacgacgaagacaagttcttcccatgagcgggtgtcatgattttggaaaagagagcggcggagcttcaaacactgc
attggacaatgtcatgattacggacgaagaggaaattaaagccactaaccctgtggccaccgaaagatttgggaccgtggcag
tcaatttcagagcagcagcagcacagacctgcgaccggagatgtgcatgctatgggagcattacctggcatggtgtggcaagat
agagacgtgtacctgcaggggtccatttgggccaaaattcctcacacagatggacactttaccctgtctctcttatggcgggctt
tggactcaagaacccgcctcctcagatcctcatcaaaaacacgcctgttctcgcgaatcctccggcggagtttcagctacaaag
tttgettcattcatcactcaatactccacaggacaagtgagcgtggaaattgaatgggagctgcagaaagaaaacagcaaacgc
tggaatcccgaagtgcagtatacatctaactatgcaaaatctgccaacgttgatttactgtggacaacaatggactttatactgag
cctcgccccattggcaccggttacctcacccgtccctgtaa;

[00135] SM 10-8 (amino acid sequence) (SEQ ID NO: 31):

[00136] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EF
QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPD
SSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLGTNTMATGS
GAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYK
QISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFPRKRL
KFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFP
ADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYLSRTDTPSGTTTQSRLQFSQAGASDIRDQSR
NWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNPAMASHK
DDEEKFFPQSGVLIFGKQGSEKTSVDIEKVMITDEEEIRTTNPVATEQYGSVSTNL
QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMG
GFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS
KRWNPEVQYTSNYNKSNNVDFTVDTNNGVYSEPRPIGTRYLTRNL;

[00137] SM 10-8 (nucleotide sequence) (SEQ ID NO: 39):

[00138] atggctgccgatgggtatcttccagattggctcgaggacactctctgaaggaataagacagtgggtggaagctcaa
acctggccccaccaccacaaagcccgagagcggcagacaggacagcaggggtctgtgcttctgggtacaaagtacctc
ggaccttcaacggactcgacaaggagagccgggtcaacgaggcagacgccgcggccctcgagcacgacaagcctatg
accggcagctcgacagcggagacaacccgtacctcaagtacaaccacgccgacgcggagtctcaggagcgccttaaagaag
atacgtcttttgggggcaacctcggacgagcagcttccaggcgaaaaagagggttcttgaacctctgggcctgggtgaggaaac
ctgttaagacggctccgggaaaaaagaggccggtagacactctctgtggagccagactcctcctcggaaccgggaaagg
cgggccagcagcctgcaagaaaaagattgaatttggcagactggagacgcagactcagctgatccccagcctctcggga
cagccaccagcagccccctctgggtctgggaactaatac gatggctacaggcagtgccgcaccaatggcagacaataacgag
ggcggcgacggagtggttaattctcgggaaattggcattgcattccacatggatggcgacagagtcaccaccagca
cccgaacctgggcctgcccacctacaacaaccctctacaacaaatttcagccaatcaggagcctcgaacgacaatcac
tactttggctacagcacccttgggggtattttgacttcaacagattccactgccacttttcaccacgtgactggcaaagactcatc
aacaacaactggggattccgaccaagagactcaagttcaagctctttaaattcaagtc aaagaggtcacgcagaatgacggt
acgacgacgattgccataaccttaccagcaggggtcaggtgttactgactcggagtaccagctcccgtatgtcctcggctcgg
cgcatcaaggatgcctcccgcgttcccagcagacgttctcatggtgccacagtatggatactcacctgaacaacgggagt
caggcagtaggacgctcttcttactgctggagtactttccttctcagatgctgcgtaccggttaacaactttaccttcagctaca
cttttgaggacgttctttccacagcagctacgctcacagccagagtctggaccgtctcatgaatcctctcatcgaccagtacgtg
tattacttgagcagaacagacactccaagtggaaaccaccacgcagtc aaaggcttcagttttcaggccggagcagtgacatt
cgggaccagctaggaactggcttctggaccctgttaccgccagcagcagtatcaaagacatctgcggataacaacaacag
tgaatactcgtggactggagctaccaagtaccacctcaatggcagagactctctggtgaatccggggccggccatggcaagcc
acaaggacgatgaagaaaagttttctcagagcgggggttctcatctttgggaagcaaggctcagagaaaacaagtgtggaca
ttgaaaaggatcatgattacagacgaagaggaaatcaggacaaccaatcccgtggctacggagcagtatggttctgtatctacca
acctccagagaggcaacagacaagcagctaccgcagatgtcaacacacaaggcgttcttccaggcatggtctggcaggaca
gagatgtgtaccttcagggggcccatctgggcaaagattccacacacggacggacattttaccctctcccctcatgggtggatt
cggacttaaacacctctccacagattctcatgaagaacccccgtacctgcgaatccttcgaccaccttcagtcggcaaa
gtttgcttcttcatcacacagtactccacgggacaggtcagcgtggagatcgagtgaggagctgcagaaggaaaacagcaaac
gctggaatcccgaagttcagctacacttccaactacaacaagtctgttaatgtggactttactgtggacactaatggcgtgtattcag
agcctcgccccattggcaccagatactgactcgtaatctgtaa;

[00139] SM 100-3 (amino acid sequence) (SEQ ID NO: 32):

[00140] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
GYKYLGPFNGLDKGEPVNEADAALEHDKAYDRQLDSGDNPYLKYNHADA EF
QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTPGKKRPVEHSPVEPD

SSSGTGKAGQQPARKRLNFGQTGDANSVPDPQPLGQPPAAPSGLGTNTMATGS
 GAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYK
 QISSQSGASNDNHYFGYSTPWGYFDFNRHFHCHFSRPDWQRLINNNWGRPKRL
 KFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFP
 ADVFMVPQYGYLTLNNGSRAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
 HSSYAHSQSLDRLMNPLIDQYLYLSRTDTPSGTTTQSRLQFSQAGASDIRDQSR
 NWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNPAMASHK
 DDEEKFFPQSGVLIFGKQGSEKTSVDIEKVMITDEEEIRTTNPVATEQYGSVSTNL
 QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMG
 GFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS
 KRWNPEVQYTSNYNKSVNVDFTVDTNGVYTEPRPIGTRYLTRNL;

[00141] SM 100-3 (nucleotide sequence) (SEQ ID NO: 40):

[00142] atggctgccgatgggtatctccagattggctcgaggacactctctgaaggaataagacagtgggtgaagctcaa
 acctgccccaccaccacaaagcccgagagcggcgataaggacgacagcaggggtctgtgcttctgggtacaagtacctc
 ggaccttcaacggactcgacaagggagagccgggtcaacgaggcagacgccgcggccctcgagcagacaaagcctatg
 accggcagctcgacagcggagacaaccgtacctcaagtacaaccacgccgacgcggagtctcaggagcgccttaaagaag
 atacgtcttttgggggcaacctcggacgagcagctctccaggcgaaaaagagggttcttgaacctctgggcctggttaggaac
 ctgttaagacggctccgggaaaaagaggccggtagagcactctctgtggagccagactcctcctcggaaccggaaagg
 cgggccagcagcctgcaagaaaaagattgaatttggcagactggagacgcaaactcagctacctgacccccagcctctcgga
 cagccaccagcagccccctctggtctgggaactaatacagtggttacaggcagtgccgcaccaatggcagacaataacgag
 ggcgccgacggagtgggtaattctcgggaaattggcattgcgattccacatggatggcgacagagtcaccaccagca
 cccgaacctgggcctgcccacctacaacaaccctctacaacaatttcagccaatcaggagcctcgaacgacaatcac
 tactttggctacagcacccttgggggtatttgaactcaacagattccactgccacttttcaccacgtgactggcaaagactcatc
 aacaacaactggggattccgacccaagagactcaagttcaagctctttaacattcaagtaagagggtcacgcagaatgacggt
 acgacgacgattgccataaccttaccagcaggggtcaggtgttactgactcggagtaccagctcccgtacgtcctcggctcg
 gcgcatcaaggatgcctcccgcgttccagcagacgttctcatgggtgccacagtatggatacctcaccctgaacaacgggag
 tcgggcagtaggacgctcttcatttactgcctggagtacttctctcagatgctgcgtaccggtaacaactttaccttcagctac
 acttttgaggacgttctttccacagcagctacgctcacagccagagctctggaccgtctcatgaatcctctcatcgaccagtacct
 gtattacttgagcagaacagacactccaagtggaaaccaccacgcagtcgaaggcttcagtttctcaggccggagcagtgatgacat
 tcgggaccagcttaggaactgggttcttgaccctgttaccgccagcagcgagtatcaaaagacatctcgggataacaacaaca
 gtgaatactcgtggactggagctaccaagtaccacctcaatggcagagactctctggtgaatccggggccggccatggcaagc
 cacaaggacgatgaagaaaagttttcctcagagcgggggtctcatctttgggaagcaaggctcagagaaaacaagtgtggac

attgaaaaggatcatgattacagacgaagaggaaatcaggacgaccaatcccggtgctacggagcagtatggttctgtatctacc
 aacctccagagaggcaacagacaagcagctaccgcagatgtcaacacacaaggcggttctccaggcatggtctggcaggac
 agagatgtgtaccttcagggggcccatctgggcaaagattccacacacggacggacattttacccctctcccctcatgggtgga
 ttcggacttaaacacctctctccacagattctcatcaagaacacccccggtacctgcgaatccttcgaccaccttcagtcggcaa
 agtttgcttctctcatcacacagtactccacgggacaggtcagcggtggagatcgagtgggagctgcagaaggaaaacagcaaa
 cgctggaatcccgaagttcagtagacttccaactacaacaagtctgttaatgtggactttactgtggacactaatggcgtgtataca
 gagcctcgccccattggcaccagatactgactcgtaatctgtaa;

[00143] SM 100-10 (amino acid sequence) (SEQ ID NO: 33):

[00144] MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLP
 GYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EF
 QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPD
 SSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPPAAPSGLGTNTMATGS
 GAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYK
 QISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGRPKRL
 KFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFP
 ADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
 HSSYAHSQSLDRLMNPLIDQYLYLSRTDAPSGTTTQSRLQFSQAGASDIRDQSR
 NWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLNVNPGPAMASHK
 DDEEKFFPQSGVLIFGKQGSEKTSVDIEKVMITDEEEIRTTNPVATEQYGSVSTNL
 QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMG
 GFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS
 KRWNPEVQYTSNKNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL;

[00145] SM 100-10 (nucleotide sequence) (SEQ ID NO: 41):

[00146] atggctgccgatgggtatcttccagattggctcgaggacactctctgaaggaataagacagtgggtggaagctcaa
 acctgccccaccaccacaaagcccgagagcggcagacagcagcaggggtctgtgcttctgggtacaagtacctc
 ggaccttcaacggactcgacaaggagagccggtcaacgagcagacgccgcggcctcgagcacgacaaagcctatg
 accggcagctcgacagcggagacaaccgtacctcaagtacaaccacgccgacgcggagtctcaggagcgccttaagaag
 atacgtcttttgggggcaacctcggacgagcagcttccaggcgaaaaagagggttctgaacctctgggcctggttgaggaa
 ctgttaagacggctccgggaaaaaaggccggtagagcactctctgtggagccagactcctcctcggaaccggaaagg
 cgggtcagcagcctgcaagaaaaagattgaatttggcagactggagacgcagactcagctacctgacccccagcctctcga
 cagccaccagcagccccctctggtctgggaactaatacatgatggctacaggcagtgccgcaccaatggcagacaataacgag
 ggcgccgacggagtgggtaattcctcgggaaattggcattgcattccacatggatggcgacagagtcaccaccagca

cccgaacctgggccctgccacactacaacaaccacctctacaaacaaattccagccaatcaggagcctcgaacgacaatcac
 tactttggctacagcacccttgggggtattttgacttaacagattccactgccactttcaccacgtgactggcaaagactcatc
 aacaacaactggggattccgacccaagagactcaagttaagctctttaacattcaagtaagaggtcacgcagaatgacggt
 acgacgacgattgccataaccttaccagcacgggtcaggtgtttactgactcggagtagcagctcccgtacgtcctcggctcg
 gcgcatcaaggatgcctcccgcgttcccagcagacgttctcatggtgccacagtatggatacctcaccctgaacaacgggag
 tcaggcagtaggacgctcttcattttactgcctggagtagtcttctctcagatgctcgtaccggtaacaactttaccttcagctac
 acttttgaggacgttctttccacagcagctacgctcacagccagagctctggaccgtctcatgaatcctctcatcgaccagtacct
 gtattacttgagcagaacagacgctccaagtggaaaccaccacgcagtcgaaggcttcagttttctcaggccggagcgagtgacat
 tcgggaccagcttaggaactggcttctggaccctgttaccgccagcagcgagtatcaaagacatctgcggataacaacaaca
 gtgaatactcgtggactggagctaccaagtaccacctcaatggcagagactctctggtgaatccggggccggccatggcaagc
 cacaaggacgatgaagaaaagttttcctcagagcgggggttctcatctttgggaagcaaggctcagagaaaacaagtgtggac
 attgaaaaggctcatgattacagacgaagaggaaatcaggacaaccaatcccgtggctacggagcagtagtgttctgtatctacc
 aacctccagagaggcaacagacaagcagctaccgcagatgtcaacacacaaggcgttcttcaggcatggtctggcaggac
 agagatgtgtaccttcagggggcccatctggggcaaagattccacacacggacggacattttcacccctctcccctcatgggtgga
 ttcgacttaaacacctctctccacagattctcatcaagaacacccccgtacctgcgaatccttcgaccaccttcagtcggcaa
 agtttgcttctctcatcacacagtactccacgggacaggtcagcgtggagatcgagtgggagctgcagaaggaaaacagcaaa
 cgctggaatcccgaagttcagtagacttccaactacaacaagtctgtaatgtggactttactgtggacactaatggcgtgtattca
 gagcctcgccccattggcaccagatacctgactcgtaatctgtaa.

NUCLEIC ACIDS AND HOST CELLS

[00147] The present disclosure provides nucleic acids comprising nucleotide sequences encoding a variant AAV capsid protein (as described above), as well as host cells comprising a subject nucleic acid. The nucleic acids and host cells are useful for generating rAAV virions (as described below).

[00148] The present disclosure provides host cells, e.g., isolated host cells, comprising a subject nucleic acid. A subject host cell can be referred to as a “genetically modified host cell” and is typically an isolated cell, e.g., a cell in *in vitro* culture. A subject host cell is useful for producing a subject rAAV virion, as described below. Where a subject host cell is used to produce a subject rAAV virion, it is referred to as a “packaging cell.” In some embodiments, a subject host cell is stably genetically modified (i.e., stably transfected) with a subject nucleic acid. In other embodiments, a subject host cell is transiently genetically modified (i.e., transiently transfected) with a subject nucleic acid.

- [00149] A subject nucleic acid is introduced stably or transiently into a host cell, using established techniques, including, but not limited to, electroporation, calcium phosphate precipitation, liposome-mediated transfection, and the like. For stable transformation, a subject nucleic acid will generally further include a selectable marker, e.g., any of several well-known selectable markers such as neomycin resistance, and the like.
- [00150] A subject host cell is generated by introducing a subject nucleic acid into any of a variety of cells, e.g., mammalian cells, including, e.g., murine cells, and primate cells (e.g., human cells). Suitable mammalian cells include, but are not limited to, primary cells and cell lines, where suitable cell lines include, but are not limited to, 293 cells, COS cells, HeLa cells, Vero cells, 3T3 mouse fibroblasts, C3H10T1/2 fibroblasts, CHO cells, and the like.
- [00151] In some embodiments, a subject host cell includes, in addition to a nucleic acid comprising a nucleotide sequence encoding a mutant capsid protein, a nucleic acid that comprises a nucleotide sequence encoding one or more AAV rep proteins. In other embodiments, a subject host cell further comprises an rAAV vector, as described below. As described in more detail below, an rAAV virion is generated using a subject host cell.

INFECTIOUS rAAV VIRIONS

- [00152] A subject infectious rAAV virion comprises a variant AAV capsid protein and a heterologous nucleic acid (described in greater detail below), and exhibits an increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein. By “increased resistance” it is meant that a subject infectious rAAV virion exhibits an increased infectivity in the presence of human anti-AAV antibodies. As described above, viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Thus in increased infectivity means an increased ratio of infectious viral particles to total viral particles. To determine resistance of an AAV to human anti-AAV antibodies, infectivity of the AAV is measured in the presence of various concentrations of human anti-AAV antibodies in order to obtain the antibody concentration (e.g., serum concentration, IVIG concentration, etc.) (mg/mL) required to reduce gene delivery efficiency (i.e., infectivity) to 50% of that in the absence of human anti-AAV antibodies. A virus that requires a higher antibody

concentration to reduce gene delivery efficiency to 50% of that in the absence of human anti-AAV antibodies is said to have increased resistance to antibody neutralization. Thus, a two-fold increase in resistance means a two-fold increase in the antibody concentration required to reduce gene delivery efficiency to 50% of that in the absence of human anti-AAV antibodies. In some embodiments, a subject infectious rAAV virion exhibits at least about 1.5-fold (e.g., at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 17-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, etc.) greater resistance to human AAV neutralizing antibodies than the resistance exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein.

[00153] A subject infectious rAAV virion can be said to exhibit increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies. In some embodiments, a subject infectious rAAV virion exhibits at least about 1.5-fold (e.g., at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 17-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, etc.) greater transduction of mammalian cells in the presence of human AAV neutralizing antibodies than the transduction exhibited by a wild type AAV (e.g., AAV2 (wild type AAV serotype 2)) or an AAV comprising a wild-type capsid protein.

[00154] In some embodiments, a subject infectious rAAV virion exhibits decreased binding to a neutralizing antibody that binds a wild-type AAV capsid protein. For example, a subject infectious rAAV virion can exhibit at least about 1.5-fold (e.g., at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 12-fold, at least about 15-fold, at least about 17-fold, at least about 20-fold, at least about 25-fold, at

least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, etc.) reduced binding (e.g., reduced affinity) to a neutralizing antibody that binds a wild-type capsid AAV protein, compared to the binding affinity of the antibody to wild-type AAV capsid protein.

[00155] In some embodiments, an anti-AAV neutralizing antibody binds to a subject infectious rAAV virion with an affinity of less than about 10^{-7} M, less than about 5×10^{-6} M, less than about 10^{-6} M, less than about 5×10^{-5} M, less than about 10^{-5} M, less than about 10^{-4} M, or lower.

[00156] In some embodiments, a subject infectious rAAV virion exhibits increased *in vivo* residence time compared to a wild-type AAV. For example, a subject infectious rAAV virion exhibits a residence time that is at least about 10%, at least about 25%, at least about 50%, at least about 100%, at least about 3-fold, at least about 5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 100-fold, or more, longer than the residence time of a wild-type AAV.

[00157] Whether a given subject infectious rAAV virion exhibits reduced binding to a neutralizing antibody and/or increased resistance to neutralizing antibody can be determined using any convenient assay known to one of ordinary skill in the art.

[00158] In some embodiments, a subject infectious rAAV virion comprises wild-type Rep78, Rep68, Rep52, and Rep40 proteins. In other embodiments, a subject infectious rAAV virion comprises, in addition to one or more variant capsid proteins, one or more mutations in one or more of Rep78, Rep68, Rep52, and Rep40 proteins.

HETEROLOGOUS NUCLEIC ACIDS

[00159] A suitable heterologous DNA molecule (also referred to herein as a “heterologous nucleic acid”) for use in a subject rAAV vector (e.g., a subject infectious rAAV virion) can be any heterologous nucleic acid. In some embodiments, the heterologous nucleic acid comprises a nucleotide sequence encoding a polypeptide (e.g., a protein that imparts some desired characteristic to the target cell, e.g., a fluorescent protein that allows for cell tracking, an enzyme that provides an activity missing or altered in the target cell, etc.). In some embodiments, the heterologous nucleic acid comprises an RNA interfering agent (as defined above).

[00160] A subject heterologous nucleic acid will generally be less than about 5 kilobases (kb) in size and will include, for example, a gene (a nucleotide sequence) that encodes a protein that is defective or missing from a recipient individual or target cell; a gene that encodes a protein having a desired biological or therapeutic effect (e.g., an antibacterial, antiviral or antitumor/anti-cancer function); a nucleotide sequence that encodes an RNA that inhibits or reduces production of a deleterious or otherwise undesired protein (e.g., a nucleotide sequence that encodes an RNA interfering agent, as defined above); and/or a nucleotide sequence that encodes an antigenic protein.

[00161] Suitable heterologous nucleic acids include, but are not limited to, those encoding proteins used for the treatment of endocrine, metabolic, hematologic, cardiovascular, neurologic, musculoskeletal, urologic, pulmonary and immune disorders, including such disorders as inflammatory diseases, autoimmune, chronic and infectious diseases, such as acquired immunodeficiency syndrome (AIDS), cancer, hypercholesterolemia, lysosomal storage diseases such as Activator Deficiency/GM2 Gangliosidosis, Alpha-mannosidosis, Aspartylglucosaminuria, Cholesteryl ester storage disease, Chronic Hexosaminidase A Deficiency, Cystinosis, Danon disease, Fabry disease, Farber disease, Fucosidosis, Galactosialidosis, Gaucher Disease, GM1 gangliosidosis, I-Cell disease/Mucopolidosis II, Infantile Free Sialic Acid Storage Disease/ISSD, Juvenile Hexosaminidase A Deficiency, Krabbe disease, Lysosomal acid lipase deficiency, Metachromatic Leukodystrophy, Mucopolysaccharidoses disorders (including Pseudo-Hurler polydystrophy/Mucopolidosis IIIA, MPSI Hurler Syndrome, MPSI Scheie Syndrome, MPS I Hurler-Scheie Syndrome, MPS II Hunter syndrome, Sanfilippo syndrome Type A/MPS III A, Sanfilippo syndrome Type B/MPS III B, Sanfilippo syndrome Type C/MPS III C, Sanfilippo syndrome Type D/MPS III D, Morquio Type A/MPS IVA, Morquio Type B/MPS IVB, MPS IX Hyaluronidase Deficiency, MPS VI Maroteaux-Lamy, MPS VII Sly Syndrome, Mucopolidosis I/Sialidosis, Mucopolidosis IIIC, and Mucopolidosis type IV), Multiple sulfatase deficiency, Niemann-Pick Disease, Neuronal Ceroid Lipofuscinoses, Pompe disease/Glycogen storage disease type II, Pycnodysostosis, Sandhoff disease/Adult Onset/GM2 Gangliosidosis, Sandhoff disease/GM2 gangliosidosis - Infantile, Sandhoff disease/GM2 gangliosidosis - Juvenile, Schindler disease, Salla disease/Sialic Acid Storage Disease, Tay-Sachs/GM2 gangliosidosis, and Wolman disease, insulin disorders

such as diabetes, growth disorders, various blood disorders including various anemias, thalassemias and hemophilia; genetic defects such as cystic fibrosis, Gaucher's Disease, Hurler's Disease, adenosine deaminase (ADA) deficiency, emphysema, or the like.

[00162] Suitable heterologous nucleic acids include, but are not limited to, those encoding any of a variety of proteins, including, but not limited to: an interferon (e.g., IFN- γ , IFN- α , IFN- β , IFN- ω ; IFN- τ); an insulin (e.g., Novolin, Humulin, Humalog, Lantus, Ultralente, etc.); an erythropoietin ("EPO"; e.g., Procrit®, Eprex®, or Epogen® (epoetin- α); Aranesp® (darbepoietin- α); NeoRecormon®, Epogin® (epoetin- β); and the like); an antibody (e.g., a monoclonal antibody) (e.g., Rituxan® (rituximab); Remicade® (infliximab); Herceptin® (trastuzumab); Humira™ (adalimumab); Xolair® (omalizumab); Bexxar® (tositumomab); Raptiva™ (efalizumab); Erbitux™ (cetuximab); Avastin® (bevacizumab); and the like), including an antigen-binding fragment of a monoclonal antibody (e.g., Lucentis® (ranibizumab)); a blood factor (e.g., Activase® (alteplase) tissue plasminogen activator; NovoSeven® (recombinant human factor VIIa); Factor VIIa; Factor VIII (e.g., Kogenate®); Factor IX; β -globin; hemoglobin; and the like); a colony stimulating factor (e.g., Neupogen® (filgrastim; G-CSF); Neulasta (pegfilgrastim); granulocyte colony stimulating factor (G-CSF), granulocyte-monocyte colony stimulating factor, macrophage colony stimulating factor, megakaryocyte colony stimulating factor; and the like); a growth hormone (e.g., a somatotropin, e.g., Genotropin®, Nutropin®, Norditropin®, Saizen®, Serostim®, Humatrope®, etc.; a human growth hormone; and the like); an interleukin (e.g., IL-1; IL-2, including, e.g., Proleukin®; IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9; etc.); a growth factor (e.g., Regranex® (beclapernin; PDGF); Fiblast® (trafermin; bFGF); Stemgen® (ancestim; stem cell factor); keratinocyte growth factor; an acidic fibroblast growth factor, a stem cell factor, a basic fibroblast growth factor, a hepatocyte growth factor; and the like); a soluble receptor (e.g., a TNF- α -binding soluble receptor such as Enbrel® (etanercept); a soluble VEGF receptor; a soluble interleukin receptor; a soluble γ/δ T cell receptor; and the like); an enzyme (e.g., α -glucosidase; Cerazyme® (imiglucarase; β -glucocerebrosidase, Ceredase® (alglucerase;); an enzyme activator (e.g., tissue plasminogen activator); a chemokine (e.g., IP-10; Mig; Gro α /IL-8, RANTES; MIP-1 α ; MIP-1 β ; MCP-1; PF-4; and the like); an angiogenic agent (e.g., vascular endothelial growth factor (VEGF) ; an anti-angiogenic agent (e.g., a soluble VEGF receptor); a

protein vaccine; a neuroactive peptide such as bradykinin, cholecystokinin, gastrin, secretin, oxytocin, gonadotropin-releasing hormone, beta-endorphin, enkephalin, substance P, somatostatin, prolactin, galanin, growth hormone-releasing hormone, bombesin, dynorphin, neurotensin, motilin, thyrotropin, neuropeptide Y, luteinizing hormone, calcitonin, insulin, glucagon, vasopressin, angiotensin II, thyrotropin-releasing hormone, vasoactive intestinal peptide, a sleep peptide, etc.; other proteins such as a thrombolytic agent, an atrial natriuretic peptide, bone morphogenic protein, thrombopoietin, relaxin, glial fibrillary acidic protein, follicle stimulating hormone, a human alpha-1 antitrypsin, a leukemia inhibitory factor, a transforming growth factor, an insulin-like growth factor, a luteinizing hormone, a macrophage activating factor, tumor necrosis factor, a neutrophil chemotactic factor, a nerve growth factor a tissue inhibitor of metalloproteinases; a vasoactive intestinal peptide, angiogenin, angiotropin, fibrin; hirudin; a leukemia inhibitory factor; an IL-1 receptor antagonist (e.g., Kineret® (anakinra)); an ion channel, e.g., cystic fibrosis transmembrane conductance regulator (CFTR); dystrophin; utrophin, a tumor suppressor; lysosomal enzyme acid α -glucosidase (GAA); and the like. Suitable nucleic acids also include those that encode a functional fragment of any of the aforementioned proteins; and nucleic acids that encode functional variants of any of the aforementioned proteins.

[00163] Suitable heterologous nucleic acids also include those that encode antigenic proteins. A subject rAAV vector that comprises a heterologous nucleic acid that encodes an antigenic protein is suitable for stimulating an immune response to the antigenic protein in a mammalian host. The antigenic protein is derived from an autoantigen, an allergen, a tumor/cancer-associated antigen, a pathogenic virus, a pathogenic bacterium, a pathogenic protozoan, a pathogenic helminth, or any other pathogenic organism that infects a mammalian host. As used herein, the term “a nucleic acid encoding an antigenic protein derived from” includes nucleic acids encoding wild-type antigenic proteins, e.g., a nucleic acid isolated from a pathogenic virus that encodes a viral protein; synthetic nucleic acids generated in the laboratory that encode antigenic proteins that are identical in amino acid sequence to a naturally-occurring antigenic protein; synthetic nucleic acids generated in the laboratory that encode antigenic proteins that differ in amino acid sequence (e.g., by from one amino acid to about 15 amino acids) from a naturally-occurring antigenic protein, but that nonetheless induce an

immune response to the corresponding naturally-occurring antigenic protein; synthetic nucleic acids generated in the laboratory that encode fragments of antigenic proteins (e.g., fragments of from about 5 amino acids to about 50 amino acids, which fragments comprises one or more antigenic epitopes), which fragments induce an immune response to the corresponding naturally-occurring antigenic protein; etc.

[00164] Similarly, an antigenic protein “derived from” an autoantigen, an allergen, a tumor/cancer-associated antigen, a pathogenic virus, a pathogenic bacterium, a pathogenic protozoan, a pathogenic helminth, or any other pathogenic organism that infects a mammalian host, includes proteins that are identical in amino acid sequence to a naturally-occurring antigenic protein, and proteins that differ in amino acid sequence (e.g., by from one amino acid to about 15 amino acids) from a naturally-occurring antigenic protein, but that nonetheless induce an immune response to the corresponding naturally-occurring antigenic protein; and fragments of antigenic proteins (e.g., fragments of from about 5 amino acids to about 100 amino acids, e.g., from about 5 to about 50 amino acids, which fragments comprises one or more antigenic epitopes), which fragments induce an immune response to the corresponding naturally-occurring antigenic protein.

[00165] In some embodiments, an immune response to an antigenic protein encoded by a subject rAAV vector will stimulate a protective immune response to a pathogenic organism that displays the antigenic protein or antigenic epitope (or a protein or an epitope that is cross-reactive with the rAAV-encoded antigenic protein or antigenic epitopes) in the mammalian host. In some embodiments, a cytotoxic T lymphocyte (CTL) response to the rAAV-encoded antigenic protein will be induced in the mammalian host. In other embodiments, a humoral response to the rAAV-encoded antigenic protein will be induced in the mammalian host, such that antibodies specific to the antigenic protein are generated. In many embodiments, a TH1 immune response to the rAAV-encoded antigenic protein will be induced in the mammalian host. Suitable antigenic proteins include tumor/cancer-associated antigens, viral antigens, bacterial antigens, and protozoal antigens; and antigenic fragments thereof. In some embodiments, the antigenic protein is derived from an intracellular pathogen. In other embodiments, the antigenic protein is a self-antigen. In yet other embodiments, the antigenic protein is an allergen.

- [00166] Tumor/cancer-specific antigens include, but are not limited to, any of the various MAGEs (Melanoma-Associated Antigen E), including MAGE 1 (e.g., GenBank Accession No. M77481), MAGE 2 (e.g., GenBank Accession No. U03735), MAGE 3, MAGE 4, etc.; any of the various tyrosinases; mutant ras; mutant p53 (e.g., GenBank Accession No. X54156 and AA494311); and p97 melanoma antigen (e.g., GenBank Accession No. M12154). Other tumor/cancer-specific antigens include the Ras peptide and p53 peptide associated with advanced cancers, the HPV 16/18 and E6/E7 antigens associated with cervical cancers, MUCII-KLH antigen associated with breast carcinoma (e.g., GenBank Accession No. J03651), CEA (carcinoembryonic antigen) associated with colorectal cancer (e.g., GenBank Accession No. X98311), gp100 (e.g., GenBank Accession No. S73003) or MART1 antigens associated with melanoma, and the PSA antigen associated with prostate cancer (e.g., GenBank Accession No. X14810). The p53 gene sequence is known (See e.g., Harris et al. (1986) Mol. Cell. Biol., 6:4650-4656) and is deposited with GenBank under Accession No. M14694. Thus, subject proteins, nucleic acids, and/or virions can be used as immunotherapeutics for cancers including, but not limited to, cervical, breast, colorectal, prostate, lung cancers, and for melanomas.
- [00167] Viral antigens are derived from known causative agents responsible for diseases including, but not limited to, measles, mumps, rubella, poliomyelitis, hepatitis A, B (e.g., GenBank Accession No. E02707), and C (e.g., GenBank Accession No. E06890), as well as other hepatitis viruses, influenza, adenovirus (e.g., types 4 and 7), rabies (e.g., GenBank Accession No. M34678), yellow fever, Japanese encephalitis (e.g., GenBank Accession No. E07883), dengue (e.g., GenBank Accession No. M24444), hantavirus, and human immunodeficiency virus (e.g., GenBank Accession No. U18552).
- [00168] Suitable bacterial and parasitic antigens include those derived from known causative agents responsible for diseases including, but not limited to, diphtheria, pertussis (e.g., GenBank Accession No. M35274), tetanus (e.g., GenBank Accession No. M64353), tuberculosis, bacterial and fungal pneumonias (e.g., Haemophilus influenzae, Pneumocystis carinii, etc.), cholera, typhoid, plague, shigellosis, salmonellosis (e.g., GenBank Accession No. L03833), Legionnaire's Disease, Lyme disease (e.g., GenBank Accession No. U59487), malaria (e.g., GenBank Accession No. X53832), hookworm, onchocerciasis (e.g., GenBank Accession No. M27807), schistosomiasis (e.g., GenBank Accession No. L08198), trypanosomiasis, leishmaniasis, giardiasis (e.g., GenBank

Accession No. M33641), amoebiasis, filariasis (e.g., GenBank Accession No. J03266), borreliosis, and trichinosis.

- [00169]** Suitable heterologous nucleic acids that encode heterologous gene products include non-translated RNAs, such as an RNAi agent (as described in greater detail above) (e.g., an antisense RNA; an siRNA; an shRNA; a double stranded RNA (dsRNA); a CRISPR agent, e.g., a Cas9 or Cas9-like protein, a crRNA-like RNA, a tracrRNA-like RNA, a single guide RNA, and/or a donor polynucleotide; and the like), a ribozyme, etc. RNAi agents can be used to inhibit gene expression. Some RNAi agents provide a tool that can be subsequently used to inhibit gene expression (e.g., a CRISPR agent such as a cas9 or cas9-like protein).
- [00170]** Target genes include any gene encoding a target gene product (RNA or protein) that is deleterious (e.g., pathological), for example, a target gene product that is malfunctioning (e.g., due to a mutation in the encoded protein sequence, due to a mutation in the non-coding sequences that control the steady state level of the gene product, etc.). Target gene products include, but are not limited to, huntingtin; hepatitis C virus; human immunodeficiency virus; amyloid precursor protein; tau; a protein that includes a polyglutamine repeat; a herpes virus (e.g., varicella zoster); any pathological virus; and the like.
- [00171]** As such a subject rAAV that includes a heterologous nucleic acid encoding an RNAi agent is useful for treating a variety of disorders and conditions, including, but not limited to, neurodegenerative diseases, e.g., a trinucleotide-repeat disease, such as a disease associated with polyglutamine repeats, e.g., Huntington's disease, spinocerebellar ataxia, spinal and bulbar muscular atrophy (SBMA), dentatorubropallidoluysian atrophy (DRPLA), etc.; an acquired pathology (e.g., a disease or syndrome manifested by an abnormal physiological, biochemical, cellular, structural, or molecular biological state) such as a viral infection, e.g., hepatitis that occurs or may occur as a result of an HCV infection, acquired immunodeficiency syndrome, which occurs as a result of an HIV infection; cancer; and the like.
- [00172]** In many embodiments, a heterologous nucleic acid encoding an RNAi agent is operably linked to a promoter. Suitable promoters are known those skilled in the art and include the promoter of any protein-encoding gene, e.g., an endogenously regulated gene or a constitutively expressed gene. For example, the promoters of genes regulated by

cellular physiological events, e.g., heat shock, oxygen levels and/or carbon monoxide levels, e.g., in hypoxia, may be operably linked to an siRNA-encoding nucleic acid.

[00173] The selected heterologous nucleotide sequence, such as EPO-encoding or nucleic acid of interest, is operably linked to control elements that direct the transcription or expression thereof in the nucleotide sequence *in vivo*. Such control elements can comprise control sequences normally associated with the selected gene (e.g., endogenous cellular control elements). Alternatively, heterologous control sequences can be employed. Useful heterologous control sequences generally include those derived from sequences encoding mammalian or viral genes. Examples include, but are not limited to, the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, an endogenous cellular promoter that is heterologous to the gene of interest, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like. In addition, sequences derived from nonviral genes, such as the murine metallothionein gene, will also find use herein. Such promoter sequences are commercially available from, e.g., Stratagene (San Diego, Calif.).

[00174] In some embodiments, cell type-specific or tissue-specific promoter will be operably linked to the heterologous nucleic acid encoding the heterologous gene product, such that the gene product is produced selectively or preferentially in a particular cell type(s) or tissue(s). In some embodiments, an inducible promoter will be operably linked to the heterologous nucleic acid.

[00175] For example, muscle-specific and inducible promoters, enhancers and the like, are useful for delivery of a gene product to a muscle cell. Such control elements include, but are not limited to, those derived from the actin and myosin gene families, such as from the myoD gene family; the myocyte-specific enhancer binding factor MEF-2; control elements derived from the human skeletal actin gene and the cardiac actin gene; muscle creatine kinase sequence elements and the murine creatine kinase enhancer (mCK) element; control elements derived from the skeletal fast-twitch troponin C gene, the slow-twitch cardiac troponin C gene and the slow-twitch troponin I gene; hypoxia-inducible nuclear factors; steroid-inducible elements and promoters, such as the

glucocorticoid response element (GRE); the fusion consensus element for RU486 induction; and elements that provide for tetracycline regulated gene expression.

[00176] The AAV expression vector which harbors the DNA molecule of interest (the heterologous DNA) bounded by AAV ITRs, can be constructed by directly inserting the selected sequence(s) into an AAV genome which has had the major AAV open reading frames ("ORFs") excised therefrom. Other portions of the AAV genome can also be deleted, so long as a sufficient portion of the ITRs remain to allow for replication and packaging functions. Such constructs can be designed using techniques well known in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published Jan. 23, 1992) and WO 93/03769 (published March 4, 1993); Lebkowski et al. (1988) *Molec. Cell. Biol.* 8:3988-3996; Vincent et al. (1990) *Vaccines 90* (Cold Spring Harbor Laboratory Press); Carter, B. J. (1992) *Current Opinion in Biotechnology* 3:533-539; Muzyczka, N. (1992) *Current Topics in Microbiol. and Immunol.* 158:97-129; Kotin, R. M. (1994) *Human Gene Therapy* 5:793-801; Shelling and Smith (1994) *Gene Therapy* 1:165-169; and Zhou et al. (1994) *J. Exp. Med.* 179:1867-1875.

[00177] Alternatively, AAV ITRs can be excised from the viral genome or from an AAV vector containing the same and fused 5' and 3' of a selected nucleic acid construct that is present in another vector using any convenient method known to one of ordinary skill in the art. For example, one suitable approach uses standard ligation techniques, such as those described in Sambrook et al., *supra*. For example, ligations can be accomplished in 20 mM Tris-Cl pH 7.5, 10 mM MgCl₂, 10 mM DTT, 33 µg/ml BSA, 10 mM-50 mM NaCl, and either 40 µM ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0 °C to 16° C (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14° C (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 30-100 µg/ml total DNA concentrations (5-100 nM total end concentration). AAV vectors which contain ITRs have been described in, e.g., U.S. Pat. No. 5,139,941. In particular, several AAV vectors are described therein which are available from the American Type Culture Collection ("ATCC") under Accession Numbers 53222, 53223, 53224, 53225 and 53226.

[00178] Additionally, chimeric genes can be produced synthetically to include AAV ITR sequences arranged 5' and 3' of one or more selected nucleic acid sequences. Preferred

codons for expression of the chimeric gene sequence in mammalian muscle cells can be used. The complete chimeric sequence is assembled from overlapping oligonucleotides prepared by standard methods. See, e.g., Edge, Nature (1981) 292:756; Nambair et al. Science (1984) 223:1299; Jay et al. J. Biol. Chem. (1984) 259:6311.

GENERATION OF SUBJECT INFECTIOUS rAAV VIRIONS

[00179] By way of introduction, it is typical to employ a host or "producer" cell for rAAV vector replication and packaging. Such a producer cell (usually a mammalian host cell) generally comprises or is modified to comprise several different types of components for rAAV production. The first component is a recombinant adeno-associated viral (rAAV) vector genome (or "rAAV pro-vector") that can be replicated and packaged into vector particles by the host packaging cell. The rAAV pro-vector will normally comprise a heterologous polynucleotide (or "transgene"), with which it is desired to genetically alter another cell in the context of gene therapy (since the packaging of such a transgene into rAAV vector particles can be effectively used to deliver the transgene to a variety of mammalian cells). The transgene is generally flanked by two AAV inverted terminal repeats (ITRs) which comprise sequences that are recognized during excision, replication and packaging of the AAV vector, as well as during integration of the vector into a host cell genome.

[00180] A second component is a helper virus that can provide helper functions for AAV replication. Although adenovirus is commonly employed, other helper viruses can also be used as is known in the art. Alternatively, the requisite helper virus functions can be isolated genetically from a helper virus and the encoding genes can be used to provide helper virus functions in trans. The AAV vector elements and the helper virus (or helper virus functions) can be introduced into the host cell either simultaneously or sequentially in any order.

[00181] The final components for AAV production to be provided in the producer cell are "AAV packaging genes" such as AAV rep and cap genes that provide replication and encapsidation proteins, respectively. Several different versions of AAV packaging genes can be provided (including rep-cap cassettes and separate rep and/or cap cassettes in which the rep and/or cap genes can be left under the control of the native promoters or operably linked to heterologous promoters. Such AAV packaging genes can be

introduced either transiently or stably into the host packaging cell, as is known in the art and described in more detail below.

1. rAAV vector

[00182] A subject rAAV virion, including the heterologous DNA of interest (where “heterologous DNA of interest” is also referred to herein as “heterologous nucleic acid”), can be produced using standard methodology, known to those of skill in the art. The methods generally involve the steps of (1) introducing a subject rAAV vector into a host cell; (2) introducing an AAV helper construct into the host cell, where the helper construct includes AAV coding regions capable of being expressed in the host cell to complement AAV helper functions missing from the AAV vector; (3) introducing one or more helper viruses and/or accessory function vectors into the host cell, wherein the helper virus and/or accessory function vectors provide accessory functions capable of supporting efficient recombinant AAV ("rAAV") virion production in the host cell; and (4) culturing the host cell to produce rAAV virions. The AAV expression vector, AAV helper construct and the helper virus or accessory function vector(s) can be introduced into the host cell, either simultaneously or serially, using standard transfection techniques.

[00183] AAV expression vectors are constructed using known techniques to at least provide as operatively linked components in the direction of transcription, control elements including a transcriptional initiation region, the DNA of interest and a transcriptional termination region. The control elements are selected to be functional in a mammalian muscle cell. The resulting construct which contains the operatively linked components is bounded (5' and 3') with functional AAV ITR sequences.

[00184] The nucleotide sequences of AAV ITR regions are known. See, e.g., Kotin, R. M. (1994) Human Gene Therapy 5:793-801; Berns, K. I. "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.) for the AAV-2 sequence. AAV ITRs used in the vectors of the invention need not have a wild-type nucleotide sequence, and may be altered, e.g., by the insertion, deletion or substitution of nucleotides. Additionally, AAV ITRs may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-7, etc. Furthermore, 5' and 3' ITRs which flank a selected nucleotide sequence in an AAV expression vector need not necessarily be identical or derived from the same

AAV serotype or isolate, so long as they function as intended, i.e., to allow for excision and rescue of the sequence of interest from a host cell genome or vector, and to allow integration of the DNA molecule into the recipient cell genome when AAV Rep gene products are present in the cell. ITRs allow replication of the vector sequence in the presence of an appropriate mixture of Rep proteins. ITRs also allow for the incorporation of the vector sequence into the capsid to generate an AAV particle.

[00185] In order to produce rAAV virions, an AAV expression vector is introduced into a suitable host cell using known techniques, such as by transfection. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*, 52:456, Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu et al. (1981) *Gene* 13:197. Particularly suitable transfection methods include calcium phosphate co-precipitation (Graham et al. (1973) *Virol.* 52:456-467), direct micro-injection into cultured cells (Capecchi, M. R. (1980) *Cell* 22:479-488), electroporation (Shigekawa et al. (1988) *BioTechniques* 6:742-751), liposome mediated gene transfer (Mannino et al. (1988) *BioTechniques* 6:682-690), lipid-mediated transduction (Felgner et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7417), and nucleic acid delivery using high-velocity microprojectiles (Klein et al. (1987) *Nature* 327:70-73).

[00186] For the purposes of this disclosure, suitable host cells for producing rAAV virions include microorganisms, yeast cells, insect cells, and mammalian cells, that can be, or have been, used as recipients of a heterologous DNA molecule. The term includes the progeny of the original cell which has been transfected. Thus, a "host cell" for producing rAAV virions generally refers to a cell which has been transfected with an exogenous DNA sequence. Cells from the stable human cell line, 293 (readily available through, e.g., the American Type Culture Collection under Accession Number ATCC CRL1573) are used in many embodiments. Particularly, the human cell line 293 is a human embryonic kidney cell line that has been transformed with adenovirus type-5 DNA fragments (Graham et al. (1977) *J. Gen. Virol.* 36:59), and expresses the adenoviral E1a and E1b genes (Aiello et al. (1979) *Virology* 94:460). The 293 cell line is readily transfected, and provides a particularly convenient platform in which to produce rAAV virions.

2. AAV Helper Functions

[00187] Host cells containing the above-described AAV expression vectors must be rendered capable of providing AAV helper functions in order to replicate and encapsidate the nucleotide sequences flanked by the AAV ITRs to produce rAAV virions. AAV helper functions are generally AAV-derived coding sequences which can be expressed to provide AAV gene products that, in turn, function in trans for productive AAV replication. AAV helper functions are used herein to complement necessary AAV functions that are missing from the AAV expression vectors. Thus, AAV helper functions include one, or both of the major AAV ORFs, namely the rep and cap coding regions, or functional homologues thereof. In the context of the instant disclosure, the cap functions include one or more mutant capsid proteins, wherein at least one capsid protein comprises at least one mutation, as described above.

[00188] By "AAV rep coding region" is meant the art-recognized region of the AAV genome which encodes the replication proteins Rep 78, Rep 68, Rep 52 and Rep 40. These Rep expression products have been shown to possess many functions, including recognition, binding and nicking of the AAV origin of DNA replication, DNA helicase activity and modulation of transcription from AAV (or other heterologous) promoters. The Rep expression products are collectively required for replicating the AAV genome. For a description of the AAV rep coding region, see, e.g., Muzyczka, N. (1992) *Current Topics in Microbiol. and Immunol.* 158:97-129; and Kotin, R. M. (1994) *Human Gene Therapy* 5:793-801. Suitable homologues of the AAV rep coding region include the human herpesvirus 6 (HHV-6) rep gene which is also known to mediate AAV-2 DNA replication (Thomson et al. (1994) *Virology* 204:304-311).

[00189] AAV cap proteins include VP1, VP2, and VP3, wherein at least one of VP1, VP2, and VP3 comprises at least one mutation, as described above.

[00190] AAV helper functions are introduced into the host cell by transfecting the host cell with an AAV helper construct either prior to, or concurrently with, the transfection of the AAV expression vector. AAV helper constructs are thus used to provide at least transient expression of AAV rep and/or cap genes to complement missing AAV functions that are necessary for productive AAV infection. AAV helper constructs lack AAV ITRs and can neither replicate nor package themselves. These constructs can be in the form of a plasmid, phage, transposon, cosmid, virus, or virion. A number of AAV

helper constructs have been described, such as the commonly used plasmids pAAV/Ad and pIM29+45 which encode both Rep and Cap expression products. See, e.g., Samulski et al. (1989) J. Virol. 63:3822-3828; and McCarty et al. (1991) J. Virol. 65:2936-2945. A number of other vectors have been described which encode Rep and/or Cap expression products. See, e.g., U.S. Pat. No. 5,139,941.

[00191] Both AAV expression vectors and AAV helper constructs can be constructed to contain one or more optional selectable markers. Suitable markers include genes which confer antibiotic resistance or sensitivity to, impart color to, or change the antigenic characteristics of those cells which have been transfected with a nucleic acid construct containing the selectable marker when the cells are grown in an appropriate selective medium. Several selectable marker genes that are useful in practicing methods of the disclosure include the hygromycin B resistance gene (encoding Aminoglycoside phosphotransferase (APH)) that allows selection in mammalian cells by conferring resistance to hygromycin; the neomycin phosphotransferase gene (encoding neomycin phosphotransferase) that allows selection in mammalian cells by conferring resistance to G418; and the like. Other suitable markers are known to those of skill in the art.

3. AAV Accessory Functions

[00192] The host cell (or packaging cell) must also be rendered capable of providing non AAV derived functions, or "accessory functions," in order to produce rAAV virions. Accessory functions are non AAV derived viral and/or cellular functions upon which AAV is dependent for its replication. Thus, accessory functions include at least those non AAV proteins and RNAs that are required in AAV replication, including those involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of Cap expression products and AAV capsid assembly. Viral-based accessory functions can be derived from any of the known helper viruses.

[00193] Particularly, accessory functions can be introduced into and then expressed in host cells using methods known to those of skill in the art. Commonly, accessory functions are provided by infection of the host cells with an unrelated helper virus. A number of suitable helper viruses are known, including adenoviruses; herpesviruses such as herpes simplex virus types 1 and 2; and vaccinia viruses. Nonviral accessory functions will also find use herein, such as those provided by cell synchronization using any of various known agents. See, e.g., Buller et al. (1981) J. Virol. 40:241-247;

McPherson et al. (1985) *Virology* 147:217-222; Schlehofer et al. (1986) *Virology* 152:110-117.

[00194] Alternatively, accessory functions can be provided using an accessory function vector. Accessory function vectors include nucleotide sequences that provide one or more accessory functions. An accessory function vector is capable of being introduced into a suitable host cell in order to support efficient AAV virion production in the host cell. Accessory function vectors can be in the form of a plasmid, phage, transposon, cosmid, or another virus. Accessory vectors can also be in the form of one or more linearized DNA or RNA fragments which, when associated with the appropriate control elements and enzymes, can be transcribed or expressed in a host cell to provide accessory functions.

[00195] Nucleic acid sequences providing the accessory functions can be obtained from natural sources, such as from the genome of an adenovirus particle, or constructed using recombinant or synthetic methods known in the art. In this regard, adenovirus-derived accessory functions have been widely studied, and a number of adenovirus genes involved in accessory functions have been identified and partially characterized. See, e.g., Carter, B. J. (1990) "Adeno-Associated Virus Helper Functions," in *CRC Handbook of Parvoviruses*, vol. I (P. Tijssen, ed.), and Muzyczka, N. (1992) *Curr. Topics. Microbiol. and Immun.* 158:97-129. Specifically, early adenoviral gene regions E1a, E2a, E4, VAI RNA and, possibly, E1b are thought to participate in the accessory process. Janik et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:1925-1929. Herpesvirus-derived accessory functions have been described. See, e.g., Young et al. (1979) *Prog. Med. Virol.* 25:113. Vaccinia virus-derived accessory functions have also been described. See, e.g., Carter, B. J. (1990), *supra.*, Schlehofer et al. (1986) *Virology* 152:110-117.

[00196] As a consequence of the infection of the host cell with a helper virus, or transfection of the host cell with an accessory function vector, accessory functions are expressed which transactivate the AAV helper construct to produce AAV Rep and/or Cap proteins. The Rep expression products excise the recombinant DNA (including the DNA of interest, e.g., the heterologous nucleic acid) from the AAV expression vector. The Rep proteins also serve to duplicate the AAV genome. The expressed Cap proteins

assemble into capsids, and the recombinant AAV genome is packaged into the capsids.

Thus, productive AAV replication ensues, and the DNA is packaged into rAAV virions.

[00197] Following recombinant AAV replication, rAAV virions can be purified from the host cell using a variety of conventional purification methods, such as CsCl gradients. Further, if infection is employed to express the accessory functions, residual helper virus can be inactivated, using known methods. For example, adenovirus can be inactivated by heating to temperatures of approximately 60° C. for, e.g., 20 minutes or more. This treatment effectively inactivates only the helper virus since AAV is extremely heat stable while the helper adenovirus is heat labile.

[00198] The resulting rAAV virions are then ready for use for DNA delivery, such as in gene therapy applications, or for the delivery of a gene product to a mammalian host.

DELIVERING A HETEROLOGOUS NUCLEIC ACID

[00199] The present disclosure further provides methods of delivering a heterologous nucleic acid to a target cell and/or to an individual in need thereof. In some embodiments, an individual in need thereof is a human who has previously been naturally exposed to AAV and as a result harbors anti-AAV antibodies (i.e., AAV neutralizing antibodies). Based on positive results in clinical trials involving AAV gene delivery to, for example, liver, muscle, and retina - all tissues affected by neutralizing antibodies against this vehicle - there are many such therapeutic applications/disease targets.

[00200] A subject method generally involves: (i) administering an effective amount of a subject rAAV virion to an individual, and/or (ii) contacting a target cell with a subject virion. Generally, rAAV virions are administered to a subject using either *in vivo* ("direct") or *in vitro* ("indirect") transduction techniques. If transduced *in vitro* ("indirectly"), a desired recipient cell (i.e., "target cell") can be removed from the individual, transduced with rAAV virions and reintroduced into the individual. Alternatively, syngeneic or xenogeneic cells can be used where those cells will not generate an inappropriate immune response in the individual.

[00201] Suitable methods for the delivery and introduction of transduced target cells into an individual have been described. For example, cells can be transduced *in vitro* by combining recombinant AAV virions with cells e.g., in appropriate media, and screening

for those cells harboring the DNA of interest using conventional techniques such as Southern blots and/or PCR, or by using selectable markers. Transduced cells can then be formulated into pharmaceutical compositions, described more fully below, and the composition introduced into the subject by various techniques, such as by intramuscular, intravenous, subcutaneous and intraperitoneal injection.

[00202] For *in vivo* (i.e., “direct”) delivery, the rAAV virions will be formulated into pharmaceutical compositions and will generally be administered parenterally (e.g., administered via an intramuscular, subcutaneous, intratumoral, transdermal, intrathecal, intravenous, etc.) route of administration.

[00203] Pharmaceutical compositions will comprise sufficient genetic material to produce a therapeutically effective amount of the gene expression product of interest, i.e., an amount sufficient to reduce or ameliorate symptoms of the disease state in question or an amount sufficient to confer the desired benefit. The pharmaceutical compositions will also contain a pharmaceutically acceptable excipient. Such excipients include any pharmaceutical agent that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) “Remington: The Science and Practice of Pharmacy,” 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

[00204] Appropriate doses will depend on the mammal being treated (e.g., human or nonhuman primate or other mammal), age and general condition of the subject to be

treated, the severity of the condition being treated, the particular therapeutic protein in question, its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art.

[00205] Thus, a "therapeutically effective amount" will fall in a relatively broad range that can be determined through clinical trials. For example, for *in vivo* injection, i.e., injection directly to skeletal or cardiac muscle, a therapeutically effective dose will be on the order of from about 10^6 to about 10^{15} of the rAAV virions, e.g., from about 10^8 to 10^{12} rAAV virions. For *in vitro* transduction, an effective amount of rAAV virions to be delivered to cells will be on the order of from about 10^8 to about 10^{13} of the rAAV virions. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

[00206] Dosage treatment may be a single dose schedule or a multiple dose schedule. Moreover, the subject may be administered as many doses as appropriate. One of skill in the art can readily determine an appropriate number of doses.

[00207] The cells of interest (i.e., "target cells") are typically mammalian, where the term refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, laboratory, sports, or pet animals, such as dogs, horses, cats, cows, mice, rats, rabbits, etc. In some embodiments, the target cell is a human cell.

[00208] Target cells of interest include any cell susceptible to infection by a subject rAAV virion. In some cases, e.g., when the method is a method of delivering a heterologous nucleic acid to a target cell, the target cell can be a cell removed from an individual (e.g., a "primary" cell), or the target cell can be a tissue culture cell (e.g., from an established cell line).

[00209] Exemplary target cells include, but are not limited to, liver cells, pancreatic cells (e.g., islet cells: alpha cells, beta cells, delta cells, gamma cells, and/or epsilon cells), skeletal muscle cells, heart muscle cells, fibroblasts, retinal cells, synovial joint cells, lung cells, T cells, neurons, glial cells, stem cells, hematopoietic progenitor cells, neural progenitor cells, endothelial cells, and cancer cells. Exemplary stem cell target cells include, but are not limited to, hematopoietic stem cells, neural stem cells, neural crest stem cells, embryonic stem cells, induced pluripotent stem cells (iPS cells), mesenchymal stem cells, mesodermal stem cells, liver stem cells, pancreatic stem cells, muscle stem cells, and retinal stem cells.

- [00210] The term “stem cell” is used herein to refer to a mammalian cell that has the ability both to self-renew, and to generate differentiated progeny (see, e.g., Morrison et al. (1997) *Cell* 88:287-298). Generally, stem cells also have one or more of the following properties: an ability to undergo asynchronous, or symmetric replication, that is where the two daughter cells after division can have different phenotypes; extensive self-renewal capacity; capacity for existence in a mitotically quiescent form; and clonal regeneration of all the tissue in which they exist, for example the ability of hematopoietic stem cells to reconstitute all hematopoietic lineages. As is appreciated by one of ordinary skill in the art, “progenitor cells” differ from stem cells in that they typically do not have the extensive self-renewal capacity, and often can generate a more restricted subset of the lineages in the tissue from which they derive, for example only lymphoid, or erythroid lineages in a hematopoietic setting. As used herein, the term “stem cell” encompasses both “stem cells” and “progenitor cells” as defined above.
- [00211] Stem cells may be characterized by both the presence of markers associated with specific epitopes identified by antibodies and the absence of certain markers as identified by the lack of binding of specific antibodies. Stem cells may also be identified by functional assays both *in vitro* and *in vivo*, particularly assays relating to the ability of stem cells to give rise to multiple differentiated progeny.
- [00212] Suitable stem cells of interest include, but are not limited to: hematopoietic stem cells and progenitor cells derived therefrom (U.S. Pat. No. 5,061,620); neural crest stem cells (see Morrison et al. (1999) *Cell* 96:737-749); neural stem cells and neural progenitor cells; embryonic stem cells; mesenchymal stem cells; mesodermal stem cells; liver stem cells, muscle stem cells, retinal stem cells, induced pluripotent stem cells (iPS cells), etc. Other hematopoietic “progenitor” cells of interest include cells dedicated to lymphoid lineages, e.g. immature T cell and B cell populations.
- [00213] Purified populations of stem or progenitor cells may be used. For example, human hematopoietic stem cells may be positively selected using antibodies specific for CD34, thy-1; or negatively selected using lineage specific markers which may include glycophorin A, CD3, CD24, CD16, CD14, CD38, CD45RA, CD36, CD2, CD19, CD56, CD66a, and CD66b; T cell specific markers, tumor/cancer specific markers, etc. Markers useful for the separation of mesodermal stem cells include FcγRII, FcγRIII, Thy-1, CD44, VLA-4α, LFA-1β, HSA, ICAM-1, CD45, Aa4.1, Sca-1, etc. Neural crest stem

cells may be positively selected with antibodies specific for low-affinity nerve growth factor receptor (LNGFR), and negatively selected for the markers sulfatide, glial fibrillary acidic protein (GFAP), myelin protein P₀, peripherin and neurofilament. Human mesenchymal stem cells may be positively separated using the markers SH2, SH3 and SH4.

[00214] Target cells which are employed may be fresh, frozen, or have been subject to prior culture. They may be fetal, neonate, adult. Hematopoietic cells may be obtained from fetal liver, bone marrow, blood, particularly G-CSF or GM-CSF mobilized peripheral blood, or any other conventional source. The manner in which stem cells are separated from other cells of the hematopoietic or other lineage is not critical to this disclosure. As described above, a substantially homogeneous population of stem or progenitor cells may be obtained by selective isolation of cells free of markers associated with differentiated cells, while displaying epitopic characteristics associated with the stem cells.

[00215] Nucleic acids that can be delivered to an individual include any of the above defined heterologous nucleic acids. Proteins that can be delivered using a subject method also include a functional fragment of any of the aforementioned proteins; and functional variants of any of the aforementioned proteins.

[00216] In some embodiments, a therapeutically effective amount of a protein is produced in the mammalian host. Whether a therapeutically effective amount of a particular protein is produced in the mammalian host using a subject method is readily determined using assays appropriate to the particular protein. For example, where the protein is EPO, hematocrit is measured.

[00217] Where the rAAV encodes an antigenic protein, suitable antigenic proteins that can be delivered to an individual using a subject method include, but are not limited to, tumor/cancer-associated antigens, autoantigens ("self" antigens), viral antigens, bacterial antigens, protozoal antigens, and allergens; and antigenic fragments thereof. In some embodiments, a cytotoxic T lymphocyte (CTL) response to the rAAV-encoded antigenic protein will be induced in the mammalian host. In other embodiments, a humoral response to the rAAV-encoded antigenic protein will be induced in the mammalian host, such that antibodies specific to the antigenic protein are generated. In many embodiments, a TH1 immune response to the rAAV-encoded antigenic protein will be

induced in the mammalian host. Whether an immune response to the antigenic protein has been generated is readily determined using well-established methods. For example, an enzyme-linked immunosorbent assay can be used to determine whether antibody to an antigenic protein has been generated. Methods of detecting antigen-specific CTL are well known in the art. For example, a detectably labeled target cell expressing the antigenic protein on its surface is used to assay for the presence of antigen-specific CTL in a blood sample.

[00218] Whether a therapeutically effective amount of a heterologous nucleic acid (e.g., a nucleic acid encoding a polypeptide, an RNAi agent, etc.) has been delivered to a mammalian host using a subject method is readily determined using any appropriate assay. For example, where the gene product is an RNAi agent that inhibits HIV, viral load can be measured.

METHODS OF GENERATING AND IDENTIFYING MODIFIED rAAV VIRIONS

[00219] The present disclosure provides a method of generating and identifying a modified infectious recombinant adeno-associated virus (rAAV) virion that comprises a variant capsid protein comprising an amino acid sequence with at least one amino acid substitution (including deletions, insertions, etc.) compared to a starter AAV capsid protein. A starter AAV capsid protein comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33.

[00220] The method generally involves generating a mutant rAAV virion library; and selecting the library for modified rAAV virions with altered properties relative to a starter rAAV virion. The starter rAAV virion comprises a variant AAV capsid protein that comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33. The present disclosure further provides libraries and compositions comprising the libraries.

[00221] In some embodiments, a given selection step is repeated two, three, four, or more times to enrich a subject AAV library for altered virion properties. In some embodiments, following selection of an AAV library, individual clones are isolated and sequenced.

Generation of a mutant AAV library

- [00222] A mutant AAV library is generated that comprises one or more mutations relative to a starter AAV *cap* gene. A starter *cap* gene is a *cap* comprising a nucleotide sequence that encodes a variant AAV capsid protein that comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33. Mutations in the rAAV *cap* gene are generated using any known method. Suitable methods for mutagenesis of a starter AAV *cap* gene include, but are not limited to, a polymerase chain reaction (PCR)-based method, oligonucleotide-directed mutagenesis, saturation mutagenesis, loop-swapping mutagenesis, fragment shuffling mutagenesis (i.e., DNA shuffling), and the like. Methods for generating mutations are well described in the art. See, e.g., Zhao et al. Nat Biotechnol. 1998 Mar;16(3):234-5; Koerber et. al.; Mol Ther. 2008 Oct;16(10):1703-9; Koerber et. al.; Mol Ther. 2009 Dec;17(12):2088-95; U.S. Patent No. 6,579,678; U.S. Patent No. 6,573,098; and U.S. Patent No. 6,582,914; all of which are hereby incorporated by reference for their teachings related to mutagenesis.
- [00223] In some embodiments, a mutant AAV library comprising mutations in the *cap* gene will be generated using a staggered extension process. The staggered extension process involves amplification of the *cap* gene using a PCR-based method. The template *cap* gene is primed using specific PCR primers, followed by repeated cycles of denaturation and very short annealing/polymerase-catalyzed extension. In each cycle, the growing fragments anneal to different templates based on sequence complementarity and extend further. The cycles of denaturation, annealing, and extension are repeated until full-length sequences form. The resulting full-length sequences include at least one mutation in the *cap* gene compared to a wild-type AAV *cap* gene.
- [00224] The PCR products comprising AAV *cap* sequences that include one or more mutations are inserted into a plasmid containing a wild-type AAV genome. The result is a library of AAV *cap* mutants. Thus, the present disclosure provides a mutant AAV *cap* gene library comprising from about 10 to about 10^{10} members, and comprising mutations in the AAV *cap* gene. A given member of the library has from about one to about 50 mutations in the AAV *cap* gene. A subject library comprises from 10 to about 10^9 distinct members, each having a different mutation(s) in the AAV *cap* gene.
- [00225] Once a *cap* mutant library is generated, viral particles are produced that can then be selected on the basis of altered capsid properties. Library plasmid DNA is transfected

into a suitable host cell (e.g., 293 cells), followed by introduction into the cell of helper virus. Viral particles produced by the transfected host cells (rAAV library particles) are collected.

Library selection

[00226] Once a library is generated, it is selected for a particular virion property (i.e., an altered property of infection). Viral particles are generated as discussed above (thus producing a library of modified rAAV virions), and subjected to one or more selection steps to identify a modified rAAV virion with an altered property of infection (relative to an infectious rAAV virion comprising a variant capsid protein that comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33). Properties of infection that are selected for can include, but are not limited to: 1) altered binding (e.g., decreased binding) to AAV neutralizing antibodies; 2) increased evasion of AAV neutralizing antibodies; 3) increased infectivity of a cell that is resistant to infection with AAV; and 4) altered heparin binding.

1. Selection for reduced binding to AAV neutralizing antibodies

[00227] In some embodiments, a subject AAV library is selected for altered (e.g., reduced) binding to neutralizing antibodies that bind to and neutralize wild-type AAV virions, compared to the binding of such antibodies to wild-type AAV virions and neutralization of wild-type AAV virions (or relative to an infectious rAAV virion comprising a variant capsid protein that comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33). AAV library particles (AAV library virion) are contacted with neutralizing antibodies and the ability of the AAV library particles to infect a permissive host cell is tested. Typically, AAV library particles are contacted with various concentrations of neutralizing antibodies. The higher the concentration of neutralizing antibodies that is required to reduce infectivity of the AAV library particles, the more resistant the AAV particles are to neutralization. Any convenient assay known to one of ordinary skill in the art may be used to directly measure the binding (e.g., measure the binding affinity) of an AAV library virion to neutralizing anti-AAV antibodies.

2. Selection for increased evasion of AAV neutralizing antibodies

[00228] In some embodiments, a subject AAV library is selected for increased evasion of neutralizing antibodies (i.e. increased resistance to human neutralizing AAV antibodies) relative to an infectious rAAV virion comprising a variant capsid protein that comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33. AAV library particles are contacted with target cells in the presence of neutralizing AAV antibodies (usually human neutralizing anti-AAV antibodies). After a suitable amount of time to allow for infection of the cells with AAV library particles, helper virus is added, and AAV library particles that successfully infected the cell(s) are harvested. In some embodiments, infectivity is measured (e.g., as described above) for those virions exhibiting successful infection. In some embodiments, the cycle of infection, addition of helper virus, and harvesting of AAV particles is repeated one, two, three, or more times. The selection can occur with varying amounts (concentrations) of neutralizing AAV antibodies to select for various degrees of evasion (e.g., each repeated round can utilize an increased concentration of antibodies relative to the previous round).

3. Selection for increased infectivity of non-permissive cells

[00229] In some embodiments, a subject AAV library is selected for increased infectivity of non-permissive cells (relative to an infectious rAAV virion comprising a variant capsid protein that comprises an amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33). AAV library particles are contacted with a non-permissive cell (e.g., a population of non-permissive cells). After a suitable amount of time to allow for infection of the cells with AAV library particles, helper virus is added, and AAV library particles that successfully infected the non-permissive cell(s) are harvested. In some embodiments, the cycle of infection, addition of helper virus, and harvesting of AAV particles is repeated one, two, three, or more times.

4. Selection for altered heparin binding

[00230] In some embodiments, a subject library is selected for altered heparin binding, including increased heparin binding and decreased heparin binding relative to wild-type AAV virion heparin binding (or relative to an infectious rAAV virion comprising a variant capsid protein that comprises an amino acid sequence set forth in one of SEQ ID

NOs: 10-13 and 26-33). AAV library particles are contacted with a heparin affinity matrix. For example, AAV library particles are loaded onto a heparin affinity column under conditions that permit binding of the AAV library particles to the heparin. Exemplary conditions include equilibration of the column with 0.15 M NaCl and 50 mM Tris at pH 7.5. After allowing the AAV library particle to bind to the heparin affinity matrix, the AAV library particle/heparin affinity matrix complex is washed with volumes of buffer containing progressively increasing concentrations of NaCl, and at each NaCl concentration, eluted AAV library particles are collected. For example, after binding the AAV library particle/heparin affinity matrix complex is washed with a volume of 50 mM Tris buffer, pH 7.5, containing 200 mM NaCl, and eluted AAV library particles are collected. The elution step is repeated with a 50 mM Tris buffer, pH 7.5, containing about 250 mM NaCl, about 300 mM NaCl, about 350 mM, about 400 mM NaCl, about 450 mM NaCl, about 500 mM NaCl, about 550 mM NaCl, about 600 mM NaCl, about 650 mM NaCl, about 700 mM NaCl, or about 750 mM NaCl.

[00231] AAV library particles that elute at NaCl concentrations lower than about 450 mM NaCl exhibit decreased heparin binding properties relative to wild-type AAV. AAV library particles that elute at NaCl concentrations higher than about 550 mM NaCl exhibit increased heparin binding properties relative to wild-type AAV.

[00232] In some embodiments, eluted AAV library particles are amplified by co-infection of permissive cells with a helper virus, and are re-fractionated on heparin affinity matrix. This step can be repeated a number of times to enrich for AAV library particles with altered heparin binding properties.

[00233] In the present methods, one or more selection steps may follow generation of AAV library particles. For example, in some embodiments, the method comprises selecting for increased heparin binding, followed by selecting for decreased binding to neutralizing antibodies. In other embodiments, the method comprises selecting for decreased binding to neutralizing antibodies, followed by selecting for increased heparin binding. In other embodiments, the method comprises selecting for decreased heparin binding, followed by selecting for decreased binding to neutralizing antibodies. In other embodiments, the method comprises selecting for decreased binding to neutralizing antibodies, followed by selecting for decreased heparin binding. In other embodiments,

the method comprises selecting for decreased binding to neutralizing antibodies, followed by selecting for increased infectivity of a stem cell. In other embodiments, the method comprises selecting for decreased binding to neutralizing antibodies, followed by selecting for increased evasion of neutralizing antibodies. In other embodiments, the method comprises selecting for increased evasion of neutralizing antibodies, followed by selecting for decreased binding to neutralizing antibodies.

[00234] Thus, the present disclosure provides an adeno-associated virus (AAV) library that includes a plurality of nucleic acids, each of which nucleic acid includes a nucleotide sequence that encodes a variant AAV capsid protein. The encoded variant AAV capsid protein includes at least one amino acid substitution relative to a sequence set forth in one of SEQ ID NOs: 10-13 and 26-33. The present disclosure provides a library of mutant adeno-associated virus (AAV) particles, including a plurality of AAV particles each of which includes an AAV capsid protein that includes at least one amino acid substitution relative to a sequence set forth in one of SEQ ID NOs: 10-13 and 26-33. Nucleic acids encoding mutant AAV capsid proteins are described above, as are the properties of the encoded mutant AAV capsid proteins.

[00235] The present disclosure further provides a library comprising at least one of: (i) two or more infectious rAAV virions, each comprising a variant adeno-associated virus (AAV) capsid protein and a heterologous nucleic acid; (ii) two or more isolated nucleic acids, each comprising a nucleotide sequence that encodes a variant AAV capsid protein; (iii) two or more host cells, each comprising a nucleic acid that comprises a nucleotide sequence that encodes a variant AAV capsid protein; and (iv) two or more variant AAV capsid proteins; where the variant AAV capsid protein of at least one member of the library comprises an amino acid sequence having at least one amino acid substitution relative to the amino acid sequence set forth in one of SEQ ID NOs: 10-13 and 26-33.

COMPOSITIONS AND KITS

[00236] Also provided are compositions and kits for use in the methods of the present disclosure. The subject compositions and kits include at least one of: a subject infectious rAAV virion, a subject rAAV vector, a subject nucleotide acid comprising a nucleotide sequence encoding a subject variant AAV capsid protein, an isolated host cell comprising a subject nucleic acid (i.e., a subject genetically modified host cell

comprising a nucleic acid that comprises a nucleotide sequence encoding a subject variant AAV capsid protein); a subject library (e.g., any of the above described libraries); and a subject variant AAV capsid protein. A composition or kit can include any convenient combination of the above. A composition or kit can also include helper virus and/or a nucleic acid comprising a nucleotide sequence that encodes a helper virus. A kit may also include reagents for the generation of nucleic acids (i.e., “mutant” nucleic acids) encoding modified variant AAV capsid proteins.

[00237] In addition to the above components, the subject kits may further include (in certain embodiments) instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, and the like. Yet another form of these instructions is a computer readable medium, e.g., diskette, compact disk (CD), flash drive, and the like, on which the information has been recorded. Yet another form of these instructions that may be present is a website address which may be used via the internet to access the information at a removed site.

[00238] The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

EXAMPLES

[00239] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used,

e.g., bp, base pair(s); kb, kilobase(s); ml, milliliter(s); µl, microliter(s); nl, nanoliter(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); i.v., intravenous(ly); and the like.

Example 1:

[00240] Adeno-associated virus (AAV) gene therapy vectors have demonstrated considerable promise in several clinical trials to date. However, circulating anti-AAV antibodies, resulting from childhood exposure or prior administration of an AAV vector, have prevented the implementation of AAV gene therapy for many potential patients. We have isolated novel AAV variants that are capable of enhanced anti-AAV antibody evasion, both in vitro and in vivo. The stringent pressure resulting from selections using low and high potency human sera pools and human IVIG evolved AAV variants capable of evasion of antibody neutralization from individual human sera, human IVIG, and mouse sera, the most broadly evasive variants to date.

Materials and Methods

Cell Lines

[00241] Cell lines were cultured at 37°C and 5% CO₂, and unless otherwise mentioned, were obtained from the American Type Culture Collection (Manassas, VA). HEK293T, HeLa, and HT1080 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). CHO K1 and CHO pgsA cells were cultured in F-12K medium (ATCC) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Invitrogen). Pro5 and Lec1 cells were cultured in MEM-alpha medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Invitrogen).

Human Sera Pools for Selection

[00242] Eighteen individual human serum samples were obtained from Innovative Research, Inc. (Southfield, MI) and the neutralizing antibody titer for wild type AAV2 was determined for each sample (**Table 2**). Since individual samples likely possess

variations in both the affinities and epitope specificities of the antibodies, three potent sera pools ($\alpha = A + F + G$, $\beta = B + H + M$, and $\gamma = I + J + N$) were generated by mixing equal volumes of individual serum samples. Selection in the presence of these variations of antibodies should result in a general enhancement of resistance to many pre-existing human antibodies. Later selections were performed in the presence of Gamimune N, 10% Human IVIG (Bayer, Elkhart IN) to select for resistance to an even broader range of antibodies.

[00243] Table 2: Neutralizing Antibody Titers of Individual Human Serum Samples

[00244] Neutralizing antibody (NAb) titers for each sample are reported as the reciprocal of the volume fraction of serum necessary to reduce infectivity to 37% of the value measured in the absence of serum. Three sera pools ($\alpha = A + F + G$, $\beta = B + H + M$, and $\gamma = I + J + N$) were then generated by mixing equivolume amounts of three individual serum samples.

Table 2

Human Serum Sample	~ NAB titer	Human Serum Sample	~ NAB titer
A	500	J	500
B	275	K	172
C	200	L	<75
D	<75	M	2200
E	<75	N	5000
F	350	O	<75
G	425	P	<75
H	450	Q	<75
I	200	R	120

Library Generation and Viral Production

[00245] To create the saturation mutagenesis library, an AAV2 *cap* library was generated by error-prone PCR followed by the staggered extension process described by Zhao *et al.* using 5'-GCGGAAGCTTCGATCAACTACGC-3' (SEQ ID NO: 14) and 5'-GGGGCGGCCGCAATTACAGATTACGAGTCAGGTATCTGGTG-3' (SEQ ID NO: 15) as forward and reverse primers, respectively. Selections using pooled individual human sera revealed a variant containing four point mutations (described in the results section) that served as the basis for the saturation mutagenesis library. The *cap* gene for

this variant was subjected to further mutagenesis by changing the amino acids at specific sites. Primer 5'-cattNNKgaccagtctaggaactgg-3' (SEQ ID NO: 16) and the corresponding reverse complement primer were used to mutagenize the R471 amino acid site. Primer 5'-

gccacaaggacgatgaagaaNNKtttttcctcagagcgggggtctcatcttgggaagcaaggctcaNNKaaaacaagtgtggacattg-3' (SEQ ID NO: 17) and the corresponding reverse complement primer were used to mutagenize the K532 and E548 amino acid sites. Primer 5'-

ccaacctccagagagcNNKagacaagcagctacc-3' (SEQ ID NO: 18) and the corresponding reverse complement primer were used to mutagenize the N587 amino acid site. Primer 5'-

ccaactacaacaagtctNNKaattgtggactttactgtggacNNKaattggcgtgtatt-3' (SEQ ID NO: 19) and the corresponding reverse complement primer were used to mutagenize the V708 and T716 amino acid sites. A library consisting of AAV2 containing randomized *cap* loop regions and a library containing shuffled DNA from the wild type AAV1, AAV2, AAV4, AAV5, AAV6, AAV8, AAV9 *cap* genes were packaged and pooled for initial selection steps (Koerber et. al.; Mol Ther. 2008 Oct;16(10):1703-9; and Koerber et. al.; Mol Ther. 2009 Dec;17(12):2088-95; both of which are hereby incorporated by reference in their entirety).

[00246] For the second and third rounds of evolution, random mutagenesis libraries were generated by subjecting *cap* genes from the Loop-Swap/Shuffle library and the Saturation Mutagenesis library to error-prone PCR using 5'-CATGGGAAAGGTGCCAGACG-3' (SEQ ID NO: 20) and 5'-ACCATCGGCAGCCATACCTG-3' (SEQ ID NO: 21) as forward and reverse primers, respectively, as previously described. The replication competent AAV libraries and recombinant AAV vectors expressing GFP under the control of a CMV promoter were packaged using HEK293T cells (ATCC) using the calcium phosphate transfection method, and the viruses were purified by iodixonal gradient centrifugation. Recombinant AAV vectors expressing GFP or luciferase under the control of a CMV promoter for use *in vivo* were further purified by Amicon filtration. DNase-resistant genomic titers were determined via quantitative PCR. (Excoffon et. al, Proc Natl Acad Sci U S A. 2009 Mar 10;106(10):3865-70; and Maheshri et al., Nat Biotechnol. 2006 Feb;24(2):198-204; both of which are hereby incorporated by reference in their entirety).

Library Selection and Evolution

[00247] One round of selection is defined as HEK293T cell infection using the AAV starting library (incubated for 30 minutes at room temperature for the pooled individual human sera or for 1 hour at 37°C with heat inactivated IVIG prior to infection), followed by adenovirus rescue and harvest of successful variants. Each round of evolution consists of mutagenesis of the *cap* gene to create the starting library and three rounds of selection. Three rounds of evolution were performed with each library, with clonal analysis performed between each round of evolution. The starting libraries for each round of evolution were generated as described above. Following the third round of selection, AAV *cap* genes were isolated from the pool of successful AAV variants and amplified via PCR. *Cap* genes were inserted into the pXX2 recombinant AAV packaging plasmid using *NotI* and *HindIII*. *Cap* genes were then sequenced at the University of California, Berkeley DNA sequencing facility, and analyzed using Geneious software (Biomatters, Auckland, New Zealand). Three-dimensional models of the AAV2 capsid (Protein Databank accession number 1LP3) were rendered in Pymol (DeLano Scientific, San Carlos, CA).

In Vitro Transduction Analysis of Antibody-Evading Variants

[00248] HEK293T were plated at a density of 3×10^4 cells/well 24 hours prior to infection. Variants were incubated at 37°C for 1 hour with heat inactivated IVIG, individual human sera, or individual mouse sera prior to infection, and cells were then infected with rAAV-GFP at a genomic MOI of 2000. The percentage of GFP positive cells was assessed 48 hours post infection using an ImageXpress Micro Cellular Imaging and Analysis System (Molecular Devices, Sunnyvale, CA) and MetaXpress Image Analysis Software, version 3.1.0, Multi Wavelength Cell Scoring Application Module (Molecular Devices).

In Vitro Transduction Analysis

[00249] To determine the relative transduction efficiencies the selected mutants compared to parental wild-type AAV serotypes, HEK293T, CHO K1, CHO pgsA (lacking all surface glycosaminoglycans), CHO Pro5 (the parental line for several glycosylation mutants, including Lec1 cells), CHO Lec1 (glycosylation defective), HeLa, and HT1080

cells (a human fibrosarcoma cell line) were plated at a density of 2.5×10^4 cells per well 24 hours prior to infection. Cells were infected with rAAV1-GFP, rAAV2-GFP, rAAV6-GFP, Shuffle 100.1-GFP, Shuffle 100.3-GFP, SM 10.2-GFP, or Shuffle 100.7-GFP at a range of MOI of 100-1000. The percentage of GFP positive cells was assessed 48 hours post infection using a Beckman-Coulter Cytomics FC500 flow cytometer (Beckman-Coulter, Brea, CA).

In Vivo Analysis of Antibody-Evading Variants

[00250] For analysis of gene expression *in vivo*, eight week old, female, Balb/c mice were primed with 4 mg IVIG per mouse or phosphate buffered saline (for control mice) via tail vein injection 24 hours prior to administration of recombinant Shuffle 100-3 (see SEQ ID NO: 12), SM 10-2 (see SEQ ID NO: 10), or AAV2 vectors. Mice were infected with 10^{11} viral genomes of recombinant AAV vectors encoding luciferase under the control of a CMV promoter via tail vein injection. For bioluminescence imaging, mice were anesthetized with 2% isoflurane and oxygen. D-luciferin substrate (GOLD Biotechnology, St. Louis, MO) was injected intraperitoneally, at a dose of 500 $\mu\text{g/g}$ of body weight. Images were generated using a VivoVision IVIS Lumina imager (Xenogen, Alameda, CA). For each mouse, ventral images were taken 7–10 minutes after the substrate injection, every week for four weeks. Five weeks post-infection, serum was collected via cardiac puncture and mice were then perfused with .9% saline solution. Heart, liver, lungs, kidney, spleen, brain, spinal cord, and hind limb muscle were harvested and frozen. Frozen tissue samples were homogenized and resuspended in reporter lysis buffer (Promega, Mannheim, Germany) for *in vitro* luciferase analysis. Lysate containing luciferase was clarified by centrifugation for 10 minutes at 10,000g. To assay the samples, 20 μL of the lysate was added to 100 μL of the luciferase assay buffer, mixed, incubated for 5 minutes, and placed in the luminometer. The signal was integrated for 30 seconds with a 2 second delay and was reported in Relative Light Units (RLU) detected by a TD 20/20 luminometer (Turner Designs, Sunnyvale, CA). The luciferase signal was normalized to the total protein content determined by a bicinchoninic acid assay (Pierce).

Results

[00251] Our results demonstrate that AAV can evolve to significantly overcome neutralization by anti-AAV antibodies, both *in vitro* and *in vivo*. Novel AAV variants were isolated that required 2- to 35-fold higher neutralizing antibody titers (using human IVIG) than wild-type AAV *in vitro*. The antibody neutralization properties also translated to enhanced transduction *in vivo* in the presence of neutralizing antibodies. The isolation of such novel clones resistant to anti-AAV antibodies allows for the broader implementation of treatments based on AAV as a nucleic acid delivery vector (including individuals with high antibody titers that are currently ineligible for AAV gene therapy).

AAV Library Generation and Selection Through Directed Evolution

[00252] **Figure 1a** shows a schematic of the directed evolution approach used to isolate novel AAV variants capable of evading human antibody neutralization. Libraries of viruses were created using the DNA mutagenesis techniques described in the following paragraphs (**Figure 1a**, steps 1 and 2). During initial selections, pools of viral libraries developed from error-prone PCR mutations to AAV2 cap genes were incubated with various dilutions of the low potency α human sera pool for 30 minutes at room temperature prior to infection of HEK293T cells (step 3). Following three rounds of selection against the low potency α human sera pool (**Figure 1a**, steps 4 and 5), several variants with enhanced resistance to this neutralizing sera pool were obtained (**Figure 1a**, step 6, **Figure 7a**). Variant 1.45, contained two point mutations (N312K, N449D), which resulted in >10-fold more resistance to neutralization by the α pool compared to wild type AAV2.

[00253] The *cap* gene from variant 1.45 was subjected to additional random mutagenesis and the resulting library was selected for three additional rounds of selection against the β and γ pools, in parallel. As only minor improvements in antibody evasion were observed (data not shown), the recovered *cap* genes were pooled and subjected to additional diversification via DNA shuffling and EP PCR. Three more rounds of selection against increasing amounts of sera from both the β and γ pools resulted in substantial enrichment in the amount of recovered virus from the viral library compared to wild type AAV2 (**Figure 7b, c**). Sequencing of the successful *cap* genes from both

pools revealed several low frequency mutants and a single dominant mutant, variant $\gamma 4.3$, which contained four point mutations (N312K, N449D, N551S, and I698V), present within both libraries. In the presence of human IVIG, variant 1.45 demonstrated a modest 1.2-fold enhanced resistance to neutralization, whereas $\gamma 4.3$ demonstrated 3.1-fold enhanced resistance to neutralization (**Figure 7d**). This observation confirms the hypothesis that pools of individual human sera can be used to isolate AAV variants capable of enhanced evasion of antibodies present in the general human population.

[00254] The moderate success of variant $\gamma 4.3$ in resisting neutralization by anti-AAV antibodies prompted the development of a library based on the $\gamma 4.3$ *cap* gene. Amino acid sites R471, K532, E548, N587, V708, T716, previously determined to be immunogenic sites on the AAV2 capsid, were subjected to saturation mutagenesis in an attempt to find amino acid mutations that may improve upon the antibody resistance of $\gamma 4.3$. This “saturation mutagenesis” library, along with a “shuffled” library composed of random *cap* chimeras of 7 parent AAV serotypes and a “loop-swap” library composed of AAV2 *cap* with substituted loop regions were subjected to three additional rounds of selection, in which the pools of viral libraries were incubated with various dilutions of human IVIG for one hour at 37°C prior to infection of HEK293T cells. Following infection with AAV libraries, and amplification of the infectious AAV variants through adenovirus superinfection, the number of viral genomes, or viral titer, from each library condition was quantified and compared to titers of wild-type AAV2 as a method for determining the success of the selection (**Figure 1b**). For each round of selection using the saturation mutagenesis and loop-swap/shuffled libraries, viral pools from the 1:10 and 1:100 IVIG dilution conditions that produced higher viral titers than wild-type AAV2 were used as the starting point for the subsequent round of selection. After three rounds of selection, the successful viral *cap* genes were isolated and tested individually to determine the virus with the most efficient gene delivery. In addition, the *cap* genes isolated from the third round of selection were subjected to additional rounds of error-prone PCR mutagenesis, and the process was repeated to iteratively increase the fitness of the virus.

[00255] **Figure 1** depicts directed Evolution of AAV for Enhanced Antibody Evasion. (a) Schematic of Directed Evolution. 1) A viral library is created by genetically diversifying the *cap* gene using several complementary approaches. 2) Viruses are packaged in

HEK293T cells using plasmid transfection, then harvested and purified. 3) The viral library is incubated with human IVIG at several concentrations and introduced to HEK293T cells in vitro. 4) Successful viruses are amplified and recovered via adenovirus superinfection. 5) Successful clones are enriched through repeated selections at lower MOIs. 6) Isolated viral DNA reveals successful cap genes. 7) Successful cap genes are mutated again to serve as a new starting point for selection. **(b)** Selection of Antibody Evading Mutants from Loop-Swap/Shuffled, and Saturation Mutagenesis libraries. HEK293T cells were infected with viral libraries for 24 hours. Viral particles that productively infected cells were amplified by adenovirus infection, and the rescued AAV was quantified by qPCR. A 1:10 dilution of IVIG corresponds to a concentration of 10 mg IVIG/mL. Error bars indicate the standard deviation ($n = 3$).

[00256] **Figure 7** demonstrates the generation of human antibody evaders based on AAV2. **(a)** Four viral clones selected after three rounds of selection against the low stringency α pool demonstrate enhanced resistance to 1 μ L of α serum at MOI of 1. Two additional rounds of diversification (i.e. mutagenesis and DNA shuffling) and selection (3 rounds of increasing serum amounts) resulted in significantly enhanced viral recovery in the presence of large amounts of highly potent **(b)** β and **(c)** γ pools. **(d)** Additionally, two viral clones (1.45 and γ 4.3) demonstrate 1.23- and 3.10-fold enhanced resistances to a highly diverse pool of pre-existing antibodies present with pooled human intravenous immunoglobulin (IVIg) from ~100,000 individuals compared to wild-type AAV2.

Increased Antibody Evasion of the Novel Evolved AAV Variants In Vitro

[00257] Of the twelve clones selected and packaged for individual analysis from the saturation mutagenesis and loop-swap/shuffled libraries after nine rounds screening against human IVIG, all twelve required higher neutralizing antibody titers than both wild-type AAV1 and AAV2 (**Figure 2a** and **Table 1**). Variant Shuffle 100-3 (see SEQ ID NO: 12), which required a 35-fold higher *in vitro* IVIG concentration for neutralization than wild-type AAV2, was still capable of transducing approximately 10% of cells in the presence of 1 mg/mL IVIG (**Figure 2b**). In addition, variant SM 10-2 from the AAV2 saturation mutagenesis library required a 7.5-fold higher *in vitro* IVIG concentration for neutralization than wild-type AAV2. Furthermore, variants Shuffle 100-3 and SM 10-2 (see SEQ ID NO: 10) showed enhanced transduction in the presence

of sera samples from individual patients excluded from a hemophilia B clinical trial (**Figure 3**) (Nathwani et al., N Engl J Med. 2011 Dec 22;365(25):2357-65).

[00258] **Figure 2** depicts the neutralization profiles of antibody evading variants. The cap genes of antibody evading mutants isolated after three rounds of evolution were used to package recombinant AAV encoding GFP and incubated with human IVIG before infection of HEK293T cells. The fraction of remaining infectious particles was determined using high content fluorescence imaging and normalized to the infectious titer in the absence of IVIG. Two clones from each library with resistance to IVIG are shown. Data for the other clones analyzed are displayed in **Table 1**. **(a)** Neutralization curves. Error bars indicate the standard deviation (n = 3). **(b)** Representative fluorescence images from several IVIG dilutions show that mutants are capable of HEK293T transduction in the presence of high concentrations of neutralizing antibodies.

[00259] **Figure 3** depicts the neutralization profiles of antibody evading variants. Human sera were acquired from individuals that were excluded from hemophilia B clinical trials due to the presence of high neutralizing antibody titers against AAV. Recombinant AAV encoding GFP was incubated with individual human serum samples before infection of HEK293T cells. The fraction of remaining infectious particles was determined using fluorescence microscopy and normalized to the infectious titer in the absence of human sera. Error bars indicate the standard deviation (n = 3).

[00260] Sequence analysis of the twelve clones revealed that the two variants with the highest neutralizing antibody resistance, Shuffle 100-3 (see SEQ ID NO: 12) and Shuffle 100-1 (see SEQ ID NO: 11), are almost identical shuffled capsids containing fragments of AAV1-4, AAV6, and AAV9 (**Figure 4**). Differences in amino acids 469 (AAV6 residue to AAV7 residue) and 598 (AAV6 residue to AAV1 residue) between the two variants translate to almost a 3-fold increase in neutralizing antibody titer for Shuffle 100-3 (see SEQ ID NO: 12) (**Table 1**). Variant Shuffle 100-7 (see SEQ ID NO: 13), which had the fourth highest neutralizing antibody resistance (**Table 1**), is also a shuffled capsid containing fragments of AAV1, AAV6, and AAV8 (**Figure 4**), which agrees well with reported data showing that wild-type AAV1 and AAV8 are effective at evading anti-AAV2 antibodies. Interestingly, variant SM 10-2 (SEE SEQ ID NO: 10) retained the point mutations acquired by variant γ 4.3 and also retained wild type residues

at the saturation mutagenesis sites. Variant SM 10-2 (SEE SEQ ID NO: 10) did acquire additional point mutations at surface residue D472N and internal residue L735Q.

Figure 4 depicts the amino acid sequences of loop-swap/shuffle and saturation mutagenesis clones. **(a)** Schematics of the capsid protein are shown for the two clones from each library with the highest neutralizing IVIG concentrations. Each region is shaded according to the parent serotype from which it is derived. Black arrows denote (from left to right) the start codons of VP1, VP2, and VP3 capsid proteins. Gray arrows denote (from left to right) surface loop regions I, II, III, IV, and V based on the AAV2 capsid. **(b)** Molecular models of the full AAV2 capsid, based on the solved structure, are shown for the two clones from each library with the highest neutralizing IVIG concentrations. Each region is shaded according to the parent serotype from which it is derived. For variant Shuffle 100-3 (see SEQ ID NO: 12), black arrows indicate differences from variant Shuffle 100-1 (see SEQ ID NO: 11). For variant SM 10-2 (SEE SEQ ID NO: 10), mutations N449D, D472N, N551S, and I698V are surface mutations (black).

[00261] Table 1: IVIG Neutralizing Antibody Titers of Library Clones and Parent Serotypes Human IVIG was used to neutralize recombinant AAV-GFP vectors with capsids from wild-type AAV1, AAV2, AAV8, and variants recovered from the loop-swap/shuffled and saturation mutagenesis libraries. The IVIG concentration (mg/mL) required to reduce gene delivery efficiency to 50% of that in the absence of IVIG is shown, and compared to the concentration required to reduce delivery of AAV2. All variants analyzed required higher concentrations of IVIG than wild-type AAV1 and AAV2. The neutralizing antibody titer was determined by fitting the curves in Figure 2 to an exponential curve. SEQ ID NOs are listed as “amino acid, nucleotide.”

Table 1

Clone	SEQ ID NO:	Neutralizing IVIG concentration	Fold Resistance Relative to AAV2
		<i>mg/ml</i>	
AAV1	1	0.026	1.757
AAV2	2	0.015	1.000
AAV8	8	0.092	6.113

Clone	SEQ ID NO:	Neutralizing IVIG concentration	Fold Resistance Relative to AAV2
Shuffle 10-2	26, 34	0.037	2.443
Shuffle 10-6	27, 35	0.028	1.842
Shuffle 10-8	28, 36	0.084	5.583
Shuffle 100-1	11, 23	0.183	12.178
Shuffle 100-2	29, 37	0.073	4.831
Shuffle 100-3	12, 24	0.529	35.227
Shuffle 100-7	13, 25	0.090	6.025
SM 10-1	30, 38	0.071	4.732
SM 10-2	10, 22	0.113	7.519
SM 10-8	31, 39	0.051	3.409
SM 100-3	32, 40	0.074	4.941
SM 100-10	33, 41	0.066	4.393

[00262] Variants Shuffle 100-3 (see SEQ ID NO: 12), Shuffle 100-1 (see SEQ ID NO: 11), and Shuffle 100-7 (see SEQ ID NO: 13) have transduction profiles that mimic the transduction profiles of parent serotypes AAV1 and AAV6 (**Figure 5**). In addition, the mutations in SM 10-2 (see SEQ ID NO: 10) do not prevent a heparin dependence (as seen in parent serotype AAV2) leading to a profile similar to AAV2 (**Figure 5**).

[00263] **Figure 5** demonstrates the in vitro tropism of novel aav variants. Recombinant AAV vectors expressing green fluorescent protein were used to transduce a panel of cell lines: CHO, pgsA (lacking all surface glycosaminoglycans), Pro5, Lec1 (lacking sialic acid), HEK293T, HeLa, and HT1080 (human fibrosarcoma cell line) to profile the transduction properties of the new AAV variants. Error bars indicate the standard deviation (n = 3).

Increased Antibody Evasion of the Novel Evolved AAV Variants In Vivo

[00264] To determine the localization pattern of variants Shuffle 100-3 and Shuffle 100-7, luciferase enzyme activity was examined in various tissues of naïve mice injected with AAV2, Shuffle 100-3, or Shuffle 100-7 (**Figure 6a**). Variant Shuffle 100-7 displayed similar *in vivo* tropism to AAV2, except for 7-fold higher transduction of the

heart, 5-fold higher transduction of the lungs, and 4.5-fold lower transduction of the liver. The Shuffle 100-3 variant exhibited over 4-fold higher transduction of the brain, over 3-fold higher transduction of the lungs, and 27-fold higher transduction of muscle than AAV2. Analysis of the serum from these mice showed that variant Shuffle 100-3 required equal or higher *in vitro* serum concentrations for neutralization than AAV1 and AAV8 for serum from mice given AAV1, AAV2, AAV8 or Shuffle 100-3 gene delivery vectors (**Figure 11**). Shuffle 100-7 required equal or higher *in vitro* serum concentrations for neutralization than AAV1 for serum from mice given AAV1, AAV2, AAV8, Shuffle 100-3, or SM 10-2 gene delivery vectors (**Figure 11**). Furthermore, both variants were less neutralized by serum from mice given AAV2 gene delivery vectors than all wild-type AAV serotypes tested. Interestingly, variant Shuffle 100-3 was also less neutralized by serum of mice immunized against it than any of the other serotypes or variants tested (**Figure 11**). This data illustrates the possibility that these variants could be used in combination with wild-type AAV serotypes or the other variant in applications requiring multiple vector administrations.

[00265] **Figure 11** shows the neutralizing antibody titers of library clones and parent serotypes in immunized mouse sera. Sera from mice administered library clones or wild-type AAV was used to neutralize recombinant AAV-GFP vectors with capsids from wild-type AAV1, AAV2, AAV8, and variants recovered from the loop-swap/shuffled and saturation mutagenesis libraries. The serum dilution required to reduce gene delivery efficiency to 50% of that in the absence of serum is shown.

[00266] To determine the ability of variants Shuffle 100-7 and Shuffle 100-3 to evade antibody neutralization *in vivo*, mice were passively immunized with human IVIG prior to AAV injection. Variant Shuffle 100-7 had significantly higher heart, liver, and muscle transduction than AAV2, as measured by luciferase enzyme activity (**Figure 6b**). Variant Shuffle 100-3 had significantly higher heart and muscle transduction compared to AAV2 (**Figure 6b**).

[00267] **Figure 6** shows the *in vivo* localization and neutralization of novel AAV variants. (a) Recombinant AAV vectors encoding luciferase were administered via tail vein injection to female BALB/c mice. After 5 weeks, levels of luciferase activity were determined and normalized to total protein for each sample analyzed. (b) Recombinant AAV vectors expressing luciferase were administered via tail vein injection to female

BALB/c mice 24 hours after tail vein injection of 4 mg of human IVIG. After 5 weeks, levels of luciferase expression were normalized to total protein for each sample analyzed. Error bars indicate the standard deviation ($n = 3$), $* = p < 0.05$. RLU, relative luciferase unit.

[00268] Variant $\gamma 4.3$, isolated from an AAV2-based error-prone library selected against a pool of individual human sera, contained four point mutations (N312K, N449D, N551S, and I698V). Interestingly, two of these positions (N449 and N551) were previously identified as immunogenic residues using other pools of human serum, demonstrating that antigenic epitopes involving these sites are targeted by many different neutralizing antibodies. Thus, these sites are interesting and valuable targets for mutation. Pairing directed evolution and rational design in the saturation mutagenesis library resulted in the isolation of variant SM 10-2, which was capable of higher antibody resistance than both AAV1 and AAV2 *in vitro*. Variant SM 10-2 incorporates two additional point mutations (D472N and L735Q) to those found on variant $\gamma 4.3$. The D472N mutation was previously shown to increase the level of capsid synthesis in HEK293 cells. Similarly, the replacement of the positively charged lysine side chain at amino acid position 735 with the uncharged glutamine side chain may function to stabilize the capsid, as it is also present in variant Shuffle 100-7 despite being located within the interior of the assembled capsid (**Figure 4**).

[00269] The creation of chimeric AAV capsids allows for the creation of viral variants that can merge desirable properties from multiple AAV serotypes. Although AAV8 and AAV9 have also been shown to be much more resistant to neutralization by IVIG than AAV2, amino acids specific to these capsids were only present in small spans on the surface of the shuffled variants isolated during our selections (**Figure 4**). The variant displaying the more efficient evasion of antibody neutralization *in vitro*, Shuffle 100-3, displayed similar *in vitro* tropism to its parental serotypes AAV1 and AAV6, but at a higher rate of infectivity than either wild-type serotype. Differences in amino acids 469 and 598 between variants Shuffle 100-1 and Shuffle 100-3 translate to almost a 3-fold increase in neutralizing antibody titer for Shuffle 100-3. A study by Lochrie *et al.* reported that the immunogenic residues recognized by human sera and IVIG are different, suggesting that different humans can produce various neutralizing antibodies to different sets of epitopes on the AAV capsid and complete escape from neutralization

is not easy (Lochrie et al., J Virol. 2006 Jan;80(2):821-34). Our work demonstrates that the use of multiple rounds of directed evolution using several different serum pools containing various amounts and potencies of anti-AAV antibodies will result in the isolation of novel AAV variants that are capable of enhanced cellular transduction, both *in vitro* and *in vivo*, in the presence of multiple anti-AAV antibody pools.

[00270] Adaptive immune responses to AAV vector components in animals and humans often prevent re-administration of AAV vectors of the same serotype, making gene delivery applications requiring multiple vector administrations difficult. In vitro neutralization assays using the serum from the mice used in the biodistribution studies demonstrate that the variants are less neutralized by these sera than wild-type AAV (**Figure 11**), which may be useful for gene therapy strategies in which vector readministration is necessary. For example, Shuffle 100-3 was not neutralized by serum from mice injected with AAV2, and AAV2 was not neutralized by serum from mice injected with Shuffle 100-3, suggesting this variant can be used in combination with wild-type AAV serotypes or in applications requiring multiple vector administrations. In conclusion, we have used directed evolution to isolate novel AAV variants that are capable of reduced neutralization by anti-AAV antibodies derived from individual human patients, pooled human serum, and mouse serum, both *in vitro* and *in vivo*.

[00271] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An infectious recombinant adeno-associated virus (rAAV) virion comprising:
 - (a) a variant adeno-associated virus (AAV) capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33; and
 - (b) a heterologous nucleic acid.
2. The infectious rAAV of Claim 1, wherein the variant AAV capsid protein comprises an amino acid sequence having at least about 95% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.
3. The infectious rAAV of Claim 1, wherein the variant AAV capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.
4. An infectious recombinant adeno-associated virus (rAAV) virion comprising:
 - (a) a variant adeno-associated virus (AAV) capsid protein that comprises an amino acid sequence having at least about 95% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to SEQ ID NO:2; and
 - (b) a heterologous nucleic acid.
5. The infectious rAAV of Claim 4, wherein the variant AAV capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 10.
6. The infectious rAAV of Claim 1 or Claim 4, wherein the rAAV exhibits increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by AAV2 (wild type AAV serotype 2).

7. The infectious rAAV of Claim 6, wherein the rAAV virion exhibits at least about 1.5-fold greater resistance to human AAV neutralizing antibodies than the resistance exhibited by AAV2.

8. The infectious rAAV of Claim 6, wherein the rAAV virion exhibits at least about 3-fold greater resistance to human AAV neutralizing antibodies than the resistance exhibited by AAV2.

9. The infectious rAAV of Claim 6, wherein the rAAV virion exhibits at least about 5-fold greater resistance to human AAV neutralizing antibodies than the resistance exhibited by AAV2.

10. The infectious rAAV of Claim 6, wherein the rAAV virion exhibits at least about 10-fold greater resistance to human AAV neutralizing antibodies than the resistance exhibited by AAV2.

11. The infectious rAAV of Claim 6, wherein the rAAV virion exhibits at least about 30-fold greater resistance to human AAV neutralizing antibodies than the resistance exhibited by AAV2.

12. The infectious rAAV of Claim 1 or Claim 4, wherein the rAAV exhibits increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction of mammalian cells exhibited by wild type AAV serotype 2 (AAV2).

13. The infectious rAAV of Claim 12, wherein the mammalian cells are liver cells, pancreatic cells, skeletal muscle cells, heart muscle cells, fibroblasts, retinal cells, synovial joint cells, lung cells, T cells, neurons, glial cells, stem cells, endothelial cells, or cancer cells.

14. The infectious rAAV of Claim 13, wherein the stem cells are hematopoietic stem cells, hematopoietic progenitor cells, neural stem cells, neural progenitor cells, neural crest stem cells, embryonic stem cells, induced pluripotent stem cells (iPS cells), mesenchymal stem cells,

mesodermal stem cells, liver stem cells, pancreatic stem cells, pancreatic progenitor cells, muscle stem cells, or retinal stem cells.

15. The infectious rAAV of Claim 1 or Claim 4, wherein the heterologous nucleic acid comprises an RNA interfering agent.

16. The infectious rAAV of Claim 1 or Claim 4, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a polypeptide.

17. An isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.

18. The isolated nucleic acid of Claim 17, wherein the encoded variant AAV capsid protein comprises an amino acid sequence having at least about 95% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.

19. The isolated nucleic acid of Claim 17, wherein the encoded variant AAV capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.

20. An isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein that comprises an amino acid sequence having at least about 95% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to SEQ ID NO:2.

21. The isolated nucleic acid of Claim 17 or Claim 20, wherein the encoded variant AAV capsid protein confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by AAV2 (wild type AAV serotype 2).

22. The isolated nucleic acid of Claim 21, wherein the increased resistance is at least about 1.5-fold greater than the resistance exhibited by AAV2.

23. The isolated nucleic acid of Claim 21, wherein the increased resistance is at least about 3-fold greater than the resistance exhibited by AAV2.

24. The isolated nucleic acid of Claim 21, wherein the increased resistance is at least about 5-fold greater than the resistance exhibited by AAV2.

25. The isolated nucleic acid of Claim 21, wherein the increased resistance is at least about 10-fold greater than the resistance exhibited by AAV2.

26. The isolated nucleic acid of Claim 21, wherein the increased resistance is at least about 30-fold greater than the resistance exhibited by AAV2.

27. The isolated nucleic acid of Claim 17 or Claim 20, wherein the variant AAV capsid protein confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by AAV2 (wild type AAV serotype 2).

28. An isolated host cell comprising the nucleic acid of Claim 17.

29. An isolated host cell comprising the nucleic acid of Claim 20.

30. The isolated host cell of Claim 28 or Claim 29, wherein the host cell is stably transfected with the nucleic acid.

31. The isolated host cell of Claim 28 or Claim 29, further comprising a nucleic acid comprising a nucleotide sequence encoding an AAV rep protein.

32. The isolated host cell of Claim 28 or Claim 29, further comprising a recombinant AAV vector.

33. A method of delivering a heterologous nucleic acid to a target cell, comprising contacting the target cell with a virion according to Claim 1 or Claim 4.

34. The method according to Claim 33, wherein the target cell is a liver cell, a pancreatic cell, a skeletal muscle cell, a heart muscle cell, a fibroblast, a retinal cell, a synovial joint cell, a lung cell, a T cell, a neuron, a glial cell, a stem cell, an endothelial cell, or a cancer cell.

35. The method according to Claim 34, wherein the stem cell is a hematopoietic stem cell, a hematopoietic progenitor cell, a neural stem cell, a neural progenitor cell, a neural crest stem cell, an embryonic stem cell, an induced pluripotent stem cell (iPS cell), a mesenchymal stem cell, a mesodermal stem cell, a liver stem cell, a pancreatic stem cell, a pancreatic progenitor cell, a muscle stem cell, or a retinal stem cell.

36. The method according to Claim 33, wherein the target cell is in vitro.

37. The method according to Claim 33, wherein the target cell is in vivo.

38. A method of delivering a gene product to an individual in need thereof, the method comprising administering to the individual an effective amount of an infectious recombinant adeno-associated virus (rAAV) virion according to Claim 1 or Claim 4.

39. The method according to Claim 38, wherein the heterologous nucleic acid comprises an RNA interfering agent.

40. The method according to Claim 38, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a polypeptide.

41. The method according to Claim 38, wherein the administering step comprises the indirect delivery of the infectious rAAV virion.

42. The method according to Claim 38, wherein the administering step comprises the direct delivery of the infectious rAAV virion.

43. A variant adeno-associated virus (AAV) capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.

44. The variant AAV capsid protein of Claim 43 comprising an amino acid sequence having at least about 95% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.

45. The variant AAV capsid protein of Claim 43 comprising the amino acid sequence set forth in one of SEQ ID NOs:11-13 and 26-33.

46. A variant adeno-associated virus (AAV) capsid protein that comprises an amino acid sequence having at least about 95% amino acid sequence identity to amino acids 203-736 of the amino acid sequence set forth in SEQ ID NO:10, and includes the amino acid substitutions N312K, N449D, D472N, N551S, I698V, and L735Q relative to SEQ ID NO:2.

47. The variant AAV capsid protein of Claim 46 comprising the amino acid sequence set forth in SEQ ID NO:10.

48. The variant AAV capsid protein of Claim 43 or Claim 46, wherein the variant AAV capsid protein confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased resistance to human AAV neutralizing antibodies compared to the resistance exhibited by AAV2 (wild type AAV serotype 2).

49. The variant AAV capsid protein of Claim 48, wherein the increased resistance is at least about 1.5-fold greater than the resistance exhibited by AAV2.

50. The variant AAV capsid protein of Claim 48, wherein the increased resistance is at least about 3-fold greater than the resistance exhibited by AAV2.

51. The variant AAV capsid protein of Claim 48, wherein the increased resistance is at least about 5-fold greater than the resistance exhibited by AAV2.

52. The variant AAV capsid protein of Claim 48, wherein the increased resistance is at least about 10-fold greater than the resistance exhibited by AAV2.

53. The variant AAV capsid protein of Claim 48, wherein the increased resistance is at least about 30-fold greater than the resistance exhibited by AAV2.

54. The variant AAV capsid protein of Claim 43 or Claim 46, wherein the variant AAV capsid protein confers to an infectious recombinant adeno-associated virus (rAAV) virion an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by AAV2 (wild type AAV serotype 2).

55. A library comprising at least one of:

(i) two or more infectious rAAV virions, each comprising a variant adeno-associated virus (AAV) capsid protein and a heterologous nucleic acid;

(ii) two or more isolated nucleic acids, each comprising a nucleotide sequence that encodes a variant AAV capsid protein;

(iii) two or more host cells, each comprising a nucleic acid that comprises a nucleotide sequence that encodes a variant AAV capsid protein; and

(iv) two or more variant AAV capsid proteins,

wherein the variant AAV capsid protein of at least one member of the library comprises an amino acid sequence having at least one amino acid substitution relative to the amino acid sequence set forth in one of SEQ ID NOs:10-13 and 26-33.

56. A method of generating and identifying a modified infectious recombinant adeno-associated virus (rAAV) virion that exhibits an altered property of infection relative to a starter virion comprising a starter capsid protein, the method comprising:

(a) generating variant adeno-associated virus (AAV) capsid proteins from the starter capsid protein, wherein the starter capsid protein comprises the amino acid sequence set forth in one of SEQ ID NOs:10-13 and 26-33, and wherein each variant AAV capsid protein comprises at least one amino acid substitution relative to the starter capsid protein;

(b) generating variant AAV virions, each comprising a variant capsid AAV protein generated in step (a); and

(c) assaying variant AAV virions generated in step (b) for the altered property of infection to identify the modified infectious rAAV virion.

57. The method of Claim 56, wherein the generation of the library of variant AAV capsid proteins comprises a method of mutagenesis selected from the group consisting of: polymerase chain reaction mutagenesis, oligonucleotide-directed mutagenesis, saturation mutagenesis, loop-swapping mutagenesis, fragment shuffling mutagenesis, and a combination thereof.

58. The method of Claim 56, wherein the altered property of infection is an increased resistance to human neutralizing AAV antibodies compared to the resistance exhibited by the starter virion.

59. The method of Claim 56, wherein the altered property of infection is an increased transduction of mammalian cells in the presence of human AAV neutralizing antibodies compared to the transduction exhibited by the starter virion.

60. The method of Claim 56, wherein modified infectious rAAV virion comprises a modified AAV capsid protein comprising an amino acid sequence having at least about 90% amino acid sequence identity to the starter capsid protein.

1 / 37

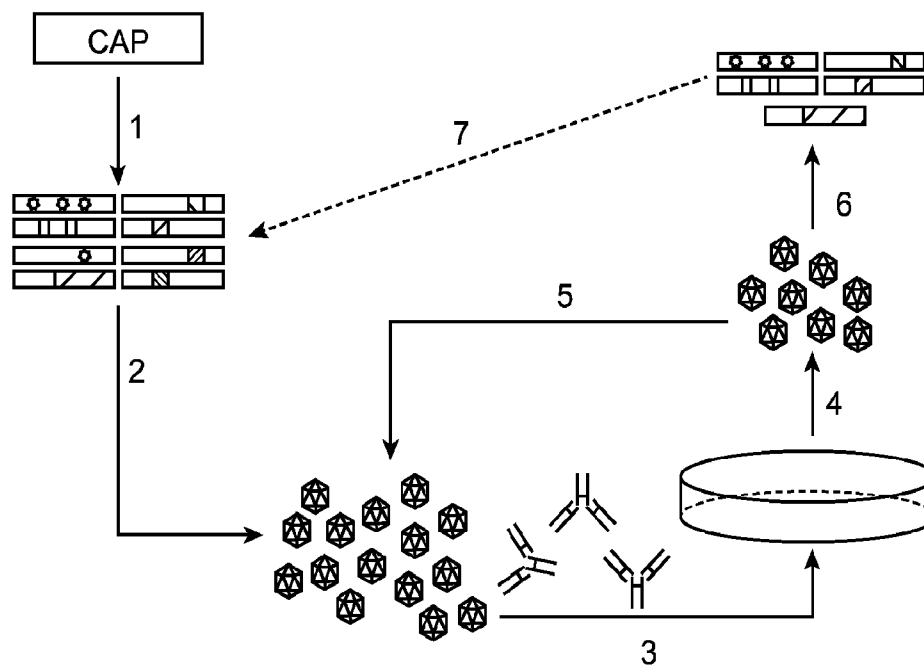


FIG. 1A

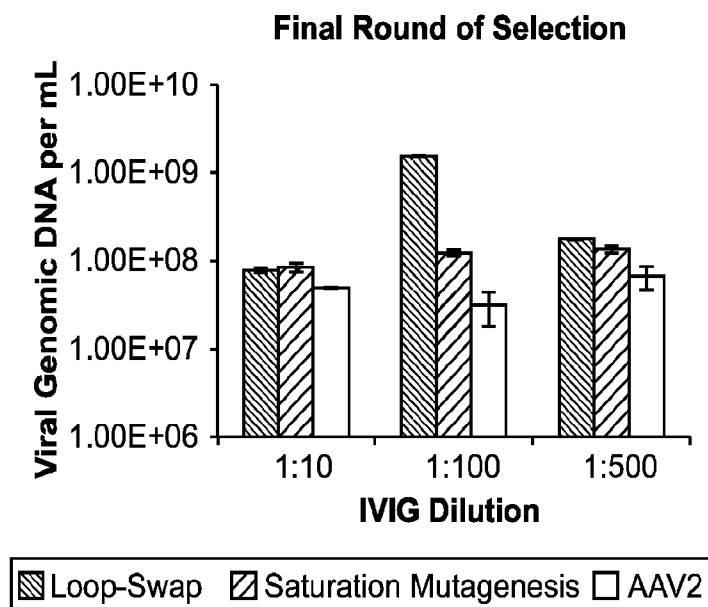


FIG. 1B

2 / 37

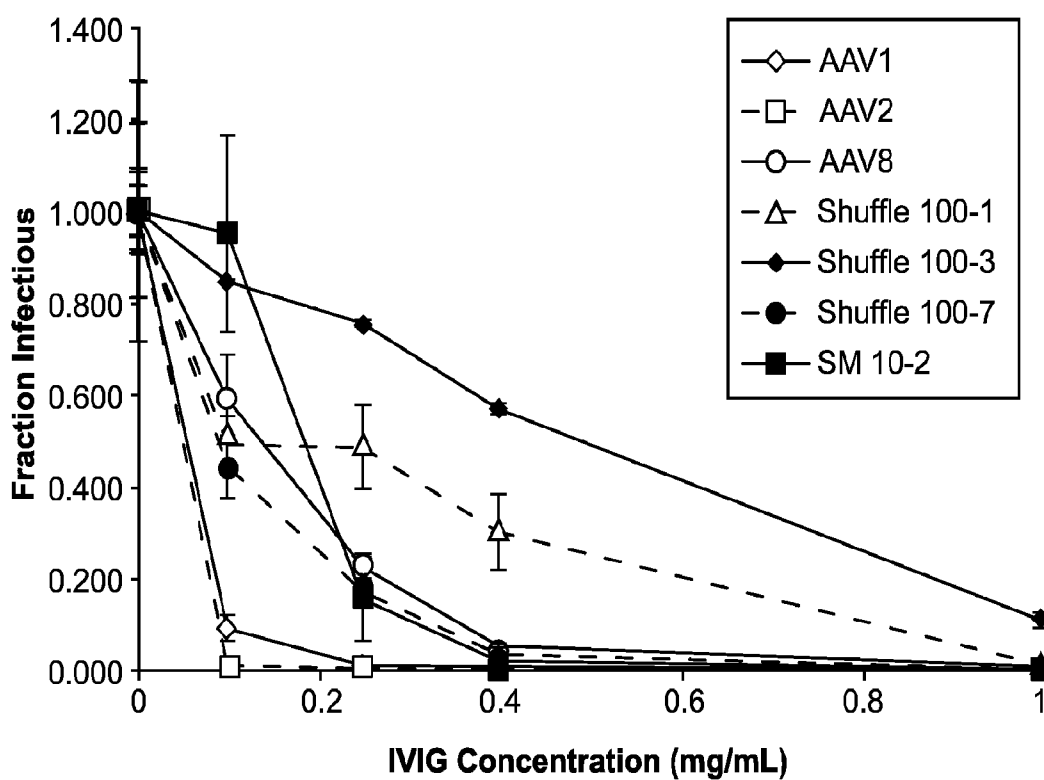


FIG. 2A

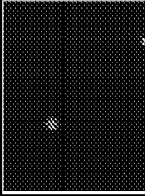
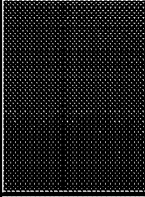
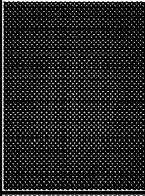
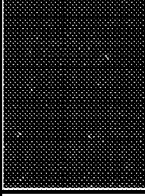
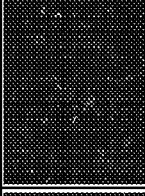
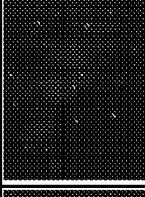
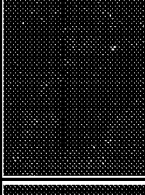
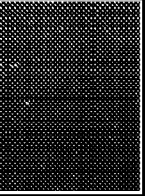
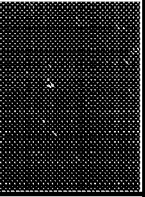
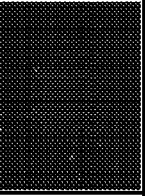
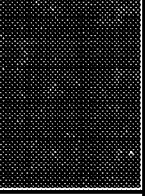
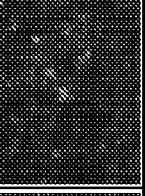
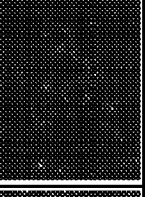
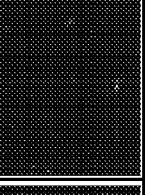
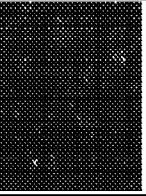

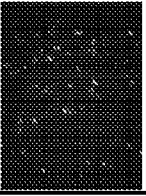
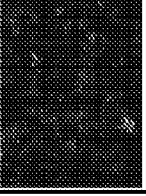
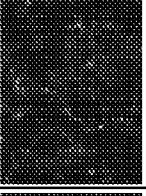
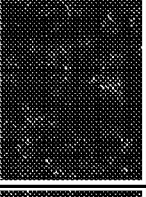
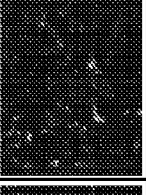
	AAV1	AAV2	AAV8	Shuffle 100-1	Shuffle 100-3	Shuffle 100-7	SM 10-2
1 mg/mL IVIG							
0.25 mg/mL IVIG							
no serum							

FIG. 2B

4 / 37

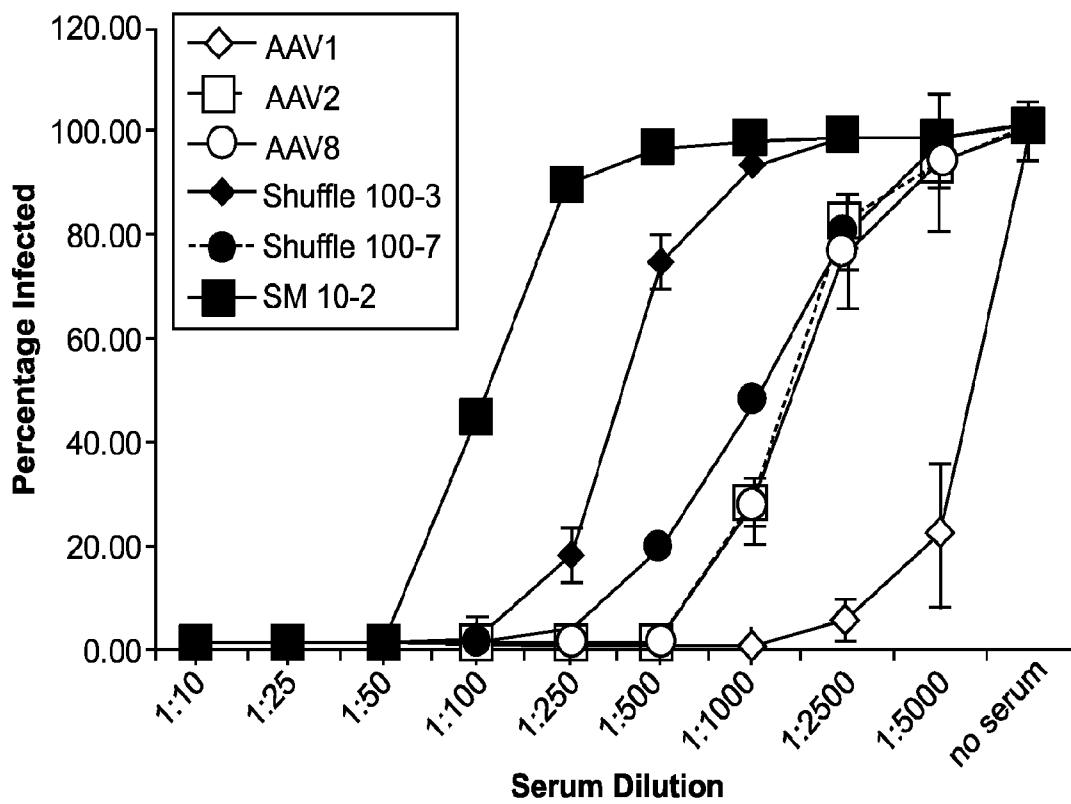


FIG. 3A

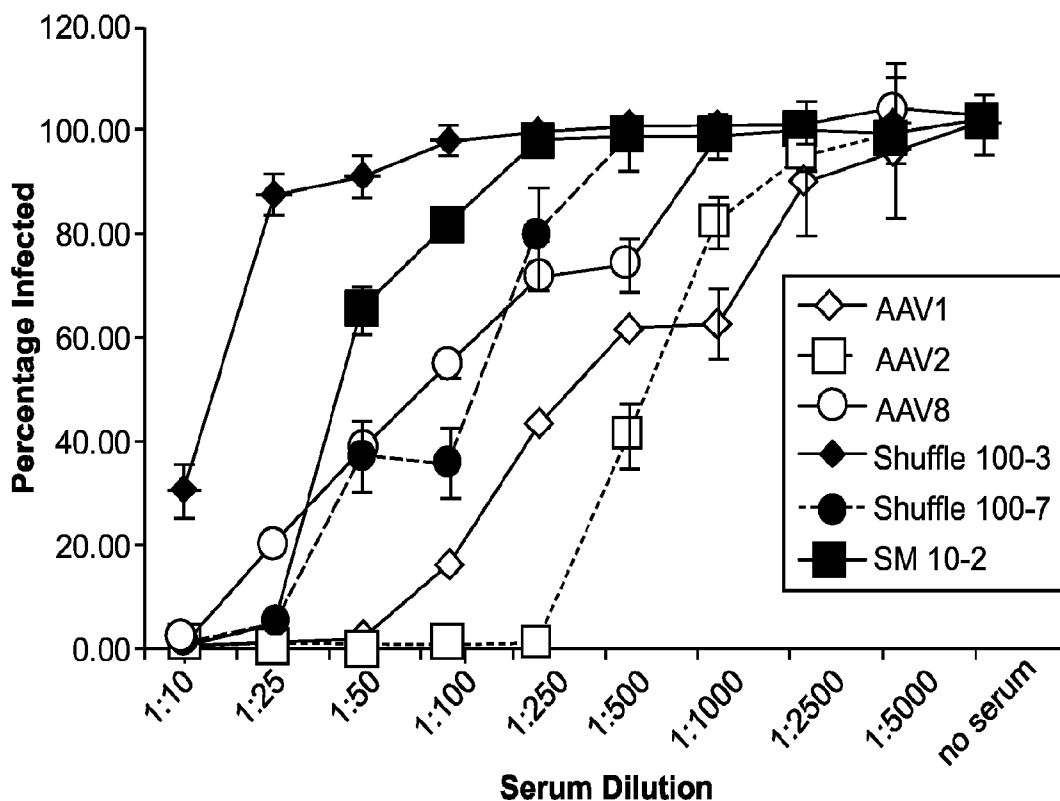


FIG. 3B

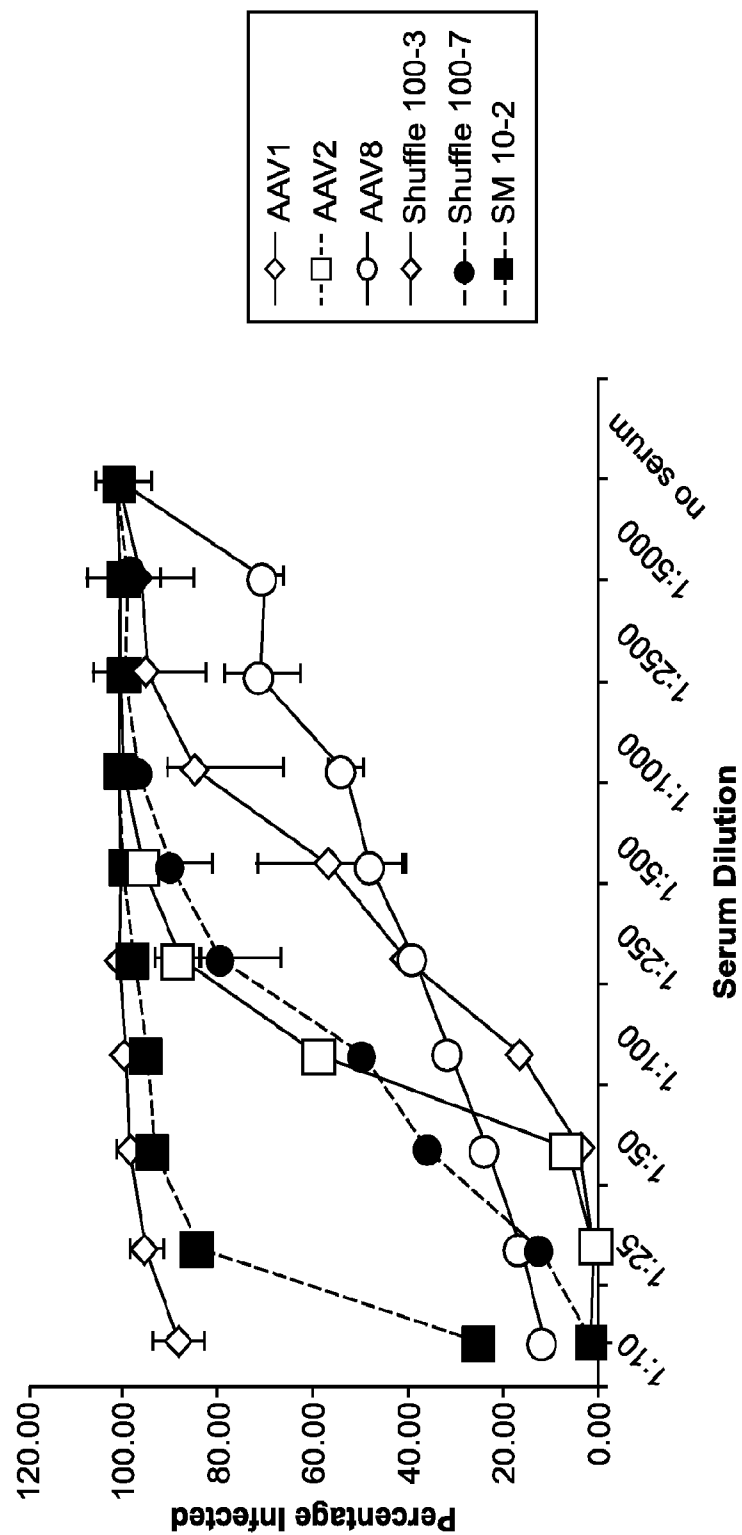


FIG. 3C

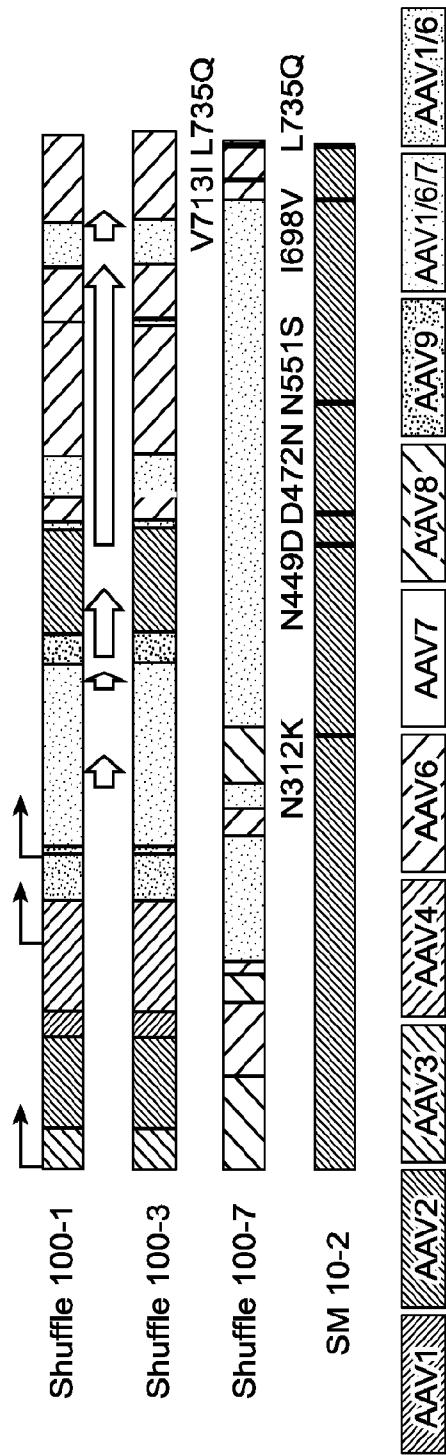


FIG. 4A

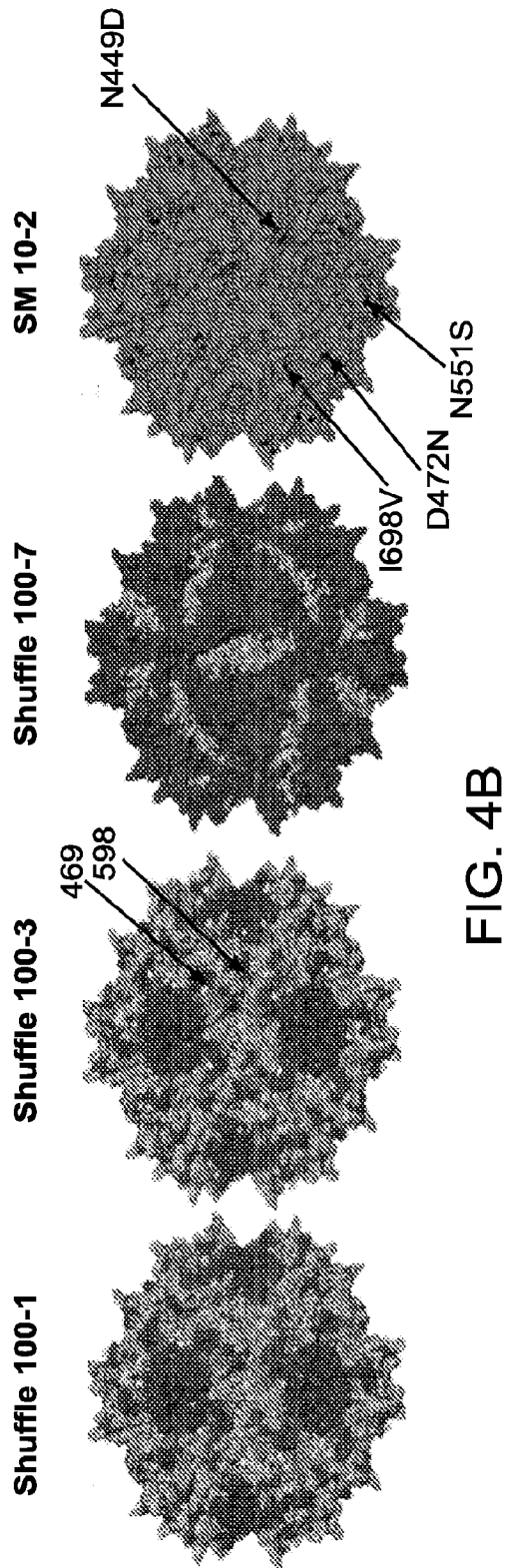


FIG. 4B

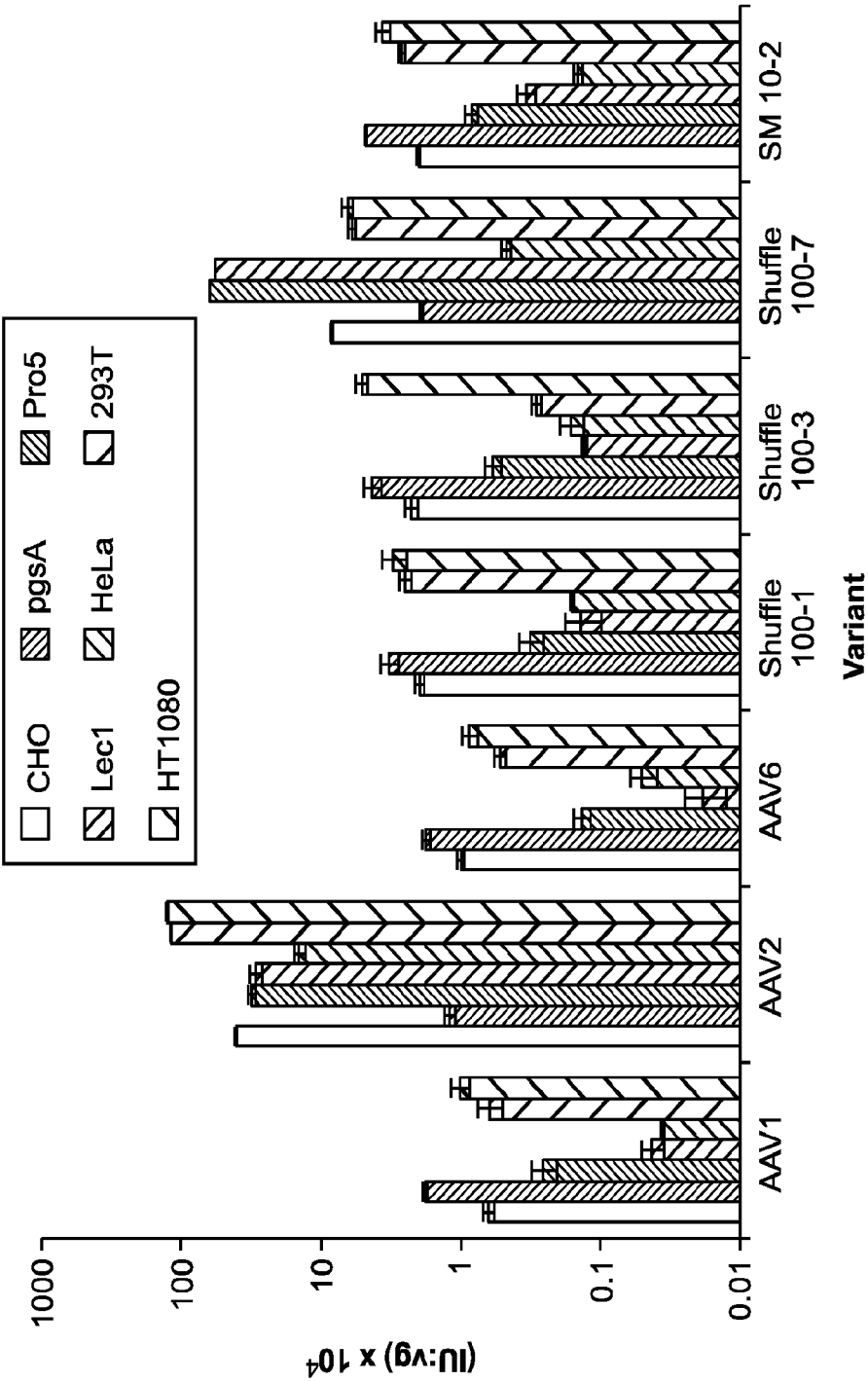


FIG. 5

8 / 37

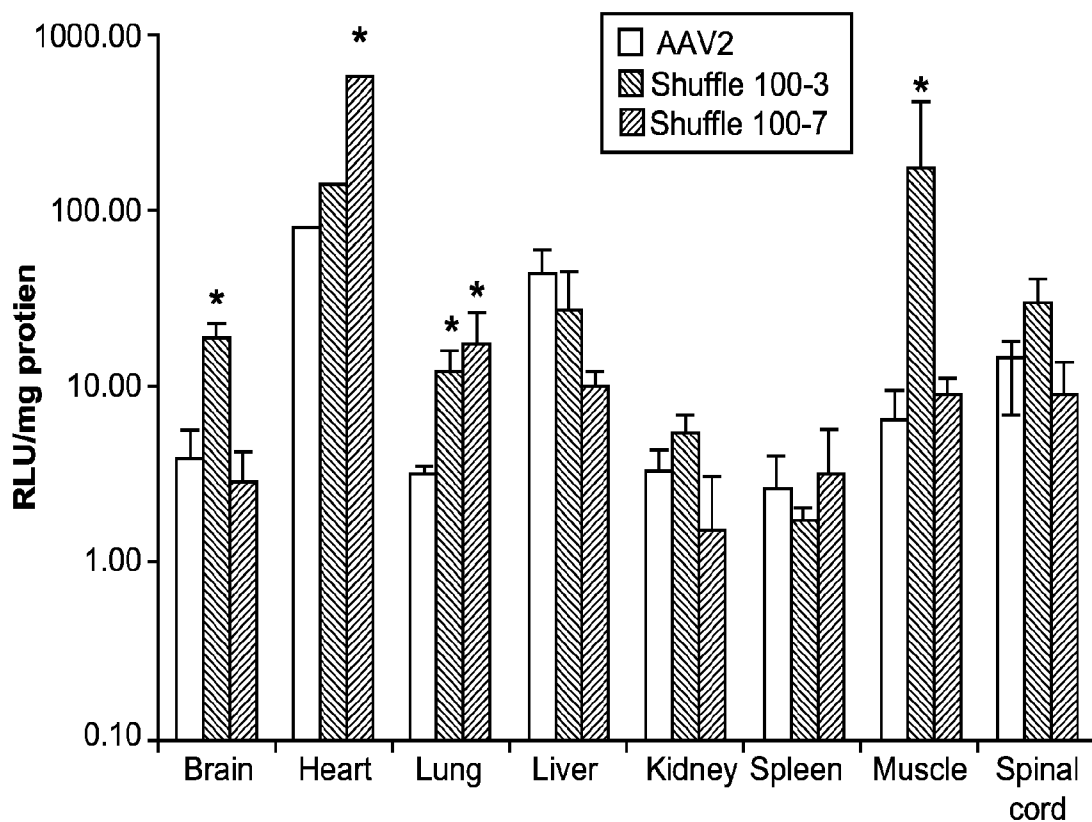


FIG. 6A

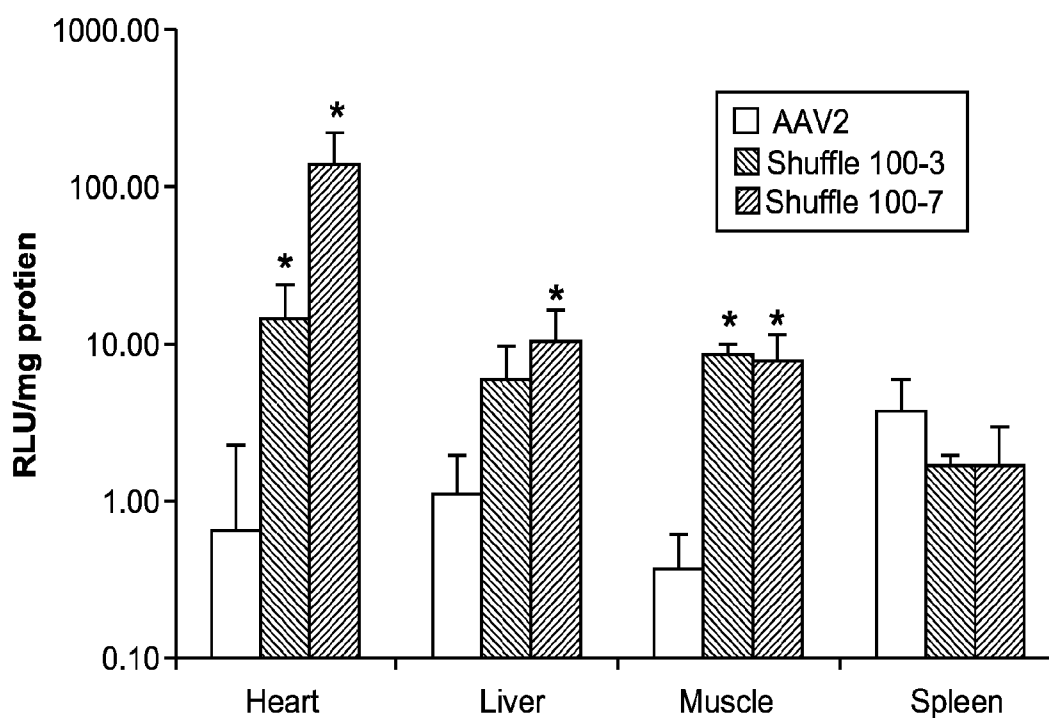


FIG. 6B

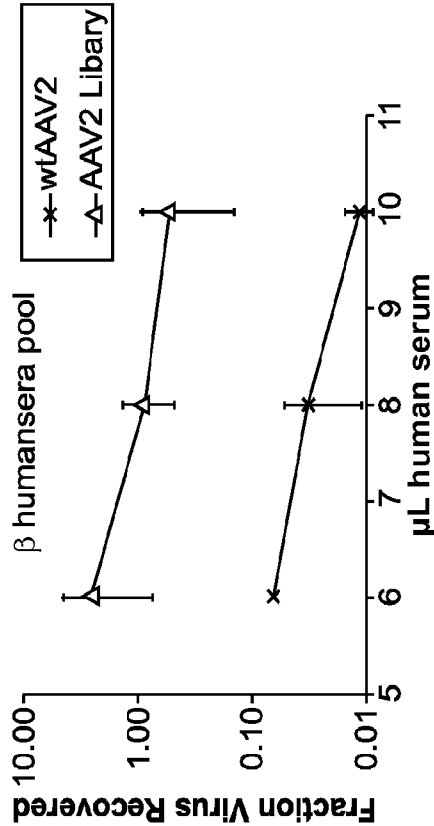


FIG. 7B

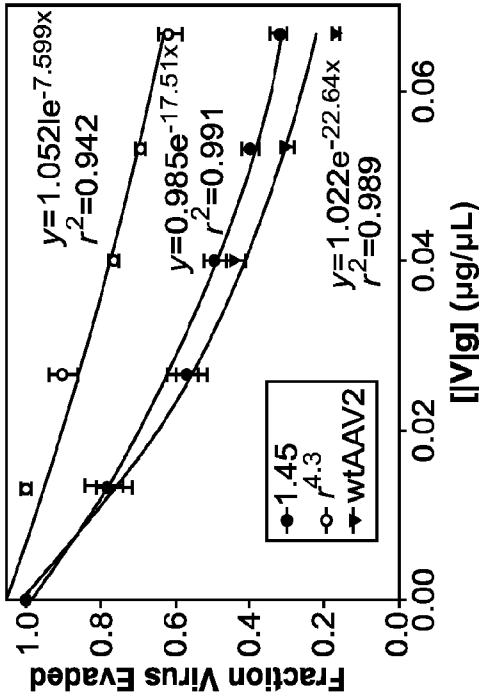


FIG. 7D

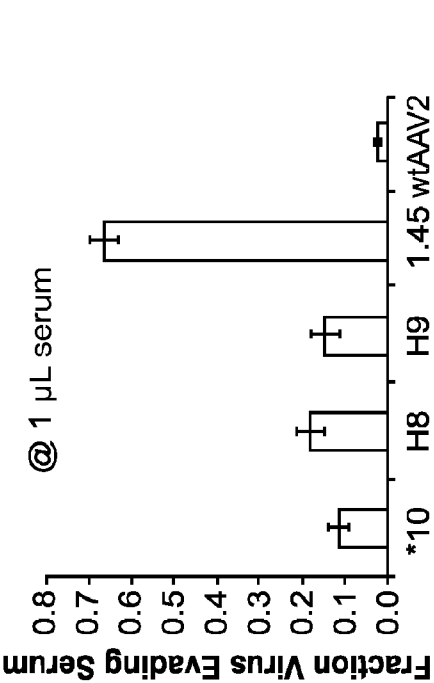


FIG. 7A

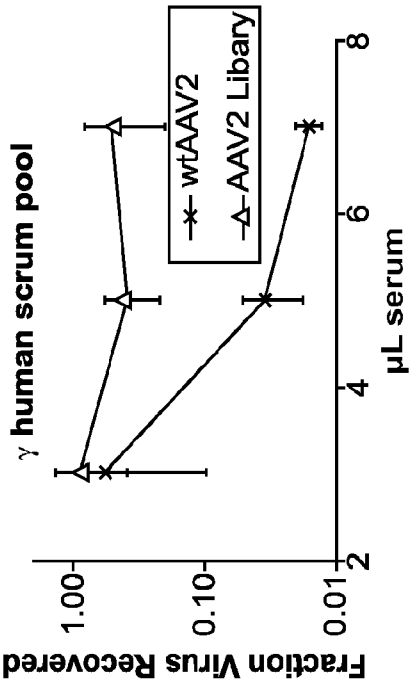


FIG. 7C

10 / 37




Consensus	1 10 20 30	MAADGYLPDWLEDNLS EGIREWWALKPGAP
Identity		
1. AAV1 translation		MAADGYLPDWLEDNLS EGIREWWDLKPGAP
2. AAV2 translation		MAADGYLPDWLEDTLSE GIRQWWKLPKPP
3. AAV3 translation		MAADGYLPDWLEDNLS EGIREWWALKPGVP
4. AAV4 translation		MTDGYLPDWLEDNLS EGVREWWALQPGAP
5. AAV5 translation		MSFVDHPPDWLEE-VGEGLREFLGLAAGPP
6. AAV6 translation		MAADGYLPDWLEDNLS EGIREWWDLKPGAP
7. AAV7 translation		MAADGYLPDWLEDNLS EGIREWWDLKPGAP
8. AAV8 translation		MAADGYLPDWLEDNLS EGIREWWALKPGAP
9. AAV9 translation		MAADGYLPDWLEDNLS EGIREWWALKPGAP
10. Shuffle 100.1 Translation		MAADGYLPDWLEDTLSE GIRQWWKLPKPP
		A... AAV
Consensus	40 50 60	KPKANQQHQDDGRGLVLPGYKYLGPFNGLD
Identity		
1. AAV1 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
2. AAV2 translation		PPKPAERHKDDSRGLVLPGYKYLGPFNGLD
3. AAV3 translation		QPKANQQHQDNRRGLVLPGYKYLGPFNGLD
4. AAV4 translation		KPKANQQHQDNARGLVLPGYKYLGPFNGLD
5. AAV5 translation		KPKPNQQHQDQARGLVLPGYNYLGPFNGLD
6. AAV6 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
7. AAV7 translation		KPKANQQKQDNGRGLVLPGYKYLGPFNGLD
8. AAV8 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
9. AAV9 translation		QPKANQQHQDNARGLVLPGYKYLGPFNGLD
10. Shuffle 100.1 Translation		PPKPAERHKDDSRGLVLPGYKYLGPFNGLD
		AAV2
Consensus	70 80 90	KGEPVNAADAAALEHDKAYDQQLKAGDNPY
Identity		
1. AAV1 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
2. AAV2 translation		KGEPVNEADAAALEHDKAYDRQLDSGDNPY
3. AAV3 translation		KGEPVNEADAAALEHDKAYDQQLKAGDNPY
4. AAV4 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
5. AAV5 translation		RGEPVNRADDEVAREHDISYNEQLEAGDNPY
6. AAV6 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
7. AAV7 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
8. AAV8 translation		KGEPVNAADAAALEHDKAYDQQLQAGDNPY
9. AAV9 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
10. Shuffle 100.1 Translation		KGEPVNEADAAALEHDKAYDQQLKAGDNPY
		AAV2 {AAV1,6,7}

FIG. 8A

11 / 37




Consensus	LKYNHADA EFQERLQEDTSFGGNLGRAVFQ
Identity	
1. AAV1 translation	LRYNHADA EFQERLQEDTSFGGNLGRAVFQ
2. AAV2 translation	LKYNHADA EFQERLKEDTSFGGNLGRAVFQ
3. AAV3 translation	LKYNHADA EFQERLQEDTSFGGNLGRAVFQ
4. AAV4 translation	LKYNHADA EFQQR LQGDTSFGGNLGRAVFQ
5. AAV5 translation	LKYNHADA EFQEK LADDTSGGNL GKAVFQ
6. AAV6 translation	LRYNHADA EFQERLQEDTSFGGNLGRAVFQ
7. AAV7 translation	LRYNHADA EFQERLQEDTSFGGNLGRAVFQ
8. AAV8 translation	LRYNHADA EFQERLQEDTSFGGNLGRAVFQ
9. AAV9 translation	LKYNHADA EFQERLKEDTSFGGNLGRAVFQ
10. Shuffle 100.1 Translation	LKYNHADA EFQQR LQGDTSFGGNLGRAVFQ
	AAV4
Consensus	AKKRVLEPLGLVEEGAKTAPGKKRPVEQSP
Identity	
1. AAV1 translation	AKKRVLEPLGLVEEGAKTAPGKKRPVEQSP
2. AAV2 translation	AKKRVLEPLGLVEEPVK TAPGKKRPVEHSP
3. AAV3 translation	AKKRILEPLGLVEEAAK TAPGKKGAVDQSP
4. AAV4 translation	AKKRVLEPLGLVEQAGETAPGKKRPLIESP
5. AAV5 translation	AKKRVLEPFG LVEEGAKTAPT GKRIDDHFP
6. AAV6 translation	AKKRVLEPFG LVEEGAKTAPGKKRPVEQSP
7. AAV7 translation	AKKRVLEPLGLVEEGAKTAPAKKRPVEPSP
8. AAV8 translation	AKKRVLEPLGLVEEGAKTAPGKKRPVEPSP
9. AAV9 translation	AKKR LLEPLGLVEEAAK TAPGKKRPVEQSP
10. Shuffle 100.1 Translation	AKKRVLEPLGLVEQAGETAPGKKRPLIESP
	AAV4
Consensus	QE-PDSSXGIGKKGQQPAKKRLNFGQTGDS
Identity	
1. AAV1 translation	QE-PDSSSGIGKTGQQPAKKRLNFGQTGDS
2. AAV2 translation	VE-PDSSSGTGKAGQQPARKRLNFGQTGDA
3. AAV3 translation	QE-PDSSSGVGKSGKQPARKRLNFGQTGDS
4. AAV4 translation	QQ-PDSS TGIGKKGKQPAKKKLVFE--DE
5. AAV5 translation	KR----KKARTEEDSKPST-----SSDA
6. AAV6 translation	QE-PDSSSGIGKTGQQPAKKRLNFGQTGDS
7. AAV7 translation	QRSPDSS TGIGKKGQQPARKRLNFGQTGDS
8. AAV8 translation	QRSPDSS TGIGKKGQQPARKRLNFGQTGDS
9. AAV9 translation	QE-PDSSAGIGKSGAQPAKKRLNFGQTGDT
10. Shuffle 100.1 Translation	QQ-PDSS TGIGKKGKQPAKKRLNFGQTGDS
	AAV4
	AAV1/6

FIG. 8B

12 / 37




Consensus	ESVPDPQPLGEPPAAP-SSVGXXTMASGGG
Identity	
1. AAV1 translation	ESVPDPQPLGEPPATP-AAVGPTTMASGGG
2. AAV2 translation	DSVPDPQPLGQPPAAP-SGLGTNTMATGSG
3. AAV3 translation	ESVPDPQPLGEPPAAP-TSLGSNTMASGGG
4. AAV4 translation	TGAGDGPPEGSTSGA--MSDDSEMRAAAGG
5. AAV5 translation	EAGPSGSQQLOIPAQPASSLGADTMSAGGG
6. AAV6 translation	ESVPDPQPLGEPPATP-AAVGPTTMASGGG
7. AAV7 translation	ESVPDPQPLGEPPAAP-SSVGS GTVAAGGG
8. AAV8 translation	ESVPDPQPLGEPPAAP-SGVGPNTMAAGGG
9. AAV9 translation	ESVPDPQPIGEPPAAP-SGVGSLTMASGGG
10. Shuffle 100.1 Translation	ESVPDPQPLGEPPATP-AAVGPTTMASGGG
	AAV1/6 >A...
Consensus	APMADNNEGADGVGNASGNWHCDS TWLGDR
Identity	
1. AAV1 translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
2. AAV2 translation	APMADNNEGADGVGNSSGNWHCDS TWMGDR
3. AAV3 translation	APMADNNEGADGVGNSSGNWHCDS QWLGDR
4. AAV4 translation	AAV-EGGQGADGVGNASGDWHCDS TWSEGH
5. AAV5 translation	GPLGDNNQGADGVGNASGDWHCDS TWMGDR
6. AAV6 translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
7. AAV7 translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
8. AAV8 translation	APMADNNEGADGVGSSSGNWHCDS TWLGDR
9. AAV9 translation	APVADNNEGADGVGSSSGNWHCDS QWLGDR
10. Shuffle 100.1 Translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
	A AAV1
Consensus	VITTSRTRTWALPTYNNHLYKQISSASX-GA
Identity	
1. AAV1 translation	VITTSRTRTWALPTYNNHLYKQISSAST-GA
2. AAV2 translation	VITTSRTRTWALPTYNNHLYKQISSQS--GA
3. AAV3 translation	VITTSRTRTWALPTYNNHLYKQISSQS--GA
4. AAV4 translation	VTTTSRTRTWVLPPTYNNHLYKRLGESL----
5. AAV5 translation	VVTKSRTRTWVLPSPYNNHQYREIKSGSVDG-
6. AAV6 translation	VITTSRTRTWALPTYNNHLYKQISSAST-GA
7. AAV7 translation	VITTSRTRTWALPTYNNHLYKQISSETA-GS
8. AAV8 translation	VITTSRTRTWALPTYNNHLYKQISNGTSGGA
9. AAV9 translation	VITTSRTRTWALPTYNNHLYKQISNSTSGGS
10. Shuffle 100.1 Translation	VITTSRTRTWALPTYNNHLYKQISSAST-GA
	AAV1

FIG. 8C

13 / 37

		280	290	300																											
Consensus		S	N	D	N	H	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
Identity		<div></div>																													
1. AAV1 translation		S	N	D	N	H	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
2. AAV2 translation		S	N	D	N	H	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
3. AAV3 translation		S	N	D	N	H	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
4. AAV4 translation		-	Q	S	N	T	Y	N	G	F	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
5. AAV5 translation		S	N	A	N	A	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	S	H	W	S	P	R	D	W
6. AAV6 translation		S	N	D	N	H	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
7. AAV7 translation		T	N	D	N	T	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
8. AAV8 translation		T	N	D	N	T	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
9. AAV9 translation		S	N	D	N	A	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
10. Shuffle 100.1 Translation		S	N	D	N	H	Y	F	G	Y	S	T	P	W	G	Y	F	D	F	N	R	F	H	C	H	F	S	P	R	D	W
		AAV1																													

		310	320	330																											
Consensus		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	N	F	K	L	F	N	I	Q	V	K	E	V	T	T	N
Identity		<div></div>																													
1. AAV1 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	N	F	K	L	F	N	I	Q	V	K	E	V	T	T	N
2. AAV2 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	N	F	K	L	F	N	I	Q	V	K	E	V	T	Q	N
3. AAV3 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	K	L	S	F	K	L	F	N	I	Q	V	R	G	V	T	Q	N
4. AAV4 translation		Q	R	L	I	N	N	N	W	G	M	R	P	K	A	M	R	V	K	I	F	N	I	Q	V	K	E	V	T	T	S
5. AAV5 translation		Q	R	L	I	N	N	Y	W	G	F	R	P	R	S	L	R	V	K	I	F	N	I	Q	V	K	E	V	T	V	Q
6. AAV6 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	N	F	K	L	F	N	I	Q	V	K	E	V	T	T	N
7. AAV7 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	K	L	R	F	K	L	F	N	I	Q	V	K	E	V	T	T	N
8. AAV8 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	S	F	K	L	F	N	I	Q	V	K	E	V	T	Q	N
9. AAV9 translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	N	F	K	L	F	N	I	Q	V	K	E	V	T	D	N
10. Shuffle 100.1 Translation		Q	R	L	I	N	N	N	W	G	F	R	P	K	R	L	N	F	K	L	F	N	I	Q	V	K	E	V	T	T	N
		AAV1																													

		340	350	360																											
Consensus		D	G	V	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	T	D	S	E	Y	Q	L	P	Y	V	L	G	S
Identity		<div></div>																													
1. AAV1 translation		D	G	V	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	S	D	S	E	Y	Q	L	P	Y	V	L	G	S
2. AAV2 translation		D	G	T	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	T	D	S	E	Y	Q	L	P	Y	V	L	G	S
3. AAV3 translation		D	G	T	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	T	D	S	E	Y	Q	L	P	Y	V	L	G	S
4. AAV4 translation		N	G	E	T	T	V	A	N	N	L	T	S	T	V	Q	I	F	A	D	S	S	Y	E	L	P	Y	V	M	D	A
5. AAV5 translation		D	S	T	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	T	D	D	D	Y	Q	L	P	Y	V	V	G	N
6. AAV6 translation		D	G	V	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	S	D	S	E	Y	Q	L	P	Y	V	L	G	S
7. AAV7 translation		D	G	V	T	T	I	A	N	N	L	T	S	T	I	Q	V	F	S	D	S	E	Y	Q	L	P	Y	V	L	G	S
8. AAV8 translation		E	G	T	K	T	I	A	N	N	L	T	S	T	I	Q	V	F	T	D	S	E	Y	Q	L	P	Y	V	L	G	S
9. AAV9 translation		N	G	V	K	T	I	A	N	N	L	T	S	T	V	Q	V	F	T	D	S	D	Y	Q	L	P	Y	V	L	G	S
10. Shuffle 100.1 Translation		D	G	V	T	T	I	A	N	N	L	T	S	T	V	Q	V	F	S	D	S	D	Y	Q	L	P	Y	V	L	G	S
		AAV1														AAV9															

FIG. 8D

14 / 37




Consensus	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
Identity	
1. AAV1 translation	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
2. AAV2 translation	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
3. AAV3 translation	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
4. AAV4 translation	GQEGSLPPFPNDVFMI PQYGYLT LN--NGS
5. AAV5 translation	GTEGCLPAFPFPQVFTLPQYGYLT LN--NGS
6. AAV6 translation	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
7. AAV7 translation	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
8. AAV8 translation	AHQGCLPPFPADVFMI PQYGYLT LN--NGS
9. AAV9 translation	AHEGCLPPFPADVFMI PQYGYLT LN--DGS
10. Shuffle 100.1 Translation	AHEGCLPPFPADVFMI PQYGYLT LN--NGS
	AAV9 AAV2
Consensus	QA-VGRSSFYCYEYFPSQMLRTGNNFTFSY
Identity	
1. AAV1 translation	QA-VGRSSFYCYEYFPSQMLRTGNNFTFSY
2. AAV2 translation	QA-VGRSSFYCYEYFPSQMLRTGNNFTFSY
3. AAV3 translation	QA-VGRSSFYCYEYFPSQMLRTGNNFQFSY
4. AAV4 translation	QQQTDRNAFYCYEYFPSQMLRTGNNFEITY
5. AAV5 translation	NP-TERSSFFCYEYFPSKMLRTGNNFEITY
6. AAV6 translation	QA-VGRSSFYCYEYFPSQMLRTGNNFTFSY
7. AAV7 translation	QS-VGRSSFYCYEYFPSQMLRTGNNFEFSY
8. AAV8 translation	QA-VGRSSFYCYEYFPSQMLRTGNNFQFTY
9. AAV9 translation	QA-VGRSSFYCYEYFPSQMLRTGNNFQFSY
10. Shuffle 100.1 Translation	QA-VGRSSFYCYEYFPSQMLRTGNNFTFSY
	AAV2
Consensus	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
Identity	
1. AAV1 translation	TFEEVPFHSSYAHSQSLDRLMNPLIDQYLY
2. AAV2 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
3. AAV3 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
4. AAV4 translation	SFEKVPFHSMYAHSQSLDRLMNPLIDQYLW
5. AAV5 translation	NFEEVPFHSSFAPSQNLFKLANPLVDQYLY
6. AAV6 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
7. AAV7 translation	SFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
8. AAV8 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
9. AAV9 translation	EFENVPFHSSYAHSQSLDRLMNPLIDQYLY
10. Shuffle 100.1 Translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
	AAV2

FIG. 8E

15 / 37

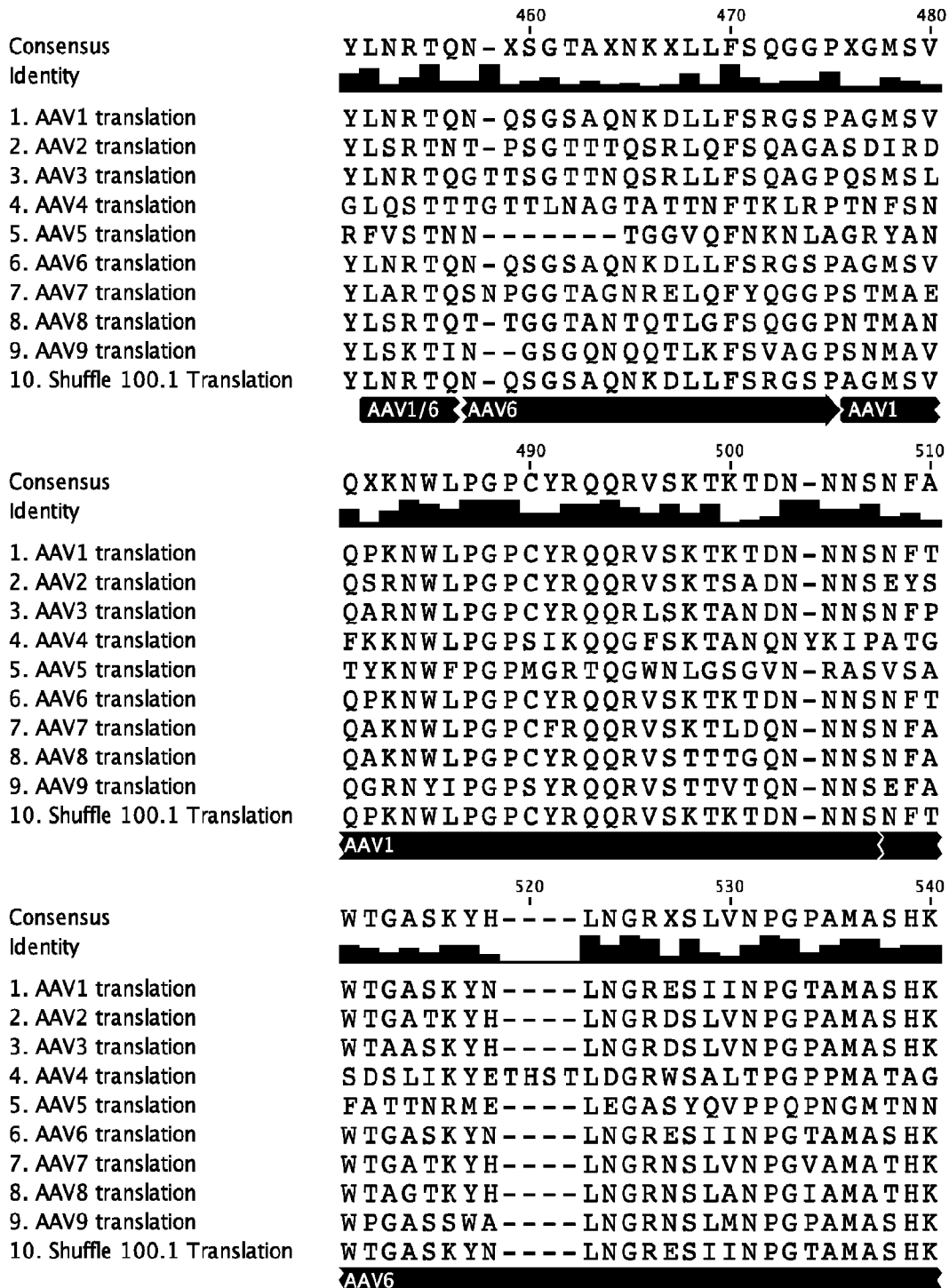


FIG. 8F

16 / 37

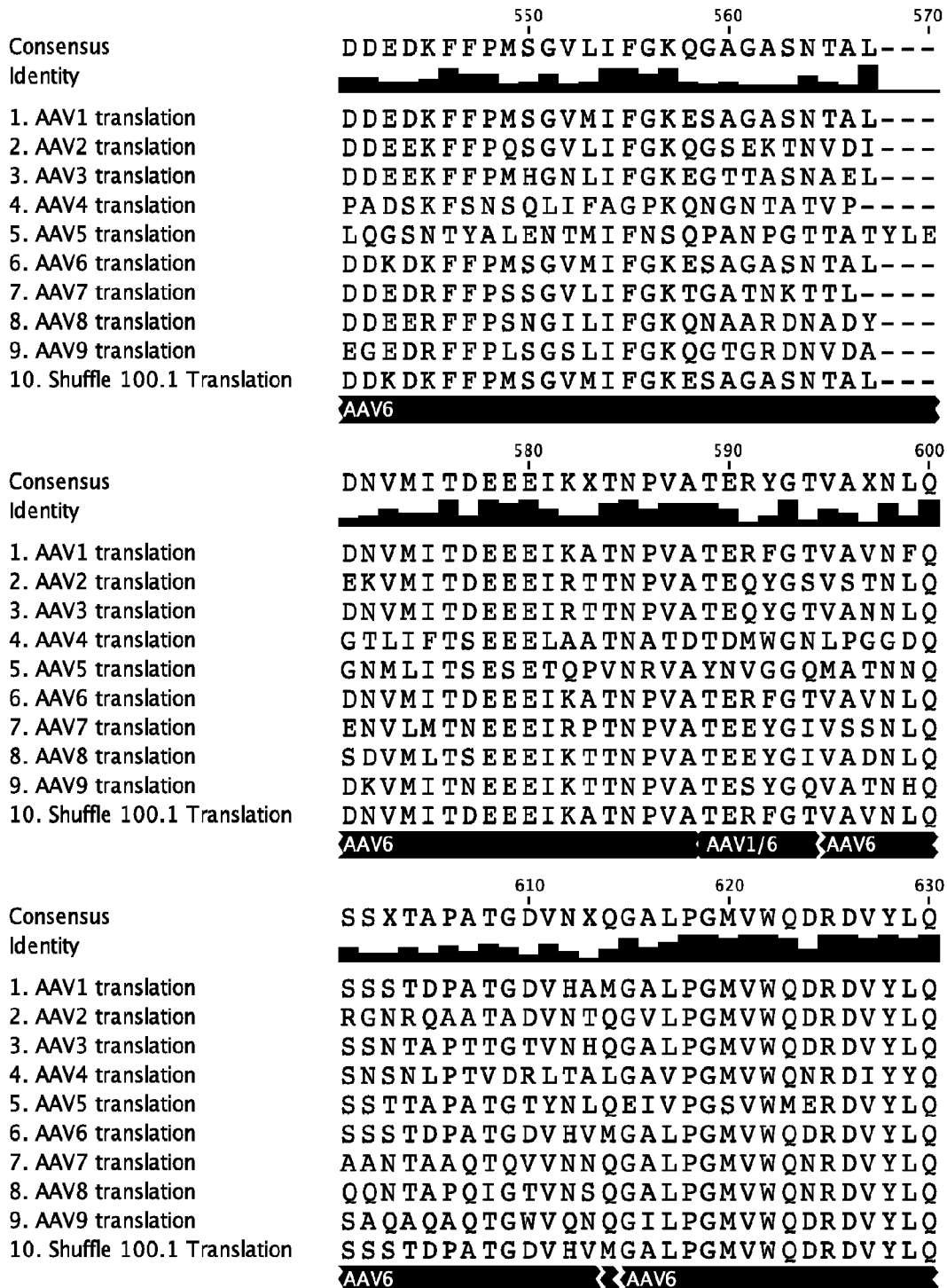


FIG. 8G

17 / 37




Consensus	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
Identity	
1. AAV1 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKNPPP
2. AAV2 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
3. AAV3 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
4. AAV4 translation	GPIWAKIPHTDGHFHPSPLIGGFGLKHPPP
5. AAV5 translation	GPIWAKIPETGAHFHPSPLMGGFGLKHPPP
6. AAV6 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
7. AAV7 translation	GPIWAKIPHTDGNFHPSPLMGGFGLKHPPP
8. AAV8 translation	GPIWAKIPHTDGNFHPSPLMGGFGLKHPPP
9. AAV9 translation	GPIWAKIPHTDGNFHPSPLMGGFGMKHPPP
10. Shuffle 100.1 Translation	GPIWAKIPHTDGHFHPSPLMGGFGLKNPPP
	AAV6 AAV1
Consensus	QILIKNTPVPANPPTTFSATKFASFITQYS
Identity	
1. AAV1 translation	QILIKNTPVPANPPAEFSATKFASFITQYS
2. AAV2 translation	QILIKNTPVPANPSTTFSAAKFASFITQYS
3. AAV3 translation	QIMIKNTPVPANPPTTFSPAKFASFITQYS
4. AAV4 translation	QIFIKNTPVPANPATTFSSTPVNSFITQYS
5. AAV5 translation	MMLIKNTPVPGN-ITSFSDVPVSSFITQYS
6. AAV6 translation	QILIKNTPVPANPPAEFSATKFASFITQYS
7. AAV7 translation	QILIKNTPVPANPPEVFTPAPKFASFITQYS
8. AAV8 translation	QILIKNTPVPADPPTTFNQSKLNSFITQYS
9. AAV9 translation	QILIKNTPVPADPPTAFNKDKLNSFITQYS
10. Shuffle 100.1 Translation	QILIKNTPVPANPPAEFSATKFASFITQYS
	AAV1
Consensus	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
Identity	
1. AAV1 translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
2. AAV2 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
3. AAV3 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
4. AAV4 translation	TGQVSVQIDWEIQKERS KRWNPEVQFTSNY
5. AAV5 translation	TGQVTVEMEWELKKENS KRWNPEIQYTNNY
6. AAV6 translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
7. AAV7 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNF
8. AAV8 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
9. AAV9 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
10. Shuffle 100.1 Translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
	AAV6

FIG. 8H

18 / 37




		730	740	750
Consensus	XKSANVDFXVDTNGVYSEPRPIGTRYLTRN			
Identity				
1. AAV1 translation	AKSANVDFTVDNNGLYTEPRPIGTRYLTRP			
2. AAV2 translation	NKS VNVDFTVD TNGVYSEPRPIGTRYLTRN			
3. AAV3 translation	NKS VNVDFTVD TNGVYSEPRPIGTRYLTRN			
4. AAV4 translation	GQQNSLLWAPDAAGKYTEPRAIGTRYLTHH			
5. AAV5 translation	NDPQFVDFAPDSTGEYRTTRPIGTRYLTRP			
6. AAV6 translation	AKSANVDFTVDNNGLYTEPRPIGTRYLTRP			
7. AAV7 translation	EKQTGVDFAVDSQGVYSEPRPIGTRYLTRN			
8. AAV8 translation	YKSTSVDFAVNTEGVYSEPRPIGTRYLTRN			
9. AAV9 translation	YKSNNVEFAVNTEGVYSEPRPIGTRYLTRN			
10. Shuffle 100.1 Translation	AKSANVDFTVDNNGLYTEPRPIGTRYLTRP			
	AAV6			
		752		
Consensus	L *			
Identity				
1. AAV1 translation	L *			
2. AAV2 translation	L *			
3. AAV3 translation	L *			
4. AAV4 translation	L *			
5. AAV5 translation	L *			
6. AAV6 translation	L *			
7. AAV7 translation	L *			
8. AAV8 translation	L *			
9. AAV9 translation	L *			
10. Shuffle 100.1 Translation	L *			
				

FIG. 8I

19 / 37




Consensus	1 10 20 30	MAADGYLPDWLEDNLSEGIREWALKPGAP
Identity		
1. AAV1 translation		MAADGYLPDWLEDNLSEGIREWDLKPGAP
2. AAV2 translation		MAADGYLPDWLEDTLSEGIQWWKLPKPGPP
3. AAV3 translation		MAADGYLPDWLEDNLSEGIREWALKPGVP
4. AAV4 translation		MTDGYLPDWLEDNLSEGVREWWALQPGAP
5. AAV5 translation		MSFVDHPPDWLEE-VGEGLRFLGLEAGPP
6. AAV6 translation		MAADGYLPDWLEDNLSEGIREWDLKPGAP
7. AAV7 translation		MAADGYLPDWLEDNLSEGIREWDLKPGAP
8. AAV8 translation		MAADGYLPDWLEDNLSEGIREWALKPGAP
9. AAV9 translation		MAADGYLPDWLEDNLSEGIREWALKPGAP
10. Shuffle 100-3 Translation		MAADGYLPDWLEDTLSEGIQWWKLPKPGPP
		A... AAV2
Consensus	40 50 60	KPKANQQHQDDGRGLVLPGYKYLGPFNGLD
Identity		
1. AAV1 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
2. AAV2 translation		PPKPAERHKDDSRGLVLPGYKYLGPFNGLD
3. AAV3 translation		QPKANQQHQDNRRGLVLPGYKYLGPFNGLD
4. AAV4 translation		KPKANQQHQDNARGLVLPGYKYLGPFNGLD
5. AAV5 translation		KPKPNQQHQDQARGLVLPGYNYLGPFNGLD
6. AAV6 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
7. AAV7 translation		KPKANQQKQDNRRGLVLPGYKYLGPFNGLD
8. AAV8 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
9. AAV9 translation		QPKANQQHQDNARGLVLPGYKYLGPFNGLD
10. Shuffle 100-3 Translation		PPKPAERHKDDSRGLVLPGYKYLGPFNGLD
		AAV2
Consensus	70 80 90	KGEPVNAADAAALEHDKAYDQQLKAGDNPY
Identity		
1. AAV1 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
2. AAV2 translation		KGEPVNEADAAALEHDKAYDRQLDSGDNPY
3. AAV3 translation		KGEPVNEADAAALEHDKAYDQQLKAGDNPY
4. AAV4 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
5. AAV5 translation		RGEPVNRADDEVAREHDISYNEQLEAGDNPY
6. AAV6 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
7. AAV7 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
8. AAV8 translation		KGEPVNAADAAALEHDKAYDQQLQAGDNPY
9. AAV9 translation		KGEPVNAADAAALEHDKAYDQQLKAGDNPY
10. Shuffle 100-3 Translation		KGEPVNEADAAALEHDKAYDQQLKAGDNPY
		AAV2 AAV1,6,7

FIG. 9A

20 / 37







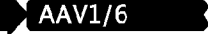
Consensus	LKYNHADA ¹⁰⁰ EFQERLQEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
Identity	
1. AAV1 translation	LRYNHADA ¹⁰⁰ EFQERLQEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
2. AAV2 translation	LKYNHADA ¹⁰⁰ EFQERLKEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
3. AAV3 translation	LKYNHADA ¹⁰⁰ EFQERLQEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
4. AAV4 translation	LKYNHADA ¹⁰⁰ EFQ ¹¹⁰ QRLQGDTS ¹²⁰ FGGNLGRAVFQ ¹²⁰
5. AAV5 translation	LKYNHADA ¹⁰⁰ EFQ ¹¹⁰ EKLADDTST ¹²⁰ FGGNLKGAVFQ ¹²⁰
6. AAV6 translation	LRYNHADA ¹⁰⁰ EFQERLQEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
7. AAV7 translation	LRYNHADA ¹⁰⁰ EFQERLQEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
8. AAV8 translation	LRYNHADA ¹⁰⁰ EFQERLQEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
9. AAV9 translation	LKYNHADA ¹⁰⁰ EFQERLKEDTS ¹¹⁰ FGGNLGRAVFQ ¹²⁰
10. Shuffle 100-3 Translation	LKYNHADA ¹⁰⁰ EFQ ¹¹⁰ QRLQGDTS ¹²⁰ FGGNLGRAVFQ ¹²⁰
	
Consensus	AKKRVLEPLGLVEEGAKTAPGKKRPVEQSP ¹³⁰
Identity	
1. AAV1 translation	AKKRVLEPLGLVEEGAKTAPGKKRPVEQSP
2. AAV2 translation	AKKRVLEPLGLVEEPPVKTAPGKKRPVEHSP
3. AAV3 translation	AKKRILEPLGLVEEAAKTAPGKKGAVDQSP
4. AAV4 translation	AKKRVLEPLGLVEEQAGETAPGKKRPLIESP
5. AAV5 translation	AKKRVLEPFGLVVEEGAKTAPTGKRIDDHFP
6. AAV6 translation	AKKRVLEPFGLVVEEGAKTAPGKKRPVEQSP
7. AAV7 translation	AKKRVLEPLGLVEEGAKTAPAKKRPVEPS
8. AAV8 translation	AKKRVLEPLGLVEEGAKTAPGKKRPVEPS
9. AAV9 translation	AKKRILLEPLGLVEEAAKTAPGKKRPVEQSP
10. Shuffle 100-3 Translation	AKKRVLEPLGLVEEQAGETAPGKKRPLIESP
	
Consensus	QE-PDSSXG ¹⁶⁰ IGKKGQQPAKKRLNFGQTGDS ¹⁷⁰
Identity	
1. AAV1 translation	QE-PDSSSGIGKTGQQPAKKRLNFGQTGDS
2. AAV2 translation	VE-PDSSSGTGKAGQQPARKRLNFGQTGDA
3. AAV3 translation	QE-PDSSSGVGKSGKQPARKRLNFGQTGDS
4. AAV4 translation	QQ-PDSSTGIGKKGKQPAKKKLVE---DE
5. AAV5 translation	KR----KKARTEEDSKPST-----SSDA
6. AAV6 translation	QE-PDSSSGIGKTGQQPAKKRLNFGQTGDS
7. AAV7 translation	QRS PDSS TGIGKKGQQPARKRLNFGQTGDS
8. AAV8 translation	QRS PDSS TGIGKKGQQPARKRLNFGQTGDS
9. AAV9 translation	QE-PDSSAGIGKSGAQPAKKRLNFGQTGDT
10. Shuffle 100-3 Translation	QQ-PDSS TGIGKKGKQPAKKRLNFGQTGDS
	 

FIG. 9B

21 / 37




Consensus	ESVPDPQPLGEPPAAP-SSVGXXTMASGGG
Identity	
1. AAV1 translation	ESVPDPQPLGEPPATP-AAVGPTTMASGGG
2. AAV2 translation	DSVPDPQPLGQPPAAP-SGLGTNTMATGSG
3. AAV3 translation	ESVPDPQPLGEPPAAP-TSLGSNTMASGGG
4. AAV4 translation	TGAGDGPPEGSTSGA--MSDDSEMRAAAGG
5. AAV5 translation	EAGPSGSQQQLQIPAQPASSLGADTMSAGGG
6. AAV6 translation	ESVPDPQPLGEPPATP-AAVGPTTMASGGG
7. AAV7 translation	ESVPDPQPLGEPPAAP-SSVGSGTVAAGGG
8. AAV8 translation	ESVPDPQPLGEPPAAP-SGVGPNTMAAGGG
9. AAV9 translation	ESVPDPQPIGEPPAAP-SGVGSLTMASGGG
10. Shuffle 100-3 Translation	ESVPDPQPLGEPPATP-AAVGPTTMASGGG
	AAV1/6 } A...
Consensus	APMADNNEGADGVGNASGNWHCDS TWLGDR
Identity	
1. AAV1 translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
2. AAV2 translation	APMADNNEGADGVGNSSGNWHCDS TWMGDR
3. AAV3 translation	APMADNNEGADGVGNSSGNWHCDS QWLGDR
4. AAV4 translation	AAV-EGGQGADGVGNASGDWHCDS TWSEGH
5. AAV5 translation	GPLGDNNQGADGVGNASGDWHCDS TWMGDR
6. AAV6 translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
7. AAV7 translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
8. AAV8 translation	APMADNNEGADGVGSSSGNWHCDS TWLGDR
9. AAV9 translation	APVADNNEGADGVGSSSGNWHCDS QWLGDR
10. Shuffle 100-3 Translation	APMADNNEGADGVGNASGNWHCDS TWLGDR
	A AAV1
Consensus	VITTSSTRTWALPTYNNHLYKQISSASX-GA
Identity	
1. AAV1 translation	VITTSSTRTWALPTYNNHLYKQISSAST-GA
2. AAV2 translation	VITTSSTRTWALPTYNNHLYKQISSQS--GA
3. AAV3 translation	VITTSSTRTWALPTYNNHLYKQISSQS--GA
4. AAV4 translation	VTTTSTRTWVLPPTYNNHLYKRLGESL----
5. AAV5 translation	VVTKSTRTWVLP SYNNHQYREIKSGSVDG-
6. AAV6 translation	VITTSSTRTWALPTYNNHLYKQISSAST-GA
7. AAV7 translation	VITTSSTRTWALPTYNNHLYKQISSETA-GS
8. AAV8 translation	VITTSSTRTWALPTYNNHLYKQISNGTSGGA
9. AAV9 translation	VITTSSTRTWALPTYNNHLYKQISNSTSGGS
10. Shuffle 100-3 Translation	VITTSSTRTWALPTYNNHLYKQISSAST-GA
	AAV1

FIG. 9C

22 / 37




Consensus	SNDNHYFGYS ²⁸⁰ TPWGYFDFNR ²⁹⁰ FHCHFS ³⁰⁰ PRDW
Identity	
1. AAV1 translation	SNDNHYFGYSTPWGYFDFNRFHCHFSPRDW
2. AAV2 translation	SNDNHYFGYSTPWGYFDFNRFHCHFSPRDW
3. AAV3 translation	SNDNHYFGYSTPWGYFDFNRFHCHFSPRDW
4. AAV4 translation	- QSN TYNGFSTPWGYFDFNRFHCHFSPRDW
5. AAV5 translation	SNANAYFGYSTPWGYFDFNRFHSHWSPRDW
6. AAV6 translation	SNDNHYFGYSTPWGYFDFNRFHCHFSPRDW
7. AAV7 translation	TNDNTYFGYSTPWGYFDFNRFHCHFSPRDW
8. AAV8 translation	TNDNTYFGYSTPWGYFDFNRFHCHFSPRDW
9. AAV9 translation	SNDNAYFGYSTPWGYFDFNRFHCHFSPRDW
10. Shuffle 100-3 Translation	SNDNHYFGYSTPWGYFDFNRFHCHFSPRDW
	AAV1
Consensus	QRLINNNWGF ³¹⁰ RPKRLNFKLFNI ³²⁰ QVKEVTTN ³³⁰
Identity	
1. AAV1 translation	QRLINNNWGF ³¹⁰ RPKRLNFKLFNI ³²⁰ QVKEVTTN
2. AAV2 translation	QRLINNNWGF ³¹⁰ RPKRLNFKLFNI ³²⁰ QVKEVTQN
3. AAV3 translation	QRLINNNWGF ³¹⁰ RPKKLSFKLFNI ³²⁰ QVRGVTON
4. AAV4 translation	QRLINNNWGM ³¹⁰ RPKAMRVKI ³²⁰ FNI ³³⁰ QVKEVTTTS
5. AAV5 translation	QRLINNYWGF ³¹⁰ RP ³²⁰ SLRVKI ³³⁰ FNI ³⁴⁰ QVKEVTVQ
6. AAV6 translation	QRLINNNWGF ³¹⁰ RPKRLNFKLFNI ³²⁰ QVKEVTTN
7. AAV7 translation	QRLINNNWGF ³¹⁰ RPKKLRFKLFNI ³²⁰ QVKEVTTN
8. AAV8 translation	QRLINNNWGF ³¹⁰ RPKRLSFKLFNI ³²⁰ QVKEVTQN
9. AAV9 translation	QRLINNNWGF ³¹⁰ RPKRLNFKLFNI ³²⁰ QVKEVTDN
10. Shuffle 100-3 Translation	QRLINNNWGF ³¹⁰ RPKRLNFKLFNI ³²⁰ QVKEVTTN
	AAV1
Consensus	DGVTTIANNL ³⁴⁰ TS ³⁵⁰ TVQVF ³⁶⁰ TDSEYQLPYVLGS
Identity	
1. AAV1 translation	DGVTTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ SDSEYQLPYVLGS
2. AAV2 translation	DGTTTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ TDSEYQLPYVLGS
3. AAV3 translation	DGTTTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ TDSEYQLPYVLGS
4. AAV4 translation	NGETTVANNLTS ³⁴⁰ TVQIFADSSYELPYVMDA
5. AAV5 translation	DSTTTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ TDDDYQLPYVVG
6. AAV6 translation	DGVTTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ SDSEYQLPYVLGS
7. AAV7 translation	DGVTTIANNLTS ³⁴⁰ TIQVF ³⁵⁰ SDSEYQLPYVLGS
8. AAV8 translation	EGTKTIANNLTS ³⁴⁰ TIQVF ³⁵⁰ TDSEYQLPYVLGS
9. AAV9 translation	NGVKTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ TDSDYQLPYVLGS
10. Shuffle 100-3 Translation	DGVTTIANNLTS ³⁴⁰ TVQVF ³⁵⁰ SDSDYQLPYVLGS
	AAV1 AAV9

FIG. 9D

23 / 37




Consensus	AHQGCLPPFPADVFMIPQYGYLTNL--NGS
Identity	
1. AAV1 translation	AHQGCLPPFPADVFMIPQYGYLTNL--NGS
2. AAV2 translation	AHQGCLPPFPADVFMVPQYGYLTNL--NGS
3. AAV3 translation	AHQGCLPPFPADVFMVPQYGYLTNL--NGS
4. AAV4 translation	GQEGSLPPFPNDVFMVPQYGYCGLVTGNTS
5. AAV5 translation	GTEGCLPAFPFPQVFTLPQYGYATLNRDNTS
6. AAV6 translation	AHQGCLPPFPADVFMIPQYGYLTNL--NGS
7. AAV7 translation	AHQGCLPPFPADVFMIPQYGYLTNL--NGS
8. AAV8 translation	AHQGCLPPFPADVFMIPQYGYLTNL--NGS
9. AAV9 translation	AHEGCLPPFPADVFMIPQYGYLTNL--DGS
10. Shuffle 100-3 Translation	AHEGCLPPFPADVFMVPQYGYLTNL--NGS
	AAV9 AAV2
Consensus	QA-VGRSSFYCLEYFPSQMLRTGNNFTFSY
Identity	
1. AAV1 translation	QA-VGRSSFYCLEYFPSQMLRTGNNFTFSY
2. AAV2 translation	QA-VGRSSFYCLEYFPSQMLRTGNNFTFSY
3. AAV3 translation	QA-VGRSSFYCLEYFPSQMLRTGNNFQFSY
4. AAV4 translation	QQQTDRNAFYCLEYFPSQMLRTGNNFEITY
5. AAV5 translation	NP-TERSSFFCLEYFPSKMLRTGNNFEFTY
6. AAV6 translation	QA-VGRSSFYCLEYFPSQMLRTGNNFTFSY
7. AAV7 translation	QS-VGRSSFYCLEYFPSQMLRTGNNFEFSY
8. AAV8 translation	QA-VGRSSFYCLEYFPSQMLRTGNNFQFTY
9. AAV9 translation	QA-VGRSSFYCLEYFPSQMLRTGNNFQFSY
10. Shuffle 100-3 Translation	QA-VGRSSFYCLEYFPSQMLRTGNNFTFSY
	AAV2
Consensus	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
Identity	
1. AAV1 translation	TFEEVPPFHSSYAHSQSLDRLMNPLIDQYLY
2. AAV2 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
3. AAV3 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
4. AAV4 translation	SFEKVPFHSMYAHSQSLDRLMNPLIDQYLW
5. AAV5 translation	NFEEVPPFHSSFAPSQNLFKLANPLVDQYLY
6. AAV6 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
7. AAV7 translation	SFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
8. AAV8 translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
9. AAV9 translation	EFENVPPFHSSYAHSQSLDRLMNPLIDQYLY
10. Shuffle 100-3 Translation	TFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
	AAV2

FIG. 9E

24 / 37

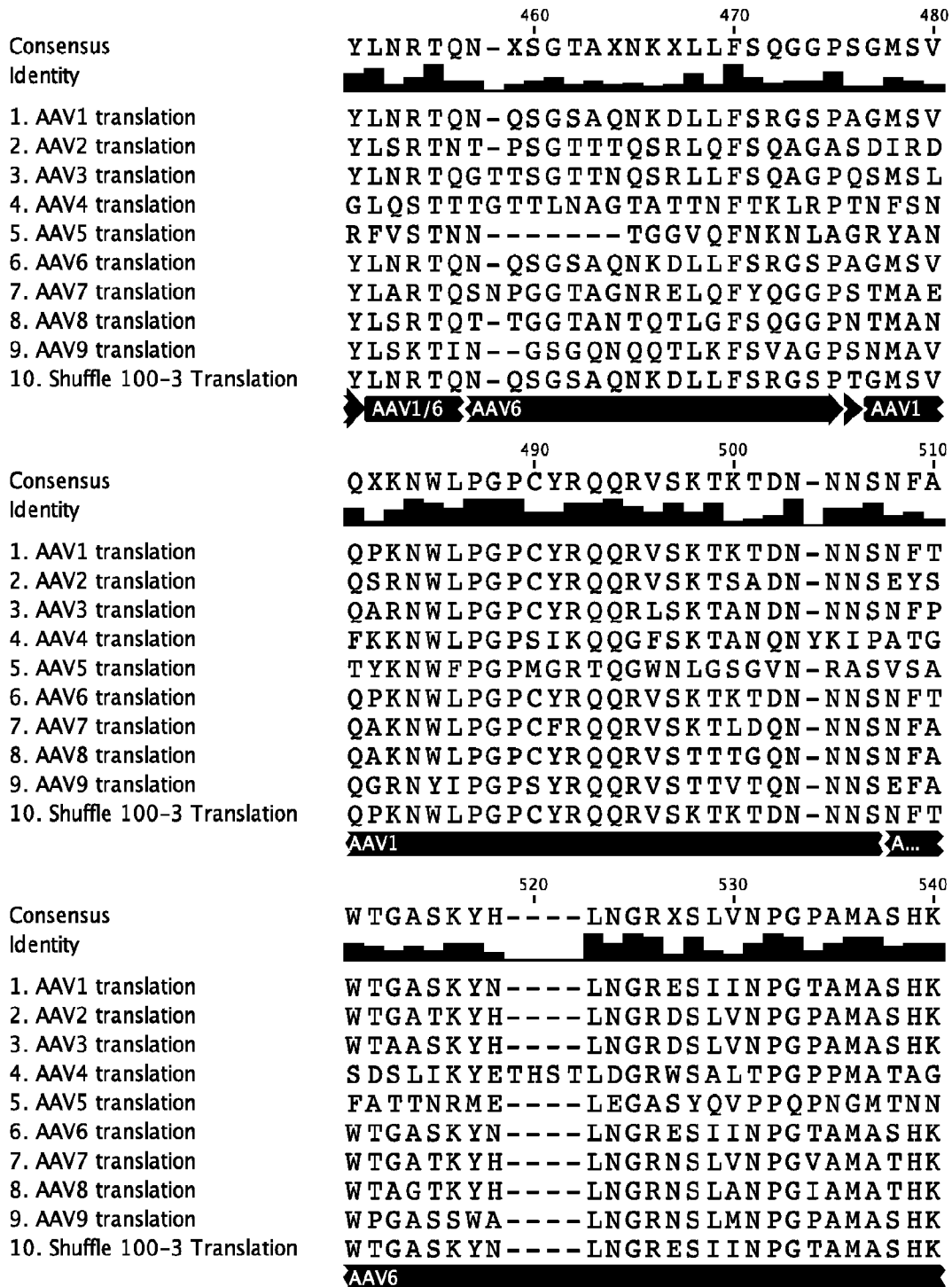


FIG. 9F

25 / 37

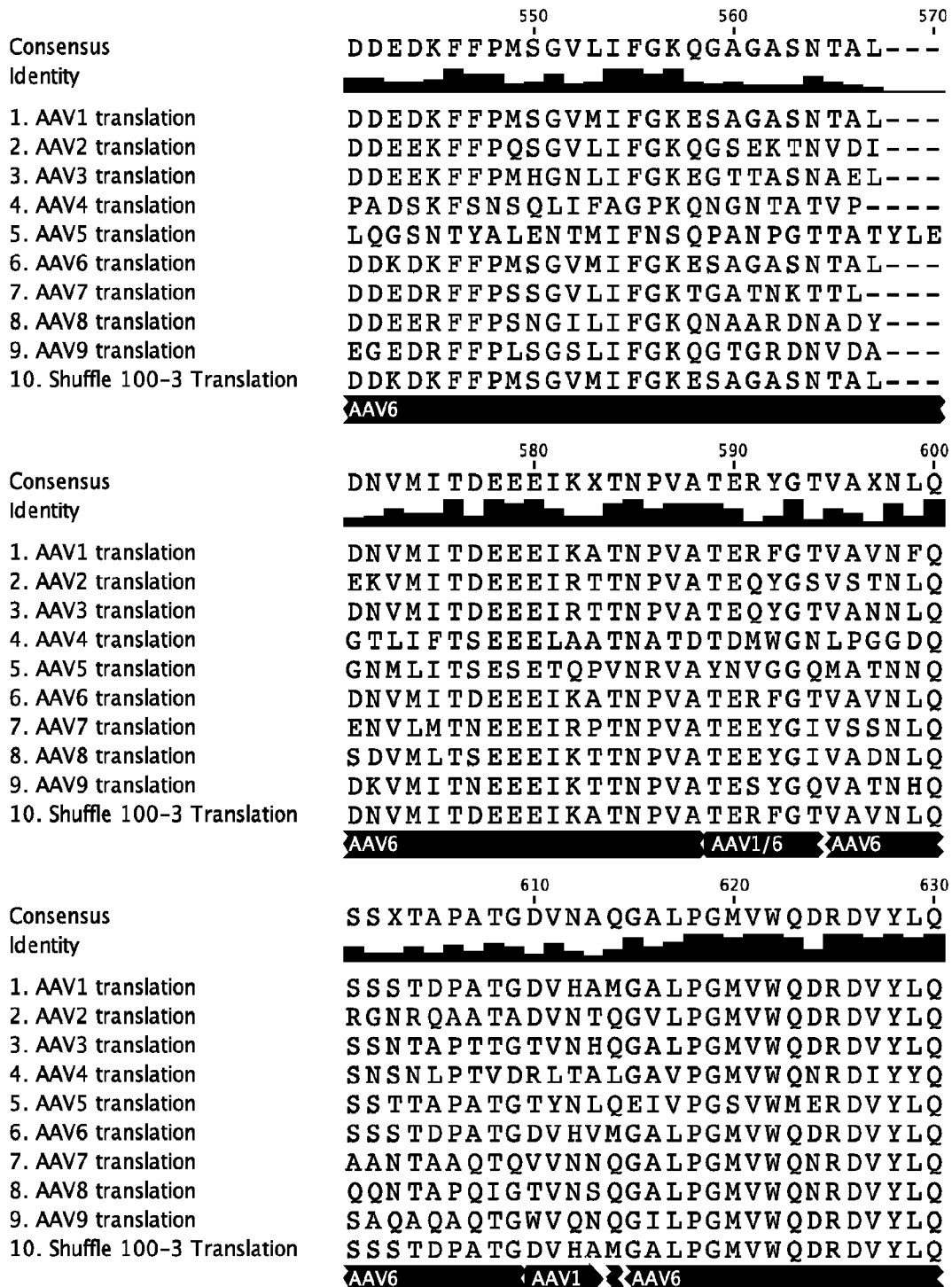


FIG. 9G

26 / 37

Consensus Identity	<div> <div>640650660</div> <div>GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP</div> <div></div> </div>
1. AAV1 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKNPPP
2. AAV2 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
3. AAV3 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
4. AAV4 translation	GPIWAKIPHTDGHFHPSPLIGGFGLKHPPP
5. AAV5 translation	GPIWAKIPETGAHFHPSPLMGGFGLKHPPP
6. AAV6 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
7. AAV7 translation	GPIWAKIPHTDGNFHPSPLMGGFGLKHPPP
8. AAV8 translation	GPIWAKIPHTDGNFHPSPLMGGFGLKHPPP
9. AAV9 translation	GPIWAKIPHTDGNFHPSPLMGGFGMKHPPP
10. Shuffle 100-3 Translation	GPIWAKIPHTDGHFHPSPLMGGFGLKNPPP
	AAV6AAV1
Consensus Identity	<div> <div>670680690</div> <div>QILIKNTPVPANPPTTFSA TKFASFITQYS</div> <div></div> </div>
1. AAV1 translation	QILIKNTPVPANPPAEFSATKFASFITQYS
2. AAV2 translation	QILIKNTPVPANPSTTFSAAKFASFITQYS
3. AAV3 translation	QIMIKNTPVPANPPTTFSPA K FASFITQYS
4. AAV4 translation	QIFIKNTPVPANPATTFSS TPVNSFITQYS
5. AAV5 translation	MMLIKNTPVPGN-ITSFSDVPVSSFITQYS
6. AAV6 translation	QILIKNTPVPANPPAEFSATKFASFITQYS
7. AAV7 translation	QILIKNTPVPANPPPEVFTP AK FASFITQYS
8. AAV8 translation	QILIKNTPVPADPPTTFNQSKLNSFITQYS
9. AAV9 translation	QILIKNTPVPADPPTAFNKDKLNSFITQYS
10. Shuffle 100-3 Translation	QILIKNTPVPANPPAEFSATKFASFITQYS
	AAV1A...
Consensus Identity	<div> <div>700710720</div> <div>TGQVSVEIEWELQKENS KRWNPEIQYTSNY</div> <div></div> </div>
1. AAV1 translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
2. AAV2 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
3. AAV3 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
4. AAV4 translation	TGQVSVQIDWEIQKERS KRWNPEVQFTSNY
5. AAV5 translation	TGQVTVEMEWELKKENS KRWNPEIQYTNNY
6. AAV6 translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
7. AAV7 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNF
8. AAV8 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
9. AAV9 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
10. Shuffle 100-3 Translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
	AAV6

FIG. 9H

27 / 37

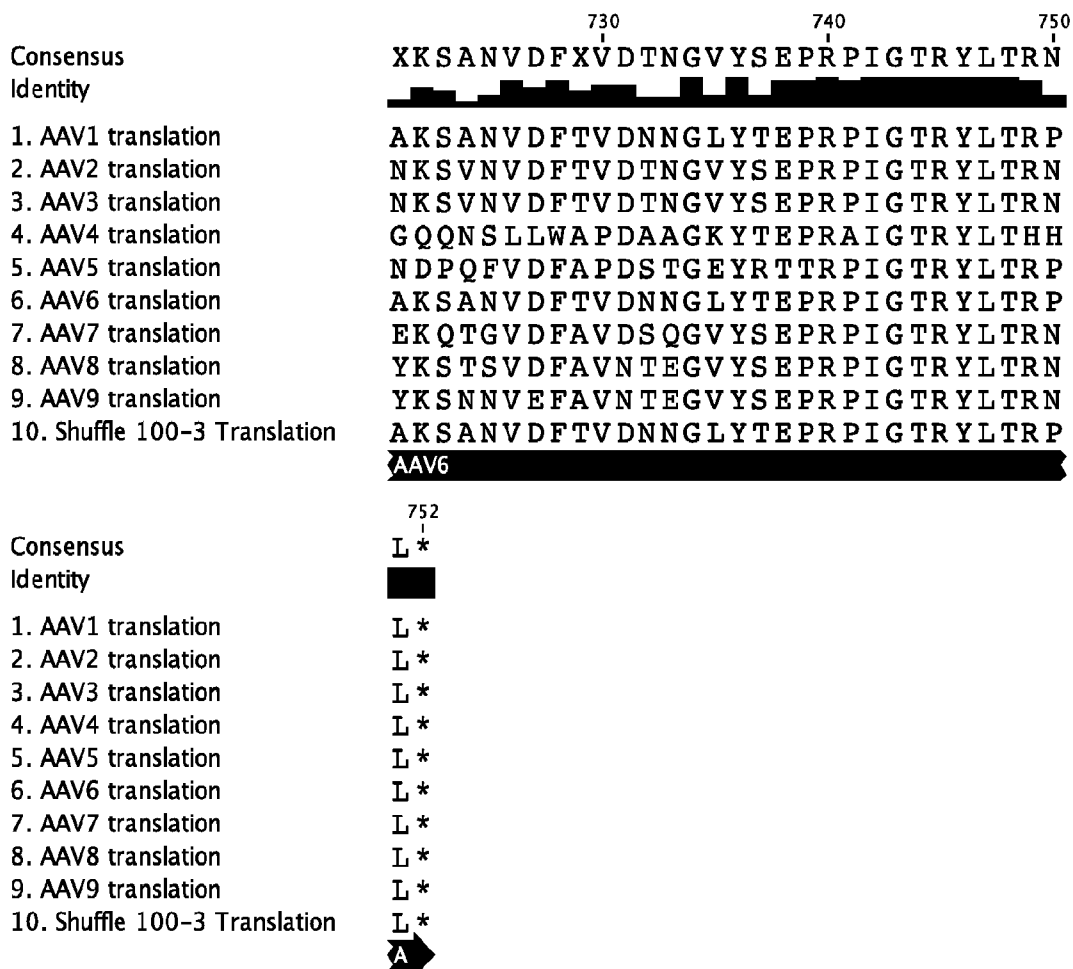


FIG. 9I

28 / 37




Consensus	1 10 20 30	MAADGYLPDWLEDNLSEGIREWALKPGAP
Identity		
1. AAV1 translation		MAADGYLPDWLEDNLSEGIREWWDLKPGAP
2. AAV2 translation		MAADGYLPDWLEDTLSEGIQWWKLKPGPP
3. AAV3 translation		MAADGYLPDWLEDNLSEGIREWALKPGVP
4. AAV4 translation		MTDGYLPDWLEDNLSEGVREWWALQPGAP
5. AAV5 translation		MSFVDHPPDWLEE-VGEGLRFLGLEAGPP
6. AAV6 translation		MAADGYLPDWLEDNLSEGIREWWDLKPGAP
7. AAV7 translation		MAADGYLPDWLEDNLSEGIREWWDLKPGAP
8. AAV8 translation		MAADGYLPDWLEDNLSEGIREWALKPGAP
9. AAV9 translation		MAADGYLPDWLEDNLSEGIREWALKPGAP
10. Shuffle 100.7 Translation		MAADGYLPDWLEDNLSEGIREWALKPGAP
		AAV8
Consensus	40 50 60	KPKANQQXQDDGRGLVLPGYKYLGPFNGLD
Identity		
1. AAV1 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
2. AAV2 translation		PPKPAERHKDDSRGLVLPGYKYLGPFNGLD
3. AAV3 translation		QPKANQQHQDNRRGLVLPGYKYLGPGNGLD
4. AAV4 translation		KPKANQQHQDNARGLVLPGYKYLGPGNGLD
5. AAV5 translation		KPKPNQQHQDQARGLVLPGYNYLGPGNGLD
6. AAV6 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
7. AAV7 translation		KPKANQQKQDNARGLVLPGYKYLGPFNGLD
8. AAV8 translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
9. AAV9 translation		QPKANQQHQDNARGLVLPGYKYLGPGNGLD
10. Shuffle 100.7 Translation		KPKANQQKQDDGRGLVLPGYKYLGPFNGLD
		AAV8
Consensus	70 80 90	KGEPVNAADAAALEHDKAYDQQQKAGDNPY
Identity		
1. AAV1 translation		KGEPVNAADAAALEHDKAYDQQQKAGDNPY
2. AAV2 translation		KGEPVNEADAAALEHDKAYDRQLDSGDNPY
3. AAV3 translation		KGEPVNEADAAALEHDKAYDQQQKAGDNPY
4. AAV4 translation		KGEPVNAADAAALEHDKAYDQQQKAGDNPY
5. AAV5 translation		RGEPVNRADDEVAREHDISYNEQLEAGDNPY
6. AAV6 translation		KGEPVNAADAAALEHDKAYDQQQKAGDNPY
7. AAV7 translation		KGEPVNAADAAALEHDKAYDQQQKAGDNPY
8. AAV8 translation		KGEPVNAADAAALEHDKAYDQQQLQAGDNPY
9. AAV9 translation		KGEPVNAADAAALEHDKAYDQQQKAGDNPY
10. Shuffle 100.7 Translation		KGEPVNAADAAALEHDKAYDQQQKAGDNPY
		AAV8 AAV6

FIG. 10A

29 / 37




Consensus	L X Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
Identity	
1. AAV1 translation	L R Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
2. AAV2 translation	L K Y N H A D A E F Q E R L K E D T S F G G N L G R A V F Q
3. AAV3 translation	L K Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
4. AAV4 translation	L K Y N H A D A E F Q Q R L Q G D T S F G G N L G R A V F Q
5. AAV5 translation	L K Y N H A D A E F Q E K L A D D T S F G G N L G K A V F Q
6. AAV6 translation	L R Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
7. AAV7 translation	L R Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
8. AAV8 translation	L R Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
9. AAV9 translation	L K Y N H A D A E F Q E R L K E D T S F G G N L G R A V F Q
10. Shuffle 100.7 Translation	L R Y N H A D A E F Q E R L Q E D T S F G G N L G R A V F Q
	AAV6
Consensus	A K K R V L E P L G L V E E G A K T A P G K K R P V E Q S P
Identity	
1. AAV1 translation	A K K R V L E P L G L V E E G A K T A P G K K R P V E Q S P
2. AAV2 translation	A K K R V L E P L G L V E E P V K T A P G K K R P V E H S P
3. AAV3 translation	A K K R I L E P L G L V E E A A K T A P G K K G A V D Q S P
4. AAV4 translation	A K K R V L E P L G L V E Q A G E T A P G K K R P L I E S P
5. AAV5 translation	A K K R V L E P F G L V E E G A K T A P T G K R I D D H F P
6. AAV6 translation	A K K R V L E P F G L V E E G A K T A P G K K R P V E Q S P
7. AAV7 translation	A K K R V L E P L G L V E E G A K T A P A K K R P V E P S P
8. AAV8 translation	A K K R V L E P L G L V E E G A K T A P G K K R P V E P S P
9. AAV9 translation	A K K R L L E P L G L V E E A A K T A P G K K R P V E Q S P
10. Shuffle 100.7 Translation	A K K R V L E P L G L V E E G A K T A P G K K R P V E Q S P
	A... AAV8 AAV6
Consensus	Q E - P D S S S G I G K X G Q Q P A K K R L N F G Q T G D S
Identity	
1. AAV1 translation	Q E - P D S S S G I G K T G Q Q P A K K R L N F G Q T G D S
2. AAV2 translation	V E - P D S S S G T G K A G Q Q P A R K R L N F G Q T G D A
3. AAV3 translation	Q E - P D S S S G V G K S G K Q P A R K R L N F G Q T G D S
4. AAV4 translation	Q Q - P D S S T G I G K K G K Q P A K K K L V F - - - E D E
5. AAV5 translation	K R - - - - K K A R T E E D S K P S T - - - - - S S D A
6. AAV6 translation	Q E - P D S S S G I G K T G Q Q P A K K R L N F G Q T G D S
7. AAV7 translation	Q R S P D S S T G I G K K G Q Q P A R K R L N F G Q T G D S
8. AAV8 translation	Q R S P D S S T G I G K K G Q Q P A R K R L N F G Q T G D S
9. AAV9 translation	Q E - P D S S A G I G K S G A Q P A K K R L N F G Q T G D T
10. Shuffle 100.7 Translation	Q E - P D S S S G I G K T G Q Q P A K K R L N F G Q T G D S
	A AAV1

FIG. 10B

30 / 37








Consensus	ESVPDPQPLGEP ¹⁹⁰ PAAP-SSV ²⁰⁰ GXX ²¹⁰ TMASGGG
Identity	
1. AAV1 translation	ESVPDPQPLGEPATP-AAVGPTTMASGGG
2. AAV2 translation	DSVPDPQPLGQPPAAP-SGLGTNTMATGSG
3. AAV3 translation	ESVPDPQPLGEP ¹⁹⁰ PAAP-TSLGSNTMASGGG
4. AAV4 translation	TGAGDGPPEGSTSGA--MSDDSEMRAAAGG
5. AAV5 translation	EAGPSGSQQ ¹⁹⁰ LQIPAQPASSLGADTMSAGGG
6. AAV6 translation	ESVPDPQPLGEPATP-AAVGPTTMASGGG
7. AAV7 translation	ESVPDPQPLGEP ¹⁹⁰ PAAP-SSVGS ²⁰⁰ GTVAAGGG
8. AAV8 translation	ESVPDPQPLGEP ¹⁹⁰ PAAP-SGVGPNTMAAGGG
9. AAV9 translation	ESVPDPQPIGEP ¹⁹⁰ PAAP-SGVGSLTMAAGGG
10. Shuffle 100.7 Translation	ESVPDPQPLGEPATP-AAVGPTTMASGGG
	AAV1  AAV1
Consensus	APMADNNEGADGVGNASGN ²²⁰ WH ²³⁰ CDS ²⁴⁰ TWLGDR
Identity	
1. AAV1 translation	APMADNNEGADGVGNASGNWHCDS ²²⁰ TWLGDR
2. AAV2 translation	APMADNNEGADGVGNSSGNWHCDS ²²⁰ TWMGDR
3. AAV3 translation	APMADNNEGADGVGNSSGNWHCDS ²²⁰ QWLGDR
4. AAV4 translation	AAV-EGGQGADGVGNASGDWHCDS ²²⁰ TWSEGH
5. AAV5 translation	GPLGDNNQGADGVGNASGDWHCDS ²²⁰ TWMGDR
6. AAV6 translation	APMADNNEGADGVGNASGNWHCDS ²²⁰ TWLGDR
7. AAV7 translation	APMADNNEGADGVGNASGNWHCDS ²²⁰ TWLGDR
8. AAV8 translation	APMADNNEGADGVGSSSGNWHCDS ²²⁰ TWLGDR
9. AAV9 translation	APVADNNEGADGVGSSSGNWHCDS ²²⁰ QWLGDR
10. Shuffle 100.7 Translation	APMADNNEGADGVGNASGNWHCDS ²²⁰ TWLGDR
	AAV1 
Consensus	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSAS ²⁷⁰ X-GA
Identity	
1. AAV1 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSAST-GA
2. AAV2 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSQS--GA
3. AAV3 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSQS--GA
4. AAV4 translation	VTTTS ²⁵⁰ TRTWVLP ²⁶⁰ TYNNHLYKRLGES-----
5. AAV5 translation	VVTKSTRTWVLP ²⁵⁰ SYNNHQYREIKSGSVDG-
6. AAV6 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSAST-GA
7. AAV7 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSETA-GS
8. AAV8 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISNGTSGGA
9. AAV9 translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISNSTSGGS
10. Shuffle 100.7 Translation	VITTS ²⁵⁰ TRTWALPTYN ²⁶⁰ NHLYKQISSAST-GA
	AAV1  AAV6 

FIG. 10C

31 / 37

Consensus Identity	<div> <div>280</div> <div>290</div> <div>300</div> <div> SNDNH YFG Y S T P W G Y F D F N R F H C H F S P R D W </div> </div>
1. AAV1 translation	SNDNH YFG Y S T P W G Y F D F N R F H C H F S P R D W
2. AAV2 translation	SNDNH YFG Y S T P W G Y F D F N R F H C H F S P R D W
3. AAV3 translation	SNDNH YFG Y S T P W G Y F D F N R F H C H F S P R D W
4. AAV4 translation	LQ S N T Y N G F S T P W G Y F D F N R F H C H F S P R D W
5. AAV5 translation	S N A N A Y F G Y S T P W G Y F D F N R F H S H W S P R D W
6. AAV6 translation	SNDNH YFG Y S T P W G Y F D F N R F H C H F S P R D W
7. AAV7 translation	T N D N T Y F G Y S T P W G Y F D F N R F H C H F S P R D W
8. AAV8 translation	T N D N T Y F G Y S T P W G Y F D F N R F H C H F S P R D W
9. AAV9 translation	SNDN A Y F G Y S T P W G Y F D F N R F H C H F S P R D W
10. Shuffle 100.7 Translation	SNDNH YFG Y S T P W G Y F D F N R F H C H F S P R D W
	AAV1 AAV8
Consensus Identity	<div> <div>310</div> <div>320</div> <div>330</div> <div> Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T T N </div> </div>
1. AAV1 translation	Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T T N
2. AAV2 translation	Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T Q N
3. AAV3 translation	Q R L I N N N W G F R P K K L S F K L F N I Q V R G V T Q N
4. AAV4 translation	Q R L I N N N W G M R P K A M R V K I F N I Q V K E V T T S
5. AAV5 translation	Q R L I N N Y W G F R P R S L R V K I F N I Q V K E V T V Q
6. AAV6 translation	Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T T N
7. AAV7 translation	Q R L I N N N W G F R P K K L R F K L F N I Q V K E V T T N
8. AAV8 translation	Q R L I N N N W G F R P K R L S F K L F N I Q V K E V T Q N
9. AAV9 translation	Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T D N
10. Shuffle 100.7 Translation	Q R L I N N N W G F R P K R L S F K L F N I Q V K E V T T N
	AAV8
Consensus Identity	<div> <div>340</div> <div>350</div> <div>360</div> <div> D G V T T I A N N L T S T V Q V F T D S E Y Q L P Y V L G S </div> </div>
1. AAV1 translation	D G V T T I A N N L T S T V Q V F S D S E Y Q L P Y V L G S
2. AAV2 translation	D G T T T I A N N L T S T V Q V F T D S E Y Q L P Y V L G S
3. AAV3 translation	D G T T T I A N N L T S T V Q V F T D S E Y Q L P Y V L G S
4. AAV4 translation	N G E T T V A N N L T S T V Q I F A D S S Y E L P Y V M D A
5. AAV5 translation	D S T T T I A N N L T S T V Q V F T D D D Y Q L P Y V V G N
6. AAV6 translation	D G V T T I A N N L T S T V Q V F S D S E Y Q L P Y V L G S
7. AAV7 translation	D G V T T I A N N L T S T I Q V F S D S E Y Q L P Y V L G S
8. AAV8 translation	E G T K T I A N N L T S T I Q V F T D S E Y Q L P Y V L G S
9. AAV9 translation	N G V K T I A N N L T S T V Q V F T D S D Y Q L P Y V L G S
10. Shuffle 100.7 Translation	D G V T T I A N N L T S T V Q V F S D S E Y Q L P Y V L G S
	AAV1

FIG. 10D

32 / 37




Consensus	A H Q G C L P P F P A D V F M I P Q Y G Y L T L N - - N G S
Identity	
1. AAV1 translation	A H Q G C L P P F P A D V F M I P Q Y G Y L T L N - - N G S
2. AAV2 translation	A H Q G C L P P F P A D V F M V P Q Y G Y L T L N - - N G S
3. AAV3 translation	A H Q G C L P P F P A D V F M V P Q Y G Y L T L N - - N G S
4. AAV4 translation	G Q E G S L P P F P N D V F M V P Q Y G Y C G L V T G N T S
5. AAV5 translation	G T E G C L P A F P P Q V F T L P Q Y G Y A T L N R D N T E
6. AAV6 translation	A H Q G C L P P F P A D V F M I P Q Y G Y L T L N - - N G S
7. AAV7 translation	A H Q G C L P P F P A D V F M I P Q Y G Y L T L N - - N G S
8. AAV8 translation	A H Q G C L P P F P A D V F M I P Q Y G Y L T L N - - N G S
9. AAV9 translation	A H E G C L P P F P A D V F M I P Q Y G Y L T L N - - D G S
10. Shuffle 100.7 Translation	A H Q G C L P P F P A D V F M I P Q Y G Y L T L N - - N G S
	AAV1
Consensus	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F T F S Y
Identity	
1. AAV1 translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F T F S Y
2. AAV2 translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F T F S Y
3. AAV3 translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F Q F S Y
4. AAV4 translation	Q Q Q T D R N A F Y C L E Y F P S Q M L R T G N N F E I T Y
5. AAV5 translation	- N P T E R S S F F C L E Y F P S K M L R T G N N F E F T Y
6. AAV6 translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F T F S Y
7. AAV7 translation	- Q S V G R S S F Y C L E Y F P S Q M L R T G N N F E F S Y
8. AAV8 translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F Q F T Y
9. AAV9 translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F Q F S Y
10. Shuffle 100.7 Translation	- Q A V G R S S F Y C L E Y F P S Q M L R T G N N F T F S Y
	AAV1
Consensus	T F E D V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
Identity	
1. AAV1 translation	T F E E V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
2. AAV2 translation	T F E D V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
3. AAV3 translation	T F E D V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
4. AAV4 translation	S F E K V P F H S M Y A H S Q S L D R L M N P L I D Q Y L W
5. AAV5 translation	N F E E V P F H S S F A P S Q N L F K L A N P L V D Q Y L Y
6. AAV6 translation	T F E D V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
7. AAV7 translation	S F E D V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
8. AAV8 translation	T F E D V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
9. AAV9 translation	E F E N V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
10. Shuffle 100.7 Translation	T F E E V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y
	AAV1

FIG. 10E

33 / 37

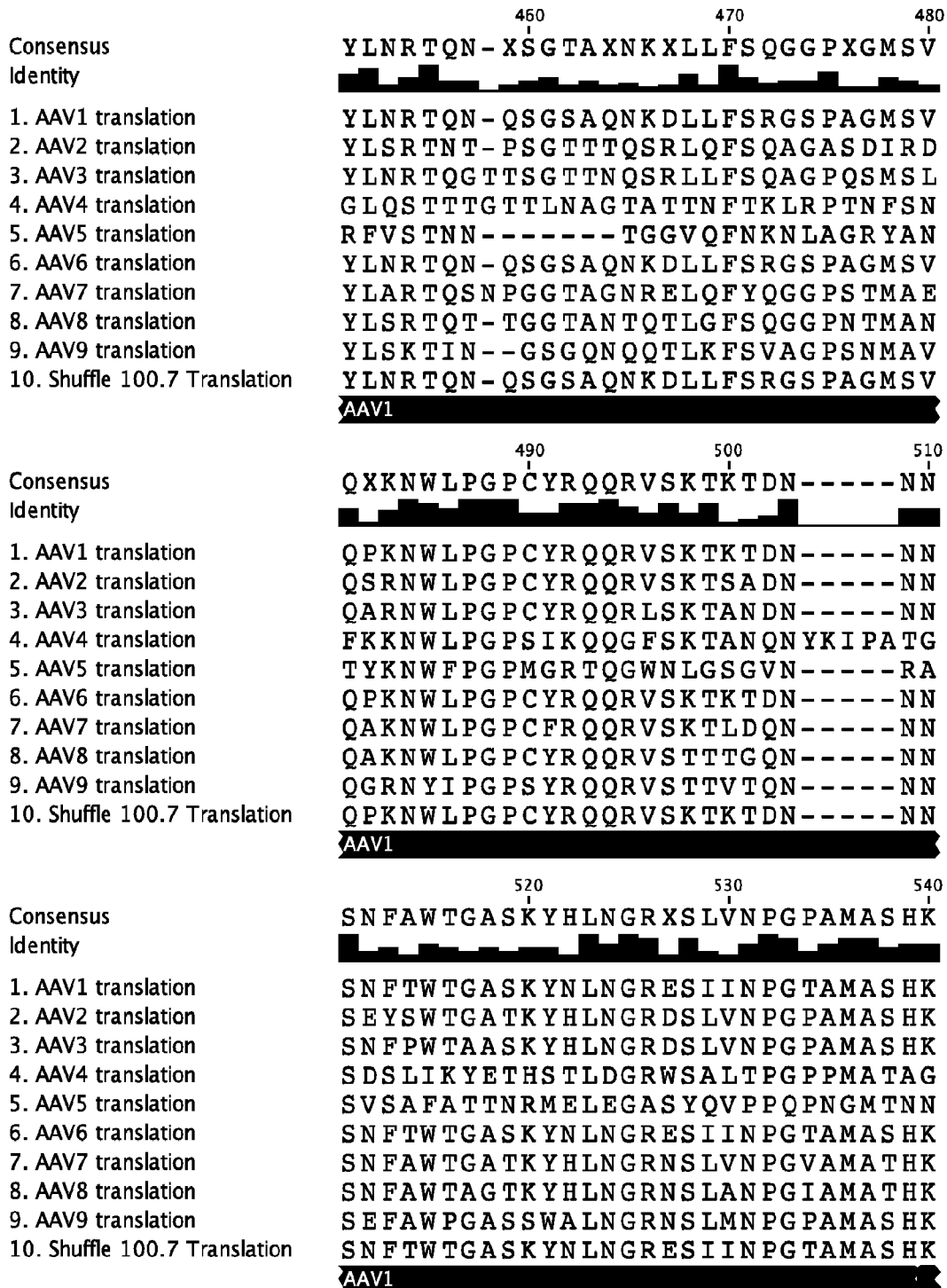


FIG. 10F

34 / 37

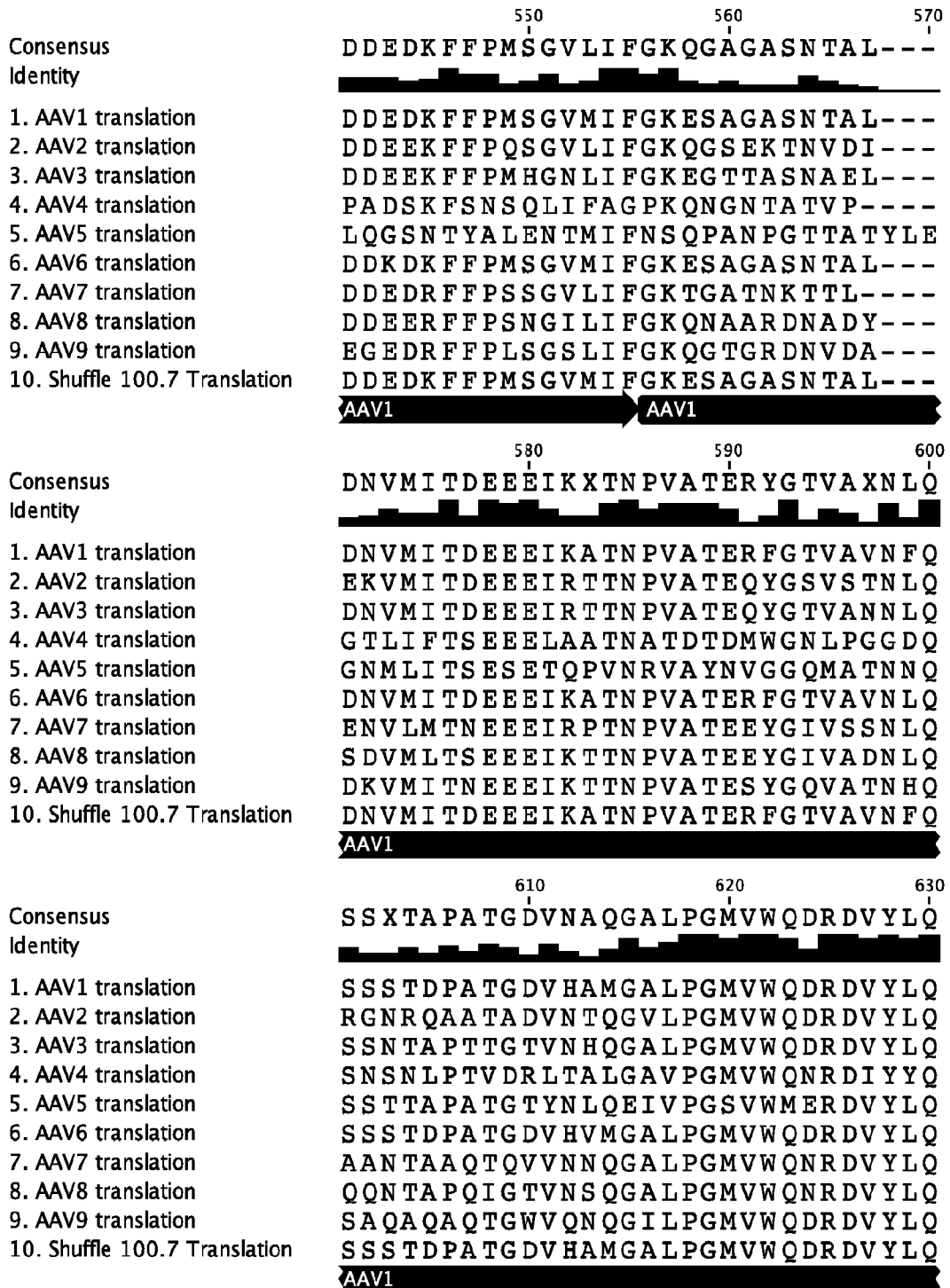


FIG. 10G

35 / 37

Consensus Identity	<div>640650660</div> <div>GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP</div> <div></div>
1. AAV1 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKNPPP
2. AAV2 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
3. AAV3 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
4. AAV4 translation	GPIWAKIPHTDGHFHPSPLIGGFGLKHPPP
5. AAV5 translation	GPIWAKIPETGAHFHPSPLMGGFGLKHPPP
6. AAV6 translation	GPIWAKIPHTDGHFHPSPLMGGFGLKHPPP
7. AAV7 translation	GPIWAKIPHTDGNFHPSPLMGGFGLKHPPP
8. AAV8 translation	GPIWAKIPHTDGNFHPSPLMGGFGLKHPPP
9. AAV9 translation	GPIWAKIPHTDGNFHPSPLMGGFGMKHPPP
10. Shuffle 100.7 Translation	GPIWAKIPHTDGHFHPSPLMGGFGLKNPPP
	AAV1
Consensus Identity	<div>670680690</div> <div>QILIKNTPVPANPPTTFSATKFASFITQYS</div> <div></div>
1. AAV1 translation	QILIKNTPVPANPPAEFSATKFASFITQYS
2. AAV2 translation	QILIKNTPVPANPSTTFSAAKFASFITQYS
3. AAV3 translation	QIMIKNTPVPANPPTTFSPAKFASFITQYS
4. AAV4 translation	QIFIKNTPVPANPATTFSSTPVNSFITQYS
5. AAV5 translation	MMLIKNTPVPGN-ITSFSDVPVSSFITQYS
6. AAV6 translation	QILIKNTPVPANPPAEFSATKFASFITQYS
7. AAV7 translation	QILIKNTPVPANPPEVFTPAPKFASFITQYS
8. AAV8 translation	QILIKNTPVPADPPTTFNQSKLNSFITQYS
9. AAV9 translation	QILIKNTPVPADPPTAFNKDKLNSFITQYS
10. Shuffle 100.7 Translation	QILIKNTPVPANPPAEFSATKFASFITQYS
	AAV1
Consensus Identity	<div>700710720</div> <div>TGQVSVEIEWELQKENS KRWNPEIQYTSNY</div> <div></div>
1. AAV1 translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
2. AAV2 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
3. AAV3 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
4. AAV4 translation	TGQVSVQIDWEIQKERS KRWNPEVQFTSNY
5. AAV5 translation	TGQVTVEMEWELKKENS KRWNPEIQYTNNY
6. AAV6 translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
7. AAV7 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNF
8. AAV8 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
9. AAV9 translation	TGQVSVEIEWELQKENS KRWNPEIQYTSNY
10. Shuffle 100.7 Translation	TGQVSVEIEWELQKENS KRWNPEVQYTSNY
	AAV1AAV6

FIG. 10H

36 / 37

																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					</
--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	----

FIG. 10I

37 / 37

		Neutralizing Serum Dilution					
		AAV1	AAV2	AAV8	Shuffle 100-3	Shuffle 100-7	SM 10-2
AAV1	Animal						
	1	1:1000	none	1:250	1:250	1:1000	none
	2	1:1000	1:25	1:250	1:250	1:1000	none
	3	1:1000	none	1:250	1:250	1:1000	none
AAV2	1	1:50	1:500	1:100	none	none	1:250
	2	1:50	1:500	1:100	none	1:25	1:500
	3	1:1000	1:1000	1:100	none	1:100	1:500
AAV8	1	1:100	none	1:250	none	1:100	none
	2	1:250	none	1:100	none	1:100	none
	3	1:250	none	1:250	none	1:250	none
Shuffle 100-3	1	1:1000	none	1:500	1:100	1:1000	none
	2	1:1000	none	1:500	1:100	1:1000	none
	3	1:1000	none	1:100	1:250	1:1000	none
Shuffle 100-7	1	1:1000	none	1:100	1:100	1:500	none
	2	1:1000	none	1:100	1:250	1:1000	none
	3	1:1000	none	1:50	1:100	1:1000	none

FIG. 11

INTERNATIONAL SEARCH REPORT

4/040083-07-11-2014
International application No.

PCT/US14/40083

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 48/00, 35/76; C12N 7/00 (2014.01)

CPC - C12N 2750/14122, 15/86; C07K 14/005

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)

IPC(8): A61K 48/00, 35/76; C12N 7/00 (2014.01)

CPC: C12N 2750/14143, 2750/14122, 15/86; C07K 14/005

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; PubMed; ProQuest; 'AAV,' 'rAAV,' 'AAV2,' variant, capsid, 'amino acid,' 'nucleic acid,' 'nucleotide, sequence, 'neutralizing antibody'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	WO 2009/137006 A2 (XIAO, X et al.); November 12, 2009; figure 6BB; page 6, lines 20-37; page 7, lines 1-5; page 9, lines 19-25; page 11, lines 1-10; page 22, lines 34-36; page 26, lines 5-20; page 35, lines 35-36; page 36, lines 10-15; page 51, lines 10-15; page 54, lines 30-36; page 55, lines 28-37; page 56, lines 1-6; page 65, lines 15-25; Table 1; Claim 18; SEQ ID NO:66	1, 2, 6/1, 12/1, 13/12/1, 14/13/12/1, 15/1, 16/1, 17, 18, 21/17, 33/1, 34/33/1, 35/34/33/1, 36/33/1, 37/33/1, 38, 39/38/1, 40/38/1, 41/38/1, 42/38/1, 43, 44, 48/43, 54/43, 55 ----- 7/6/1, 8/6/1, 9/6/1, 10/6/1, 11/6/1, 22/21/17, 23/21/17, 24/21/17, 25/21/17, 26/21/17, 27/17, 28/17, 30/28/17, 31/28/17, 32/28/17, 49/48/43, 49/48/46, 50/48/43, 50/48/46, 51/48/43, 51/48/46, 52/48/43, 52/48/46, 53/48 ----- 3-5, 6/4, 7/6/4, 8/6/4, 9/6/4, 10/6/4, 11/6/4, ... Next Page ...

☒ Further documents are listed in the continuation of Box C.

* 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

10 October 2014 (10.10.2014)

Date of mailing of the international search report

07 NOV 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

4/040083 07-11-2014

International application No.

PCT/US14/40083

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	... Continued from Previous Page Continued from Previous Page ... 12/4, 13/12/4, 14/13/12/4, 15/4, 16/4, 19, 20, 21/20, 22/21/20, 23/21/20, 24/21/20, 25/21/20, 26/21/20, 27/21/20, 29, 30/29, 31/29, 32/29, 33/4, 34/33/4, 35/34/33/4, 36/33/4, 37/33/4, 38/4, 39/38/4, 40/38/4, 41/38/4, 42/38/4, 45-47, 48/46, 49/48/46, 50/48/46, 51/48/46, 52/48/46, 53/48/46, 54/46, 56-60
Y	US 2009/0202490 A1 (SCHAFER, DV et al.); August 13, 2009; paragraphs [0067], [0082], [0112], [0114]; SEQ ID NO:5	7/6/1, 8/6/1, 9/6/1, 10/6/1, 10/6/4, 11/6/1, 22/21/17, 22/21/20, 23/21/17, 23/21/20, 24/21/17, 24/21/20, 25/21/17, 25/21/20, 26/21/17, 26/21/20, 27/17, 27/21/20, 28/17, 30/28/17, 30/29, 31/28/17, 32/28/17, 49/48/43, 49/48/46, 50/48/43, 50/48/46, 51/48/43, 51/48/46, 52/48/43, 52/48/46, 53/48/43, 53/48/46 3-5, 6/4, 7/6/4, 8/6/4, 9/6/4, 10/6/4, 11/6/4, 12/4, 13/12/4, 14/13/12/4, 15/4, 16/4, 19, 20, 21/20, 22/21/20, 23/21/20, 24/21/20, 25/21/20, 26/21/20, 27/21/20, 29, 30/29, 31/29, 32/29, 33/4, 34/33/4, 35/34/33/4, 36/33/4, 37/33/4, 38/4, 39/38/4, 40/38/4, 41/38/4, 42/38/4, 46, 48/46, 49/48/46, 50/48/46, 51/48/46, 52/48/46, 53/48/46, 54/46, 56-60
A	WO 2005/033321 A2 (WILSON, JM et al.) April 14, 2005; SEQ ID NOs:27, 221, 224	3, 4, 19, 20, 45-47, 56 3, 4, 19, 20, 45-47, 56 3, 4, 19, 20, 45-47, 56 3, 4, 19, 20, 45-47, 56 3, 4, 19, 20, 45-47, 56
A	WO 2006/110689 A2 (VANDENBERGHE, L et al.); October 19, 2006; SEQ ID NOs: 27, 29, 45	3, 4, 19, 20, 45-47, 56
A	WO 2011/117258 A2 (VOLT, T et al.); September 29, 2011; SEQ ID NO: 4	
A	WO 2004/108922 A2 (RADER, DJ et al.); December 16, 2004; SEQ ID NOs: 1, 4	
A	WO 2010/138263 A2 (GAO, G et al.); December 2, 2010; SEQ ID NO: 182	
A	US 5773700 A (GRINSVEN, MQJMV et al.); June 30, 1998; SEQ ID NO: 27	
A	US 7368428 B2 (SERRERO, G); May 6, 2008; SEQ ID NO: 4	