

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 3872085 T5**

(12) **Rettet oversættelse af
europæisk patentskrift**

-
- (51) Int.Cl.: **C 07 K 14/005 (2006.01)** **C 12 N 15/86 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2024-08-12**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2023-03-01**
- (86) Europæisk ansøgning nr.: **21161372.4**
- (86) Europæisk indleveringsdag: **2016-07-29**
- (87) Den europæiske ansøgnings publiceringsdag: **2021-09-01**
- (30) Prioritet: **2015-07-30 US 201562199059 P** **2015-08-10 US 201562203002 P**
- (62) Stamansøgningsnr: **16831443.3**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **STAM-VIRUSSEKVENSER OG ANVENDELSER HERAF**
- (56) Fremdragne publikationer:
WO-A1-2014/160092
WO-A2-2005/033321
WO-A2-2015/054653
J SANTIAGO-ORTIZ ET AL: "AAV ancestral reconstruction library enables selection of broadly infectious viral variants", GENE THERAPY, vol. 22, no. 12, 17 July 2015 (2015-07-17) , pages 934-946, XP055349475, GB ISSN: 0969-7128, DOI: 10.1038/gt.2015.74
ERIC ZINN ET AL: "In Silico Reconstruction of the Viral Evolutionary Lineage Yields a Potent Gene Therapy Vector", CELL REPORTS, vol. 12, no. 6, 1 August 2015 (2015-08-01) , pages 1056-1068, XP055524241, ISSN: 2211-1247, DOI: 10.1016/j.celrep.2015.07.019
ERIC ZINN ET AL: "Adeno-associated virus: fit to serve", CURRENT OPINION IN VIROLOGY, vol. 8, 1 October 2014 (2014-10-01), pages 90-97, XP055172036, ISSN: 1879-6257, DOI: 10.1016/j.coviro.2014.07.008
DANIEL J. SCHUSTER ET AL: "Biodistribution of adeno-associated virus serotype 9 (AAV9) vector after intrathecal and intravenous delivery in mouse", FRONTIERS IN NEUROANATOMY, vol. 8, 10 June 2014 (2014-06-10), XP055524232, DOI: 10.3389/fnana.2014.00042

DESCRIPTION

TECHNICAL FIELD

[0001] This disclosure generally relates to viruses.

BACKGROUND

[0002] Circumventing and avoiding a neutralizing or toxic immune response against a gene therapy vector is a major challenge with all gene transfer vector types. Gene transfer to date is most efficiently achieved using vectors based on viruses circulating in humans and animals, e.g., adenovirus and adeno-associated virus (AAV). However, if subjects have been naturally infected with a virus, a subsequent treatment with a vector based on that virus leads to increased safety risks and decreased efficiency of gene transfer due to cellular and humoral immune responses. Capsid antigens are primarily responsible for the innate and/or adaptive immunity toward virus particles, however, viral gene-encoded polypeptides also can be immunogenic.

SUMMARY

[0003] This disclosure describes methods of predicting and synthesizing ancestral viral sequences or portions thereof, and also describes virus particles containing such ancestral viral sequences. The methods described herein were applied to adeno-associated virus (AAV); thus, this disclosure describes predicted ancestral AAV sequences and AAV virus particles containing such ancestral AAV sequences. This disclosure also describes the seroprevalance exhibited by virus particles containing ancestral sequences relative to virus particles containing contemporary sequences.

[0004] In one aspect, an adeno-associated virus (AAV) capsid polypeptide having the amino acid sequence shown in SEQ ID NO: 42 is provided. In some embodiments, such an AAV capsid polypeptide, or a virus particle comprising such an AAV capsid polypeptide, exhibits about the same or a lower seroprevalance than does an AAV9 capsid polypeptide or a virus particle comprising an AAV9 capsid polypeptide. In some embodiments, such an AAV capsid polypeptide, or a virus particle comprising the AAV capsid polypeptide, is neutralized to a similar or lesser extent by human serum than is an AAV9 capsid polypeptide or a virus particle comprising an AAV9 capsid polypeptide. In some embodiments, such an AAV capsid polypeptide is purified. In some embodiments, such an AAV capsid polypeptide is encoded by the nucleic acid sequence shown in SEQ ID NO: 43.

[0005] Also provided is a purified virus particle that includes such an AAV capsid polypeptide.

In some embodiments, such a purified virus particle further includes a transgene.

[0006] In another aspect, a nucleic acid molecule encoding an adeno-associated virus (AAV) capsid polypeptide having the nucleic acid sequence shown in SEQ ID NO: 43 is provided. In some embodiments, a vector is provided that includes such a nucleic acid molecule. In some embodiments, a host cell is provided that includes such a vector.

[0007] In another aspect, a method of gene transfer and/or vaccination with a transgene is provided. Such a method typically includes administering a virus particle as described herein to a subject in need of gene transfer or vaccination, wherein the virus particle exhibits about the same or a lower seroprevalence than does an AAV9 virus particle. In some embodiments, such a virus particle is neutralized to the same or to a lesser extent by human serum than is an AAV9 virus particle.

[0008] In another aspect, a method of vaccinating a subject is provided. Such a method typically includes administering a target antigen operably linked to an AAV capsid polypeptide as described herein to a subject in need of vaccination, wherein the AAV capsid polypeptide exhibits about the same or a lower seroprevalence than does an AAV9 capsid polypeptide. In some embodiments, such an AAV capsid polypeptide is neutralized to the same or to a lesser extent by human serum than is an AAV9 capsid polypeptide.

[0009] Thus, the present disclosure provides ancestral viruses or portions thereof that exhibit reduced susceptibility to pre-existing immunity in current day human populations than do contemporary viruses or portions thereof. Generally, the reduced susceptibility to pre-existing immunity exhibited by the ancestral viruses or portions thereof in current day human populations is reflected as a reduced susceptibility to neutralizing antibodies.

[0010] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods and compositions of matter belong.

[0011] In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

DESCRIPTION OF DRAWINGS

[0012]

Figure 1 is a schematic showing the relationships between ancestral/contemporary viral infections and ancestral/contemporary host immune response.

Figures 2A to 2D are a series of schematics showing an example of an ancestral reconstruction procedure. Data shown are excerpted from a full dataset and represent

residues 564-584 (AAV2-VP1 numbering).

Figure 3 illustrates a phylogenetic tree of AAV contemporary sequences generated using the methods described herein.

Figure 4 illustrates an alignment of ancestral AAV VP1 polypeptides.

Figures 5A and 5B together illustrate an alignment of functional ancestral AAV VP1 polypeptides and contemporary AAV VP1 polypeptides.

Figure 6 is an electrophoretic gel demonstrating that ancestral AAV VP1 sequences are transcribed and alternately spliced in a manner similar to that for contemporary AAV VP1 sequences.

Figure 7 is a graph showing the luciferase activity in HEK293 cells transduced with ancestral AAV vectors.

Figure 8 is a graph showing the sequence comparison (% up from diagonal, # of aa differences below) between the Anc80 library and Anc80L65.

Figures 9A-D are images of experimental results demonstrating that Anc80L65 is capable of assembling and yielding particles of high titer. Panel A shows that Anc80L65 is able to produce vector yields equivalent to AAV2; Panel B is a TEM image of virus particles that include Anc80L65; Panel C shows that virus particles that include Anc80L65 are able to produce AAV cap VP1, 2 and 3 proteins based on SDS-PAGE gel under denaturing conditions; and Panel D shows a Western blot of Anc80L65 using the AAV capsid antibody, B1.

Figures 10A-C are images of experimental results demonstrating that Anc80L65 is able to infect cells in vitro on HEK293 cells using GFP as readout (Panel A) or luciferase (Panel B) versus AAV2 and/or AAV8 controls and also is efficient at targeting liver following an IV injection of AAV encoding a nuclear LacZ transgene (top row, Panel C: liver), following direct IM injection of an AAV encoding GFP (middle row, Panel C: muscle), and following sub-retinal injection with AAV encoding GFP (bottom row, Panel C: retina).

Figures 11A and 11B are sequence identity matrices produced using MAFFT that show the amino acid sequences of the VP1 proteins of ancestral vectors aligned with those of representative extant AAVs (Figure 11A), and the amino acid sequences of the VP3 proteins of ancestral vectors aligned with those of representative extant AAVs (Figure 11B).

Figure 12 is a graph that demonstrates that AAV vectors were produced in triplicate in small scale (6-well dishes). Crude viruses were assessed via qPCR to determine the absolute production of each vector.

Figure 13 is a table showing the titers of each vector, averaged and compared, to those of AAV8.

Figure 14 are photographs that show the results of experiments in which 1.9×10^3 GC/cell of each vector was added to HEK293 cells (except for Anc126, in which case MOIs of 2.5×10^2 - 3.1×10^2

GC/cell were achieved). Sixty hours later, infectivity was assessed using fluorescence microscopy.

Figure 15 is a graph showing the results of experiments in which the same cells from Figure 14 were lysed and assayed for luciferase expression. As in Figure 14, Anc126 was not titer controlled with the other vectors, but rather ranged from an MOI of $2.5E2$ - $3.1E2$ GC/cell.

Figure 16 is a table showing the luminescence of cells transduced by each vector, which were averaged and compared to those of AAV8.

Figure 17 is a chart that provides a summary of *in vitro* experiments to determine the relative production and infectivity of the ancestral AAV vectors described herein.

Figure 18 is a phylogeny and ASR of the AAV evolutionary lineage created using maximum-likelihood phylogeny and 75 different isolates of AAV. Red circles represent evolutionary intermediates reconstructed through ASR. The blue circle represents a library of probabilistic space built around Anc80. Subclades are collapsed for clarity. The full phylogeny is presented in Figure 24.

Figure 19 shows the sequence and structural analysis of Anc80 vectors. Panel A is a sequence structure alignment of Anc80 (SEQ ID NO:37), AAV2 (SEQ ID NO:38) and AAV8 (SEQ ID NO:39) VP3 proteins. A structural alignment derived from the crystal structures of AAV2 (PDB 1LP3) and AAV8 (PDB 2QA0) VP3 and the predicted structure of Anc80L65 VP3, generated with UCSF Chimera (Pettersen et al., 2004, J. Comp. Chem., 25:1605-12) is shown in black print. The blue region is a non-structural alignment of the VP1/VP2 domains of AAV2, AAV8 and Anc80 (Notredame et al., 2000, J. Mol. Biol., 302:205-17). The ambiguous residues in the Anc80 library are represented in red, the lower position corresponding to Anc80L65 residues. Beta-strands and alpha-helices are represented in green and yellow, respectively. The positions of the nine beta-strands forming the AAV antiparallel beta-barrel are depicted with plain arrows, whereas the position of the conserved core alpha-helix is depicted with a dotted arrow. The approximate positions of variable regions (VR) I-IX are represented by the roman numerals above the sequence alignment. Panel B shows an AAV Cap sequence divergence matrix. Above the diagonal, the matrix represents the percent sequence divergence from selected AAV serotypes, as well as rh.10, the most homologous VP1 sequence as determined by BLAST. Below the diagonal, the number of amino-acid differences per position is presented. Panel C shows the superimposition of AAV2 and AAV8 VP3 crystal structures with Anc80L0065 VP3 predicted structure. The color code depicts the amino acid conservation between the 3 aligned sequences of panel A (red: highest conservation; blue: lowest conservation). Variable regions I-IX and C/M-termini are indicated in black. The approximate positions of the two, three and five-fold axis are represented by the black ellipse, triangle and pentagon, respectively. Panel D is the structural mapping of amino-acid changes as compared to AAV2 (left) and AAV8 (right) on VP1 trimer, visualizing the external (top) and internal (bottom) of the virion. Colored residues are divergent in Anc80. Red colored residues are ambiguous via ASR and, therefore, dimorphic in Anc80Lib.

Figure 20 are the results of biophysical and biochemical characterization of Anc80L65. Panel A

shows negative staining Transmission Electron Microscopy (TEM) of Anc80L65, demonstrating that Anc80L65 forms particles of approximately 20-25 nm in diameter. Panel B is the Anc80L65 VP composition. Purified preps of Anc80L65 and three extant viruses were analyzed by SDS-PAGE. Anc80 demonstrates similar incorporation levels of monomers VP1, 2, and 3. Panel C shows an Empty:Full particle composition of purified AAV preparations. Sedimentation coefficient distributions were derived from the sedimentation profiles acquired with the refractive index optical measurement systems during analytical ultracentrifugation of preps of AAV8 and Anc80L65. Panel D shows the AAV thermostability. Intrinsic tryptophan fluorescence measurement of AAV particles under different temperatures illustrates distinct melting temperatures of AAV serotypes as compared to Anc80L65.

Figure 21 are results from the *in vivo* evaluation of Anc80L65. Panel A, top panel, shows liver transduction and lacZ transgene expression comparison of AAV-2, AAV-8 and Anc80L65.TBG.nLacZ in liver 28 days after intraperitoneal delivery at a dose of 7.2×10^{10} GC. Panel A, middle panel, shows muscle tropism of AAV2, AAV8 and Anc80L65 28 days following an intramuscular delivery at a dose of 1×10^{10} GC to the rear-right thigh (gastrocnemius/biceps femoris muscle). Panel A, lower panel, shows a comparison of eGFP transgene expression between AAV2, AAV8, and Anc80L65 in the retina after subretinal delivery at a dose of 2×10^9 GC. AAV2 shows high affinity for RPE cells, while both RPE and photoreceptors are targeted using AAV8 and Anc80L65 vectors, with Anc80L65 showing higher transduction efficiency compared to AAV2 and AAV8. Panel B is a qualitative dose response eGFP-expression analysis at 10^{11} (top panel), 10^{10} (middle panel), and 10^{09} (bottom panel) GC comparing AAV-8 and Anc80L65 by retro-orbital sinus intravenous delivery. Both AAV8 and Anc80L65 show comparable eGFP expression at equal doses throughout the dose ranging. Panel C shows a quantitative AAV dose response analysis measuring mouse serum levels of recombinant human alpha 1-antitrypsin (hA1AT) transgene expression from AAV-8 (black symbols: square- 10^{11} GC, circle- 10^{10} GC, and four-square- 10^9 GC) and Anc80L65 (grey symbols: diamond- 10^{11} GC, square- 10^{10} GC, and triangle- 10^9 GC). Panel D is a graph of the Rhesus macaque liver gene transfer of AAV-8 and Anc80L65 expressing Rhesus chorionic-gonadotropin (rhCG) following saphenous vein injection of a dose of 1×10^{12} GC/kg. Genomic DNA was harvested from macaque liver-lobes and viral genome (vg) per diploid genome (dpg) was measured by qPCR assay. One AAV8 and all three Anc80L65 animals successfully received ~1-3 vg per diploid cell of the caudal liver lobe, while 2 AAV8 animals likely had low level NAB resulting in vector neutralization and limited liver gene transfer. Panel E is a graph showing transgene mRNA expression of AAV8 and Anc80L65 in NHP caudal, right, left and middle liver-lobes by TaqMan probe-specific, quantitative reverse-transcriptase PCR (qRT-PCR). Quantitation of rhCG transcript was normalized with endogenous GAPDH mRNA levels.

Figure 22 are results from experiments in which Anc80L65 was immunologically characterized. Panel A is a graph showing rabbit anti-AAV serum cross-reactivity: rabbit antiserum raised against AAV serotypes (Y-axis) was tested for NAB to Anc80L65 versus the homologous AAV serotype in order to assess sero-cross-reactivity. Values (X-axis) represent smallest dilution at which 50% neutralization is achieved. The phylogenetic relationship between immunizing

serotypes is depicted on the left. Panel B are Tables showing mouse *in vivo* gene transfer cross-neutralization: C57B1/6 mice received an IV injection of AAV8 or Anc80L65.CAS1.EGFP.2A.A1AT 25 days following an IM injection with either saline or AAV8.TBG.nLacZ. 14 days following the second injections, serum was titrated by ELISA for hA1AT expression. The Tables present the relative hA1AT levels of the pre-immunized mice versus the non-immunized for each vector (% control), and the NAB titer dilutions for AAV8 (NAB8) and Anc80L65 (NAB80) 24 h prior to the second injection in the immunized group (n=5). Grey diverging arrow in Panels A and B schematically illustrate AAV2 and AAV8 lineage phenotypic evolution. Panel C is a non-structural multiple sequence alignment between Anc80, Anc126, Anc127 and AAV2 VP3 sequences was generated using the T-coffee alignment package. AAV2 trimer structure was generated using UCSF Chimera. The blue residues represent the variable residues relative to Anc80. The orange residues represent previously defined T and B-cell epitopes on AAV2. The green residues are overlaps between mutations relative to Anc80L65 and B/T-cell epitopes.

Figure 23 is data showing that AAV lineage reconstruction modulates production, infectivity, and thermostability. Panel A is a graph showing the production of nine ancestral and two extant viral vectors containing a luciferase reporter gene driven by a CMV promoter, as determined by qPCR. Error bars represent standard deviation of three biological replicates. Panel B is a graph showing that ancestral and extant viral vectors were used to transduce HEK293 cells at a particle-to-cell ratio of 1.9×10^3 . Error bars represent standard deviation of three distinct lots of vector. *Anc126 was added at ratios between 2.1×10^2 and 3.5×10^2 GC/cell due to low vector yield. Panel C shows a sypro-orange thermostability assay indicating denaturation temperatures of selected ancestral and extant AAV vectors.

Figure 24 shows eGFP expression after viral vector intramuscular injection (see, also, Figure 21 above). For muscle-targeted eGFP experiments, mice received a single injection in the gastrocnemius muscle. eGFP expression was observed in transversal and longitudinal muscle sections (first and second columns). Blue staining marks nuclei (DAPI). The morphology of muscle was unchanged as seen in haematoxylin and eosin (H&E) stained sections (third column).

Figure 25 is a multiple sequence alignment of the VP1 polypeptides from AAV isolates used in the ancestral sequence reconstruction (see, also, Figures 18 and 23 above). AAV2 (SEQ ID NO:31); AAV5 (SEQ ID NO:40); AAV7 (SEQ ID NO:34); And 13 (SEQ ID NO:13); AAV8 (SEQ ID NO:27); Anc83 (SEQ ID NO:7); Anc84 (SEQ ID NO:9); rh10 (SEQ ID NO:41); Anc82 (SEQ ID NO:5); And 10 (SEQ ID NO:42); Anc81 (SEQ ID NO:3); Anc80 (SEQ ID NO:1); Anc126 (SEQ ID NO:15); AAV3 (SEQ ID NO:32); AAV3B (SEQ ID NO:33); Anc127 (SEQ ID NO:17); AAV6 (SEQ ID NO:29); AAV1 (SEQ ID NO:30); AAV9 (SEQ ID NO:28); AAV4 (SEQ ID NO:44); rh32.33 (SEQ ID NO:45).

Figure 26 shows a full phylogeny and reconstructed nodes of the AAV evolutionary lineage (see, also, Figure 18 above). Maximum-likelihood phylogeny relating 75 isolates of AAV. Red circles represent evolutionary intermediates reconstructed through ASR. Blue circle represents a library of probabilistic space built around Anc80.

Figure 27 is a graph showing luciferase liver transduction of Anc80, Anc81, Anc82, and And 10 in comparison to AAV9 after IV administration in C57Bl/6 mice.

DETAILED DESCRIPTION

[0013] Gene transfer, either for experimental or therapeutic purposes, relies upon a vector or vector system to shuttle genetic information into target cells. The vector or vector system is considered the major determinant of efficiency, specificity, host response, pharmacology, and longevity of the gene transfer reaction. Currently, the most efficient and effective way to accomplish gene transfer is through the use of vectors or vector systems based on viruses that have been made replication-defective.

[0014] Seroprevalence studies, however, indicate that significant proportions of worldwide human populations have been pre-exposed (e.g., by natural infection) to a large number of the viruses currently used in gene transfer and, therefore, harbor pre-existing immunity. Neutralizing antibodies toward the viral vector in these pre-exposed individuals are known to limit, sometimes significantly, the extent of gene transfer or even re-direct the virus away from the target. See, for example, Calcedo et al. (2009, *J. Infect. Dis.*, 199:381-90) and Boutin et al. (2010, *Human Gene Ther.*, 21 :704-12). Thus, the present disclosure is based on the recognition that ancestral viruses or portions thereof exhibit reduced susceptibility to pre-existing immunity (e.g., reduced susceptibility to neutralizing antibodies) in current day human populations than do contemporary viruses or portions thereof.

[0015] Figure 1 is a schematic showing the relationships between ancestral and contemporary viral infections and ancestral and contemporary host immune response. Figure 1 shows how ancestral AAVs can be refractory to contemporary pre-existing immunity. A contemporary, extant virus (Vc) is presumed to have evolved from an ancestral species (Vane), primarily under evolutionary pressures of host immunity through mechanisms of immune escape. Each of these species, Vane and Vc, have the ability to induce adaptive immunity including B and T cell immunity (Ianc and Ic, respectively). It was hypothesized, and confirmed herein, that immunity induced by contemporary viruses does not necessarily cross-react with an ancestral viral species, which can be substantially different in terms of epitope composition than the extant virus.

[0016] This disclosure provides methods of predicting the sequence of an ancestral virus or a portion thereof. One or more of the ancestral virus sequences predicted using the methods described herein can be generated and assembled into a virus particle. As demonstrated herein, virus particles assembled from predicted ancestral viral sequences can exhibit less, sometimes significantly less, seroprevalence than current-day, contemporary virus particles. Thus, the ancestral virus sequences disclosed herein are suitable for use in vectors or vector systems for gene transfer.

Methods of Predicting and Synthesizing an Ancestral Viral Sequence

[0017] To predict an ancestral viral sequence, nucleotide or amino acid sequences first are compiled from a plurality of contemporary viruses or portions thereof. While the methods described herein were exemplified using adeno-associated virus (AAV) capsid sequences, the same methods can be applied to other sequences from AAV (e.g., the entire genome, rep sequences, ITR sequences) or to any other virus or portion thereof. Viruses other than AAV include, without limitation, adenovirus (AV), human immunodeficiency virus (HIV), retrovirus, lentivirus, herpes simplex virus (HSV), measles, vaccinia virus, pox virus, influenza virus, respiratory syncytial virus, parainfluenza virus, foamy virus, or any other virus to which pre-existing immunity is considered a problem.

[0018] Sequences from as few as two contemporary viruses or portions thereof can be used, however, it is understood that a larger number of sequences of contemporary viruses or portions thereof is desirable so as to include as much of the landscape of modern day sequence diversity as possible, but also because a larger number of sequences can increase the predictive capabilities of the algorithms described and used. For example, sequences from 10 or more contemporary viruses or portions thereof can be used, sequences from 50 or more contemporary viruses or portions thereof can be used, or sequences from 100 or more contemporary viruses or portions thereof can be used.

[0019] Such sequences can be obtained, for example, from any number of public databases including, without limitation, GenBank, UniProt, EMBL, International Nucleotide Sequence Database Collaboration (INSDC), or European Nucleotide Archive. Additionally or alternatively, such sequences can be obtained from a database that is specific to a particular organism (e.g., HIV database). The contemporary sequences can correspond to the entire genome, or only a portion of the genome can be used such as, without limitation, sequences that encode one or more components of the viral capsid, the replication protein, or the ITR sequences.

[0020] Next, the contemporary sequences are aligned using a multiple sequence alignment (MSA) algorithm. Figure 2(a) is a schematic showing an alignment of multiple sequences. MSA algorithms are well known in the art and generally are designed to be applied to different size datasets and different inputs (e.g., nucleic acid or protein), and to align the sequences in a particular manner (e.g., dynamic programming, progressive, heuristic) and apply different scoring schemes in the alignment (e.g., matrix-based or consistency-based, e.g., minimum entropy, sum of pairs, similarity matrix, gap scores). Well known MSA algorithms include, for example, ClustalW (Thompson et al., 1994, Nuc. Acids Res., 22:4673-90), Kalign (Lassmann et al., 2006, Nuc. Acids Res., 34:W596-99), MAFFT (Kato et al., 2005, Nuc. Acids Res., 33:511-8), MUSCLE (Edgar, 2004, BMC Bioinform., 5:113), and T-Coffee (Notredame et al., 2000, J. Mol. Biol., 302:205-17).

[0021] As described herein, one of the main features when selecting a MSA algorithm for use in the methods described herein is the manner in which the algorithm treats a gap in the alignment. Gaps in a sequence alignment can be assigned a penalty value that is either dependent or independent on the size of the gap. In the present methods, it is preferred that the MSA algorithm used in the methods described herein apply phylogenetic information to predict whether a gap in the alignment is a result of a deletion or an insertion as opposed to a biased, non-phylogenetic treatment of gaps due to, e.g., insertions and/or deletions. A suitable method of treating gaps in alignments and evolutionary analysis is described in Loytynoja and Goldman, 2008, *Science*, 320:1632-5, and commercially available algorithms that apply gaps in alignments in a manner that is suitable for use in the methods described herein is a Probabilistic Alignment Kit (PRANK; Goldman Group Software; Loytynoja and Goldman, 2005, *PNAS USA*, 102:10557-62), and variations of the PRANK algorithm.

[0022] An evolutionary model is then applied to the resulting alignment to obtain a predicted ancestral phylogeny (see Figure 2(b)). There are a number of evolutionary models available in the art, each of which apply slightly different matrices of replacement rates for amino acids. Without limitation, algorithms for applying models of evolution include the Dayhoff models (e.g., PAM120, PAM160, PAM250; Dayhoff et al., 1978, In *Atlas of Protein Sequence and Structure* (ed. Dayhoff), pp. 345-52, National Biomedical Research Foundation, Washington D.C.), the JTT model (Jones et al., 1992, *Comp. Appl. Biosci.*, 8:275-82), the WAG model (Whelan and Goldman, 2001, *Mol. Biol. Evol.*, 18:691-9), and the Blosum models (e.g., Blosum45, Blosum62, Blosum80; Henikoff and Henikoff, 1992, *PNAS USA*, 89:10915-9).

[0023] In addition, the constraints that structure and function impose on an evolutionary model can themselves be modeled, for example, by considering that some positions are invariant ("I"; Reeves, 1992, *J. Mol. Evol.*, 35:17-31), that some positions undergo different rates of change ("G"; Yang, 1993, *Mol. Biol. Evol.*, 10:1396-1401), and/or that equilibrium frequencies of nucleotides or amino acids are the same as those in the alignment ("F"; Cao et al., 1994, *J. Mol. Evol.*, 39:519-27).

[0024] The fitness of one or more models of evolution can be evaluated using the Aikake Information Criterion (AIC; Akaike, 1973, In *Second International Symposium on Information Theory*, Petrov and Csaki, eds., pp 267-81, Budapest, Akademiai Kiado), the Bayesian Information Criterion (BIC; Schwarz, 1978, *Ann. Statist.* 6:461-4), or variations or combinations thereof. In addition, AIC, BIC, or variations or combinations thereof can be used to evaluate the relative importance of including one or more parameters (e.g., the constraints discussed above) in the evolutionary model.

[0025] As explained in the Example section below, ProTest3 (Darriba et al., 2011, *Bioinformatics*, 27(8):1164-5) can be used to determine, based on the lowest AIC, that a JTT+G+F algorithm was the most suitable model for AAV evolution. It would be understood by a skilled artisan that a JTT+G+F algorithm also may be used to predict ancestral viral sequences other than AAV capsid polypeptides, however, it also would be understood by a skilled artisan that, depending on the dataset and the fitness score, a different model of

evolution may be more suitable.

[0026] Once a model of evolution has been selected and its fitness determined, a phylogenetic tree of the virus sequences or portions thereof can be constructed. Constructing phylogenetic trees is known in the art and typically employs maximum likelihood methods such as those implemented by PhyML (Guindon and Gascuel, 2003, *Systematic Biology*, 52:696-704)), MOLPHY (Adachi and Hasegawa, 1996, ed. Tokyo Institute of Statistical Mathematics), BioNJ (Gascuel, 1997, *Mol. Biol. Evol.*, 14:685-95), or PHYLIP (Felsenstein, 1973, *Systematic Biology*, 22:240-9). A skilled artisan would understand that a balance between computational complexity and the goodness of fit is desirable in a model of amino acid substitutions.

[0027] If desired, the phylogenetic tree can be assessed for significance. A number of statistical methods are available and routinely used to evaluate the significance of a model including, without limitation, bootstrap, jackknife, cross-validation, permutation tests, or combinations or variations thereof. Significance also can be evaluated using, for example, an approximate likelihood-ratio test (aLRT; Anisimova and Gascuel, 2006, *Systematic Biology*, 55:539-52)).

[0028] At any phylogenetic node of the phylogeny (e.g., an interior phylogenetic node), the sequence can be reconstructed by estimating the evolutionary probability of a particular nucleotide or amino acid residue at each position of the sequence (Figure 2(c)). A phylogenetic node refers to an intermediate evolutionary branch point within the predicted ancestral phylogeny. As used herein, "evolutionary probability" refers to the probability of the presence of a particular nucleotide or amino acid at a particular position based on an evolutionary model as opposed to a model that does not take into account, for example, an evolutionary shift in the codon usage. Exemplary models that take into account the evolutionary probability of a particular nucleotide or amino acid residue at a particular position can be estimated using, for example, any number of maximum likelihood methods including, without limitation, Phylogenetic Analysis by Maximum Likelihood (PAML; Yang, 1997, *Comp. Applic. BioSci.*, 13:555-6) or Phylogenetic Analysis Using Parsimony (PAUP; Sinauer Assoc., Inc., Sunderland, MA).

[0029] Based on the estimated evolutionary probability of a particular nucleotide or amino acid residue at each position, the predicted sequence of an ancestral virus or portion thereof can be assembled to form a complete or partial synthetic nucleic acid or polypeptide sequence. If desired, the likelihood that any residue was in a given state at a given node along the node can be calculated, and any position along the sequence having a calculated posterior probability beneath a particular threshold can be identified (Figure 2(d)). In this manner, an ancestral scaffold sequence can be generated, which can include variations at those positions having a probability below the particular threshold.

[0030] If the ancestral sequence that is predicted using the methods herein is a nucleic acid sequence, the sequence then can be codon optimized so that it can be efficiently translated into an amino acid sequence. Codon usage tables for different organisms are known in the art.

Optionally, however, a codon usage table can be designed based on one or more contemporary sequences that has homology (e.g., at least 90% sequence identity) to the ancestral scaffold sequence, and an ancestral sequence as described herein can be codon optimized toward mammalian (e.g., human) codon usage.

[0031] Any or all of the steps outlined herein for predicting an ancestral viral sequence can be performed or simulated on a computer (e.g., *in silico*) using a processor or a microprocessor.

Ancestral Adeno-Associated Virus (AAV) Scaffold Sequences

[0032] The methods described herein were applied to adeno-associated virus (AAV) using contemporary capsid sequences (described in detail in the Examples below). AAV is widely considered as a therapeutic gene transfer vector and a genetic vaccine vehicle, but exhibits a high seroprevalence in human populations. Using the methods described herein, a phylogenetic tree was assembled using contemporary AAV sequences (see Figure 3) and predicted ancestral scaffold sequences were obtained at the designated phylogenetic node (Table 1). As used herein, an ancestral scaffold sequence refers to a sequence that is constructed using the methods described herein (e.g., using evolutionary probabilities and evolutionary modeling) and is not known to have existed in nature. As used herein, the ancestral scaffold sequences are different from consensus sequences, which are typically constructed using the frequency of nucleotides or amino acid residues at a particular position.

Table 1.

Node	Polypeptide (SEQ ID NO)	Nucleic Acid (SEQ ID NO)
Anc80	1	2
Anc81	3	4
Anc82	5	6
Anc83	7	8
Anc84	9	10
Anc94	11	12
Anc113	13	14
Anc126	15	16
Anc127	17	18
Anc110	42	43

[0033] The sequences of the scaffold polypeptide and nucleic acid, as well as the set of possible nucleotides or residues at positions of probability, are shown in the Sequence Listing. For example, the scaffold sequence of the Anc80 polypeptide is shown in SEQ ID NO: 1, which is encoded by the scaffold sequence of the Anc80 nucleic acid shown in SEQ ID NO:2. As shown in the Sequence Listing, the scaffold sequence of Anc80 contains 11 positions at which

either of two residues were probable. Therefore, the Anc80 scaffold sequence represents 2048 (2^{11}) different sequences. Additional scaffold sequences of the Anc81, Anc82, Anc83, Anc84, Anc94, Anc113, Anc126, Anc127, and Anc110 polypeptides are shown in SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, and 42; these polypeptides are encoded by the scaffold sequence of the Anc81, Anc82, Anc83, Anc84, Anc94, Anc113, Anc126, Anc127, and Anc110 nucleic acids, respectively, shown in SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, and 43. For each ancestral sequence, the set of possible nucleotides or residues at each position of probability is indicated.

[0034] To demonstrate the effectiveness of the methods described herein for predicting the ancestral sequence of a virus or portion thereof, a library of the 2048 predicted ancestral sequences at the AAV Anc80 node was generated and, as described herein, demonstrated to form viable virus particles exhibiting less seroprevalence, in some instances, significantly less seroprevalence, than virus particles assembled with contemporary capsid polypeptides.

Methods of Making Ancestral Virus Particles

[0035] After the predicted ancestral sequence of a virus or portion thereof has been obtained, the actual nucleic acid molecule and/or polypeptide(s) can be generated. Methods of generating a nucleic acid molecule or polypeptide based on a sequence obtained, for example, *in silico*, are known in the art and include, for example, chemical synthesis or recombinant cloning. Additional methods for generating nucleic acid molecules or polypeptides are known in the art and are discussed in more detail below.

[0036] Once an ancestral polypeptide has been produced, or once an ancestral nucleic acid molecule has been generated and expressed to produce an ancestral polypeptide, the ancestral polypeptide can be assembled into an ancestral virus particle using, for example, a packaging host cell. The components of a virus particle (e.g., rep sequences, cap sequences, inverted terminal repeat (ITR) sequences) can be introduced, transiently or stably, into a packaging host cell using one or more vectors as described herein. One or more of the components of a virus particle can be based on a predicted ancestral sequence as described herein, while the remaining components can be based on contemporary sequences. In some instances, the entire virus particle can be based on predicted ancestral sequences.

[0037] Such ancestral virus particles can be purified using routine methods. As used herein, "purified" virus particles refer to virus particles that are removed from components in the mixture in which they were made such as, but not limited to, viral components (e.g., rep sequences, cap sequences), packaging host cells, and partially- or incompletely-assembled virus particles.

[0038] Once assembled, the ancestral virus particles can be screened for, e.g., the ability to replicate; gene transfer properties; receptor binding ability; and/or seroprevalence in a

population (e.g., a human population). Determining whether a virus particle can replicate is routine in the art and typically includes infecting a host cell with an amount of virus particles and determining if the virus particles increase in number over time. Determining whether a virus particle is capable of performing gene transfer also is routine in the art and typically includes infecting host cells with virus particles containing a transgene (e.g., a detectable transgene such as a reporter gene, discussed in more detail below). Following infection and clearance of the virus, the host cells can be evaluated for the presence or absence of the transgene. Determining whether a virus particle binds to its receptor is routine in the art, and such methods can be performed *in vitro* or *in vivo*.

[0039] Determining the seroprevalence of a virus particle is routinely performed in the art and typically includes using an immunoassay to determine the prevalence of one or more antibodies in samples (e.g., blood samples) from a particular population of individuals. Seroprevalence is understood in the art to refer to the proportion of subjects in a population that is seropositive (i.e., has been exposed to a particular pathogen or immunogen), and is calculated as the number of subjects in a population who produce an antibody against a particular pathogen or immunogen divided by the total number of individuals in the population examined. Immunoassays are well known in the art and include, without limitation, an immunodot, Western blot, enzyme immunoassays (EIA), enzyme-linked immunosorbent assay (ELISA), or radioimmunoassay (RIA). As indicated herein, ancestral virus particles exhibit less seroprevalence than do contemporary virus particles (i.e., virus particles assembled using contemporary virus sequences or portions thereof). Simply by way of example, see Xu et al. (2007, *Am. J. Obstet. Gynecol.*, 196:43.e1-6); Paul et al. (1994, *J. Infect. Dis.*, 169:801-6); Sauerbrei et al. (2011, *Eurosurv.*, 16(44):3); and Sakhria et al. (2013, *PLoS Negl. Trop. Dis.*, 7:e2429), each of which determined seroprevalence for a particular antibody in a given population.

[0040] As described herein, ancestral virus particles are neutralized to a lesser extent than are contemporary virus particles. Several methods to determine the extent of neutralizing antibodies in a serum sample are available. For example, a neutralizing antibody assay measures the titer at which an experimental sample contains an antibody concentration that neutralizes infection by 50% or more as compared to a control sample without antibody. See, also, Fisher et al. (1997, *Nature Med.*, 3:306-12) and Manning et al. (1998, *Human Gene Ther.*, 9:477-85).

[0041] With respect to the ancestral AAV capsid polypeptides exemplified herein, the seroprevalence and/or extent of neutralization can be compared, for example, to an AAV8 capsid polypeptide or virus particle that includes an AAV8 capsid polypeptide, or an AAV2 capsid polypeptide or virus particle that includes an AAV2 capsid polypeptide. It is generally understood in the art that AAV8 capsid polypeptides or virus particles exhibit a seroprevalence, and a resulting neutralization, in the human population that is considered low, while AAV2 capsid polypeptide or virus particles exhibit a seroprevalence, and a resulting neutralization, in the human population that is considered high. Obviously, the particular seroprevalence will depend upon the population examined as well as the immunological methods used, but there

are reports that AAV8 exhibits a seroprevalence of about 22% up to about 38%, while AAV2 exhibits a seroprevalence of about 43.5% up to about 72%. See, for example, Boutin et al., 2010, "Prevalence of serum IgG and neutralizing factors against AAV types 1, 2, 5, 6, 8 and 9 in the healthy population: implications for gene therapy using AAV vectors," Hum. Gene Ther., 21:704-12. See, also, Calcedo et al., 2009, J. Infect. Dis., 199:381-90.

Predicted Adeno-Associated Virus (AAV) Ancestral Nucleic Acid and Polypeptide Sequences

[0042] A number of different clones from the library encoding predicted ancestral capsid polypeptides from the Anc80 node were sequenced, and the amino acid sequences of representative AAV predicted ancestral capsid polypeptides are shown in SEQ ID NO: 19 (Anc80L27); SEQ ID NO: 20 (Anc80L59); SEQ ID NO: 21 (Anc80L60); SEQ ID NO: 22 (Anc80L62); SEQ ID NO: 23 (Anc80L65); SEQ ID NO: 24 (Anc80L33); SEQ ID NO: 25 (Anc80L36); and SEQ ID NO: 26 (Anc80L44). Those skilled in the art would appreciate that the nucleic acid sequence encoding each amino acid sequence readily can be determined.

[0043] In addition to the predicted ancestral capsid polypeptides having the sequences shown in SEQ ID NOs: 19, 20, 21, 22, 23, 24, 25 or 26, polypeptides are provided that have at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to the predicted ancestral capsid polypeptides having the sequences shown in SEQ ID NOs: 19, 20, 21, 22, 23, 24, 25 or 26. Similarly, nucleic acid molecules are provided that have at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity) to the nucleic acid molecules encoding the ancestral capsid polypeptides.

[0044] In calculating percent sequence identity, two sequences are aligned and the number of identical matches of nucleotides or amino acid residues between the two sequences is determined. The number of identical matches is divided by the length of the aligned region (i.e., the number of aligned nucleotides or amino acid residues) and multiplied by 100 to arrive at a percent sequence identity value. It will be appreciated that the length of the aligned region can be a portion of one or both sequences up to the full-length size of the shortest sequence. It also will be appreciated that a single sequence can align with more than one other sequence and hence, can have different percent sequence identity values over each aligned region.

[0045] The alignment of two or more sequences to determine percent sequence identity can be performed using the algorithm described by Altschul et al. (1997, Nucleic Acids Res., 25:3389-3402) as incorporated into BLAST (basic local alignment search tool) programs, available at ncbi.nlm.nih.gov on the World Wide Web. BLAST searches can be performed to determine percent sequence identity between a sequence (nucleic acid or amino acid) and any other sequence or portion thereof aligned using the Altschul et al. algorithm. BLASTN is the program used to align and compare the identity between nucleic acid sequences, while BLASTP is the program used to align and compare the identity between amino acid

sequences. When utilizing BLAST programs to calculate the percent identity between a sequence and another sequence, the default parameters of the respective programs generally are used.

[0046] Representative alignments are shown in Figures 4A and 4B and Figures 5A and 5B. Figures 4A and 4B show an alignment of ancestral AAV VP1 capsid polypeptides, designated Anc80L65 (SEQ ID NO: 23), Anc80L27 (SEQ ID NO: 19), Anc80L33 (SEQ ID NO: 24), Anc80L36 (SEQ ID NO: 25), Anc80L44 (SEQ ID NO: 26), Anc80L59 (SEQ ID NO: 20), Anc80L60 (SEQ ID NO: 21), and Anc80L62 (SEQ ID NO: 22). The alignment shown in Figures 4A and 4B confirms the predicted variation at each of the 11 sites, and a single non-synonymous mutation at position 609E of Anc80L60 (SEQ ID NO: 21), which may be a cloning artifact. Figures 5A and 5B shows an alignment between ancestral AAV VP1 capsid polypeptides (Anc80L65 (SEQ ID NO: 23), Anc80L27 (SEQ ID NO: 19), Anc80L33 (SEQ ID NO: 24), Anc80L36 (SEQ ID NO: 25), Anc80L60 (SEQ ID NO: 21), Anc80L62 (SEQ ID NO: 22), Anc80L44 (SEQ ID NO: 26), and Anc80L59 (SEQ ID NO: 20)) and contemporary AAV VP1 capsid polypeptides (AAV8 (SEQ ID NO: 27), AAV9 (SEQ ID NO: 28), AAV6 (SEQ ID NO: 29), AAV1 (SEQ ID NO: 30), AAV2 (SEQ ID NO: 31), AAV3 (SEQ ID NO: 32), AAV3B (SEQ ID NO: 33), and AAV7 (SEQ ID NO: 34)). The alignment in Figures 5A and 5B shows that the ancestral AAV sequences have between about 85% and 91% sequence identity to contemporary AAV sequences.

[0047] Vectors containing nucleic acid molecules that encode polypeptides also are provided. Vectors, including expression vectors, are commercially available or can be produced by recombinant technology. A vector containing a nucleic acid molecule can have one or more elements for expression operably linked to such a nucleic acid molecule, and further can include sequences such as those encoding a selectable marker (e.g., an antibiotic resistance gene), and/or those that can be used in purification of a polypeptide (e.g., 6xHis tag). Elements for expression include nucleic acid sequences that direct and regulate expression of nucleic acid coding sequences. One example of an expression element is a promoter sequence. Expression elements also can include one or more of introns, enhancer sequences, response elements, or inducible elements that modulate expression of a nucleic acid molecule. Expression elements can be of bacterial, yeast, insect, mammalian, or viral origin and vectors can contain a combination of expression elements from different origins. As used herein, operably linked means that elements for expression are positioned in a vector relative to a coding sequence in such a way as to direct or regulate expression of the coding sequence.

[0048] A nucleic acid molecule, e.g., a nucleic acid molecule in a vector (e.g., an expression vector, a viral vector) can be introduced into a host cell. The term "host cell" refers not only to the particular cell(s) into which the nucleic acid molecule has been introduced, but also to the progeny or potential progeny of such a cell. Many suitable host cells are known to those skilled in the art; host cells can be prokaryotic cells (e.g., *E. coli*) or eukaryotic cells (e.g., yeast cells, insect cells, plant cells, mammalian cells). Representative host cells can include, without limitation, A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, WI38, HeLa, 293 cells, Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast,

hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. Methods for introducing nucleic acid molecules into host cells are well known in the art and include, without limitation, calcium phosphate precipitation, electroporation, heat shock, lipofection, microinjection, and viral-mediated nucleic acid transfer (e.g., transduction).

[0049] With respect to polypeptides, "purified" refers to a polypeptide (i.e., a peptide or a polypeptide) that has been separated or purified from cellular components that naturally accompany it. Typically, the polypeptide is considered "purified" when it is at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, or 99%) by dry weight, free from the polypeptides and naturally occurring molecules with which it is naturally associated. Since a polypeptide that is chemically synthesized is, by nature, separated from the components that naturally accompany it, a synthetic polypeptide is considered "purified," but further can be removed from the components used to synthesize the polypeptide (e.g., amino acid residues). With respect to nucleic acid molecules, "isolated" refers to a nucleic acid molecule that is separated from other nucleic acid molecules that are usually associated with it in the genome. In addition, an isolated nucleic acid molecule can include an engineered nucleic acid molecule such as a recombinant or a synthetic nucleic acid molecule.

[0050] Polypeptides can be obtained (e.g., purified) from natural sources (e.g., a biological sample) by known methods such as DEAE ion exchange, gel filtration, and/or hydroxyapatite chromatography. A purified polypeptide also can be obtained, for example, by expressing a nucleic acid molecule in an expression vector or by chemical synthesis. The extent of purity of a polypeptide can be measured using any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. Similarly, nucleic acid molecules can be obtained (e.g., isolated) using routine methods such as, without limitation, recombinant nucleic acid technology (e.g., restriction enzyme digestion and ligation) or the polymerase chain reaction (PCR; see, for example, PCR Primer: A Laboratory Manual, Dieffenbach & Dveksler, Eds., Cold Spring Harbor Laboratory Press, 1995). In addition, isolated nucleic acid molecules can be chemically synthesized.

Methods of Using Ancestral Viruses or Portions Thereof

[0051] An ancestral virus or portion thereof as described herein, particularly those that exhibit reduced seroprevalence relative to contemporary viruses or portions thereof, can be used in a number of research and/or therapeutic applications. For example, an ancestral virus or portion thereof as described herein can be used in human or animal medicine for gene therapy (e.g., in a vector or vector system for gene transfer) or for vaccination (e.g., for antigen presentation). More specifically, an ancestral virus or portion thereof as described herein can be used for gene addition, gene augmentation, genetic delivery of a polypeptide therapeutic, genetic vaccination, gene silencing, genome editing, gene therapy, RNAi delivery, cDNA delivery, mRNA delivery, miRNA delivery, miRNA sponging, genetic immunization, optogenetic gene therapy, transgenesis, DNA vaccination, or DNA immunization.

[0052] A host cell can be transduced or infected with an ancestral virus or portion thereof *in vitro* (e.g., growing in culture) or *in vivo* (e.g., in a subject). Host cells that can be transduced or infected with an ancestral virus or portion thereof *in vitro* are described herein; host cells that can be transduced or infected with an ancestral virus or portion thereof *in vivo* include, without limitation, brain, liver, muscle, lung, eye (e.g., retina, retinal pigment epithelium), kidney, heart, gonads (e.g., testes, uterus, ovaries), skin, nasal passages, digestive system, pancreas, islet cells, neurons, lymphocytes, ear (e.g., inner ear), hair follicles, and/or glands (e.g., thyroid).

[0053] An ancestral virus or portion thereof as described herein can be modified to include a transgene (in *cis* or *trans* with other viral sequences). A transgene can be, for example, a reporter gene (e.g., beta-lactamase, beta-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent polypeptide (GFP), chloramphenicol acetyltransferase (CAT), or luciferase, or fusion polypeptides that include an antigen tag domain such as hemagglutinin or Myc) or a therapeutic gene (e.g., genes encoding hormones or receptors thereof, growth factors or receptors thereof, differentiation factors or receptors thereof, immune system regulators (e.g., cytokines and interleukins) or receptors thereof, enzymes, RNAs (e.g., inhibitory RNAs or catalytic RNAs), or target antigens (e.g., oncogenic antigens, autoimmune antigens)).

[0054] The particular transgene will depend, at least in part, on the particular disease or deficiency being treated. Simply by way of example, gene transfer or gene therapy can be applied to the treatment of hemophilia, retinitis pigmentosa, cystic fibrosis, leber congenital amaurosis, lysosomal storage disorders, inborn errors of metabolism (e.g., inborn errors of amino acid metabolism including phenylketonuria, inborn errors of organic acid metabolism including propionic academia, inborn errors of fatty acid metabolism including medium-chain acyl-CoA dehydrogenase deficiency (MCAD)), cancer, achromatopsia, cone-rod dystrophies, macular degenerations (e.g., age-related macular degeneration), lipopolypeptide lipase deficiency, familial hypercholesterolemia, spinal muscular atrophy, Duchenne's muscular dystrophy, Alzheimer's disease, Parkinson's disease, obesity, inflammatory bowel disorder, diabetes, congestive heart failure, hypercholesterolemia, hearing loss, coronary heart disease, familial renal amyloidosis, Marfan's syndrome, fatal familial insomnia, Creutzfeldt-Jakob disease, sickle-cell disease, Huntington's disease, fronto-temporal lobar degeneration, Usher syndrome, lactose intolerance, lipid storage disorders (e.g., Niemann-Pick disease, type C), Batten disease, choroideremia, glycogen storage disease type II (Pompe disease), ataxia telangiectasia (Louis-Bar syndrome), congenital hypothyroidism, severe combined immunodeficiency (SCID), and/or amyotrophic lateral sclerosis (ALS).

[0055] A transgene also can be, for example, an immunogen that is useful for immunizing a subject (e.g., a human, an animal (e.g., a companion animal, a farm animal, an endangered animal)). For example, immunogens can be obtained from an organism (e.g., a pathogenic organism) or an immunogenic portion or component thereof (e.g., a toxin polypeptide or a by-product thereof). By way of example, pathogenic organisms from which immunogenic polypeptides can be obtained include viruses (e.g., picornavirus, enteroviruses,

orthomyxovirus, reovirus, retrovirus), prokaryotes (e.g., Pneumococci, Staphylococci, Listeria, Pseudomonas), and eukaryotes (e.g., amebiasis, malaria, leishmaniasis, nematodes). It would be understood that the methods described herein and compositions produced by such methods are not to be limited by any particular transgene.

[0056] An ancestral virus or portion thereof, usually suspended in a physiologically compatible carrier, can be administered to a subject (e.g., a human or non-human mammal). Suitable carriers include saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline), lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, and water. The ancestral virus or portion thereof is administered in sufficient amounts to transduce or infect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to an organ such as, for example, the liver or lung, orally, intranasally, intratracheally, by inhalation, intravenously, intramuscularly, intraocularly, subcutaneously, intradermally, transmucosally, or by other routes of administration. Routes of administration can be combined, if desired.

[0057] The dose of the ancestral virus or portion thereof administered to a subject will depend primarily on factors such as the condition being treated, and the age, weight, and health of the subject. For example, a therapeutically effective dosage of an ancestral virus or portion thereof to be administered to a human subject generally is in the range of from about 0.1 ml to about 10 ml of a solution containing concentrations of from about 1×10^1 to 1×10^{12} genome copies (GCs) of ancestral viruses (e.g., about 1×10^3 to 1×10^9 GCs). Transduction and/or expression of a transgene can be monitored at various time points following administration by DNA, RNA, or protein assays. In some instances, the levels of expression of the transgene can be monitored to determine the frequency and/or amount of dosage. Dosage regimens similar to those described for therapeutic purposes also may be utilized for immunization.

[0058] The methods described herein also can be used to model forward evolution, so as to modify or ablate one or more immunogenic domains of a virus or portion thereof.

[0059] In accordance with the present invention, there may be employed conventional molecular biology, microbiology, biochemical, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. The invention will be further described in the following examples, which do not limit the scope of the methods and compositions of matter described in the claims.

EXAMPLES

Example 1-Computational Prediction of Ancestral Sequences

[0060] A set of 75 different amino acid sequences of AAV capsids was obtained from a number of public databases including GenBank, and the sequences were aligned using the PRANK-MSA algorithm, version 121002, with the option "-F".

[0061] ProtTest3 (see, for example, Darriba et al., 2011, *Bioinformatics*, 27(8):1164-5; available at darwin.uvigo.es/software/prottest3 on the World Wide Web) was used to evaluate different models of polypeptide evolution (e.g., those included in ProTest3, namely, JTT, LG, WAG, VT, CpRev, RtRev, Dayhoff, DCMut, FLU, Blosum62, VT, HIVb, MtArt, MtMam) under different conditions (e.g., those included in ProTest3, namely, "+1", "+F", "+ G", and combinations thereof). The JTT model (Jones et al., 1992, *Comp. Appl. Biosci.*, 8:275-82) with +G and +F (Yang, 1993, *Mol. Biol. Evol.*, 10:1396-1401; and Cao et al., 1994, *J. Mol. Evol.*, 39:519-27) was selected based on its Akaike Information Criterion (AIC; Hirotugu, 1974, *IEEE Transactions on Automatic Control*, 19:716-23) score as implemented in ProTest3.

[0062] A phylogeny of AAV evolution was constructed using PhyML (Guindon and Gascuel, 2003, *Systematic Biology*, 52:696-704). See Figure 3. The tree was generated using the JTT + F substitution model with 4 discrete substitution categories and an estimated Gamma shape parameter. The resultant trees were improved via Nearest Neighbor Interchange (NNI) and Subtree Pruning and Re-Grafting (SPR), and assessed for significance via bootstrap and approximate likelihood-ratio test (aLRT; Anisimova and Gascuel, 2006, *Systematic Biology*, 55:539-52) using the "SH-Like" variant.

[0063] The phylogenetic tree constructed above was then used to estimate the ancestral states of the AAV capsid at every node interior to the phylogeny. The ancestral capsid sequences were reconstructed using maximum likelihood principles through the Phylogenetic Analysis by Maximum Likelihood (PAML) software (Yang, 1997, *Comp. Applic. BioSci.*, 13:555-6; available at abacus.gene.ucl.ac.uk/software/paml.html on the World Wide Web) wrapped in Lazarus (Sourceforge at sf.net). More specifically, the Lazarus/PAML reconstruction was set to generate an amino acid reconstruction using the JTT+F substitution model using 4 gamma-distributed categories. AAV5 was used as an outgroup. Finally, the "I" option was added to place indels (i.e., coded binarily and placed via Maximum Parsimony using Fitch's algorithm) after the PAML reconstruction was done.

[0064] Because the reconstruction was done in a maximum-likelihood fashion, the likelihood that any residue was in a given position at a given node can be calculated. To do this, an additional script was written to identify all positions along the sequence with a calculated posterior probability beneath a certain threshold. A threshold of 0.3 was selected, meaning that any amino acid with a calculated posterior probability of greater than 0.3 was included in the synthesis of the library. These residues were selected to be variants of interest in the library.

[0065] To finalize the sequence, an additional utility had to be coded to select codons. A script was written to derive codons similar to those of another AAV sequence (AVVRh10, which has about 92% sequence identity to the Anc80 scaffold sequence) and apply a novel algorithm to

substitute codons where there were sequence mismatches based on a codon-substitution matrix. The novel algorithm is shown below:

Given: amino acid sequence, Pt, with corresponding nucleotide sequence, Nt, where Nt codes for Pt; and protein sequence, Pi, where Pi exhibits strong homology to Pt.

Align Pi with Pt using Needleman-Wunsch using the Blosom62 table for scoring. Generate a new nucleotide sequence, Ni, by stepping through the protein alignment, using the corresponding codon from Nt,

where the amino acid in Pt exactly matches that in Pi,

the "best scoring" codon from the Codon-PAM matrix (Schneider et al., 2005, BMC Bioinform., 6:134) where there is a substitution,

a gap where there exists a gap in Pi aligned against an amino-acid in Pt, and

the most frequently occurring nucleotide in the Nt (coding for a given amino acid) where there exists an amino-acid in Pi aligned against a gap in Pt.

[0066] In addition, two single nucleotide changes were made to ablate transcription of assembly-activating protein (AAP), which is encoded out of frame within the AAV capsid gene in the wild type AAV. Since the coding of AAP (contemporary or ancestral) was not a part of this reconstruction, the expression of AAP was ablated by making a synonymous mutation in the cap sequence, and the AAP sequence was provided in trans during viral production.

Example 2-Expression of Ancestral AAV VP1 Sequences

[0067] Experiments were performed to determine whether predicted ancestral AAV capsid sequences can be used to make viral vectors.

[0068] A number of the predicted ancestral AAV capsid sequences were cloned. The library of ancestral capsids was transferred to a rep-cap expression plasmid to enable viral particle formation in transient transfection. To maintain appropriate expression levels and splicing of VP1, VP2, and VP3, library cap genes were cloned by cutting *HindIII*, located 5' of cap in the rep coding sequence, and *SpeI*, which was engineered between the cap stop codon and the polyadenylation signal. Consequently, to clone the ancestral capsids into a more conventional "REP/CAP" construct, the passaging-plasmid was digested with *HindIII* and *SpeI*, gel purified, and ligated into a similarly digested rep/cap plasmid.

[0069] The expressed polypeptides were resolved on a 10% SDS gel. As shown in Figure 6, the capsid polypeptides were appropriately expressed and spliced into VP1, VP2, and VP3 from a number of ancestral AAV sequences (Anc80L44, Anc80L27, and Anc80L65) as well as

from a contemporary AAV sequence, AAV2/8.

Example 3-Viral Titration

[0070] AAV was produced in HEK293 cells via transient co-transfection of plasmids encoding all elements required for viral particle assembly. Briefly, HEK293 cells were grown to 90% confluency and transfected with (a) the viral genome plasmid encoding the luciferase transgene (expressed by the CMV promoter) flanked by AAV2 ITRs, (b) the AAV packaging plasmid encoding AAV2 rep and the synthesized capsid proteins disclosed herein, (c) AAV2-AAP expressing capsid, and (d) adenoviral helper genes needed for AAV packaging and assembly. Cells were incubated at 37°C for 2 days, and cells and media were harvested and collected.

[0071] The cell-media suspension was lysed by 3 consecutive freeze-thaw cycles. Next, the lysate was cleared by centrifugation and treated with an enzyme under conditions to perform exhaustive DNA digestion, here Benzonase™, to digest any DNA present outside of the virus particle. The AAV preparation was diluted to fall within the linear measurement range of a control DNA template, in this case linearized plasmid with identical TaqMan™ primer and probe binding sequence as compared to the vector genome. TaqMan™ PCR was performed with primers and probe annealing to the viral vector genome of choice. Titer was calculated based on the TaqMan™ measurement in genome copies (GC) per milliliter (ml) as shown in Table 2 below.

Table 2

Titers (GC/ml)	Small scale #1	Small scale #2
AAV2/2	1.12×10^9	1.99×10^9
AAV2/8	4.17×10^{10}	5.91×10^{10}
Anc80L27	8.01×10^8	1.74×10^9
Anc80L44	1.52×10^9	1.43×10^9
Anc80L65	1.42×10^9	2.05×10^9
No capsid control	5.23×10^5	7.25×10^5

[0072] Small scale vector production results on ancestrally reconstructed AAV capsid particles demonstrated yields that were similar to AAV2, but reduced relative to AAV8, both of which are vector preparations based on contemporary AAVs.

Example 4- In Vitro Viral Transduction

[0073] *In vitro* viral transductions were performed to evaluate the ability of viruses containing the predicted ancestral AAV sequences to infect cells.

[0074] Following high throughput vector production using the Anc80 library of sequences, HEK293 cells were transduced with each viral vector. In addition to an Anc80 sequence, each viral vector contained a luciferase transgene. Luciferase was measured by quantification of bioluminescence in a 96 well plate reader following addition of luciferin substrate to the transduced cells or cell lysate. Following quantification, a heat map of luciferase expression in four concatenated 96-well plates was produced (excluding a column of controls in each plate). Due to the large number of insertions, deletions, and transitions associated with the process of high throughput vector production, many of the vectors were non-functional. For purposes herein, only viruses that were functional in this assay (i.e., able to transduce HEK293 cells and express the transgene) were evaluated further.

[0075] HEK293 cells were transduced, at equal multiplicity of infection (MOI) of 1×10^4 genome copies (GC) per cell, with two contemporary AAV vectors (AAV2/2 and AAV2/8) and three predicted ancestral AAV vectors (Anc80L27, Anc80L44, and Anc80L65). Each vector contained either a luciferase-encoding transgene or an eGFP-encoding transgene. Cells were imaged 60 hours later using the GFP channel of an AMG EvosFI Optical Microscope. Figure 7 shows the luciferase expression following the *in vitro* transduction. Each of the ancestral AAV viruses demonstrated efficient transduction of HEK293 cells.

Example 5-*In Vivo* Retinal Transduction

[0076] Retinal transductions were performed to determine whether or not the ancestral AAV vectors are able to target murine retinal cells *in vivo*.

[0077] Murine eyes were transduced with 2×10^8 genome copies (GC) of three different ancestral AAVs (Anc80L27, Anc80L44, and Anc80L65) and a contemporary AAV (AAV2/8), all of which included an eGFP-encoding transgene. For transductions, each AAV vector was surgically delivered below the retina by generating a space between the photoreceptor and retinal pigment epithelium layer through delivery of a vector bolus with an injection device. The vector bolus was left in the sub-retinal space and the sub-retinal detachment resolved over time. GFP expression was monitored non-invasively by fundus photography of the retina of the animal following pupil dilation with Tropicamide™. All of the presented retinas demonstrated varying degrees of successful targeting of ancestral AAVs to the retina.

[0078] Retinal histology also was performed and visualized under fluorescent microscopy to identify the transduced cell type(s). Histology was performed on a murine retina transduced with the Anc80L65 ancestral AAV vector as described above. Anc80L65-mediated eGFP expression was evident in the outer nuclear layer (ONL), the inner segments (IS), and the retinal pigment epithelium (RPE), indicating that the ancestral Anc80L65 vector targets murine

photoreceptors and retinal pigment epithelial cells.

Example 6-Neutralizing Antibody Assay

[0079] Neutralizing antibody assays are performed to evaluate whether or not an ancestral AAV virus is more resistant to antibody-neutralization than a contemporary AAV virus. Neutralizing antibody assays measure the antibody concentration (or the titer at which an experimental sample contains an antibody concentration) that neutralizes an infection by 50% or more as compared to a control in the absence of the antibody.

[0080] Serum samples or IVIG stock solution (200 mg/ml) are serially diluted by 2-fold, and undiluted and diluted samples are co-incubated with an ancestral AAV virus, Anc80L65, and a contemporary AAV virus, AAV2/8, at a MOI of 10^4 for about 30 minutes at 37°C. Each virus includes a luciferase transgene. The admixed vector and an antibody sample then are transduced into HEK293 cells. For these experiments, the antibody sample used is intravenous immunoglobulin (IVIG), pooled IgGs extracted from the plasma of over one thousand blood donors (sold commercially, for example, as Gammagard™ (Baxter Healthcare; Deerfield, IL) or Gamunex™ (Grifols; Los Angeles, CA)). 48 hours following initiation of transduction, cells are assayed by bioluminescence to detect luciferase. Neutralizing antibody titer is determined by identifying the dilution of sample for which 50% or more neutralization (transduction of sample/ transduction of control virus in absence of sample) is reached.

Example 7-Characterization of Anc80

[0081] Based on the methods described herein, the most probable Anc80 sequence (as determined through posterior probability) was obtained and designated Anc80L1 (SEQ ID NO:35 shows the nucleic acid sequence of the Anc80L1 capsid and SEQ ID NO:36 shows the amino acid sequence of the Anc80L1 VP1 polypeptide). The Anc80 probabilistic library also was synthesized using the sequences described herein by a commercial company and sub-cloned into expression vectors.

[0082] The Anc80 library was clonally evaluated for vector yield and infectivity in combined assays. Out of this screening, Anc80L65 (SEQ ID NO:23), as well as several other variants, were further characterized.

[0083] The Anc80 library and Anc80L65 were compared in terms of sequence difference (Figure 8; % up from diagonal, # of amino acid differences below). Using NCBI-BLAST, the closest publically available sequence to Anc80L65 is rh10 (GenBank Accession No. AAO88201.1).

[0084] Figure 9 shows that Anc80L65 produced vector yields equivalent to AAV2 (Panel A),

generated virus particles under Transmission Electrosopy (TEM) (Panel B), and biochemically produced the AAV cap and the VP1, 2 and 3 proteins based on SDS page under denaturing conditions (Panel C) and Western Blotting using the AAV capsid antibody, B1 (Panel D). These experiments are described in more detail in the following paragraphs.

[0085] Briefly, AAV2/8, AAV2/2, AAV2/Anc80L27, AAV2/Anc80L44, and AAV2/Anc80L65 vectors were produced in small scale containing a reporter construct comprised of eGFP and firefly luciferase under a CMV promoter were produced in small scale. Titers of these small scale preparations of viruses were then obtained via qPCR. Based on these experiments, Anc80L27, Anc80L44, and Anc80L65 vectors were found to produce viral levels comparable to that of AAV2 (Figure 9A).

[0086] To confirm that the Anc80L65 capsid proteins assembled into intact virus-like-particles of the proper size and conformation, micrographs were obtained using transmission electron microscopy (TEM). A large scale, purified preparation of Anc80-L065 was loaded onto formvar coated copper grids and was then stained with uranyl acetate. Micrographs revealed intact, hexagonal particles with diameters between 20 and 25 nm (Figure 9B).

[0087] In order to determine whether the synthetic ancestral capsid genes were properly processed (i.e. spliced and expressed), large-scale purified preparations of AAV2/8, AAV2/2, and AAV2/Anc80L65 vectors were loaded onto an SDS-PAGE gel (1E10 GC/well) under denaturing conditions. Bands representing viral capsid proteins VP1, VP2, and VP3 were clearly present for each vector preparation (Figure 9C). Western blotting with the AAV capsid antibody B1 further confirmed that these bands represented the predicted proteins (Figure 9D).

[0088] In addition, Figure 10 shows that Anc80L65 infected mammalian tissue and cells *in vitro* on HEK293 cells at MOI 10E4 GC/cell using GFP as readout (Panel A) or luciferase (Panel B) versus AAV2 and/or AAV8 controls. Anc80L65 also was efficient at targeting liver following an IV injection of the indicated AAV encoding a nuclear LacZ transgene (top row, Panel C), following direct intramuscular (IM) injection of the indicated AAV encoding GFP (middle row, Panel C), and following subretinal injection with the indicated AAV encoding GFP (bottom row, Panel C). These experiments are described in more detail in the following paragraphs.

[0089] To obtain a relative measure of the infectivity of ancestral virions, crude preparations of AAV2/2, AAV2/8, AAV2/Anc80L65, AAV2/Anc80L44, AAV2/Anc80L27, AAV2/Anc80L121, AAV2/Anc80L122, AAV2/Anc80L123, AAV2/Anc80L124, and AAV2/Anc80L125 containing a bicistronic reporter construct that includes an eGFP and firefly luciferase sequences under control of a CMV promoter were produced. 96-well plates confluent with HEK293 cells were then subjected to transduction with each vector at an MOI of 1E4 GC/cell (titers obtained via qPCR as above). 48 hours later, fluorescent microscopy confirmed the presence of GFP in transduced cells (Figure 10A). Cells were then assayed for the presence of luciferase (Figure 10B), which determined that expression of luciferase in cells transduced with Anc80-derived vectors was in-between that of cells transduced with AAV8 (lower level of transduction) and

AAV2 (higher level of transduction).

[0090] To assess the relative efficiency of gene transfer in an in vivo context, purified high-titer preparations of AAV2/2, AAV2/8, and AAV2/Anc80L65 were obtained. 3.9×10^{10} GC of each vector, encapsidating a transgene encoding nuclear LacZ under control of a TBG promoter, were injected into C57BL/6 mice (3 mice per condition) via IP injection following general anesthetization. 28 days post-injection, mice were sacrificed and tissues were collected. Livers were sectioned via standard histological techniques and stained for beta-galactosidase. Sections were then imaged under a microscope and representative images are shown in Figure 10C, top row.

[0091] Vectors of the same serotypes were then obtained containing a bicistronic transgene encoding eGFP and hA1AT under control of a pCASI promoter. To assess the ability of Anc80L65 to transduce murine skeletal muscle, 1×10^{10} GC of each vector was injected into skeletal muscle of C57BL/6 mice (5 mice per condition) following general anesthetization. 28 days post-injection, mice were sacrificed, tissues were cryosectioned, and the presence of eGFP was assessed using fluorescent confocal microscopy (blue is DAPI, green is eGFP). Representative images are shown in Figure 10C, middle row. These experiments demonstrated that Anc80L65 vectors were capable of transducing murine skeletal muscle via intramuscular injection.

[0092] Vectors of the same serotypes were obtained, this time encapsidating constructs encoding only an eGFP transgene under control of a CMV promoter. 2×10^9 particles were injected sub-retinally into C57BL/6 mice following general anesthetization. 28 days post-injection, mice were sacrificed and the eyes were collected, cryosectioned, and the presence of eGFP was assessed using fluorescent confocal microscopy (blue is DAPI, green is eGFP). Representative images are shown in Figure 10C, bottom row. These experiments demonstrate that Anc80L65 vectors are able to transduce murine retina at a level that is comparable to AAV8 vectors.

[0093] Briefly, purified, high titer preparations of AAV2/8, AAV2/2, AAV2/rh32.33, and AAV2/Anc80L65 viral vectors encapsidating a bicistronic transgene that includes eGFP and firefly luciferase under control of a CMV promoter are obtained. These vectors are then either incubated with two-fold serial dilutions of IVIG (10mg, 5mg, 2.5mg, etc.) or incubated without IVIG (1×10^9 GC per condition). Following incubation, vectors are used to transduce HEK293 cells at an MOI of 1×10^4 per well (one dilution per well). 48 hours later, the relative amounts of luciferase is assayed via luminescence assay.

Example 8-Generation of Additional Ancestral AAV Capsids

[0094] The most probable ancestral AAV capsid sequences (as determined through posterior probability) were then synthesized through a commercial lab (Gen9) and provided as linear dsDNA. These amino acid sequences were then compared to those of extant AAVs in order to

ascertain the degree to which they differ (Figure 11). Each ancestral VP1 protein differs from those of selected representative extant AAVs by between 3.6% and 9.3% (Figure 11A), while the ancestral VP3 proteins differ by between 4.2 and 9.4% (Figure 11B). At 89% sequence identity for VP1, And 10 is the closest reconstructed ancestral vector to AAV9, a potent CNS transducing vector. These capsids were each subcloned into AAV production plasmids (pAAVector2/Empty) via restriction enzyme digestion (HindIII & SpeI) and T4 ligation. These clones were confirmed via restriction digestion and Sanger sequencing, and medium scale preparations of plasmid DNA were then produced.

[0095] Each of these plasmids were then used to produce AAV vectors containing a reporter gene encoding both eGFP and firefly luciferase. These vectors were produced in triplicate in small scale as previously described. Crude preparations of the virus were then titrated via qPCR and were found to produce between 2.71% and 183.1% viral particles relative to AAV8 (Figures 12 and 13). The production and infectivity numbers of And 10 are similar to those reported for AAV9. These titers were then used to set up a titer controlled experiment to assess relative infectivity. Anc126 was not titer controlled due to its significantly depressed production, and consequently, the data regarding the infectivity of Anc126 cannot be accurately compared to the infectivity of the other viruses in the experiment. The other vectors were used to transduce HEK293 cells at a multiplicity of infection (MOI) of 1.9E3 GC/cell.

[0096] 60 hours post transduction, cells were assessed for GFP expression via fluorescence microscopy. eGFP positive cells were detected under each of the conditions except for the negative control (Figure 14). This indicates that each of the ancestral sequences that were predicted, synthesized, and cloned, including And 10, is capable of producing viable, infectious virus particles. To get an idea of the relative levels of infectivity, luciferase assays also were performed on the same cells. The results indicate that each of the ancestral vectors is capable of transducing HEK293 cells between 28.3% and 850.8% relative to AAV8 (Figures 15 and 16). It is noted that the transduction efficiency of And 10 is similar to that reported for AAV9. Anc126 was excluded from the analysis of relative transduction since it was not titer-controlled.

[0097] In summary, eight novel ancestral AAV capsid genes were synthesized and used in the production of functional viral vectors along with AAV8, AAV2, Anc110, and the previously described Anc80L65 vectors. Production and infectivity were assessed in vitro and a summary of those findings is shown in Figure 17. The in vitro production and infectivity of And 10 was within the range that would be expected for AAV9 and other viruses that are able to pass through the blood-brain barrier.

Example 9-Vectored Immunoprophylaxis

[0098] In vectored immunoprophylaxis, gene therapy vehicles (such as AAV) are used to deliver transgenes encoding broadly neutralizing antibodies against infectious agents. See, for example, Balazs et al. (2013, Nat. Biotechnol., 31:647-52); Limberis et al. (2013, Sci. Transl. Med., 5:187ra72); Balazs et al. (2012, Nature, 481:81-4); and Deal et al. (2014, PNAS USA,

111:12528-32). One advantage of this treatment is that the host produces the antibodies in their own cells, meaning that a single administration has the potential to confer a lifetime of protection against etiologic agents.

Example 10-Drug Delivery Vehicles

[0099] LUCENTIS (ranibizumab) and AVASTIN (bevacizumab) are both anti-angiogenesis agents based on the same humanized mouse monoclonal antibodies against vascular endothelial growth factor A (VEGF-A). Although bevacizumab is a full antibody and ranibizumab is a fragment (Fab), they both act to treat wet age-related macular degeneration through the same mechanism - by antagonizing VEGF. See, for example, Mao et al. (2011, Hum. Gene Ther., 22:1525-35); Xie et al. (2014, Gynecol. Oncol., doi: 10.1016/j.ygyno.2014.07.105); and Watanabe et al. (2010, Gene Ther., 17:1042-51). Because both of these molecules are proteins, they can be encoded by DNA and produced in cells transduced with vectors containing a transgene, and are small enough to be packaged into AAV vectors.

Example 11-Ancestral Sequence Reconstruction of AAV Capsids

[0100] Ancestral capsid sequences were reconstructed using maximum-likelihood methods as in Finnigan et al. (2012, Nature, 481:360-4). An alignment of 75 AAV capsids (GenBank Accession Numbers provided herein) was generated using PRANK v.121002 using the -F option (Loytynoja & Goldman, 2005, PNAS USA, 102:10557-62; Loytynoja & Goldman, 2008, Science, 320:1632-5) and the JTT+F+G model was determined to be the phylogenetic model of best fit through the Aikake Information Criterion as implemented in ProtTest3 (Darriba et al., 2011, Bioinform., 27:1164-5). The full alignment can be seen in Figure 25. The alignment and best-fit model were then used to infer a phylogeny through PhyML 3.0 (Guindon et al., 2010, System. Biol., 59:307-21), which was evaluated through the approximate likelihood-ratio test (aLRT) (Anisimova & Gascuel, 2006, Syst. Biol., 55:539-52) as implemented in PhyML. A detailed version of the phylogeny with all AAVs included in the analysis is shown in Figure 26. Ancestral capsid sequences were then inferred using PAML 4.6 (Yang, 2007, Mol. Biol. Evol., 24:1586-91) through the Lazarus package developed by the Thornton group. As indicated herein, the And 10 reconstructed ancestral vector is evolutionarily close to AAV9 and Rh.8, both of which are known to be potent CNS transducing vectors.

[0101] In order to compensate for the uncertainty inherent to the reconstruction, a script was written to assess the computed posterior probabilities to identify ambiguously reconstructed sites. All positions along the ancestrally reconstructed capsid having more than one amino acids with posterior probabilities greater than 0.3 were included. Eleven such sites were identified, each with two probable amino acids. These eleven dimorphic sites were then incorporated into a DNA library using the codons from a modern virus (rh. 10). Because the

reconstruction did not consider the coevolution of AAP and the capsid, the AAP open-reading frame was ablated by changing the non-canonical CTG start codon to CAG during library design. In addition, another downstream ATG also in the AAP ORF was ablated by changing the codon to AAG. These modifications did not alter the amino acids in the cap ORF. The DNA library was then synthesized by DNA2.0 and subsequently sub-cloned into expression vectors via restriction enzyme digest and ligation.

Example 12-Vectors and Sequences

[0102] Adeno-associated viral vectors were pseudotyped with either extant or ancestral viral capsids. Extant capsids include AAV1 (GenBank Accession No. AAD27757.1), AAV2 (GenBank Accession No. AAC03780.1), AAV5 (GenBank Accession No. AAD13756.1), AAV6.2 (GenBank Accession No. EU368910), Rh.10 (GenBank Accession No. AAO88201.1), AAV8 (GenBank Accession No. AAN03857.1), AAV9 (GenBank Accession No. AAS99264.1), and Rh32.33 (GenBank Accession No. EU368926). Ancestral AAV capsids include Anc80L65, Anc81, Anc82, Anc83, Anc84, And 10, Anc113, Anc126, and Anc127 (submissions to GenBank pending). Vector transgene cassettes included CMV.eGFP.T2A.ffLuciferase.SVPA, CMV.ffLuciferase.SVPA (*in vitro* studies), TBG.LacZ.RBG (liver), TBG.eGFP.WPRE.bGH (liver and muscle immunization study), CASI.hA1AT.FF2A.eGFP.RBG (liver, muscle), and CMV.eGFP.WPRE (retina).

Example 13-Sequence-Structure Analysis

[0103] A pseudoatomic model of Anc80L65 VP3 was generated with the SWISS-MODEL structure homology modeling server (Biasini et al., 2014, Nucl. Acids Res., 42:W252-8), using AAV8 crystal structure (PDB 2QA0) as a template. AAV2 (PDB 1LP3), AAV8 (PDB 2QA0) and Anc80 VP3 structures were further superimposed and color-coded according to residue conservation, using the UCSF Chimera package (Pettersen et al., 2004, J. Comp. Chem., 25:1605-12). A structural alignment of Anc80, AAV2 and AAV8 VP3 was then generated and completed by a non-structural alignment of the VP1/2 domains of these three serotypes, generated with the T-coffee alignment package (Notredame et al., 2000, J. Mol. Biol., 302:205-17). The spatial distribution of the mutations separating Anc80L65 and AAV8 was also visualized at the inner and outer surface of AAV8 trimer structure, where the variable residues in the structural alignment of Anc80L65 and AAV8 VP3 were represented in blue, and polymorphic residues in red.

Example 14-*In vitro* Characterization of AAV Ancestral Lineage Vectors

[0104] To identify and characterize functional AAV capsids within the Anc80Lib, individual clones from the subcloned DNA library were isolated and used to produce luciferase-containing

vectors in either 6-well or 96-well with AAP2 provided in *trans*. Crude vector was isolated by filtering cell lysate through a 0.4 µm filter after 48 hours had elapsed since transfection. Next, equal volumes of this crude vector preparation were added to 96-well plates confluent with HEK293 cells which were evaluated for their luciferase activity an additional 48 hours later. In total, 776 clones were evaluated. Crude preparations of vector containing a CMV driven luciferase were produced by triple transfection in a 6-well format, supplementing AAP in *trans* to ancestral AAV vectors. In total, three different independent biologic replicates were produced per vector. DNaseI resistant transgenes were quantified as above. These crude preparations of virus were each then evaluated for their ability to transduce HEK293 cells in technical triplicates at an MOI of 1.9×10^3 GC/cell with the exception of Anc126, which was added at MOIs between 2.1×10^2 and 3.5×10^2 GC/cell. After 48 hours had elapsed, the transduced cells were assessed for luciferase via luminescence assay.

Example 15-AAV Vector Preparation

[0105] Large-scale polyethylenimine (PEI) transfections of AAV *cis*, AAV *trans*, and adenovirus helper plasmid were performed in a 10-layer hyperflask (Corning) with near confluent monolayers of HEK 293 cells. Plasmids were transfected at a ratio of 2:1:1 (260 µg of adenovirus helper plasmid / 130 µg of *cis* plasmid / 130 µg of *trans* plasmid). Transfections for production of Anc vectors were supplemented with pAAP2 in equivalent amounts as the AAV *cis* plasmid. PEI Max (Polysciences, Warrington, PA) / DNA ratio was maintained at 1.375:1 (w/w). The transfection and downstream purification process were performed as previously described (Lock et al., 2010, Hum. Gene Ther., 21:1259-71). DNaseI-resistant vector genomes copies were used to titrate AAV preparations by TaqMan qPCR amplification (Applied Biosystems 7500, Life Technologies) with primers and probes detecting promoter, transgene, or poly-adenylation signal coding regions of the transgene cassette. The purity of the large-scale preparations was evaluated by SDS-PAGE gel electrophoresis.

Example 16-Structural and Biophysical Vector Characterization

[0106] Anc80L65 particle morphology was assessed by transmission electron microscopy loading 5 µL of a purified preparation of Anc80L65 vector onto formvar-coated 400-mesh copper grids and staining with uranyl acetate. Empty / Full particle ratios were determined through analytical ultracentrifugation. The content of a 500 µL of 10-30 µg/mL, glycerol-free Anc80L65 sample was analyzed using the Beckman Coulter ProteomeLab XL-1 analytical ultracentrifuge available at the MIT biophysical facility. The experiment was conducted at 20°C, 15,000 rpm, using an eight-hole (50 Ti) rotor. Sedimentation profiles were acquired at regular time points by refractive index optical measurements. The Lamm equation was solved using the software SEDFIT (Schuck et al., 2002, Biophys. J., 82:1096-111), and a sedimentation coefficient distribution analysis was run to identify the different species contained in the AAV sample. The thermal stability of Anc80L065 was evaluated by UV fluorescence spectroscopy

and Differential Scanning Fluorescence (DSF). For tryptophan fluorescence (Ausar et al., 2006, J. Biol. Chem., 281:19478-88) each serotype, six 4.5 μ L aliquots were prepared in 200 μ L Eppendorf tubes, incubated for 5 min at 30°C, 45°C, 60°C, 75°C, 90°C or 99°C, spun down, cooled down at room temperature for 5 min and loaded in duplicates (2 μ L each) onto a Take 3™ Micro Volume Plate (Bio-Tek). Samples were irradiated at 293 nm and emission spectra were acquired from 323 to 400 nm with a resolution of 1 nm, using a Synergy HI Hybrid Plate Reader (Bio-Tek). Sample and blank emission spectra were further smoothed using a moving average filter (span: 15). After background subtraction, the maximum emission wavelength was determined for each serotype and for each temperature condition. These wavelength values were subsequently plotted as a function of the temperature to derive the thermal stability profiles of the different AAV serotypes. For differential scanning fluorescence (Rayaprolu et al., 2013, J. Virol., 87:13150-60), 25 μ L of each AAV was supplemented with 5X SYPRO® Orange (Life Technologies) were loaded into a 96-well PCR plate (Denville Scientific Inc.) and spun down for 2 min at 2000 rpm, exposed to a temperature gradient (30-99°C, 0.1°C/6 s) while monitoring the fluorescence of the SYPRO® Orange dye, using a Replex 2S MasterCycler Real-Time PCR machine (Eppendorf) (excitation: 450 nm; emission: 550 nm). In each assay, 25 μ L FFB (21-031, Corning) and 25 μ L of a 0.25 mg/mL lysozyme solution (Sigma-Aldrich), both supplemented with 5X SYPRO® Orange, were used as negative and positive controls, respectively. The fluorescence of 25 μ L AAV vectors was also monitored in the absence of the dye for fluorescence background subtraction. Fluorescence intensity was further normalized between 0 and 100% and plotted as a function of the temperature.

Example 17-Murine Experiments

[0107] C57BL/6 male mice (6-8 weeks old) were purchased from Charles River Laboratories (Wilmington, MA) and kept at the Schepens Eye Research Institute (SERI) Animal Facility. All animal procedures were performed in accordance with protocols approved by the Institute of Animal Care and Use Committees at SERI. For liver-targeted gene transfer studies received 100 μ L single intraperitoneal injection or a single retro-orbital sinus vein injection in 150 μ L. For muscle-targeted eGFP experiments, 50 μ L was injected into the rear-right gastrocnemius. GoldenRod animal lancets (MEDpoint, Inc.) were used for submandibular mouse bleed. Brown capped tubes (Micro tube 1.1 ml Z-Gel, Sarstedt) were used for serum collections. Vector biodistribution studies were performed on tissues including liver, heart, kidney, lung, and spleen from mice sacrificed at 28 dpi of vector administration. To visualize eGFP expression in liver, tissues were fixed overnight in 4% Para-formaldehyde (PFA), washed in phosphate-buffered saline PBS for 30 min, sequentially incubated in 10%, 20% and 30% sucrose gradients and frozen in O.C.T compound (Sakura Finetek USA, Torrance, CA). Mouse liver expression of lacZ was measured using β -Gal Staining kit (Life Technologies). 4% Para-formaldehyde fixed liver tissue was sectioned at 10 μ m. Tissue sections were washed with PBS to remove residual fixative and stained at 37°C using commercial staining solutions (400 mM Potassium ferricyanide, 400 mM Potassium ferrocyanide, 200 mM magnesium chloride, X-gal 95-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) for 0.5-2 h. Cryosections were prepared

at 10 μm . To visualize eGFP expression in muscle, tissues were mounted on cork disks holding 10% Gum Tragacanth (Sigma-Aldrich Cat. No. G1128) and flash frozen using liquid nitrogen -150 c cooled Isopentane (Sigma-Aldrich 27,034-2). Muscle cryosections were prepared at 10 μm . Subretinal injections were performed with a volume of 2 μl and absolute dose per animal of 2×10^9 GC. Each vector was injected in a total of 4 eyes per serotype analysed. Animals were euthanized at 4 weeks post injection and eyes were collected for histological analysis. Enucleated eyes were fixed in 4% paraformaldehyde (PFA) for 1 hour on ice and then embedded in OCT and frozen prior to cryosectioning. Retinal sections were stained with DAPI (1 $\mu\text{g/ml}$) for 10 minutes and slides mounted for confocal imaging.

Example 18-Non-Human Primates Models

[0108] Experiments with rhesus monkeys were performed at New England Primate Research Center (Harvard Medical School). All experimental procedures were approved by the Office for Research Subject Protection, Harvard Medical Area (HMA) Standing Committee on Animals, the Harvard Medical School Institutional Animal Care and Use Committee. Animals were sedated with ketamine or telazol in combination with dexdomitor. Viral vectors expressing a secreted rhCG were administered intravenously in a 20 ml volume at a rate of 1 ml / min. After recovering from the injection, the animals were monitored clinically for general wellbeing and followed for 2 months. During this time, phlebotomies were performed at regular intervals to evaluate immune response to AAV and toxicity. After 70 days monkeys were euthanized, and liver samples were harvested.

Example 19-Quantification of Human Alpha1-Antitrypsin (hA1AT)

[0109] The expression level of hA1AT in the serum samples was quantified using ELISA. Plates were coated with primary coating rabbit anti-A1AT antibody (Sigma) at 1000 ng/well and incubated at 4°C overnight. Plates were washed and blocked for 2 hours. Serum samples were diluted five-fold and incubated at 4°C overnight. HRP-conjugated goat anti-human A1AT antibody (Abeam) was incubated for 2 hours. ABTS peroxidase substrate was added; OD_{405nm} values were measured using a spectrophotometer plate-reader within 1 hour.

Example 20-Tissue Biodistribution

[0110] Snap frozen tissue was proteinase K digested and genomic DNA (gDNA) was extracted using Blood & Cell Culture DNA Mini kit (Qiagen) as indicated. Isolated gDNA was quantified using the BioTek plate reading spectrophotometer (Biotek Instruments, Inc. Winooski, VT). Viral genome (vg) distribution in diploid cells were detected and quantified by QPCR using Applied Biosystems® 7500 Real-Time PCR Systems with TaqMan® PCR master mix reagents

(Applied Biosystems®) and transgene-specific primer/probes as previously described (Wang et al., 2010, Mol. Ther., 18:118-25).

Example 21-mRNA Expression

[0111] Total RNA was isolated using Qiagen RNeasy mini kit (Qiagen). Total RNA (1 µg) was DNase treated and reverse-transcribed into cDNA using Qiagen QuantiTect Reverse Transcription Kit (Qiagen). Real-time mRNA expression was detected and quantified using Applied Biosystems® 7500 Real-Time PCR Systems with TaqMan® PCR master mix reagents with specific primer/probe reaction mixtures; GAPDH (Rh02621745_g1), rhesus Chorionic Gonadotropin (Rb02821983_g1). TaqMan custom primer / probe suggested reaction conditions were applied.

Example 22-Neutralizing Antibody Assay

[0112] NABs were assessed *in vitro* as previously described (Calcedo et al., 2009, J. Infect. Dis., 199:381-90) with the following modifications. Serum from rabbits pre-immunized with AAV1, AAV2, AAV5, AAV6, AAV8, AAV9, rh.10 and rh32.33 (a kind gift from Dr. Roberto Calcedo and James M. Wilson, UPenn) (Gao et al., 2004, J. Virol., 78:6381-88) was serially diluted 1:40 to 1:20,971,520 and incubated with 10^9 GC particles of either matching serotype or Anc80L65 carrying a CMV.*luciferase2*.SVPA transgenic construct for 1 h at 37°C. The mixture was then added to HEK-293 cells on a 96-well plate infected with MOI (multiplicity of infection) = 20 of human adenovirus 5 (hAd5) 24 h prior. The cells were incubated for 48 h after which D-luciferin containing buffer was added and luminescence was measured using Synergy H1 microplate reader (BioTek; Winooski, VT). Luminescence was normalized against control cells infected with AAV incubated without serum. A neutralizing titer was determined at the dilution at which luminescence was <50% compared with control wells.

Example 23-*In silico* Ancestral Sequence Reconstruction of AAV Capsid Protein

[0113] In lieu of attempting to isolate an intact ancestral viral sequence from proviral DNA or archeological samples, contemporary AAV sequence data was integrated through phylogenetic analysis and maximum-likelihood ASR in order to infer the putative ancestral amino-acid sequence for the AAV Cap. A total of 75 sequences AAV serotype isolates and variants from previous biomining efforts (Gao et al., 2003, PNAS USA, 100:6081-6; Gao et al., 2004, J. Virol., 78:6381-8; Gao et al., 2005, Current Gene Ther., 5:285-97) led to a robust AAV Cap phylogeny generated with PHYML (Guindon et al., 2010, System. Biol., 59:307-21) with AAV5 as an outlier. Only full length AAV capsids were included in this analysis that were (a) naturally occurring in primate populations, (b) previously demonstrated to assemble and infect

efficiently, and (c) not known to have arisen through recombination events in its natural history, as traditional phylogenetic analysis and ASR do not account for horizontal evolutionary events. The dendrogram in Figure 18 models the evolutionary path of AAV with early speciation of AAV4, and 5 serotypes, parallel to a single node, named Anc80, from which most known contemporary AAVs evolved. These serotypes include AAV1, 2, 8 and 9, currently under clinical development in gene therapy trials. Nodes in this phylogeny were named Anc and numbered sequentially. To validate the approach described herein of ASR on AAV, Anc80 was chosen as a node to develop into a recombinant virus for possible use as a gene therapy vector.

[0114] Anc80 was chosen in part because this reconstruction of this node was highly informed by the abundance of naturally occurring AAV clinically relevant descendants from this evolutionary intermediate. Furthermore, Anc80 is embedded in the phylogeny of the *Dependoparvoviridae* with known helper-dependent primate AAVs that arose prior to Anc80's speciation (Figure 18) making it more likely that the ancestrally reconstructed particle retains the basic properties shared within this family. Using maximum-likelihood methods, a protein sequence prediction was derived for Anc80 based on calculated posterior probabilities for each residue in a particular position. In order to account for the uncertainty in selecting the appropriate amino-acid in each position, the aim was to generate all possible sequence permutations for positions with individual amino-acid posterior probabilities with $p \geq 0.3$. A representation of this library, Anc80Lib, is illustrated in Figure 19A in a part-structural alignment with an AAV2 and AAV8 reference capsid sequence. Practically, this led to a probabilistic sequence space as illustrated in Figure 18: for all but 11 of the 736 Anc80 capsid amino-acid positions a unique residue prevailed in ASR, while for those 11 positions 2 amino-acid options were provided, resulting in a sequence space encompassing $2^{11}=2048$ permutations.

[0115] Structural and sequence alignment of Anc80Lib with extant AAVs and their X-ray crystallography data highlight significant divergence from currently known circulating AAV. The closest homologue as determined via BLAST search is rh.10, a rhesus macaque isolate within Clade E of the primate *Dependoparvoviridae*, which differs from Anc80Lib by minimally 8.0% which accounts for 59 divergent amino-acid positions (Figure 19B). AAV8 and AAV2 differ 9.5% and 12.0%, respectively and those 70-87 variable sites are spread over the entire VP1 protein, including the VP1, 2 unique domains (Figure 19A, 19B). Divergence is highest in the hypervariable domains I, IV, VII, and VIII, both in terms of sequence as well as based on structural modeling of Anc80Lib clones in overlay with AAV2 and 8 monomeric structures (Figure 19A, 19C). Mapping of the variable Anc80 residues onto trimeric X-ray crystallography models of AAV2 and AAV8 in Figure 19D highlight most changes to occur on peak and flanks of the protrusions around the 3-fold axis of symmetry on the external surface of the virion. However, a significant number of variable residues were also noted on the surface exposed domains outside of the 3-fold axis in addition to a smaller number of variations on the internal surface of the particle and on regions of Cap that are not resolved in the X ray structures.

Example 24-Anc80 Synthesis and Basic Characterization

[0116] Anc80Lib protein sequences were subsequently reverse translated and generated by gene synthesis in pooled library format. Capsid genes were cloned into an AAV packaging plasmid encoding AAV2 Rep into pAnc80Lib following which the library was deconvoluted clonally. Individual clones (named pAnc80LX, with X a consecutive number) were evaluated in isolation to avoid potentially interfering competitive interactions in a minimally divergent library population. A portion of individual Anc80 clones were Sanger sequenced verifying integrity and complexity requirements. Clonal Anc80 plasmids were co-transfected with a Δ F6 adenoviral helper plasmid, an expression construct for AAP derived from AAV2 (AAP2), and ITR flanked expression construct encoding luciferase. A total of 776 library clones were produced and inoculated at equal volume of producer cell lysate on HEK293 cells in a semi-high-throughput assay aiming to assess combined particle assembly and transduction efficiency. Approximately 50% of the Anc80 clones led to detectable signal over background in this rudimentary screening assay. Several lead candidates with highest luciferase signal progressed to sequencing confirmation and titration for DNase resistant genome particles (GC) and infectivity on HEK293 cells. Based on these results, Anc80L65, the 65th Anc80Lib clone that was evaluated, was selected for further characterization. Anc80L65 vector yields from cell lysate are between 82-167% of AAV2 yields, yet were depressed compared to the high yielding AAV8 (3-5% relative AAV8 yields). *In vitro* infectivity on HEK293 is inferior to AAV2, however, superior to AAV8 on a particle per cell basis.

[0117] Anc80L65 vector preparations were produced and purified on an iodixanol gradient at scale following traditional protocols and subjected to a variety of biochemical, biophysical, and structural analyses. Particles within a purified preparation of Anc80L65 were visualized under negative staining by electron microscopy (EM) (Figure 20A). Anc80L65 virions present as relatively uniform hexagonally shaped particles with a diameter of approximately 20-25 nm, not unlike other AAV capsomers. Denatured particles resolved under SDS electrophoresis into 3 bands of 60, 72, and 90 kDa, in an approximate ratio of 1:1:10 corresponding to the VP 1-3 proteins from AAV2 and AAV8 particles (Figure 20B). Analytical ultracentrifugation (AUC) allowed the determination of the sedimentation coefficient of genome containing or full Anc80L65 at 88.9 S, slightly increased from AAV8's (85.9 S) (Figure 20C). This analysis permitted further determination of the relative abundance of empty or lower density assembled particles, presumed to be lacking a vector genome, as well as overall purity. One concern was that inaccurate modeling of the ancestral capsid sequence may have resulted in a structure deficient in its ability to package genomes and would result in a skewed empty versus : full ratio in Anc80L65 preparations. Results indicated approximately 16% empty versus 85% full particles in the preparation, in line with observations with AAV8 (Figure 20C). Additionally, it was hypothesized that particle stability may be reduced due to suboptimal modeling of the ancestral capsid composition, and subjected the particle to heat stability assays which determined, against the indicated expectations, that Anc80L65 to be 15-30°C more heat stable than its presumed AAV2 and AAV8 (Figure 20D).

Example 25-*In vivo* Gene Transfer and Transduction of Anc80L65 in Murine Model

[0118] Next, the ability of Anc80L65-packaged transgenes to be delivered and expressed was evaluated from 3 clinically relevant target tissues and routes of administration (ROA) in the C57B1/6 mouse: (a) liver following a systemic injection, (b) skeletal muscle following direct intramuscular injection, and (c) a subretinal injection for outer retina targeting. Large scale preparations of Anc80L65 were produced alongside with AAV2 and AAV8 controls with reporter genes and were injected at equal doses for liver, muscle and retina directed gene transfer in adult male C57B1/6 mice. Expression, presented in Figure 21, was monitored qualitatively (eGFP and/or LacZ) for all three target tissues and quantitatively via serum ELISA measurement of the secreted hAIAT (liver) at various time points. Liver-directed gene transfer was observed to be robust via two routes of administration and transgenes (Figure 21A, 21B, 21C). Analogously to AAV8, hepatocytes were targeted efficiently as observed by LacZ and GFP staining surpassing the limited permissively described for AAV2 (Nakai et al., 2005, J. Virol., 79:214-24; Nakai et al., 2002, J. Virol., 76:11343-9). Quantitatively, Anc80 demonstrated similar efficiency of transduction to AAV8 by intracellular reporter and a secreted serum protein transgene product. Dose ranging studies demonstrated a linearity of gene transfer with dose above 10^{10} GC/mouse but a threshold below which linearity was not maintained for hAIAT (and less obvious by eGFP) (Figure 21B, 21C). A bio-distribution study at the high dose of 5×10^{11} GC/mouse was conducted at day 7 and 28 post-injection to evaluate tissue distribution of vector genomes in liver, heart, spleen, kidney, and lung of Anc80L65, alongside AAV8 as a control (Table 3). Results show similar ranges of gene transfer of Anc80 to AAV8 in the tissues tested, with moderate increases for Anc80L65 in spleen, heart, and lung. Via direct skeletal intramuscular injection, Anc80 efficiently targeted myofibers proximal to the injection site and longitudinally extending across the fiber (Figure 21A and Figure 24). Retinal transduction after subretinal injection is efficient in targeting the retina pigment epithelium (RPE), as was the case in AAV2 and AAV8 as previously noted. Photoreceptor targeting, a more difficult cell target, as is documented for AAV2, was observed with AAV8 and Anc80L65. While both AAV8 and Anc80L65 targeted the majority of photoreceptor cells, transduction with Anc80L65 leads consistently to higher expression levels per cell. A limited number of cells in the inner retina were also observed to be GFP positive by Anc80L65 transduction (Figure 21A).

Table 3. Vector Genome Distribution in Mouse Liver, Heart, Spleen, Kidney, and Lung

	7 dpi		28 dpi	
	AAV8	Anc80L65	AAV8	Anc80L65
Liver	31.04 ± 7.04	24.19 ± 0.51	8.59 ± 3.1	8.47 ± 1.35
Lung	0.77 ± 0.07	2.2 ± 0.46	0.16 ± 0.04	1.32 ± 0.78
Kidney	0.63 ± 0.06	1.2 ± 0.16	0.22 ± 0.06	0.86 ± 0.26
Heart	0.17 ± 0.06	0.53 ± 0.04	0.1 ± 0.04	0.7 ± 0.32
Spleen	0.02 ± 0	0.19 ± 0.12	0.02 ± 0.01	0.21 ± 0.15

Example 26-Anc80L65 Gene Transfer and Expression in Non-Human Primate Liver

[0119] Given the robust hepatotropism of Anc80L65 in mice, it was an aim to evaluate gene transfer of Anc80L65 in a large animal model. Six female rhesus macaques that were enrolled in prior studies unrelated to AAV were injected via saphenous vein with either AAV8 or Anc80L65 at a clinically relevant dose of 10^{12} GC/kg (Table 4). A rhCG reporter was used to express the rhesus cDNA for the β subunit of the chorionic gonadotropin, a transgene product that the animals are tolerize for in order to avoid a non-self transgene immune response. Animals enrolled in this experiment were prescreened for NAB to AAV8 and Anc80L65. NAB serum levels weeks prior to injection were below 1/4 titer to be enrolled in this study. Gene transfer was assessed by Taqman qPCR for vg of total liver DNA (caudal lobe) 70-71 days following injection (Figure 21D). Surprisingly, 2 out of 3 control AAV8 injected animal had underwhelming gene transfer (<0.1 vg/dg) likely due to low level NAB at the time of injection undetectable by standard NAB assays as reported in previous studies. One AAV8 animal, presumably with no or minimal NAB to AAV8, demonstrated gene transfer levels for liver within the expected range of 0.81 vg/dg. Anc80L65 gene transfer apparently was unhindered by NAB (no Anc80L65 NAB detected pre-injection) and the 3 animals yielded hepatic transgene copy numbers ranging from 0.73 - 3.56 vg/dg. Liver expression was monitored via quantitative RT-PCR (Figure 21E): Anc80L65 gave rise to expression superior to the AAV8, and achieved rhCG transcript levels between 13-26% of total GAPDH mRNA amounts in all liver lobes.

Table 4. Characteristic and Previous Clinical History of Rhesus Macaques Treated with Viral Vectors Injected Via Saphenous Vein

Animal ID	Age	Sex	Weight (kg)	Experiment (days)	Previous History	Treatment
AP19	13.5	F	7.8	71	Inoculated with MVA-HIV vaccine; in 2011, diagnosed with early endometriosis	IV Anc80
AP18	9.5	F	7.2	71	Inoculated with CMV; received anti-CD4 antibody	IV Anc80
AP17	19.5	F	8.3	71	Inoculated with MVA-HIV vaccine	IV Anc80
AP16	15.5	F	6.3	70	Inoculated with MVA-HIV vaccine	IV AAV8
AP15	5	F	5	70	Inoculated with CMV; received anti-CD4 antibody	IV AAV8
AP14	5.5	F	5.2	70	Inoculated with CMV; recent weight loss	IV AAV8

Example 27-Safety, Immunology, and Toxicology of Anc80L65

[0120] The consideration to use any efficient gene delivery vector system for therapeutic application requires extensive evaluation of its safety for clinical use. In addition, the use of a

novel agent which may approximate an ancestral state of a *Dependoparvovirus* may further raise those concerns. Here, in a non-formal preclinical setting, several important aspects were examined that may limit Anc80L65 from a safety perspective. Animal expression studies (Figure 21) were monitored for obvious signs of toxicity during the in-life phase of the study and, to the extent possible, for target tissue-specific toxicity. No notable adversity was found to be associated with the vector injection. Briefly, vector administration following intraperitoneal (maximum dose tested [mdt]: 3.9×10^{10} GC/mouse), retro-orbital vein injection (mdt: 5×10^{11} GC/mouse), subretinal (mbt: 2×10^9 GC/eye), intravitreal (mbt: 2×10^9 GC/eye), and direct intramuscular (10^{10} GC/mouse) were not observed to have overt toxicity. A more direct assessment was performed in a high dose intravenous injection of 5×10^{11} GC/mouse (approximately 2×10^{13} GC/kg) of Anc80L65.TBG.eGFP alongside the following controls: (a) AAV8 with the same transgene cassette, and (b) an equal volume saline injection. Mice were phlebotomized pre-injection, 2 h, 1 d, 3 d, 7 d, 14 d, and 28 d post injection and blood was analyzed for Cell Blood Counts (CBC) and Serum Chemistry (Chem) (Tables 5 and 6), which were within range or comparable to controls for Anc80L65, and therefore, raised no significant concerns. Serum from the 2 h, 24 h, 3 d, and 7 d time points were further evaluated for cytokines as a measure of innate immune response to the vector antigens by multiplex 23 cytokine analysis (Table 7). Cytokines for Anc80L65 were overall concordant with those for saline and AAV8 control serum, and no major cytokine elevations or decreases were observed, however in some instances were moderately outside the ranges set by the saline control values in a manner that was more apparent for Anc80L65 than AAV8. Similar analyses were performed on the blood from the rhesus studies described in Figure 21D and 21E. Analogous to the mouse studies, from CBC and Chem values obtained, signs of toxicity related to the AAV8 or Anc80L65 test article were not identified (Tables 8 and 9).

[0121] Pre-existing immunity to AAV serotypes is known to block gene transfer, and may put the patient at risk for adversity due to recall of memory T-cells toward vector antigens shared with the naturally occurring wild type virus involved in the primary infection. High titer rabbit antiserum raised against AAV serotypes 1, 2, 5, 6.2, 8, 9, and rh.32.33 was used. rh.10 also was included, as its sequence is most closely homologous to Anc80L65, differing in 8.0% of residues. In Figure 22A, sera were tested for their ability to neutralize Anc80L65 versus the homologous vector capsid it was raised against. Results demonstrated no cross-reactivity to AAV5 and rh32.33, structurally highly divergent AAVs, while AAV2, 6.2, and 8, presumed descendants of Anc80L65, demonstrated low level cross-reactivity, albeit at levels that were 16-fold or lower than homologous anti-serum titers. Among Anc80 lineage members, no cross-reactivity was observed above the limit of sensitivity for AAV9 and rh.10. Next, it was an aim to validate these results in an *in vivo* model for neutralization by pre-immunizing animals for AAV8 via intramuscular route, and assessing the neutralization of Anc80L65 following intravenous injection in comparison to AAV8, 25 days following the immunization (Figure 22B). Neutralization was complete for AAV8 in the AAV8 pre-immunized animals. Anc80L65 was neutralized in 215 animals, yet demonstrated between 60-117% of transduction in 3/5 animals, notwithstanding demonstrated AAV8 NAB in those animals. These results demonstrate partial cross-reactivity of Anc80L65 with AAV8 in rabbit and mouse.

Example 28-AAV Lineage Analysis and Reconstruction

[0122] Strengthened by the successful synthesis of Anc80L65 based on ASR and its demonstration as producible, stable, and highly infectious agent for gene therapy, it was an aim to provide additional validation of the approach and modeling methodology by reconstructing the lineage of AAV further. The ambition with generating this additional set of reagents was to provide structural intermediates of Anc80 and extant AAVs that would enable empirical evaluation of the structure-function relationship within this viral family and highlight important epistatic couplings informative to future AAV rational design approaches. A total of 8 additional evolutionary intermediates of AAV were reconstructed by ASR and synthesized in the laboratory (Figure 18): Anc81, Anc82, Anc83, Anc84, And 10, and And 13 were resolved in the branching leading toward AAV7, 8, and/or 9, while Anc126 and Anc127 are positioned in the natural history of AAV1, 2, and/or 3. For each of these, the sequence was determined by selecting the amino acid with the highest posterior probability per position. First, GC viral vector yields were determined in a HEK293 standard triple transfection of vector components and adenoviral help using Taqman qPCR for vector genomes. Results, shown in Figure 23A, demonstrate increased productivity from Anc80 as the putative ancestor in the AAV7-9 lineage, in line with the higher production yields of those serotypes such as AAV8. The AAV1-3 branch did not present yield increases, and a very poor particle yield was observed for Anc126. It is possible that Anc126 yields can be improved upon through leveraging the statistical space, as was the case for Anc80, however, it is equally likely that Anc126 ASR is less informed due to undersampling of this branch of the AAV phylogeny. Infectivity of the produced particles at equal particle doses was further tested *in vitro* on HEK293 by GFP and luciferase. All newly synthesized Anc vectors demonstrated infectivity, however, at varying degrees (Figure 23B). In the AAV7-9 lineage, infectious titers were overall depressed and more similar to the AAV8 phenotype than that of Anc80. Anc127, the only intermediate in the Anc80 to AAV2 lineage that could be tested at equal dose demonstrated declined transduction efficiency as compared to both Anc80 and AAV2. The heat stability profile of selected evolutionary intermediates in both branches of this lineage was further tested (Figure 23C). Interestingly, Anc81 and Anc82 demonstrated high, yet moderately decreased melting temperature in a thermostability assay compared to Anc80L65, suggesting maybe a gradual reduction of thermostability with evolutionary age in this branch. In contrast, Anc127 demonstrated an even further increase from the already highly thermostable Anc80L65 vector.

[0123] Lastly, the ability of ASR to disrupt known epitopes to AAV2 was explored. Only few B or T-cell epitopes have been mapped on AAV2 to date, all of which were mapped onto Anc80L65, Anc126, Anc127, and AAV2, representing the AAV2 lineage. The introduction of the sequential mutations between these putative evolutionary intermediates highlights, in Figure 22C, the overlap between the mutations and 2/4 human T-cell epitopes and 2/2 mouse B-cell epitopes. These data highlight the potential of ASR to be used as a method to eliminate or modulate antigenic regions onto the AAV capsid, and may suggest immunity was a major selective pressure in the natural history of AAV.

Example 29-*In vivo* Functionality of And 110

[0124] Experiments were performed to evaluate liver transduction of luciferase by Anc80, Anc81, Anc82, and And 10 compared to AAV9 in C57B1/6 mice. The results in Figure 27 demonstrate that, following intravenous injection of the indicated vector, And 10 demonstrated equivalent levels of liver targeting as AAV9 in C57B1/6 mice based on transgene expression of the luciferase reporter gene. Notably, AAV9 is currently in clinical studies.

Table 5 Complete Blood Count Values for Mice Injected with AAV9 and Anc80/15. The values outside the reference range were highlighted in red (above) and yellow (below).

Sex	Test Name	Control 2h		AAV9 2h		AAV9Anc80/15 2h		Control 7 days		AAV9 7 days		AAV9Anc80/15 7 days		Control 14 days		AAV9 14 days		AAV9Anc80/15 14 days		Control 28 days		AAV9 28 days		AAV9Anc80/15 28 days		Test Lines		Ref Range Low		Ref Range High	
		WBC	RBC	HGB	HCT	PLT	MCV	WBC	RBC	HGB	HCT	PLT	MCV	WBC	RBC	HGB	HCT	PLT	MCV	WBC	RBC	HGB	HCT	PLT	MCV	WBC	RBC	HGB	HCT	PLT	MCV
♂	WBC	4.1	5.1	1.1	1.1	1.1	1.1	8.1	5.1	5.9	7.1	8.1	7.1	7.1	8.1	7.1	7.1	7.1	7.1	6.1	5.1	5.1	5.1	5.1	10.3/μl	1.1	1.1	1.1	1.1	1.1	
♂	RBC	3.1	3.1	0.1	0.1	0.1	0.1	7.1	4.1	4.1	4.1	4.1	4.1	6.1	6.1	6.1	6.1	6.1	6.1	5.1	4.1	4.1	4.1	4.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	HGB	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	10.3/μl	0.1	0.1	0.1	0.1	0.1
♂	HCT	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	10.3/μl	0.1	0.1	0.1	0.1	0.1
♂	PLT	7.1	7.1	7.1	7.1	7.1	7.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	MCV	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	RDW	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	RDW	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	10.3/μl	0.1	0.1	0.1	0.1	0.1	
♂	PLT	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1													

Table 6 Serum Biochemistry Values for Mice Injected with AAV6 and AAV6/AnSOL6S. The values outside the reference range were highlighted in red (above) and yellow (below).

Species	Test Name	control 2h		AAV2/AnSOL6S 2h		control 24h		AAV2/AnSOL6S 24h		control 72h		AAV2/AnSOL6S 72h		control 7 days		AAV2/AnSOL6S 7 days		control 14 days		AAV2/AnSOL6S 14 days		control 28 days		AAV2/AnSOL6S 28 days		Test Units		Ref Range Low		Ref Range High		
		Mouse	Phosphorus	Mouse	ALP [U/L]	Mouse	Total Bilirubin	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	Mouse	ALP [U/L]	
Mouse	Phosphorus	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8	8.0	0.8		
Mouse	ALP [U/L]	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	
Mouse	Total Bilirubin	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	0.8	0.5	0.4	
Mouse	ALP	130	114	137	130	102	127	103	94	83	104	95	90	69	95	101	75	76	93	75	76	93	75	76	93	75	76	93	75	76	93	
Mouse	Albumin	2.8	2.4	2.6	2.8	2.6	2.5	2.8	2.7	2.6	2.6	2.7	2.9	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	
Mouse	IGF	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	
Mouse	Creatinine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
Mouse	BUN	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	24.5	21.4	23.7	
Mouse	Cholesterol	103	71	64	103	71	64	103	71	64	103	71	64	103	71	64	103	71	64	103	71	64	103	71	64	103	71	64	103	71	64	103
Mouse	Total Protein	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	5.4	5.1	5.7	
Mouse	Glucose	171	212	225	171	212	184	193	150	180	143	171	169	176	167	161	157	150	138	157	150	138	157	150	138	157	150	138	157	150	138	
Mouse	Calcium	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3	9.9	8.3	8.3
Mouse	Hemolysis	Mod	Slight	Slight	Slight	Mod	Slight	Slight	Mod	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight

Hemolysis may cause interference with the following results: false increase in Tbil, TP, false decrease in ALP.

* Not enough serum to do test

Blank below detection limit

were highlighted in green.

r	24 hr	3 day	7 day
208	271	271	271
247	200.5	253	202
280	218	212	249
140	158.5	188	111
222	188	218.5	109
130	130	175	130
283	234	154	112
287	259	283	154
283	286	198	175
785	765	845	785
84.5	88	23.5	75.5
127	125	125	91.5
962	824	824	267.5
183	183.5	194	183
205	13.5	218	153
223	259	259	223
310	75	284	174.5
783	794	111.5	180
823	136	154	108
33.5	539	543	453.5
73.5	160	380.5	115
804	804	804	679
60.5	220	215	187

The values outside the reference range were highlighted in red (above) and

IL1s, reference values 40-58pg									
Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final		
53.23	56.42	55.28	51.31	31.85	55.71	48.64	46.83		
79.98	72.31	67.16	56.11	58.25	61.49	52.2	59.89		
24.43	25.22	57.87	51.93	73.83	67.31	57.03	58.38		
	56.42	58.45	58.41	76.3	59	57.49	45.03		
58.32	57.44	57.53	57.43	57.84	54.19	52.23	51.77		
72.68	75.38	73.48	72.38	60.34	64.01	59.89	55.86		

IL2s, reference values 1-5.4pg									
Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final		
2.53	1.81	1.32	3.34	3.32	2.23	3.1	3.9		
0.77	0.63	3.26	3.64	2.06	2.62	1.7	2.57		
3.62	8.23	2.52	3.21	1.86	2.01	2.16	1.5		
2.04	7.3	1.07	3.05	2.29	2.3	1.63	1.18		
1.15	4.31	2.85	2.43	1.88	1.47	1.23	3.26		
1.77	1.94	0.37	1.52	1.22	0.79	3.84	2.39		

reference values 11.7-14.7 p/dl									
Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final		
13.2	13.8	13.2	13.8	13.3	13.4	12.7	12.8		
12.2	12.8	11.8	12.1	12.4	12.2	13.6	13		
14.8	18.4	13.9	13.1	14.3	13.4	13.3	11		
13.1	13.7	12.4	12.2	13.8	13.4	13.3	13		
13.3	13.7	12.8	11.8	12.2	13.9	13.2	12		
11.3	12.5	11	12.1	12.4	11.3	12.2	12		

reference values 69-79.4									
Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final		
74.1	73.8	74.9	74.2	73.9	75.2	75.1			
67.7	67.6	67.3	68.7	67.5	68.6	67.7	66		
68.3	68.6	68.3	68.5	69.6	69.7	70	70		
71.4	71.8	70.7	70.7	71	70.2	71.1	71		
72.8	72.3	72.7	73.2	72.7	73.6	73	72		
67.3	67.6	68.1	67	67.6	67.5	67.3	66		

reference values 8.9-16.1									
Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final		
14.1	13.9	12.8	12.2	13.6	13.1	12.2	12		
9.6	10.9	9.7	9.7	9.2	9	8.2	10		
8.3	8.3	8.9	9.9	9.7	9.7	8	11		
9.3	10.4	10.1	10.1	10.4	9.3	9.6	11		
13.7	15.4	15.1	11.6	9.7	12.4	11.3	11		
10.3	11.1	10.7	10.2	8.9	9.3	9	10		

Table 7. Levels of Serum Cytokines Measured at Different Timepoints in Mice Injected with Saline, AAV8 and Anc80165. The values within the saline reference range

Cytokines (control)									
2 hr	24 hr	3 day	7 day	2 hr	24 hr	3 day	7 day		
IL-1alpha	215.5	226	135	176.5	255	227	248	Cytokines (AAV8)	
IL-1beta	244	213	227.5	222	252	233	256	IL-1alpha	
IL-2	152	73	143	100	265	138	216.5	IL-1beta	
IL-3	119	142	115	124	161	191	133	IL-2	
IL-4	158	218	289	200.5	257	306	253.5	IL-3	
IL-5	98	126	99.5	107	133.5	184	122.5	IL-4	
IL-6	228.5	146	124.5	130.5	268	274	241	IL-5	
IL-9	239	264.5	277	225	278	200.5	175.8	IL-6	
IL-10	175	234.5	135	156	288	283	243	IL-9	
IL-12p40	764	707	671	641	772	794	776	IL-10	
IL-12p70	280	331.5	204	271.5	380	385	258	IL-12p40	
IL-13	97.5	117	109	102.5	144.5	130	126	IL-12p70	
IL-17A	813	729	605.5	660	1033	931	1678	IL-13	
Rotaxin	171.5	193	177	178	210	135	173	IL-17A	
G-CSF	339	186	171	303	338	163.5	131	Rotaxin	
GM-CSF	263	244	272	236	284.5	234.5	207	G-CSF	
IFN-gamma	271	278	220	248	334	340	254	GM-CSF	
KC	594	293	288	243.5	335	304	199	IFN-gamma	
MCP-1	123	148	120.5	115	175	131	117.5	KC	
MP-1alpha	511.5	531.5	527	504	555	533.5	506	MCP-1	
MP-1beta	121	144.5	129.5	126	196	123	113.1	MP-1alpha	
RANTES	576.5	653	505.5	531	600	690	731.5	MP-1beta	
TNF-alpha	193	211	189	128	304	304	357	RANTES	
								TNF-alpha	

Table 3 Complete Blood Count Values for Non-Human Primates Injected with AAV6 and AAV6L35.

WBC reference values 3.4-11.2 K/ μ l									
	Baseline	1 day	3 day	7 day	15 day	30 day	50 Day	Final	
AP19	5.44	7.78	5.02	5.52	10.9	5.14	5.72	5.86	Neutrophils
AP18	5.92	7.22	5.4	4.02	7.06	6.8	7.86	7.14	
AP17	8.04	8.04	5.76	6.36	3.32	7.65	3.45	9.38	
AP16	6.36	5.64	5	8.3	4.96	4.9	4.50	6.76	
AP15	5.52	6.48	6.6	5.94	6.6	7.37	9.7	7.47	Monocytes
AP14	7.86	10.94	8.32	8.76	7.62	9.05	14.8	8.2	
AP13									
AP12									
AP11									Hgb ref
AP10									
AP9									
AP8									
AP7									MCV ref
AP6									
AP5									
AP4									
AP3									MPV ref
AP2									
AP1									
AP0									

Table 9 Serum Biochemistry Values for Non-Human Primates injected with AA-V₂ and Aves01.55. The values outside the reference range were highlighted in red (above) and yellow (below).

AST reference values 0.38 U/L												
Baseline	1 day	3 day	7 day	15 day	30 day	45 day	60 day	75 day	90 day	105 day	120 day	135 day
AP00	40	42	42	37	38	40	41	42	43	44	45	46
AP01	39	41	41	36	37	39	40	41	42	43	44	45
AP02	38	40	40	35	36	38	39	40	41	42	43	44
AP03	37	39	39	34	35	37	38	39	40	41	42	43
AP04	36	38	38	33	34	36	37	38	39	40	41	42
AP05	35	37	37	32	33	35	36	37	38	39	40	41
AP06	34	36	36	31	32	34	35	36	37	38	39	40
AP07	33	35	35	30	31	33	34	35	36	37	38	39
AP08	32	34	34	29	30	32	33	34	35	36	37	38
AP09	31	33	33	28	29	31	32	33	34	35	36	37
AP10	30	32	32	27	28	30	31	32	33	34	35	36
AP11	29	31	31	26	27	29	30	31	32	33	34	35
AP12	28	30	30	25	26	28	29	30	31	32	33	34
AP13	27	29	29	24	25	27	28	29	30	31	32	33
AP14	26	28	28	23	24	26	27	28	29	30	31	32
AP15	25	27	27	22	23	25	26	27	28	29	30	31
AP16	24	26	26	21	22	24	25	26	27	28	29	30
AP17	23	25	25	20	21	23	24	25	26	27	28	29
AP18	22	24	24	19	20	22	23	24	25	26	27	28
AP19	21	23	23	18	19	21	22	23	24	25	26	27
AP20	20	22	22	17	18	20	21	22	23	24	25	26
AP21	19	21	21	16	17	19	20	21	22	23	24	25
AP22	18	20	20	15	16	18	19	20	21	22	23	24
AP23	17	19	19	14	15	17	18	19	20	21	22	23
AP24	16	18	18	13	14	16	17	18	19	20	21	22
AP25	15	17	17	12	13	15	16	17	18	19	20	21
AP26	14	16	16	11	12	14	15	16	17	18	19	20
AP27	13	15	15	10	11	13	14	15	16	17	18	19
AP28	12	14	14	9	10	12	13	14	15	16	17	18
AP29	11	13	13	8	9	11	12	13	14	15	16	17
AP30	10	12	12	7	8	10	11	12	13	14	15	16
AP31	9	11	11	6	7	9	10	11	12	13	14	15
AP32	8	10	10	5	6	8	9	10	11	12	13	14
AP33	7	9	9	4	5	7	8	9	10	11	12	13
AP34	6	8	8	3	4	6	7	8	9	10	11	12
AP35	5	7	7	2	3	5	6	7	8	9	10	11
AP36	4	6	6	1	2	4	5	6	7	8	9	10
AP37	3	5	5	0	1	3	4	5	6	7	8	9
AP38	2	4	4	0	0	2	3	4	5	6	7	8
AP39	1	3	3	0	0	1	2	3	4	5	6	7

Sequence Listing**[0125]****SEQ ID NO:1: Anc80 VP1 polypeptide**

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNFYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQOPAXIKRLNFGQTGDSESVDPDQPLGEP
PAAPSGVGSNTMX2AGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNN
HLYKQISSQSGX3STNDNTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFRPKX4LNFKL
FNIQVKEVTINDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLT
LNNGSQAVGRSSFYCLEYFPSQMLRTGNNF5FSYTFEDVPFHSSYAHSSQSLDRLMNPLIDQYL
YYLSRTQTTSGTAGNRX6LQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTX7NQNNNSNFAWTG
ATKYHLNGRDSLVNPGPAMATHKDEDEKFFPMGVLIFGKQGAGNSNVDLDNVMITX8EEEIKT
TNPVATEX9YGT VATNLQ SX10NTAPATGTVNSQ GALPGMVWQX11RDVYLQGP IWAKI PHTDG
HFHPSPLMGGFGLKHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKEN
SKRWNP EIQYTSNYNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

X1 = K/R; X2 = A/S; X3 = A/G; X4 = R/K; X5 = E/Q; X6 = T/E; X7 = A/T; X8 = S/N; X9 = Q/E; X10
= S/A; X11 = N/D

SEQ ID NO:2: Anc80 VP1 DNA

ATGGCTGCCGATGGTTATCTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTGCGGAGT
GGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCG
GGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCC
GTCAACCGCGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCCTACGACCAGCAGCTCAAAGCGG
GTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
CTCGGTCTGGTTGAGGAAGCGCTAAGACGGCTCCTGGAAGAAGAGACCGGTAGAGCAATCAC
CCAGGAACCCAGACTCCTCTTCGGGCATCGGCAAGAAAGGCCAGCAGCCCCGCGXXX1AAGAGAC
TCAACTTTGGGCAGACAGGCGACTCAGAGTCAGTGCCCGACCCCTCAACCACTCGGAGAACCCCC
CGCAGCCCCCTCTGGTGCTGGGATCTAATAACAATGXXX2GCAGGCGGTGGCGCTCCAATGGCAGA
CAATAACGAAGGCGCGGAGTGGGTAACGCCCTCAGGAAATTGGCATTGCGATTCCACATGG
CTGGGGCAGACAGATCATCACCACCAGCACCCGAACCTGGGGCCCTCCCCACCTACAACAACCACC
TCTACAAGCAAATCTCCAGCCAATCGGGAXXX3AGCACCAACGACAACACCTACTTTCGGCTACA
GCACCCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCA
GGGACTCATCAACAACAACCTGGGGATTCCGGCCCCAAGXXX4CTCAACTTCAAGCTCTTCAACAT
CCAGGTCAAGGAGGTACGACGAATGATGGCACCACGACCATCGCCAATAACCTTACCAGCACG
GTTTCAGGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTCTCTCGGCTCTGCGCACCAGGGCT
GCCTGCCTCCGTTCGCCGGCGGACGTCTTCATGATTTCCTCAGTACGGGTACCTGACTCTGAACAA
TGGCAGTCAGGCCGTGGGCCGTTCCTCCTTCTACTGCCTGGAGTACTTTCCTTCTCAAATGCTG
AGAACGGGCAACAACCTTTXXX5TTCAGCTACACGTTTGAGGACGTGCCTTTTCACAGCAGCTAC
GCGCACAGCCAAAGCCTGGACCGGCTGATGAACCCCTCATCGACCAGTACCTGTACTACCTGT
CTCGGACTCAGACCACGAGTGGTACCGCAGGAAATCGGXXX6TTGCAATTTTCTCAGGCCGGGC
CTAGTAGCATGGCGAATCAGGCCAAAACTGGCTACCCGGGCCCTGCTACCGGCAGCAACGCGT
CTCCAAGACAXXX7AATCAAAAATAACAACAGCAACTTTGCCTGGACCGGTGCCACCAAGTATCA
TCTGAATGGCAGAGACTCTCTGGTAAATCCCGGTCCCGCTATGGCAACCCACAAGGACGACGAA
GACAAATTTTTTCCGATGAGCGGAGTCTTAATATTTGGGAAACAGGGAGCTGGAAATAGCAACG
TGGACCTTGACAACGTTATGATAACCCXXX8GAGGAAGAAATTAACACCACCAACCCAGTGGCCA

CAGAAXXX9TACGGCACGGTGGCCACTAACCTGCAATCGXXX10AACACCGCTCCTGCTACAGG
GACCGTCAACAGTCAAGGAGCCTTACCTGGCATGGTCTGGCAGXXX11CGGGACGTGTACCTGC
AGGGTCTTATCTGGGCCAAGATTCTCACACGGACGGACACTTTCATCCCTCGCCGCTGATGGG
AGGCTTTGGACTGAAACACCCGCCTCCTCAGATCCTGATTAGAATACACCTGTTCCCGCGAAT

CCTCCAACTACCTTCAGTCCAGCTAAGTTTGGCTCGTTTCATCACGCAGTACAGCACCGGACAGG
TCAGCGTGGAAATTGAATGGGAGCTGCAGAAAGAAAACAGCAAACGCTGCAACCCAGAGATTCA
ATACACTTCCAACATAACAAATCTACAAATGTGGACTTTGCTGTGACACAAATGGCGTTTAT
TCTGAGCCTCGCCCCATCGGCACCCGTTACCTCACCCGTAATCTG

XXX1 = AAG/AAA; XXX2 = GCA/AGC; XXX3 = GCA/GGC; XXX4 = AGA/AAG;

XXX5 = GAG/CAG; XXX6 = ACG/GAG; XXX7 = GCG/ACC; XXX8 = AGT/AAC;

XXX9 = CAG/GAG; XXX10 = TCA/GCC; XXX11 = AAC/GAC

SEQ ID NO:3: Anc81 VP1 polypeptide

MAADGYLPDWLEDNLSEGTREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEF
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGNLGRAVFAQKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSX1GIGKKQQPAX2KRLNFGQTGDSESVDPDQPLGE
PPAAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVITSTRTWALPTYNN
HLYKQISX3X4QSGGSTNDNTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKX5LNF
KLFINIQVKEVTTNDGTTTIANNLTSVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGY
LTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNGFX6FSYTFEDVPFHSSYAHSSQLDRIMNPLIDQ
YLYYLSRTQTTGGTAGNX7X8LQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTTNQNNNSNFAW
TGATKYHLNGRDSLVPNPGVAMATHKDEDEDRFFPSGVLIFGKQGAGNX9NVDX10X11NVMITX
12EEEIKTTNPVATEEYGX13VATNLQSX14NTAPQTGTVNSQGALPGMVWQNRDVLQGPiWA
KIPHTDGNFHPSPLMGGFGLKHPPQILIKNTVPANPPTTFX15PAKFASFITQYSTGQVSVE
IEWELQKENSkrwnPEIQYTSNynKSTNVDFAVDTEGVYSEPRPIGTRYLTRNL

X1=T/S; X2=K/R; X3=N/S; X4=S/H; X5=R/K; X6=E/Q; X7=R/Q; X8=T/E;

X9=D/S; X10=L/Y; X11=D/S; X12=S/N; X13=V/I; X14=A/S; X15=S/T

SEQ ID NO:4: Anc81 VP1 DNA

ATGGCTGCCGATGGTTATCTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTTCGCGAGT
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GGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCCTCAACGGACTCGACAAGGGGGAGCCC
GTCAACCGCGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCCTACGACCAGCAGCTCAAAGCGG
GTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
CTCGGTCTGGTTGAGGAAGCGCTAAGACGGCTCCTGGAAGAAGAGACCGGTAGAGCAATCAC
CCAGGAACCCAGACTCCTCTXXX1GGCATCGGCAAGAAAGGCCAGCAGCCCGCGXXX2AAGAGA
CTCAACTTTGGGCAGACTGGCGACTCAGAGTCAGTGCCCCGACCCTCAACCACTCGGAGAACCCC
CCGACGCCCCCTCTGGTGTGGGATCTAATAACAATGGCTGCAGGCGGTGGCGCTCCAATGGCAGA
CAATAACGAAGGCCCGACCGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCACATGG
CTGGGGCAGAGAGTCATCAACCACGACCCGAACCTGGGCCCTCCCCACCTACAACAACCAACC
TCTACAAGCAAATCTCCXXX3XXX4CAATCGGGAGGAAGCACCACGACAACACCTACTTCGGC
TACAGCACCCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGACT
GGCAGCGACTCATCAACAACAACCTGGGGATTTCGGCCCCAAGXXX5CTCAACTTCAAGCTCTTCA
ACATCCAGGTCAAGGAGGTACGACGAATGATGGCACCACGACCATCGCCAATAACCTTACCAG

CACGGTTCAGGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTCTCTGGGCTCTGCGCACCAG
GGCTGCCTGCCTCCGTTCCCGGCGGACGTCTTCATGATTCCCTCAGTACGGGTACCTGACTCTGA
ACAATGGCAGTCAGGCCGTGGGCCGTTCTCTCTACTGCCTGGAGTACTTTCTTCTCAAAT
GCTGAGAACGGCAACAACCTTTXXX6TTCAGCTACACGTTTGAGGACGTGCCTTTTCACAGCAG
CTACGCGCACAGCCAAAGCCTGGACCGGCTGATGAACCCCTCATCGACCAGTACCTGTACTAC
CTGTCTCGGACTCAGACCAAGGGAGGTACCGCAGGAAATXXX7XXX8TTGCAATTTTCTCAGGC
CGGGCCTAGTAGCATGGCGAATCAGGCCAAAACCTGGCTACCCGGGGCCCTGCTACCGGCAGCAA
CGCGTCTCCAAGACAACGAATCAAAATAACAACAGCAACTTTGCCTGGACCGGTGCCACCAAGT
ATCATCTGAATGGCAGAGACTCTCTGGTAAATCCCGGTGTGCTATGGCAACCCACAAGGACGA
CGAAGACCGATTTTTTCCGTCCAGCGGAGTCTTAATATTTGGGAAACAGGGAGCTGGAAATXXX
9AAGCTGGACXXX10XXX11AACGTTATGATAACCXXX12GAGGAAGAAATTAAAACCAAC

CCAGTGGCCACAGAAGAGTACGGCXXX13GTGGCCACTAACCTGCAATCGXXX14AACACCGCT
 CCTCAAACAGGGACCGTCAACAGTCAAGGAGCCTTACCTGGCATGGTCTGGCAGAACCGGGACG
 TGTACCTGCAGGGTCTTATCTGGGCCAAGATTCTCACACGGACGGAACCTTTCATCCCTCGCC
 GCTGATGGGAGGCTTTGGACTGAAACACCCGCCTCCTCAGATCCTGATTAAGAATACACCTGTT
 CCCGCGAATCCTCCAACCTACCTTCXXX15CCAGCTAAGTTTGCCTCGTTCATCACGCAGTACAG
 CACCGGACAGGTGAGCGTGGAATTTGAATGGGAGCTGCAGAAAGAAAACAGCAAACGCTGGAAC
 CCAGAGATTCAATACACTTCCAACCTACAACAAATCTACAAATGTGGACTTTGCTGTTGACACAG
 AAGGCGTTTATTCTGAGCCTCGCCCCATCGGCACCCGTTACCTCACCCGTAATCTG

XXX1 = ACG/AGC; XXX2 = AAA/AAG; XXX3 = AAC/AGT; XXX4 = AGC/CAC;

XXX5 = A.GA/AAG; XXX6 = GAG/CAG; XXX7 = CGG/CAG; XXX8 = ACG/GAG;

XXX9 = GAC/AGC; XXX10 = CTT/TAC; XXX11 = GAC/AGC; XXX12 =

AGT/AAC; XXX13 = GTG/ATC; XXX14 = GCA/AGC; XXX15 = AGT/ACC

SEQ ID NO:5: Anc82 VP1 polypeptide

MAADGYLPDWLEDNLSEGTREWWDLKFGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEF
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 LGLVEEAKTAPGKKRPVEQSPQREPDSX1GIGKKQQPAX2KRLNFGQTGDSESVDPQPLG
 EPPAAPSGVGSNTMAAGGCAPMADNNEGADGVGNSSGNWHCDSTWLGDRIITSTRTWALPTYN
 NHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKL
 FNIQVKEVTTNEGTKTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLT
 LNNGSQAVGRSSFYCLEYFPPSQMLRTGNNFQFSYTFEDVPEHSS'YAHSQSLDRLMNPLIDQYLY
 YLSRTQTTGGTAGTQTLQF'SQAGPSSMANQAKNWLPGPCYRQQRVSTTTNQNNSNFAWTGATK
 YHLNGRDSLVPNGVAMATHKDDDEDRFFPSSGVLI FGKQGAGNDNDVYSNVMITX3EEEIKTTNP
 VATEEYGVVATNLQSANTAPQTGTVNSQGALPGMVWQNRDVYLGQPIWAKIPHTDGNFHPSPLM
 GGFGLKHPPQILIKNTVPVADPPTTFNQAKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEI
 QYTSNYKSTNVDFAVNTEGVYSEPRPIGTRYLTRLN

X1=T/S; X2=K/R; X3=S/N

SEQ ID NO:6: Anc82 VP1 DNA

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTGCGGAGT
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 GGTCTGGTGCTTCTGGCTACAAGTACCTCGGACCTTCAACGGACTCGACAAGGGGGAGCCC
 GTCAACGCGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGG
 GTGACAATCCGTACCTGCGGTATAATCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA

TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
 CTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCAGTCAC
 CACAGCGTGAGCCCGACTCCTCCXXX1GGCATCGGCAAGAAAGGCCAGCAGCCCGCCXXX2AAG
 AGACTCAATTTTCGGTCAGACTGGCGACTCAGAGTCAGTCCCCGACCCTCAACCTCTCGGAGAAC
 CTCCAGCAGCGCCCTCTGGTGTTGGGATCTAATAACAATAGGCTGCAGGCGGTGGCGACCAATGGC
 AGACAATAACGAAGGTGCCGAGGTGGGTAAATTCCTCGGGAAATTTGGCATTGCGATTCCACA
 TGGCTGGGCGACAGAGTCATCACCAACAGCACCCGAACCTGGGCCCTGCCACCTACAACAACC
 ACCTCTACAAGCAAATCTCCAACGGGACCTCGGGAGGCAGCACCAACGACAACACCTACTTTGG
 CTACAGCACCCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTCTCACCAAGTAC
 TGGCAGCGACTCATCAACAACAACCTGGGGATTCCGGCCCAAGAGACTCAACTTCAAGCTCTTCA
 ACATCCAGGTCAAAGAGGTACGACGAATGAAGGCACCAAGACCATCGCCAATAACCTCACCAG
 CACCGTCCAGGTGTTTACGGACTCGGAATACCAGCTGCCGTACGTCTCGGCTCTGCCACACAG
 GGCTGCCTGCCTCCGTTCCCGGCGGACGTCTTCATGATTCTCAGTACGGCTACCTGACTCTCA
 ACAACGGTAGTCAGGCCGTGGGACGTTCTCTTCTACTGCCTGGAGTACTTCCCCTCTCAGAT
 GCTGAGAACGGGAACAACCTTCAATTCAGCTACACTTTTCGAGGACGTGCCTTTCCACAGCAGC
 TACGCGCACAGCCAGAGTTTGGACAGGCTGATGAATCTCTCATCGACCAGTACCTGTACTACC
 TGTCAGAACCAGACTACGGGAGGCACAGCGGGAACCCAGACGTTGCAGTTTCTCAGGCCGG
 GCCTAGCAGCATGGCGAATCAGGCCAAAACTGGCTGCCTGGACCTGCTACAGACAGCAGCGC
 GTCTCCACGACAACGAATCAAAAACAACAACAGCAACTTTGCCCTGGACTGGTGCCACCAAGTATC

ATCTGAACGGCAGAGACTCTCTGGTGAATCCGGGCGTCGCCATGGCAACCCACAAGGACGACGA
 GGACCGCTTCTTCCCATCCAGCGGCGTCTCATATTTGGCAAGCAGGGAGCTGGAAATGACAAC
 GTGGACTATAGCAACGTGATGATAACCXXX3GAGGAAGAAATCAAGACCACCAACCCCGTGGCC
 ACAGAAGAGTATGGCGTGGTGGCTACTAACCTACAGTCGGCAAAACACCGCTCCTCAAACGGGGA
 CCGTCAACAGCCAGGGAGCCTTACCTGGCATGGTCTGGCAGAACCGGGACGTGTACCTGCAGGG
 TCCTATTTGGGCCAAGATTCTCACAACAGATGGCAACTTTCACCCGCTCTCCTTTAATGGGCGGC
 TTTGGACTTAAACATCCGCCTCCTCAGATCCTCATCAAAAACACTCCTGTTCCTGCGGATCCTC
 CAACAACGTTCAACCAGGCCAAGCTGAATTCTTTTCATCACGCAGTACAGCACCGGACAAGTCAG
 CGTGGAGATCGAGTGGGAGCTGCAGAAGGAGAACAGCAAGCGCTGGAACCCAGAGATTAGTAT
 ACTTCCAACACTACAAATCTACAAATGTGGACTTTGCTGTAAATACTGAGGGTGTTTACTCTG
 AGCCTCGCCCCATTGGCACTCGTTACCTCACCCGTAATCTG
 XXX1 = ACG/AGC; XXX2 = AAA/AGA; XXX-3 = AGC/AAC

SEQ ID NO:7: Anc83 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWWDLPKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEF
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 LGLVEEGAKTAPGKKRPVEQSPQREPDSX1GIGKKGQQPAX2KRLNFGQTGDSESVDPDQPLG
 EPPAAPSGVGSNTMAAGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTTSTRTWALPTYN
 NHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRHFCHFSRWDQRLINNNWGFPRKRLX3FK
 LFNIQVKEVTQNEGKTIANNLSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYL
 TLNNGSQAVGRSSFYCLEYFPSQMLRTGNNEFX4FSYTFEDVPHSSYAHSSQLDRLMNPIDQY
 LYYLSRTQTIGGTAGTQTLQFSQAGPSX5MANQAKNWLPGPCYRQQRVSTTTTQNNNSNFAWTG
 ATKYHLNGRDSLVPNGVAMATHKDDX7RFFPSSGX7LIFGKQGAGKDNVDYSNVMLTSEEEIK
 TTNPVATEEYGVVADNLQQQNIAPQX8GTVNSQGALPGMVWQNRDVYLQGPWAKIPHTDGNFH
 PSPLMGGFGLKHPPFPQILIKNTPVPADPPTTFNQAKLNSFITQYSTGQVSVEIEWELQKENSKR
 WNPEIQYTSNYYKSTNVDFAVNTEGVYSEPRPIGTRYLTRNL

X1=T/S; X2=R/K; X3=N/S; X4=Q/E; X5=N/T/S; X6=D/E; X7=I/V; X8=I/V

SEQ ID NO:8: Anc83 VP1 DNA

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTCGCGAGT
 GGTGGGACCTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAGCAGGACGACGGCCG
 GGGTCTGGTGCTTCTCTGGCTACAAGTACCTCGGACCTTCAACGGACTCGACAAGGGGGAGCCC
 GTCAACGCGGGGACGACGCGGCCCTCGAGCAGACAAGGCCCTACGACCAGCAGCTCAAAGCGG
 GTGACAATCCGTACCTGCGGTATAATCAGCGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
 TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTCTCTGAACCT
 CTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCAGTCAC
 CACAGCGTGAGCCCGACTCCTCCXXX1GGCATCGGCAAGAAAGGCCAGCAGCCCGCCXXX2AAG
 AGACTCAATTTTCGGTCAGACTGGCGACTCAGAGTCAGTCCCCGACCTCAACCTCTCGGAGAAC
 CTCCAGCAGCGCCCTCTGGTGTGGGATCTAATACAATGGCTGCAGGCGGTGGCGCACCAATGGC
 AGACAATAACGAAGGTGCCGACGGAGTGGGTAGTTTCTCGGGAAATTGGCATTGCGATTCCACA
 TGGCTGGGCGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCACCTACAACAACC
 ACCTCTACAAGCAAATCTCCAACGGGACCTCGGGAGGCAGCACCACGACAACACCTACTTTGG
 CTACAGCACCCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGAC
 TGGCAGCGACTCATCAACAACAACCTGGGGATTCCGGCCCAAGAGACTCXXX3TTCAAGCTCTTC
 AACATCCAGGTCAAAGAGGTACGCGAGAATGAAGGCACCAAGACCATCGCCAATAACCTCACCA
 GCACCATCCAGGTGTTTACGGACTCGGAATACCAGCTGCCGTACGTCTCGGCTCTGCCACCA
 GGGCTGCCTGCCTCCGTTCCTGGCGGACGTCTTCATGATTCTCAGTACGGCTACCTGACTCTC
 AACACGGTAGTACAGCCGTGGGACGTTCCTCTTCTACTGCCTGGAGTACTTCCCTCTCAGA
 TGCTGAGAACGGGCAACAACCTTTXXX4TTTCAGCTACACTTTGAGGACGTGCCTTTCCACAGCA
 GCTACGCGCACAGCCAGAGTTTGGACAGGCTGATGAATCCTCTCATCGACCAGTACCTGTACTA
 CCTGTCAAGAACCAGACTACGGGAGGCACAGCGGGAACCCAGACGTTGCAGTTTCTCAGGCC
 GGGCTAGCXXX5ATGGCGAATCAGGCCAAAACCTGGCTGCCTGGACCCTGCTACAGACAGCAG
 CGGTCTCCACGACAACGTGCAAAAACAACAACAGCAACTTTGCCTGGACTGGTGCCACCAAGT
 ATCATCTGAACGGCAGAGACTCTCTGGTGAATCCGGCGCTCGCCATGGCAACCCACAAGGACGA
 CGAGXXX6CGCTTCTTCCCATCCAGCGGCXXX7CTCATATTTGCCAAGCAGGGAGCTGGAAAG
 ACAACGTGGAGATATAGCAACGTGATGCTAACACGCGAGGAAGAAATCAAGACCACCAACCCGT
 GGCCACAGAAAGATATGGCGTGGTGGCTGATAACCTACAGCAGCAAAACACCGCTCTCAAXXX
 8GGGACCGTCAACAGCCAGGAGCCTTACCTGAGCATGGTCTGGCAGAACCGGGACGTGTACCTG
 CAGGGTCTTATTTGGGCCAAGATTCTCACAACAGATGGCAACTTTCACCCGCTCTCCTTTAATGG

GCGGCTTTGGACTTAAACATCCGCTCCTCAGATCCTCATCAAAAACACTCCTGTTCTCCTGCGGA
 TCCTCCAACAACGTTCAACCAGGCCAAGCTGAATTCTTTTCATCACGCAGTACAGCACCGGACAA
 GTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAGAACAGCAAGCGCTGGAACCCAGAGATTC
 AGTATACTTCCAATACTACTACAAATCTACAAATGTGGACTTTGCTGTTAATACTGAGGGTGTTTA
 CTCTGAGCCTCGCCCCATTGGCACTCGTTACCTCACCCGTAATCTG

XXX1 = ACG/AGC; XXX2 = AGA/AAG; XXX3 = AAC/AGC; XXX4 = CAA/GAA;

XXX5 = AAC/ACC/AGC; XXX6 = GAC/GAG; XXX7 = ATC/GTC; XXX8 = ATA/GTA

SEQ ID NO:9: Anc84 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWWDLPKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEP
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 LGLVEEGAKTAPGKKRPVEPSPQRSFDSSTGIGKKGQOPAXIKRLNFGQTGDSESVDPQPIGE

PPAAPSGVSGTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRTVITSTRTWALPTYNN
 HLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRHFCHFSPRDWQRLINNNWGFPRKRLX2FKL
 FNIQVKEVTQNEGKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLT
 LNNGSQAVGRSSFYCLEYFPSQMLRTGNNEFESYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLY
 YLSRTQSTGGTAGTQQLLFSQAGPSNMSAQAKNWLPGPCYRQORVSTTLSONNNSNFAWTGATK
 YHLNGRDSLNVNPGVAMATHKDDX3RFFPSSGX4LMFGKQAGKDNVDYSNVMLTSEEEIKTTN
 PVATEQYGVVADNLQQONTAPIVGAVNSQALPGMVWQNRDVYLGPIWAKIPHTDGNFHPSP
 MGGFGLKHPPPQILIKNTVPVADPPTTFNQAKLNSFTQYSTGQVSVEIEWELQKENSKRWNPE
 IQYTSNYYKSTNVDFAVNTEGVYSEPRPIGTRYLTRNL

X1=R/K; X2=N/S; X3=D/E; X4=I/V

SEQ ID NO:10: Anc84 VP1 DNA

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 GGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCC
 GTCAACGCGGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGG
 GTGACAATCCGTACCTGCGGTATAATCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
 TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
 CTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCCGTCAC
 CACAGCGTTCCCCCGACTCCTCCACGGGCATCGGCAAGAAAGGCCAGCAGCCCGCCXXX1AAGA
 GACTCAATTTCCGTACAGACTGGCGACTCAGAGTCAGTCCCCGACCCTCAACCTATCGGAGAACC
 TCCAGCAGCGCCCTCTGGTGTGGGATCTGGTACAATGGCTGCAGGCGGTGGCGCACCAATGGCA
 GACAATAACGAAGGTGCCGACGGAGTGGGTAGTTCTCGGGAAATTTGGCATTGCGATTCCACAT
 GGCTGGGCGACAGAGTCATCACCAACAGCACCCGAACCTGGGCCCTGCCCACCTACAACAACCA
 CCTCTACAAGCAAATCTCCAACGGGACCTCGGGAGGCAGCAACCAACGACAACACCTACTTTGGC
 TACAGCACCCCTGGGGGTATTTGACTTTTAACAGATTCCAATGCCACTTCTCACCACGTGACT
 GGCAGCGACTCATCAACAACAACCTGGGGATTCCGGCCCAAGAGACTCXXX2TTCAAGCTCTTCA
 ACATCCAGGTCAAAGAGGTACGCAGAATGAAGGCACCAAGACCATCGCCAATAACCTCACCAG
 CACCATCCAGGTGTTTACGGACTCGGAATACCAGCTGCCGTACGTCTCGGCTCTGCCACCCAG
 GGCTGCCCTGCCCTCCGTTCCCGGCGGACGTCTTCATGATTCTCAGTACGGCTACCTGACTCTCA
 ACAACGGTAGTCAGGCCCGTGGGACGTTCTCTCTACTGCCCTGGAGTACTTCCCCTCTCAGAT
 GCTGAGAACGGGCAACAACCTTTGAGTTTACGTACACTTTCGAGGACGTGCCTTTCCACAGCAGC
 TACGCGCACAGCCAGAGTTTGGACAGGCTGATGAATCCTCTCATCGACCAGTACCTGTACTACC
 TGTCAAGAACCAGTCTACGGGAGGCACAGCGGGAACCCAGCAGTTGCTGTTTTCTCAGGCCGG
 GCCTCAAGAACATGTCCGGCTCAGGCCAAAACACTGGCTGCCTGGACCTGCTACAGACAGCGC
 GTCTCCACGACACTGTCCGCAAAACAACAACAGCAACTTTGCCTGGACTGGTGGCCACCAAGTATC
 ATCTGAACGGCAGAGACTCTCTGGTGAATCCGGGCGTCGCCATGGCAACCCACAAGGACGACGA
 GXXX3CGCTTCTTCCCATCCAGCGGCXXX4CTCATGTTTGGCAAGCAGGGAGCTGGAAAAGACA
 ACGTGGACTATAGCAACGTGATGCTAACCAGCGAGGAAGAAATCAAGACCACCAACCCCGTGGC
 CACAGAACAGTATGGCGTGGTGGCTGATAACCTACAGCAGCAAAACACCGCTCCTATTGTGGGG
 GCCGTCAACAGCCAGGGAGCCTTACCTGGCATGGTCTGGCAGAACCGGGACGTGTACCTGCAGG
 GTCTATTTTGGGCCAAGATTCTTCACACAGATGGCAACTTTCACCCGTCTCCTTTAATGGGCGG
 CTTTGGACTTAAACATCCGCTCCTCAGATCCTCATCAAAAACACTCCTGTTCTCCTGCGGATCCT

CCAACAACGTTCAACCAGGCCAAGCTGAATTCTTTTCATCACGCAGTACAGCACCGGACAAGTCA
GCGTGGAGATCGAGTGGGAGCTGCAGAAGGAGAACAGCAAGCGCTGGAACCCAGAGATTCAGTA

TACTTCCAATACTACTACAAATCTACAAATGTGGACTTTGCTGTTAATACTGAGGGTGTCTTACTCT
GAGCCTCGCCCCATTGGCACTCGTTACCTCACCCGTAATCTG

XXX1 = AGA/AAA; XXX2 = AAC/AGC; XXX3 = GAC/GAG; XXX4 = ATC/GTC

SEQ ID NO:11: Anc94 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEF
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEPSPQRSPTSSTGIGKKGQOPAKKRLNFGQTGDSESVDPQPIGEP
PAGPSGLSGTMAAGGGAPMADNNEGADGVSSSGNWHCDSTWLGDRVITSTRTWALPTYNNH
LYKQISNGTSGGSTNDNTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFN
IQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGLPPFPADVFMIPQYGYLTNLN
NGSQAVGRSSFYCLEYFPSQMLRTGNNFEFSYTFEDVPEHSSYAHSSQLDRIMNPLIDQYLYYL
SRTQSTGGTAGTQQLLFSQAGPXINMSAQAKNWLPGPCYRQQRVSTLSQNNNSNFANTGATKY
HLNGRDSLVNPGVAMATHKDDDEERFFPSSGVLMFGKQAGKDNVDYSSVMLTSEEEIKTTNPVA
TEQYGVVADNLQQQNTAPIVGAVNSQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPMLGG
FGLKHPPQILIKNTPVPADPPTTFSQAKLASFITQYSTGQVSVEIEWELQKENS KRWNPEIQY
TSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL

X1=S/N

SEQ ID NO:12: Anc94 VP1 DNA

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTGCGGAGT
GGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCG
GGGTCTGGTGCTTCTCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCC
GTCAACCGCGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCCTACGACCAGCAGCTCAAAGCGG
GTGACAACTCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
CTCGGTCTGGTTGAGGAAGCGCTAAGACGGCTCCTGGAAAGAAGAGACCAGGATAGCCATCAC
CCGAGCGTTCTCCAGACTCCTCTACGGGCATCGGCAAGAAAGGCCAGCAGCCCGCGAAAAAGAG
ACTCAACTTTGGGCAGACTGGCGACTCAGAGTCAGTGCCCCGACCTCAACCAATCGGAGAACCC
CCCGCAGGCCCTCTGGTCTGGGATCTGGTACAATGGCTGCAGGCGGTGGCGCTCCAATGGCAG
ACAATAACGAAGGCGCCGACGGAGTGGGTAGTTCCCTCAGGAAATTGGCATTGCGATTCCACATG
GCTGGGCGACAGAGTCATCACCAACCAGCACCCGAACCTGGGCCCTCCCCACCTACAACAACCAC
CTCTACAAGCAAATCTCCAACGGGACTTCGGGAGGAAGCACCAACGACAACACCTACTTCGGCT
ACAGCACCCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTCTCACACGCTGACTG
GCAGCGACTCATCAACAACAACCTGGGGATTCCGGCCCAAGAGACTCAACTTCAAGCTCTTCAAC
ATCCAGGTCAAGGAGGTCAAGCAGAATGAAGGCACCAAGACCATCGCCAATAACCTTACCAGCA
CGATTCAAGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTCTCGGCTCTGCGCACCAGGG
CTGCCCTGCCCTCCGTTCCCGCGGACGTCTTCATGATTCTCTCAGTACGGGTACCTGACTCTGAAC
AATGGCAGTCAGGCCGTGGGCCGTTCTCTCTTCTACTGCCTGGAGTACTTTCCTTCTCAAATGC
TGAGAACGGGCAACAACCTTTGAGTTCAGCTACACGTTTGAGGACGTGCCTTTTTCACAGCAGCTA
CGCGCACAGCCAAAGCCTGGACCGGTGATGAACCCCTCATCGACCAGTACCTGTACTACCTG
TCTCGGACTCAGTCCACGGGAGGTACCGCAGGAACCTCAGCAGTTGCTATTTTCTCAGGCCGGGC
CTYXXAACATGTGCGCTCAGGCCAAAACTGGCTACCCGGGCCCTGCTACCGGCAGCAACGCGT
CTCCACGACACTGTGCAAAATAACAACAGCAACTTTGCCTGGACCGGTGCCACCAAGTATCAT
CTGAATGGCAGAGACTCTCTGGTAAATCCCGGTGTGCTATGGCAACCCACAAGGACGACGAAG

AGCGATTTTTTCCGTCCAGCGGAGTCTTAATGTTTGGGAAACAGGGAGCTGGAAAAGACAACGT
GGACTATAGCAGCGTTATGCTAACCAAGTGAAGGAAGAAATTAAAACCAACCAACCCAGTGGCCACA
GAACAGTACGGCGTGGTGGCCGATAACCTGCAACAGCAAAACACCGCTCCTATTGTAGGGGGCCG
TCAACAGTCAAGGAGCCTTACCTGGCATGGTCTGGCAGAACCGGACGTGTACCTGCAGGGTCC
TATCTGGGCCAAGATTCTCTCACACGGACGGAAACTTTTCATCCCTCGCCGCTGATGGGAGGCTTT
GGACTGAAACACCCGCTCCTCAGATCCTGATTAGAATACACCTGTTCCCGCGGATCCTCCAA
CTACCTTCAGTCAAGCTAAGCTGGCGTCTTTCATCACGAGTACAGCACCGGACAGGTGACGCT
GGAAATTGAATGGGAGCTGCAGAAAGAAAACAGCAAAACGCTGGAACCCAGAGATTCATACACT
TCCAATACTACTACAAATCTACAAATGTGGACTTTGCTGTTAACACAGAAGGCACTTATTCTGAGC

CTCGCCCCATCGGCACCCGTTACCTCACCCGTAATCTG
XXX1 = AGT/AAT

SEQ ID NO:13: Anc113 VP1 polypeptide

MAADGYLPDWLEDNLSEGTREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFAQKKRVLEP
LGLVEEGAKTAPGKKRPVEX1SPQRSPDSSTGIGKKGQQPAX2KRLNFGQTGDSESVDPDQPLG
EPPAAPSGVSGTMAAGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYN
NHLYKQISSQSAGSTNDNTYFGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFPRPKKLX3FKL
FNIQVKEVTTNDGVTTIANNLSTVQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLT
LNNGSQSVGRSSFYCLEYFPPSOMLRTGNNEFESYTFEDVPPHSSYAHSSQSLDRIMNPLIDQYLY
YLARTQSTTGGTAGNRELQFX4QAGPSTMAEQAKNWLPGPCYRQQRVSKTLDQNNNSNFAWTGA
TKYHLNGRNSLVNPGVAMATHKDEDEDRFFPSSGVLI FGKTGAANKTTLENVLMTX5EEEIKTTN
PVATEEYGX6VS3NLQ8X7NTAPQTQTVNSQGALPGMVWQNRDVYLOQPIWAKIPHTDGNFHPS
PLMGGFGLKHPPPQILIKNTVPVNPANPPEVFTPAKFASFITQYSTGQVSVEIEWELQKENSKRWN
PEIQYTSNYDKSTNVDFAVDSEGVYSEPRPIGTRYLTRNL

X1=P/Q; X2=K/R; X3=R/N; X4=Y/H; X5=N/S; X6=V/I; X7=A/S

SEQ ID NO:14: Anc113 VP1 DNA

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTTCGCGAGT
GGTGGGACCTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCG
GGGTCTGGTGCTTCTCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCC
GTCAACCGCGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCCTACGACCAGCAGCTCAAAGCGG
GTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
TACGTCAATTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTCTCGAACCT
CTCGGTCTGGTTGAGGAAGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGXXX1TCA
CCTCAGCGTTCCCCCGACTCCTCCACGGGCATCGGCAAGAAAGGCCAGCAGCCCGCCXXX2AAG
AGACTCAATTTTCGGTCAGACTGGCGACTCAGAGTCAGTCCCCGACCCTCAACCTCTCGGAGAAC
CTCCAGCAGCGCCCTCTGGTGTGGGATCTGGTACAATGGCTGCAGGCGGTGGCGCACCAATGGC
AGACAATAACGAAGGTGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCATTCGCAATTCCACA
TGGCTGGGCGACAGAGTCATTACCACCAGCACCCGAACCTGGGCCCCGCCCACCTACAACAACC
ACCTCTACAAGCAAATCTCCAGTCAAAGTGCAGGTAGTACCAACGACAACACCTACTTTCGGCTA
CAGCACCCCCCTGGGGGTATTTTGACTTTAACAGATTCCTACTGCCACTTCTCACCACGTGACTGG
CAGCGACTCATCAACAACAACCTGGGGATTCCGGCCCCAAGAAGCTGXXX3TTCAAGCTCTTCAAC
ATCCAGGTCAAGGAGGTACGACGAATGACGGCGTTACGACCATCGCTAATAACCTTACCAGCA
CGGTTTCAGGTATTCTCGGACTCGGAATACCAGCTGCCGTACGTCTCTCGGCTCTGCGCACCCAGGG

CTGCCTGCCTCCGTTCCCGGCGGACGTCCTTCATGATTCTCAGTACGGCTACCTGACTCTCAAC
AATGGCAGTCAGTCTGTGGGACGTTCTCTCTTCTACTGCCTGGAGTACTTCCCCTCTCAGATGC
TGAGAACGGGCAACAACCTTTGAGTTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAGCTA
CGCACACAGCCAGAGCCTGGACCGGCTGATGAATCCCCCTCATCGACCAGTACTTGTACTACCTG
GCCAGAACACAGAGTACCACAGGAGGCACAGCTGGCAATCGGGAACTGCAGTTTXXX4CAGGCC
GGGCCCTTCAACTATGGCCGAACAAGCCAAGAATTGGTTACCTGGACCTTGCTACCGGCAACAAA
GAGTCTCCAAAACGCTGGATCAAAAACAACAACAGCAACTTTGCTTGGACTGGTGCCACCAAATA
TCACCTGAACGGCAGAACTCGTTGGTTAATCCCGGCGTCGCCATGGCAACTCACAAGGACGAC
GAGGACCGCTTTTTTCCCATCCAGCGGAGTCTGATTTTTGGAAAACTGGAGCAGCTAACAAAA
CTACATTGGAAAATGTGTTAATGACAXXX5GAAGAAGAAATTAATACTACTAATCCTGTAGCCA
CGGAAGAATACGGGXXX6GTCAGCAGCAACTTACAATCGXXX7AATACTGCACCCACAGACACAA
ACTGTCAACAGCCAGGGAGCCTTACCTGGCATGGTCTGGCAGAACCGGGACGTGTACCTGCAGG
GTCCCCTCTGGGCCAAGATTCCCTCACACGGATGGCAACTTTCACCCGTCTCCTTTGATGGGCGG
CTTTGGACTTAAACATCCGCCTCCTCAGATCCTGATCAAGAACACTCCCGTTCCCGCTAATCCT
CCGGAGGTGTTTACTCCTGCCAAGTTTGCTTCGTTTCATCACACAGTACAGCACCGGACAAGTCA
GCGTGGAAATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATTTCAGTA
CACCTCCAACATATGATAAGTCGACTAATGTGGACTTTGCCGTTGACAGCGAGGGTGTCTTACTCT
GAGCCTCGCCCTATTGGCACTCGTTACCTCACCCGTAATCTG

XXX1 = CCG/CAG; XXX2 = AAA/AGA; XXX3 = CGG/AAC; XXX4 = TAC/CAC;

XXX5 = AAT/AGT; XXX6 = GTA/ATA; XXX7 = GCT/TCT

SEQ ID NO:15: Anc126 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKX1GQQPAX2KRLNFGQTDSESVDPDQPLGE
PPAAPSGVGSNTMASGGGAPMADNNEGADGVGNX3SGNWHCDSTWLGDRTVITSTRTWALPTYN
NHLYKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSFRDWQRLINNNWGFPRKX4LNFKLF
NIQVKEVTTNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPFPADVFMIPQYGYLTL
NNGSQAVGRSSFYCLEYFPSQMLRTGNFX5FSYTFEDVFPFHSSYAHSSQLDRLMNLIDQYLY
YLY6RTQTTSQTAQNRELX7FSQAGPSSMX8NQAKNWLPGPCYRQQRVSKTANDNNNSNFAWTG
ATKYHLNGRDSLVNPGFAMASHKDEDEKFFPMISGLIFGKQAGASNVLDLNMVITDEEEIKTT
NPVATEQYGTATNLQSSNTAPATGTVNSQALPGMVWQDRDVYLQGPFWAKIPHTDGHFHPSP
LMGGFGLKHPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNF
EIQYTSNYNKSX9NVDFTVDTNGVYSEPRPIGTRYLTRNL

X1=S/T; X2=K/R; X3=A/S; X4=R/K; X5=T/Q; X6=S/N; X7=Q/L; X8=A/S;

X9=A/T

SEQ ID NO:16: Anc126 VP1 DNA

ATGGCTGCCGATGGTTATCTTCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTGCGGAGT
GGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCG
GGGTCTGGTGCTTCTCGGCTACAAGTACCTCGGACCTTCAACGGACTCGACAAGGGGGAGCCC
GTCAACGCGGGCGGATGCAGCGGCCCTCGAGCAGACAAGGCCCTACGACCAGCAGCTCAAAGCGG
GTGACAATCCGTACCTGCGGTATAACACGCGGACGCGGAGTTTCAGGAGCGTCTGCAAGAAGA
TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGAGGGTTCTCGAACCT
CTTGGTCTGGTTGAGGAAGGTGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCCG

CACAAGAGCCAGACTCCTCCTCGGGCATTGGCAAGXXX1GGCCAGCAGCCCGCTXXX2AAGAGA
CTCAATTTTGGTCAGACTGGCGACTCAGAGTCAGTCCCCGACCCACAACCTCTCGGAGAACCTC
CAGCAGCCCCCTCTGGTGTGGGATCTAATACAATGGCTTCAGGCGGTGGCGCACCATGGCAGA
CAATAACGAAGGCGCGGACGGAGTGGGTAATXXX3TCAGGAAATTGGCATTGCGATTCCACATG
GCTGGGCGACAGAGTCATCACCAACCAGCACCAGAACATGGGCCTTGCCCACCTATAACAACCAC
CTCTACAAGCAAATCTCCAGTCAATCAGGGGCCAGCAACGACAACCCTACTTTCGGCTACAGCA
CCCCCTGGGGGTATTTTGATTTCACAGATTCCACTGCCATTTCTCACCACGTGACTGGCAGCG
ACTCATCAACAACAATTGGGGATTCCGGGCCAAGXXX4CTCAACTTCAAGCTCTTCAACATCCA
AGTCAAGGAGGTACGACGAATGATGGCACCACGACCATCGCTAATAACCTTACCAGCAGGTT
CAAGTCTTACGGACTCGGAGTACCAGTTGCCGTACGTCTCGGCTCTGCGCACCAGGGCTGCC
TCCCTCCGTTCCCGGGCGGACGTGTTTCATGATTCCGCAGTACGGCTACCTAACGCTCAACAATGG
CAGCCAGGCAGTGGGACGGTCATCCTTTTACTGCCTGGAATATTTCCCATCGCAGATGCTGAGA
ACGGGCAATAACTTTXXX5TTCAGCTACACCTTCGAGGACGTGCCTTTCACAGCAGCTACGCG
CACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCACTACCTGTATTACCTGXXX6
AGAACTCAGACTACGTCCGGAACGCCCCAAAACAGGGAGTTGXXX7TTAGCCAGGCGGGTCCA
TCTAGCATGXXX8AATCAGGCCAAAAAAGTGGCTACCTGGACCTGTTACCGGCAGCAGCGCGTT
TCTAAAACAGCAAATGACAACAACAACAGCAACTTTGCCTGGACTGGTGTACAAAATATCACC
TTAATGGGCGTGATTCTTTAGTCAACCCTGGCCCTGCTATGGCCTCACACAAAGACGACGAAGA
CAAGTCTTTCCCATGAGCGGTGTCTTGATTTTGGAAAGCAGGGCGCCGGAGCTTCAAACGTT
GATTTGGACAATGTCATGATCACAGACGAAGAGGAAATCAAAACCACTAACCCCGTGGCCACCG
AACAATATGGGACTGTGGCAACCAATCTCCAGAGCAGCAACACAGCCCCTGCGACCGGAAGTGT
GAATTTCTCAGGGAGCCTTACCTGGAATGGTGTGGCAAGACAGAGACGTATACCTGCAGGGTCTT
ATTTGGGCCAAAATTCCTCACACGGATGGACACTTTCACCCGCTCCTCTCATGGGCGGCTTTG
GACTTAAGCACCCGCCCTCCTCAGATCCTCATCAAAAACACGCCCTGTTCTTCCGAATCCTCCGAC
AACGTTTTTCGCTGCAAAGTTTGCTTCATTCATCACCCAGTATTCACAGGACAAGTGAGCGTG
GAGATTGAATGGGAGCTGCAGAAAGAAAACAGCAAACGCTGGAATCCCGAAATACAGTATACAT
CTAATATAATAAATCTXXX9AACGTTGATTTCACTGTGGACACCAATGGAGTTTATAGTGAGC
CTCGCCCCATTGGCACCCGTTACCTCACCCGTAACCTG

XXX1 = TCA/ACA; XXX2 = AAA/AGA; XXX3 = GCC/TCC; XXX4 = AGA/AAA;

XXX5 = ACC/CAG; XXX6 = AGC/AAC; XXX7 = CAG/CTG; XXX8 = GCT/TCT;

XXX9 = GCC/ACC

SEQ ID NO:17: Anc127 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPQPKANQOHQDDX1RGLVLPGYKYLGPFGNLDKGE
PVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEPQERLQEDTSFGGNLGRAVFQAKKRVLE
PLGLVEEAAKTAPGKKRPVEQSPQEPDSSSGIGKSGQOPAX2KRLNFGQTDGSESVDPDQPLGE
PPAAPSGVGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSTWLGDRVITTTSTRTWALPTYNN
HLYKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSFRDWQRLINNNWGFPRPX3LNFKLEFN
IQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLN
NGSQAVGRSSFYCLEYFPSQMLRTGNNFX4FSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYY
LX5RTQTTSGETTQQSRLX6FSQAGPSSMX7QQAX8NWLPGPCYRQQRVSKTANDNNNSNFAWTX
9ATKYHLNGRDSLVPNPGPAMASHKDEEKFFPMHGX10LIFGKQCTGASNVLDLNVMITDEEEI
RTTNPVATEQYGTVATNLQSSNTAPATGTVNSQCALPGMVWQDRDVYLQGPWAKIPHTDGHFH
PSPLMGGFGLKHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKR
WNPEIQYTSNYSKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

X1=G/S; X2=R/K; X3=K/R; X4=T/Q; X5=S/R; X6=Q/L; X7=A/S; X8=K/R;

X9=G/A; X10=V/N

SEQ ID NO:18: Anc127 VP1 DNA

ATGGCTGCTGACGGTTATCTTCCAGATTGGCTCGAGGACAACCTTTCTGAAGGCATTCTGTGAGT
GGTGGGATCTGAAACCTGGAGCCCCCAACCCAAAGCGAACCAACACCAGGACGACXXX1C
GGGGTCTTGTGCTTCCGGGTACAAATACCTCGGACCCTTTAACGGACTCGACAAAGGAGAGCC
GGTCAACGAGGCGGACGCGGCAGCCCTCGAACACGACAAAGCTTACGACCAGCAGCTCAAGGCC
GGTGACAACCCGTACCTCAAGTACAACCACGCGGACGCGGAGTTTACGGAGCGTCTTCAAGAAG
ATACGTCTTTTGGGGGCAACCTTGGCAGAGCAGTCTTCCAGGCCAAAAAGAGGGTCTTTGAGCC
TCTTGGTCTGGTTGAGGAAGCAGCTAAAACGGCTCCTGGAAAGAAGAGGCCTGTAGAACAGTCT
CCTCAGGAACCGGACTCATCATCTGGTATTGGCAAATCGGGCCAAACAGCCTGCCXXX2AAAAGA
CTAAATTTGGTTCAGACTGGAGACTCAGAGTCAGTCCCAGACCCTCAACCTCTCGGAGAACCAC
CAGCAGCCCCCTCAGGTGTGGGATCTAATACAATGGCTTCAGGCGGTGGCGCACCAATGGCAGA
CAATAACGAGGGTGCCGATGGAGTGGGTAAATCCTCAGGAAATTGGCATTGCGATTCCACATGG
CTGGGCGACAGAGTCATCACCACCAGCACCAGAACCTGGGCCCTGCCACTTACAACAACCATC
TCTACAAGCAAATCTCCAGCCAATCAGGAGCTTCAAACGACAACCACTACTTTGGCTCAGCAGC
CCCTTGGGGGTATTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCA
CTCATTAACAACAACATTCGGGATTCGGGCCAAGXXX3CTCAACTCAAGCTCTTCAACATCCAA
GTTAAAGAGGTACAGCAGAACGATGGCAGCAGACTATTGCCAATAACCTTACCAGCAGGGTTC
AAGTGTTTACGGACTCGGAGTATCAGCTCCCGTACGTGCTCGGGTCGGCGCACCAAGGCTGTCT
CCCGCCGTTTCCAGCGGACGTCTTCATGATCCCTCAGTATGGATACCTCACCTGAACAACGGA
AGTCAAGCGGTGGGACGCTCATCCTTTTACTGCCTGGAGTACTTCCCTTCGCAGATGCTAAGGA
CTGGAAATAACTTCXXX4TTCAGCTATACCTTCGAGGATGTACCTTTTCACAGCAGCTACGCTC
ACAGCCAGAGTTTGGATCGCTTGATGAATCCTCTTATTGATCAGTATCTGTACTACCTGXXX5A
GAACGCAAACAACCTCTGGAACAACCCAAACAATCACGGCTGXXX6TTTAGCCAGGCTGGGCCTT
CGTCTATGXXX7CAGCAGGCCXXX8AATTGGCTACCTGGGCCCTGCTACCGGCAACAGAGAGTT
TCAAAGACTGCTAACGACAACAACAACAGTAACCTTTGCTTGGACAXXX9GCCACCAAATATCAT
CTCAATGGCCGCGACTCGCTGGTGAATCCAGGACCAGCTATGGCCAGTCACAAGGACGATGAAG
AAAAATTTTCCCTATGCACGGCXXX10CTAATATTTGGCAAACAAGGGACAGGGGCAAGTAAC
GTAGATTTAGATAATGTAATGATTACGGATGAAGAAGAGATTCTGACCACCAATCCTGTGGCAA
CAGAGCAGTATGGAACGTGGCAACTAATTGCAGAGCTCAAATACAGCTCCCGCGACTGGAAC
TGTCAATAGTCAGGGGGCCTTACCTGGCATGGTGTGGCAAGATCGTGACGTGTACCTTCAAGGA
CCTATCTGGGCAAAGATTCCCTCACACGGATGGACACTTTCATCCTTCTCCTCTGATGGGAGGCT
TTGGACTGAAACATCCGCCTCCTCAAATCTTGATCAAAAATACTCCGGTACCGGCAAATCCTCC
GACGACTTTTCAGCCCCGCCAAGTTTGCTTCATTTATCACTCAGTACTCCACTGGACAGGTCAGC
GTGGAAATTGAGTGGGAGCTACAGAAAGAAAACAGCAAACGTTGGAATCCAGAGATTCAGTACA

CTTCCAACACTACAACAAGTCTGTTAATGTGGACTTTACTGTAGACACTAATGGTGTTTATAGTGA
ACCTCGCCCTATTGGAACCCGGTATCTCACACGAAACTTG

XXX1 = GGT/AGT; XXX2 = AGA/AAA; XXX3 = AAA/AGA; XXX4 = ACA/CAG;

XXX5 = AGC/AGA; XXX6 = CAA/CTC; XXX7 = GCT/TCT; XXX8 = AAA/AGA;

XXX9 = GGG/GCG; XXX10 = GTT/GAC

SEQ ID NO:19: Anc80L27 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWWDLKPAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPARKRLNFGQTGDSESVDPDQPLGEP
AAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFSFRDWQRLINNNWGFPRKRLNFKLFNIQ
VKEVTTNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVEMIPOQYGYLTLNNG
SQAVGRSSFYCLEYFSPQMLRTGNNFEFSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYLSR
TQTTSGTAGNRTLQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTANQNNNSNFAWTGATKYHLN
GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITNEEEIKTTNPVATEQ
YGT VATNLQSANTAPATGTVNSQGALPGMVWQDRDVYLQGP IWAKI PHTDGHFHPSPLMGGFGL
KHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:20: Anc80L59 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWWDLKPAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPARKRLNFGQTGDSESVDPDQPLGEP
AAPSGVGSNTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
YKQISSQSGASTNDNTYFGYSTPWGYFDENRFHCHFSFRDWQRLINNNWGFPRKRLNFKLFNIQ
VKEVTTNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVEMIPOQYGYLTLNNG
SQAVGRSSFYCLEYFSPQMLRTGNNFQFSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYLSR
TQTTSGTAGNRELQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTANQNNNSNFAWTGATKYHLN
GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITNEEEIKTTNPVATEE
YGT VATNLQSANTAPATGTVNSQGALPGMVWQNRDVYLQGP IWAKI PHTDGHFHPSPLMGGFGL
KHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:21: Anc80L60 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWWDLKPAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPARKRLNFGQTGDSESVDPDQPLGEP
AAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFSFRDWQRLINNNWGFPRKRLNFKLFNIQ
VKEVTTNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVEMIPOQYGYLTLNNG
SQAVGRSSFYCLEYFSPQMLRTGNNFEFSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYLSR
TQTTSGTAGNRELQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTANQNNNSNFAWTGATKYHLN
GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITSEEEIKTTNPVATEE
YGT VATNLQSSNTAPATGTVNSQGALPGMVWQERDVYLQGP IWAKI PHTDGHFHPSPLMGGFGL
KHPPPQILIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:22: Anc80L62 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWWDLKPAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPARKRLNFGQTGDSESVDPDQPLGEP
AAPSGVGSNTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFSFRDWQRLINNNWGFPRKRLNFKLFNIQ
VKEVTTNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVEMIPOQYGYLTLNNG

VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAFAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 SQAVGRSSFYCLEYFPSQMLRTGNNFEFSYTFEDVFPFHSSYAHSSQSLDRLMNPLIDQYLYYLSR
 TQTTSGTAGNRELQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTNNNNNSNFAWTGATKYHLN
 GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITSEEEIKTTNPVATEE
 YGT VATNLQSANTAPATGTVNSQGALPGMVWQDRDVYLQGP I WAKI PHTDGHFHPSPLMGGFGL
 KHPPPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
 YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:23: Anc80L65 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAFAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPARKRLNFGQTGDSESVDPQPLGEPP
 AAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
 YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKKLNFKLFNIQ
 VKEVTTNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNG
 SQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVFPFHSSYAHSSQSLDRLMNPLIDQYLYYLSR
 TQTTSGTAGNRTLQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTNNNNNSNFAWTGATKYHLN
 GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITNEEEIKTTNPVATEE
 YGT VATNLQSANTAPATGTVNSQGALPGMVWQDRDVYLQGP I WAKI PHTDGHFHPSPLMGGFGL
 KHPPPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
 YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:24: Anc80L33 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAFAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPAKKRLNFGQTGDSESVDPQPLGEPP
 AAPSGVGSNTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
 YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKKLNFKLFNIQ
 VKEVTTNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNG
 SQAVGRSSFYCLEYFPSQMLRTGNNFEFSYTFEDVFPFHSSYAHSSQSLDRLMNPLIDQYLYYLSR
 TQTTSGTAGNRTLQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTANQNNNSNFAWTGATKYHLN
 GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITSEEEIKTTNPVATEQ
 YGT VATNLQSSNTAPATGTVNSQGALPGMVWQNRDVYLQGP I WAKI PHTDGHFHPSPLMGGFGL
 KHPPPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
 YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:25: Anc80L36 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAFAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPAKKRLNFGQTGDSESVDPQPLGEPP
 AAPSGVGSNTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
 YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGERPKKLNFKLFNIQ
 VKEVTTNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNG
 SQAVGRSSFYCLEYFPSQMLRTGNNFEFSYTFEDVFPFHSSYAHSSQSLDRLMNPLIDQYLYYLSR
 TQTTSGTAGNRTLQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTANQNNNSNFAWTGATKYHLN
 GRDSLVPNGPAMATHKDDDEKFFPMSGVLI FGKQGAGNSNVDLDNVMITSEEEIKTTNPVATEE
 YGT VATNLQSSNTAPATGTVNSQGALPGMVWQNRDVYLQGP I WAKI PHTDGHFHPSPLMGGFGL
 KHPPPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
 YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:26: Anc80L44 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEP
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAFAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKKGQQPAKKRLNFGQTGDSESVDPQPLGEPP
 AAPSGVGSNTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTTSTRTWALPTYNNHL
 YKQISSQSGGSTNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKKLNFKLFNIQ
 VKEVTTNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNG
 SQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVFPFHSSYAHSSQSLDRLMNPLIDQYLYYLSR

TQTTSGTAGNRELQFSQAGPSSMANQAKNWLPGPCYRQQRVSKTTNQNNSNFAWTGATKYHLN
GRDSLVPNGPAMATHKDEDDKFFPMSGVLI FGKQGAGNSNVDLDNVMITNEEEIKTTNPVATEQ
YGT VATNLQSANTAPATGTVNSQGALPGMVWQDRDVYLOGPIWAKIPHTDGHFHPSPLMGGFGL
KHPPPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
YNKSTNVDFAVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:27: AAV8 VP1 polypeptide (YP 077180.1)

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEP
VNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEPSPQSPDSSTGIGKKGQOPARKRLNFGQTGDSESVDPQPLGEP
PAAPSGVGPNMTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTTSTRTWALPTYNNH
LYKQISNGTSGGATNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLSKFLFN
IQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTNLN
NGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPEFHSSYAHSSQLDRLMNPLIDQYLYYL
SRTQTGTGTANTQTLGFSQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYH
LNGRNSLANPGIAMATHKDDDEERFFPSNGILIFGKQNAARDNADYSVMLTSEEEIKTTNPVAT
EEYGIVADNLQOONTAPQIGTVNSQGALPGMVWQNRDVYLOGPIWAKIPHTDGNFHPSPLMGGF
GLKHPPPQILIKNTVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
SNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO:28: AAV9 VP1 polypeptide (AAS99264.1)

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPFGNLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRLLEP
LGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVDPDPQPIGEPP
AAPSGVGS LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGD RVITTTSTRTWALPTYNNHL
YKQISNSTSGGSSNDNAYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLNFKLENI
QVKEVTDNNGVKTIANNLSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTNLND
GSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSSQLDRLMNPLIDQYLYYLS
KTINGSGQNQQT LKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALN
GRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATES
YQGVATNHQSAQAQAQTGWVQNGILPGMVWQDRDVYLOGPIWAKIPHTDGNFHPSPLMGGFGM
KHPPPQILIKNTVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN
YYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO:29: AAV6 VP1 polypeptide (AAB95450.1)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
FGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQOPAKKRLNFGQTGDSESVDPDPQPLGEPP
ATPAAVGPTTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGD RVITTTSTRTWALPTYNNHL
YKQISSASTGASNDNHYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLNFKLFNIQ
VKEVTTNDGVTTIANNLSTVQVFS DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTNLNG
SQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPEFHSSYAHSSQLDRLMNPLIDQYLYYLN
TQNGSGSAQNKD LLSRGS PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLN
GRESIINPGTAMASHKDDKDKFFPMSGVMI FGKESAGASNTALDNVMITDEEEIKATNPVATER
FGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLOGPIWAKIPHTDGHFHPSPLMGGFGL
KHPPPQILIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSN
YAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

SEQ ID NO:30: AAV1 VP1 polypeptide (NP 049542.1)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEP
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQOPAKKRLNFGQTGDSESVDPDPQPLGEPP
ATPAAVGPTTMAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGD RVITTTSTRTWALPTYNNHL
YKQISSASTGASNDNHYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLNFKLFNIQ
VKEVTTNDGVTTIANNLSTVQVFS DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTNLNG
SQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEEVPEFHSSYAHSSQLDRLMNPLIDQYLYYLN
TQNGSGSAQNKD LLSRGS PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLN
GRESIINPGTAMASHKDEDDKFFPMSGVMI FGKESAGASNTALDNVMITDEEEIKATNPVATER
FGTVAVNFQSSSTDPATGDVHAMGALPGMVWQDRDVYLOGPIWAKIPHTDGHFHPSPLMGGFGL
KHPPPQILIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSN

KNPPFQILILKNTFVPANFPALFSATKFASTFIQYISIGQVSVLEIWEELQKENS KRWNPEIQYTSN
YAKSANVDFTVDNNGLYTEPRPIGTRYLTRNL

SEQ ID NO:31: AAV2 VP1 polypeptide (YP 680426.1)

MAADGYLPDWLEDLTLSEGIQWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFGNLDKGEF
VNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEEFQERLKEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEPVKTA PGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPP
AAPSGLGNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWALPTYNNHL
YKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFPRKRLNFKLFNIQV
KEVTQNDGTTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGS
QAVGRSSFYCLEYFPSQMLRTGNNTFSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYYLSRT
NTPSGTTTQSRLLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNG
RDSL VNPGPAMASHKDDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQY
GDSVTLNQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGP IWAKI PHTDGHFHPS PLMGGFGLK
HPPFQILIKNTFVPANPSTTFSAAKFASFITQYSTGQVSVLEIWEELQKENS KRWNPEIQYTSN
NKS VNVDFTVD TNGVYSEPRPIGTRYLTRNL

SEQ ID NO:32: AAV3 VP1 polypeptide (NP 043941.1)

MAADGYLPDWLEDNLSEGIREWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPFGNLDKGEF
VNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRILEP
LGLVEEAAKTAPGKKGAVDQSPQEPDSSSGVGKSGKQ PARKRLNFGQTGDSESVDPDQPLGEPP
AAPTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWL GDRVITTTSTRTWALPTYNNHL

YKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFPRKKLSFKLFNIQV
RGVTQNDGTTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGS
QAVGRSSFYCLEYFPSQMLRTGNNTFSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYYLNRT
QGTTS GTTNQSRLLFSQAGPQSMQLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLN
GRDSL VNPGPAMASHKDDDEEKFFPMHGNLI FGKEGTTASNAELDNVMITDEEEIRTTNPVATEQ
YGTVANNLQSSNTAPTGTVNHQGALPGMVWQDRDVYLQGP IWAKI PHTDGHFHPS PLMGGFGL
KHPPFQIMIKNTFVPANPPTTFSPAKFASFITQYSTGQVSVLEIWEELQKENS KRWNPEIQYTSN
YNKSVNVDFTVD TNGVYSEPRPIGTRYLTRNL

SEQ ID NO:33: AAV3B VP1 polypeptide (3KIC A)

MAADGYLPDWLEDNLSEGIREWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPFGNLDKGEF
VNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRILEP
LGLVEEAAKTAPGKKRPVDQSPQEPDSSSGVGKSGKQ PARKRLNFGQTGDSESVDPDQPLGEPP
AAPTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWL GDRVITTTSTRTWALPTYNNHL
YKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFPRKKLSFKLFNIQV
KEVTQNDGTTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGS
QAVGRSSFYCLEYFPSQMLRTGNNTFSYTFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYYLNRT
QGTTS GTTNQSRLLFSQAGPQSMQLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLN
GRDSL VNPGPAMASHKDDDEEKFFPMHGNLI FGKEGTTASNAELDNVMITDEEEIRTTNPVATEQ
YGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGP IWAKI PHTDGHFHPS PLMGGFGL
KHPPFQIMIKNTFVPANPPTTFSPAKFASFITQYSTGQVSVLEIWEELQKENS KRWNPEIQYTSN
YNKSVNVDFTVD TNGVYSEPRPIGTRYLTRNL

SEQ ID NO:34: AAV7 VP1 polypeptide (YP 077178.1)

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDNRRGLVLPGYKYLGPFGNLDKGEF
VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
LGLVEEGAKTAPAKKRPVEPSPQRS PDSSGTIGKKGQQPARKRLNFGQTGDSESVDPDQPLGEF
PAAPSSVSGS TVAAGGAPMADNNEGADGVGNASGNWHCDSTWL GDRVITTTSTRTWALPTYNNH
LYKQISSETAGSTNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFPRKKLRFKLFNI
QVKEVTNDGVTITIANNTSTIQVFS DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNN
GSQS VGRSSFYCLEYFPSQMLRTGNNTFSYSFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYYLA
RTQSNPGGTAGNRELQFYQGGPSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYH
LNGRNSLVNPGVAMATHKDDDEDRFFPSSGVLI FGKTGATNKTTLENVMTNEEEIRPTNPVATE
EYGVSSNLQAANTAAQTQVVNNQGALPGMVWQNRDVYLQGP IWAKI PHTDGNFHPS PLMGGFG
LKHPFQILIKNTFVPANPPEVTFPAKFASFITQYSTGQVSVLEIWEELQKENS KRWNPEIQYTS
NFEKQTGVDFAVDSQGVYSEPRPIGTRYLTRNL

SEQ ID NO:35: Anc80L1 VP1

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTTCGCGAGT
 GGTGGGACTTGAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCG
 GGGTCTGGTGCTTCCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCC
 GTCAACGCGGCGGACGACGCGGCCCTCGAGCAGCACAAGGCCCTACGACCAGCAGCTCAAAGCGG
 GTGACAATCCGTACCTGCGGTATAACCACGCGGACGCGGAGTTTCAGGAGCGTCTGCAAGAAGA
 TACGTCCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
 CTCGGTCTGGTTGAGGAAGGGCGTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCAATCAC
 CCCAGGAACCAGACTCCTCTTCGGGCATCGGCAAGAAAGGCCAGCAGCCCCGCAAAAAGAGACT
 CAACTTTGGGCAGACAGGCGACTCAGAGTCAGTGGCCGACCCTCAACCACTCGGAGAACCCCCC

GCAGCCCCCTCTGGTGTGGGATCTAATACAATGGCTGCAGGCGGTGGCGCTCCAATGGCAGACA
 ATAACGAAGGCGCCGACGGAGTGGGTAAACGCCCTCAGGAAATTGGCATTGCGATTCCACATGGCT
 GGGCGACAGAGTCATCACCACCAGCACCCGAACCTGGGGCCCTCCCCACCTACAACAACCACCTC
 TACAAGCAAATCTCCAGCCAATCGGGAGCAAGCACCAACGACAACACCTACTTCGGCTACAGCA
 CCCCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCG
 ACTCATCAACAACAACCTGGGGATTCCGGCCCCAAGAGACTCAACTTCAAGCTCTTCAACATCCAG
 GTCAAGGAGGTACGACGAATGATGGCACCACGACCATCGCCAATAACCTTACCAGCAGCGTTTC
 AGGTCTTTACGGAATACCAGCTCCCGTACGTCTCGGCTCTGCGCACCAGGGCTGCCT
 GCCTCCGTTCCCGGCGGACGTCTTCATGATTCTCAGTACGGGTACCTGACTCTGAACAATGGC
 AGTCAGGCGGTGGGCGGTTCTCTCTTCTACTGCGTGGAGTACTTTCCTTCTCAAATGCTGAGAA
 CGGGCAACAACCTTGAGTTTACGCTACACGTTTGGAGACGTGCCTTTTCACAGCAGCTACGCGCA
 CAGCCAAAGCTTGGACCGGCTGATGAACCCCTCATCGACCAGTACCTGTACTACCTGTCTCGG
 ACTCAGACCACGAGTGGTACCGCAGGAAATCGGACGTTGCAATTTTCTCAGGCCGGGCGCTAGTA
 GCATGGCGAATCAGGCCAAAACTGGCTACCCGGGCCCTGCTACCGGCAGCAACGCGTCTCCAA
 GACAGCGAATCAAAATAACAACAGCAACTTTGCCTGGACCGGTGCCACCAAGTATCATCTGAAT
 GGCAGAGACTCTCTGGTAAATCCCGGTCCCGCTATGGCAACCCACAAGGACGACGAAGACAAAT
 TTTTTCCGATGAGCGGAGTCTTAATATTTGGGAAACAGGGAGCTGGAAATAGCAACGTGGACCT
 TGACAACGTTATGATAACCAGTGAGGAAGAAATTAACCAACCAACCCAGTGGCCACAGAACAG
 TACGGCACGTTGGCCACTAACCTGCAATCGTCAAACACCGCTCCTGCTACAGGGACCGTCAACA
 GTCAAGGAGCCTTACCTGGCATGGTCTGGCAGAACCAGGACGTGTACCTGCAGGGTCTTATCTG
 GGCCAAGATTCTCTACACGGACGGACACTTTCATCCCTCGCCGCTGATGGGAGGCTTTGGACTG
 AAACACCCGCTCCTCAGATCCTGATTAAGAATACACCTGTTCCCGCGAATCCTCCAACCTACCT
 TCAGTCCAGCTAAGTTTGGCTCGTTCATCACGCAGTACAGCACCGGACAGGTACAGCGTGGAAAT
 TGAATGGGAGCTGCAGAAAGAAAAACAGCAAACGCTGGAACCCAGAGATTCAATACACTTCCAAC
 TACAACAAATCTACAAATGTGGACTTTGCTGTTGACACAAATGGCGTTTATTCTGAGCCTCGCC
 CCATCGGCACCCGTTACCTCACCCTAATCTGTAA

SEQ ID NO:36: Anc80L1 VP1

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEF
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRVLEP
 LGLVEEGAKTAPGKKRPVEPSPQRS PDSSTGIGKKGQPAKKRLNFGQTGDSESVDPFPQIGEP
 PAGPSGLSGTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTTSTRTWALPTYNNH
 LYKQISNGTSGGSTNDNTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFN
 IQVKEVTQNEGTCTIANNLSTIQVFTDSEYQLPYVLGSAHQGCLPFPADVFMI PQYGYLTLN
 NGSQAVGRSSFYCLEYFPSQMLRTGNNFESYQFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYYL
 SRTQSTGGTAGTQQLLFSQAGPNMMSAQAKNWLPGPCYRQQRVSTTLNQNNNSNFAWTGATKYH
 LNGRDSLVPNGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVAT
 EQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGF
 GLKHPPPQILIKNTPVPADPPTTFSQAKLASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYT
 SNYYKSTNVDFAVNTDGTYSERPIGTRYLTRNL

SEQ ID NO:37: Anc80 VP3 polypeptide

MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQISNGTSG
 GSTNDNTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNEG
 TKTIANNLSTIQVFTDSEYQLPYVLGSAHQGCLPFPADVFMI PQYGYLTLNNGSQAVGRSSF
 YCLEYFPSQMLRTGNNFESYQFEDVFPFHSSYAHSSQLDRLMNPLIDQYLYYLSRTQSTGGTAG
 TQQLLFSQAGPNMMSAQAKNWLPGPCYRQQRVSTTLNQNNNSNFAWTGATKYHLNGRDSLVPNG

VAMATHKDDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQ
 QQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGFGLKHPPPQILI
 KNTFVPADPPTTFSQAKLASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSTNVDF
 AVNTDGTYSEPRPIGTRYLTRNL

SEQ ID NO:38: AAV2 VP3 polypeptide (GenBank Accession No. AAC03779.1)

MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMDRVITTTSTRTWALPTYNNHLYKQISSQSGA
 SNDNHYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTT
 TIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTNNNGSQAVGRSSFYC
 LEYFPSQMLRTGNNTFSYTFEDVPHSSYAHSSQLDRLMNPLIDQYLYLSRTINTPSGTTTQS
 RLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNGPA
 MASHKDDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRG
 NRQAATADVNTQGVLPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKN
 TPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTV
 DTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:39: AAV2 VP3 polypeptide

SEQ ID NO:39: AAV8 VP3 polypeptide

MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQISNGTSG
 GATNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEG
 TKTIANNLSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTNNNGSQAVGRSSF
 YCLEYFPSQMLRTGNNTFQTYTFEDVPHSSYAHSSQLDRLMNPLIDQYLYLSRTQTTGGTAN
 TQTLGFSQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPG
 IAMATHKDDDEERFFPSNGILIFGKQNAARDNADYSDVMLTSEEEIKTTNPVATEEYGVVADNLQ
 QQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGFGLKHPPPQILI
 KNTFVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSTSVDF
 AVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO:40: AAV5 VP1 polypeptide (GenBank Accession No. AAD13756.1)

MSFVDHPPDWLEEVGEGLEREFLGLLEAGPPKPKPNQHQDQARGLVLPGYNYLPGPNGLDRGEPV
 NRADEVAREHDISYNEQLEAGDNPYLKYNHADA EFQEKLADDTSCGNL GKAVFQAKKRVLEPF
 GLVEEGAKTAPTGKRIDDHFFKRRKARTEEDSKPSTSSDAEAGPSCSQQLQIPAPASSLGADT
 MSAGGGGGLGDNNQADGVGNASGDWHCDSTWMDRVVTKSTRTWVLPSSYNNH QYREIKSGSVD
 GSNANAYFGYSTPWGYFDENRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTQDST
 TTIANNLSTVQVFTDDDYQLPYVVGNGTEGCLPAFPQVFTLPQYGYATLNRDNTENPTERS
 FFCLEYFPSKMLRTGNNEFTYNFEEVPHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQ
 FNKNLAGRYANTYKNWFP GPMGRTOGWNLGSGVNRASVSATATTNRMELEGASYQVPPQPNGMT
 NNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSS
 TTAPATGTYNLQEI VPGSVWMERDVYLQGPWAKIPETGAH FHPSPAMGGFGLKHPPPMMLIKN
 TPVPGNITSFSDVPVSSFITQYSTGQVTVMEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPD
 STGEYRTRRPIGTRYLTRPL

SEQ ID NO:41: rhIO VP1 polypeptide (GenBank Accession No. AAO88201.1)

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNGLDKGEP
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADA EFQERLQEDTSFGGNLGRAVFQAKKRVLEP

LGLVEEGAKTAPGKKRPVEFPSPQRSPDSSGTGIGKKGQPAKKRLNFGQTGDSESVDPDPPIGEP
 PAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTTSTRTWALPTYNNH
 LYKQISNGTSGGSTNDNTYFGYSTPWGYFDENRFHCHFS PRDWQRLINNNWGFRPKRLNFKLFN
 IQVKEVTQNEGTKTIANNLSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTNN
 NGSQAVGRSSFYCLEYFPSQMLRTGNNEFFSYQFEDVPHSSYAHSSQLDRLMNPLIDQYLYYL
 SRTQSTGGTAGTQQLLFSQAGPNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYH
 LNRDLSLVNPGVAMATHKDDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVAT
 EQYGVVADNLQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGF
 GLKHPPPQILIKNTFVPADPPTTFSQAKLASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYT
 SNYYKSTNVDFAVNTDGTYSEPRPIGTRYLTRNL

SEQ ID NO:42: Anc110 VP1 polypeptide

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNGLDKGEP

PPAADGTEFDWLEEDNESESTREWWEDENFGATFENANQXQXQLEDSNGDVLFEFSTRTDGFTHGLEDGEF
 VNAADAAALEHDKAYDQQLKAGDNMPYLRYNHADAEFQERLQEDTSFGGNLGRAVFPQAKKRVLEP
 LGLVEEGAKTAPGKKRPVEQSPQEPDSSX₁GIGKTGQQPAX₂KRLNFGQTDSESVDPDPQPLGEP
 PAAPSGVGSNTMASGGGAPMADNNEGADGVCNSSGNWHCDSTWLGDRVITTSTRTWALFTYNNH
 LYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFSRQDWQRLINNNWGFPRKRLNFKLFN
 IQVKEVTTNEGKTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLN
 NGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVPFHSSYAHSQSLDRMLNPLIDQYLYYL
 SRTQTGTGX₃GQTFLX₄FSQAGPSSMANQARNWVPGPCYRQQRVSTTTNQNNNSNFANTGAX₅KX₆
 X₇LNGRDSLMLNPGVAMASHKDDDEDRFFPSSGVLI₁FGKQAGANDNDVYSX₈VMITNEEEIKTTNPV
 ATEEYGA VATNX₉QX₁₀ANTQAQTGLVHNQGVLPGMVWQNRDVYLQGPWAKIPHTDGNFHPSP
 MGGFGLKHPPFPQILIKNTVPADPPTTFNQAKLNSFITQYSTGQVSVEIEWELQKENS KRWNPE
 IQYTSNYYKSTNVDFAVNTEGVYSEPRPIGTRYLTRNL

X1 = S/T; X2 = K/R; X3 = A/G; X4 = Q/A; X5 = T/A; X6 = Y/F; X7 = H/K; X8 = Q/N; X9 = N/H;
 X10 = S/A

SEQ ID NO:43: Anc110 VP1 DNA

ATGGCTGCCGATGTTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTTCGCGAGT
 GGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACACAGCAAAAGCAGGACGACGGCCG
 GGGTCTGGTGCTTCTCGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCC
 GTCAACGCGCGCGGACGCAGCGGCCCTCGAGCACGACAAAGCCTACGACCAGCAGCTCAAAGCGG
 GTGACAATCCGTACCTGCGGTATAATCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGA
 TACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCT
 CTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGGCGGGTAGAGCAGTCCG
 CACAAGAGCCAGACTCCTCCXX₁GGCATCGGCAAGACAGGCCAGCAGCCCGCTXXX₂AAGAGA
 CTCAATTTTGGTTCAGACTGGCGACTCAGAGTCAGTCCCCGACCCACAACCTCTCGGAGAACCTC
 CAGCAGCCCCCTCAGGTGTGGGATCTAATACAATGGCTTCAGGCGGTGGCGCTCCAATGGCAGA
 CAATAACGAAGGCGCCGACGGAGTGGGTAATTCCTCGGGAAATTGGCATTGCGATTCCACATGG
 CTGGGGGACAGAGTCATCACACCAGCACCCGAACCTGGGCCCTGCCACCTACAACAACCACC
 TCTACAAGCAAATCTCCAACGGCACCTCGGGAGGAAGCACCAACGACAACACCTATTTTGGCTA
 CAGCACCCCCCTGGGGGTATTTTGACTTCAACAGATTCCACTGTCACTTTTCACCACGTGACTGG
 CAACGACTCATCAACAACAATTGGGGATTCCGGCCCCAAAAGACTCAACTTCAAGCTGTTCAACA
 TCCAGGTCAAGGAAGTCACGACGAACGAAGGCACCAAGACCATCGCCAATAATCTCACCAGCAC
 CGTGCAGGTCTTTACGGACTCGGAGTACCAGTTACCGTACGTGCTAGGATCCGCTCACCAGGGA
 TGTCTGCCCTCCGTTCCCGGCGGACGTCTTCATGATTCTCTCAGTACGGCTATTTAACTTTAAACA

ATGGAAGCCAAGCGTGGGACGTTCCCTCCTTCTACTGTCTGGAGTATTTCCCATCGCAGATGCT
 GAGAACCGGCAACAACCTTCAGTTTCAGCTACACCTTCGAGGACGTGCCCTTCCACAGCAGCTAC
 GCGCACAGCCAGAGCCTGGACAGGCTGATGAATCCCTCATCGACCAGTACCTGTACTACCTGT
 CCAGAACGCAAACGACTGGAACXXX₃GGGACGCAGACTCTGXXX₄TTTCAGCCAAGCGGGTCTCT
 AGCTCAATGGCCAACAGGCTAGAAATTGGGTGCCCGGACCTTGCTACCGGCAGCAGCGCGTCT
 CCACGACAACCAACAGAACAAACAGCAACTTTGCCTGGACGGGAGCTXXX₅AAGXXX₆XXX₇
 7CTGAACGGCCGAGACTCTCTAATGAATCCGGGCGTGGCAATGGCTTCCACAAAGGATGACGAG
 GACCGCTTCTTCCCTTCGAGCGGGGTCTGATTTTGGCAAGCAAGGAGCCGGGAACGATAATG
 TGGATTACAGCXXX₈GTGATGATTACAAATGAGGAAGAAATCAAGACTACCAACCCCGTGGCCA
 CAGAAGAATATGGAGCAGTGGCCACCAACXXX₉CAGXXX₁₀GCCAATACGCAGGCGCAGACCGG
 ACTCGTGACACAACAGGGGGTGTCTCCCGGCATGGTGTGGCAGAATAGAGACGTGTACCTGCAG
 GGTCCCATCTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCGTCTCCCTGATGGGCG
 GCTTTGGACTGAAGCACCCGCTCCTCAAATTTCTCATCAAGAACACACCGGTTCCAGCGGACCC
 GCCGACTACCTTCAACAGGCCAAGCTGAACCTTTCTCATCACGACGTACAGCACCGGACAGGTC
 AGCGTGGAAATCGAGTGGGAGCTGCAGAAAGAAAACAGCAAACGCTGGAATCCAGAGATTCAAT
 ACACTTCCAATACTACAAATCTACAAATGTGGACTTTGCTGTCAACACGGAGGGGTTTATAG
 CGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGCAACCTGTAA

XXX1 = TCG/ACG; XXX2 = AAA/AGA; XXX3 = GCA/GGA; XXX4 = CAA/GCA;

XXX5 = ACC/GCC; XXX6 = TAT/TTT; XXX7 = CAC/AAA; XXX8 = CAA/AAC;

XXX9 = AAC/CAC; XXX10 = TCC/GCC

SEQ ID NO:44: AAV4 VP1 polypeptide (GenBank Accession No. NP 044927.1)

MTDGYLPDWLEDNLSEGVREWWALQPGAPKPKANQQHQDNARGLVLPQYKYLGPNGLDKGEFV
 NAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEEFQORLQGDTSFGGNLGRAVFAQKKRVLEPL
 GLVEQAGETAPGKKRPLIESPQQPDSSTGIGKKGKQPAKKKLVEFEDTGAGDGPPEGSTSGAMS
 DDSEMRAAAGGAAVEGGQGADGVGNASGDWHCDSTWSEGHVTTTSTRTWVLPFTYNNHLYKRLGE
 SLQSNFYNGFSTPWGYFDFNRFHCHFSRWDQRLINNNWGMRPKAMRVKIFNIQVKEVTTSNGE
 TTVANNLTSTVQIFADSSYELPYVMDAGQEGSLPFPNDVFMVFPQYGYCGLVTGNTSQOQTDNRN
 AFYCLEYFSPQMLRTGNNFETIYSFEKVPFHSMYAHSQSLDRIMNPLIDQYLWGLQSTTTGTTL
 NAGTATTNFTKLRPNTFNSNFKKNWLPGPSIKQQGFSTANQNYKIPATGSDSLIKYETHSTLDG
 RWSALTPGPPMATAGPADSKFSNSQLIFAGPKQNGNTATVPGLTIFTSEEELAATNATDMDMWG
 NLPGGDQSNLPTVDRLTALGAVPGMVWQNRDIYYQGPIWAKIPHDTGHFHPSPPLIGGFGLKH
 PPPQIFIKNTPVPANPATTFSSTPVNSFITQYSTGQVSVQIDWEIQKERSKRWNPEVQFTSNYG
 QQNSLLWAPDAAGKYTEPRAIGTRYLTHHL

SEQ ID NO:45: rh32.33 VP1 polypeptide (GenBank Accession No. EU368926)

MAADGYLPDWLEDNLSEGTREWWDLKPGAPKPKANQQKQDDGRGLVLPQYKYLGPFGNLDKGEF
 VNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEEFQERLQEDTSFGGNLGRAVFAQKKRVLEP
 LGLVEEGAKTAPGKKRPLESPQEPDSSSGIGKKGKQPAKKRLNFEEDTGAGDGPPEGSDTSAMS
 SDIEMRAAPGGNAVDAGQSGDGVGNASGDWHCDSTWSEGVTTTSTRTWVLPFTYNNHLYLRLGT
 TSNSNTYNGFSTPWGYFDFNRFHCHFSRWDQRLINNNWGLRPAKAMRVKIFNIQVKEVTTSNGE
 TTVANNLTSTVQIFADSSYELPYVMDAGQEGSLPFPNDVFMVFPQYGYCGIVTGENQNQTDNRN
 FYCLEYFSPQMLRTGNNFEMAYNFEKVPFHSMYAHSQSLDRIMNPLIDQYLWHLQSTTSGETLN

QGNAAATTFGKIRSGDFAFYRKNWLPGPCVKQQRFSKTASQNYKIPASGGNALLKYDTHYTLNNR
 WSNIAPGPPMATAGPSDCDFSNQQLIFPGPSVTGNTTTSANNLLETSEEIAATNPRDMDMFCQ
 IADNNQNATTAPITGNVTAMGVLPGMVWQNRDIYYQGPIWAKIPHADGHFHPSPPLIGGFGLKHP
 PPQIFIKNTPVPANPATTFTAARVDSFITQYSTGQVAVQIEWEIEKERSKRWNPEVQFTSNYGN
 QSSMLWAPDITGKYTEPRVIGSRYLTNHL

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Non-patent literature cited in the description

- **PETTERSEN et al.**J. Comp. Chem., 2004, vol. 25, 1605-12 [0012] [0103]
- **NOTREDAME et al.**J. Mol. Biol., 2000, vol. 302, 205-17 [0012] [0020] [0103]
- **CALCEDO et al.**J. Infect. Dis., 2009, vol. 199, 381-90 [0014] [0041] [0112]

- BOUTIN et al. Human Gene Ther., 2010, vol. 21, 704-12 [0014]
- THOMPSON et al. Nuc. Acids Res., 1994, vol. 22, 4673-90 [0020]
- LASSMANN et al. Nuc. Acids Res., 2006, vol. 34, W596-99 [0020]
- KATOH et al. Nuc. Acids Res., 2005, vol. 33, 511-8 [0020]
- EDGAR BMC Bioinform., 2004, vol. 5, 113- [0020]
- LOYTYNOJAGOLDMAN Science, 2008, vol. 320, 1632-5 [0021] [0100]
- LOYTYNOJAGOLDMAN PNAS USA, 2005, vol. 102, 10557-62 [0021] [0100]
- DAYHOFF et al. Atlas of Protein Sequence and Structure 19780000345-52 [0022]
- JONES et al. Comp. Appl. Biosci., 1992, vol. 8, 275-82 [0022] [0061]
- WHELANGOLDMAN Mol. Biol. Evol., 2001, vol. 18, 691-9 [0022]
- HENIKOFF HENIKOFF PNAS USA, 1992, vol. 89, 10915-9 [0022]
- REEVES J. Mol. Evol., 1992, vol. 35, 17-31 [0023]
- YANG Mol. Biol. Evol., 1993, vol. 10, 1396-1401 [0023]
- CAO et al. J. Mol. Evol., 1994, vol. 39, 519-27 [0023] [0061]
- AKAIKE Second International Symposium on Information Theory 19730000267-81 [0024]
- SCHWARZ Ann. Statist., 1978, vol. 6, 461-4 [0024]
- DARRIBA et al. Bioinformatics, 2011, vol. 27, 81164-5 [0025] [0061]
- GUINDONGASCUEL Systematic Biology, 2003, vol. 52, 696-704 [0026] [0062]
- GASCUEL Mol. Biol. Evol., 1997, vol. 14, 685-95 [0026]
- FELSENSTEIN Systematic Biology, 1973, vol. 22, 240-9 [0026]
- ANISIMOVAGASCUEL Systematic Biology, 2006, vol. 55, 539-52 [0027] [0062]
- YANG Comp. Applic. BioSci., 1997, vol. 13, 555-6 [0028] [0063]
- XU et al. Am. J. Obstet. Gynecol., 2007, vol. 196, 431-6 [0039]
- PAUL et al. J. Infect. Dis., 1994, vol. 169, 801-6 [0039]
- SAUERBREI et al. Eurosurv, 2011, vol. 16, 443- [0039]
- SAKHRIA et al. PLoS Negl. Trop. Dis., 2013, vol. 7, e2429- [0039]
- FISHER et al. Nature Med., 1997, vol. 3, 306-12 [0040]
- MANNING et al. Human Gene Ther., 1998, vol. 9, 477-85 [0040]
- BOUTIN et al. Prevalence of serum IgG and neutralizing factors against AAV types 1, 2, 5, 6, 8 and 9 in the healthy population: implications for gene therapy using AAV vectors Hum. Gene Ther., 2010, vol. 21, 704-12 [0041]
- ALTSCHUL et al. Nucleic Acids Res., 1997, vol. 25, 3389-3402 [0045]
- PCR Primer: A Laboratory Manual Cold Spring Harbor Laboratory Press 19950000 [0050]
- YANG Mol. Biol. Evol., 1993, vol. 10, 1396-1401 [0061]
- HIROTUGUI IEEE Transactions on Automatic Control, 1974, vol. 19, 716-23 [0061]
- SCHNEIDER et al. BMC Bioinform., 2005, vol. 6, 134- [0065]
- BALAZS et al. Nat. Biotechnol., 2013, vol. 31, 647-52 [0098]
- LIMBERIS et al. Sci. Transl. Med., 2013, vol. 5, 187ra72- [0098]
- BALAZS et al. Nature, 2012, vol. 481, 81-4 [0098]
- DEAL et al. PNAS USA, 2014, vol. 111, 12528-32 [0098]
- MAO et al. Hum. Gene Ther., 2011, vol. 22, 1525-35 [0099]
- XIE et al. Gynecol. Oncol., 2014, [0099]
- WATANABE et al. Gene Ther., 2010, vol. 17, 1042-51 [0099]

- **FINNIGAN et al.** Nature, 2012, vol. 481, 360-4 [\[0100\]](#)
- **DARRIBA et al.** Bioinform, 2011, vol. 27, 1164-5 [\[0100\]](#)
- **GUINDON et al.** System. Biol., 2010, vol. 59, 307-21 [\[0100\]](#) [\[0113\]](#)
- **ANISMOVAGASCUEL** Syst. Biol., 2006, vol. 55, 539-52 [\[0100\]](#)
- **YANG** Mol. Biol. Evol., 2007, vol. 24, 1586-91 [\[0100\]](#)
- **BIASINI et al.** Nucl. Acids Res., 2014, vol. 42, W252-8 [\[0103\]](#)
- **LOCK et al.** Hum. Gene Ther., 2010, vol. 21, 1259-71 [\[0105\]](#)
- **SCHUCK et al.** Biophys. J., 2002, vol. 82, 1096-111 [\[0106\]](#)
- **AUSAR et al.** J. Biol. Chem., vol. 281, 19478-88 [\[0106\]](#)
- **RAYAPROLU et al.** J. Virol., 2013, vol. 87, 13150-60 [\[0106\]](#)
- **WANG et al.** Mol. Ther., 2010, vol. 18, 118-25 [\[0110\]](#)
- **GAO et al.** J. Virol., 2004, vol. 78, 6381-88 [\[0112\]](#)
- **GAO et al.** PNAS USA, 2003, vol. 100, 6081-6 [\[0113\]](#)
- **GAO et al.** J. Virol., 2004, vol. 78, 6381-8 [\[0113\]](#)
- **GAO et al.** Current Gene Ther., 2005, vol. 5, 285-97 [\[0113\]](#)
- **NAKAI et al.** J. Virol., 2005, vol. 79, 214-24 [\[0118\]](#)
- **NAKAI et al.** J. Virol., 2002, vol. 76, 11343-9 [\[0118\]](#)

STAM-VIRUSSEKVENSER OG ANVENDELSER HERAF**PATENTKRAVENE ER:**

1. Et adeno-associeret virus (AAV) kapsid-polypeptid med aminosyresekvensen vist i SEQ ID NO: 42.
- 5 2. AAV kapsid-polypeptidet fra krav 1, hvor AAV kapsid-polypeptidet er rensset.
3. AAV kapsid-polypeptidet fra krav 1 kodet af nukleinsyresekvensen vist i SEQ ID NO: 43.
4. Et nukleinsyremolekyle, som koder et adeno-associeret virus (AAV) kapsid-polypeptid med nukleinsyresekvensen vist i SEQ ID NO: 43.
- 10 5. En vektor omfattende nukleinsyremolekylet fra krav 4.
6. En værtscelle omfattende vektoren fra krav 5.
7. En rensset viruspartikel omfattende AAV kapsid-polypeptidet fra krav 1.
8. Den rensede viruspartikel fra krav 7, yderligere omfattende et transgen.
9. Et transgen til anvendelse i en metode til genoverførsel og/eller vaccination,
15 hvor metoden omfatter
administrering af viruspartiklen fra krav 8 til et subjekt med behov for
genoverførsel eller vaccination, hvor viruspartiklen fremviser omtrent samme
eller lavere seroprævalens, som en AAV9 viruspartikel gør det.
10. Et transgen til anvendelse fra krav 9, hvor viruspartiklen neutraliseres i samme
20 eller mindre udstrækning af humant serum, som en AAV9 viruspartikel bliver
det.
11. Et target antigen operativt forbundet med AAV kapsid-polypeptidet fra krav 1 til
anvendelse i en metode til vaccinering af et subjekt, hvor metoden omfatter

administrering af et target antigen operativt forbundet med AAV kapsid-polypeptidet fra krav 1 til et subjekt med behov for vaccination, hvor AAV kapsid-polypeptidet fremviser omtrent samme eller lavere seroprævalens som et AAV9 kapsid-polypeptid.

DRAWINGS

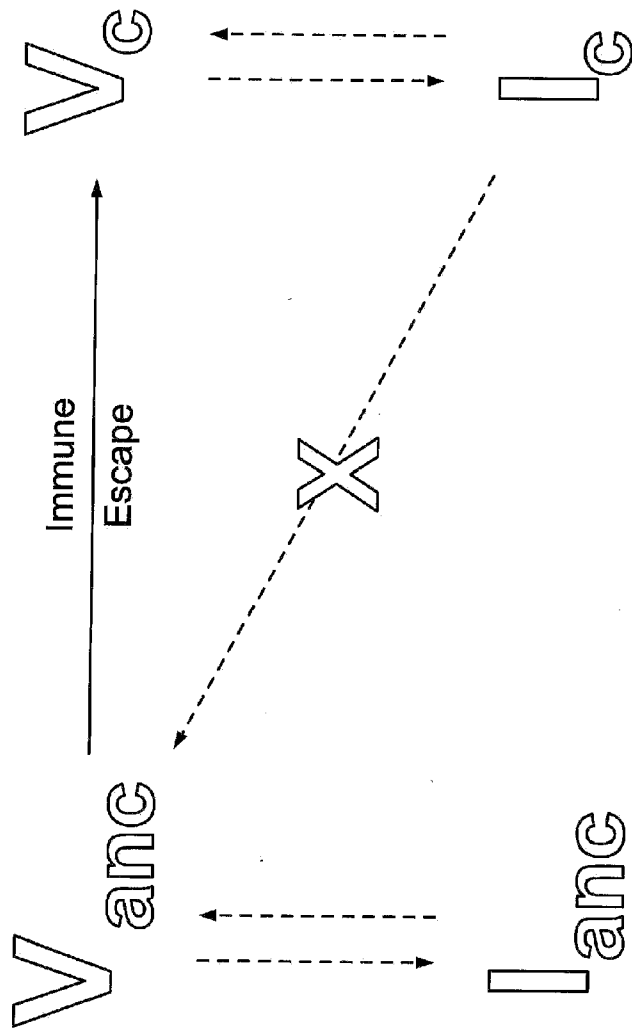


FIG. 1

○	E	I	K	A	T	N	P	V	A	T	E	R	F	G	T	V	A	V	N	F	Q
○	E	I	K	A	T	N	P	V	A	T	E	R	F	G	T	V	A	V	N	L	Q
○	E	I	R	T	T	N	P	V	A	T	E	Q	Y	G	S	V	S	T	N	L	Q
○	E	I	R	T	T	N	P	V	A	T	E	Q	Y	G	T	V	A	N	N	L	Q

FIG. 2A

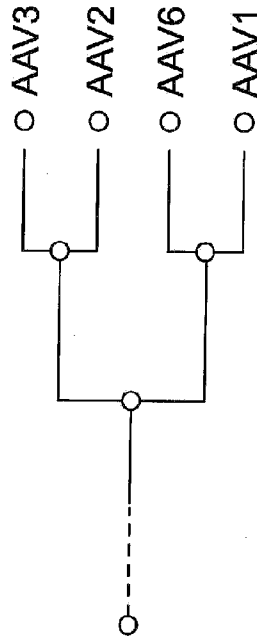


FIG. 2B

○	E	I	K	A	T	N	P	V	A	T	E	R	F	G	T	V	A	V	N	L	Q
○	E	I	R	T	T	N	P	V	A	T	E	Q	Y	G	T	V	A	T	N	L	Q
○	E	I	K	T	T	N	P	V	A	T	E	Q	Y	G	T	V	A	T	N	L	Q

FIG. 2C

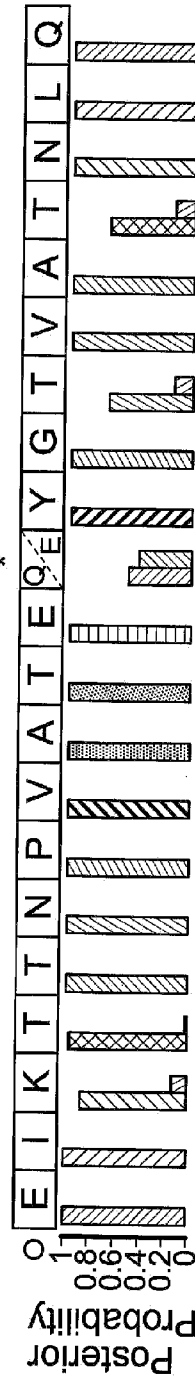


FIG. 2D

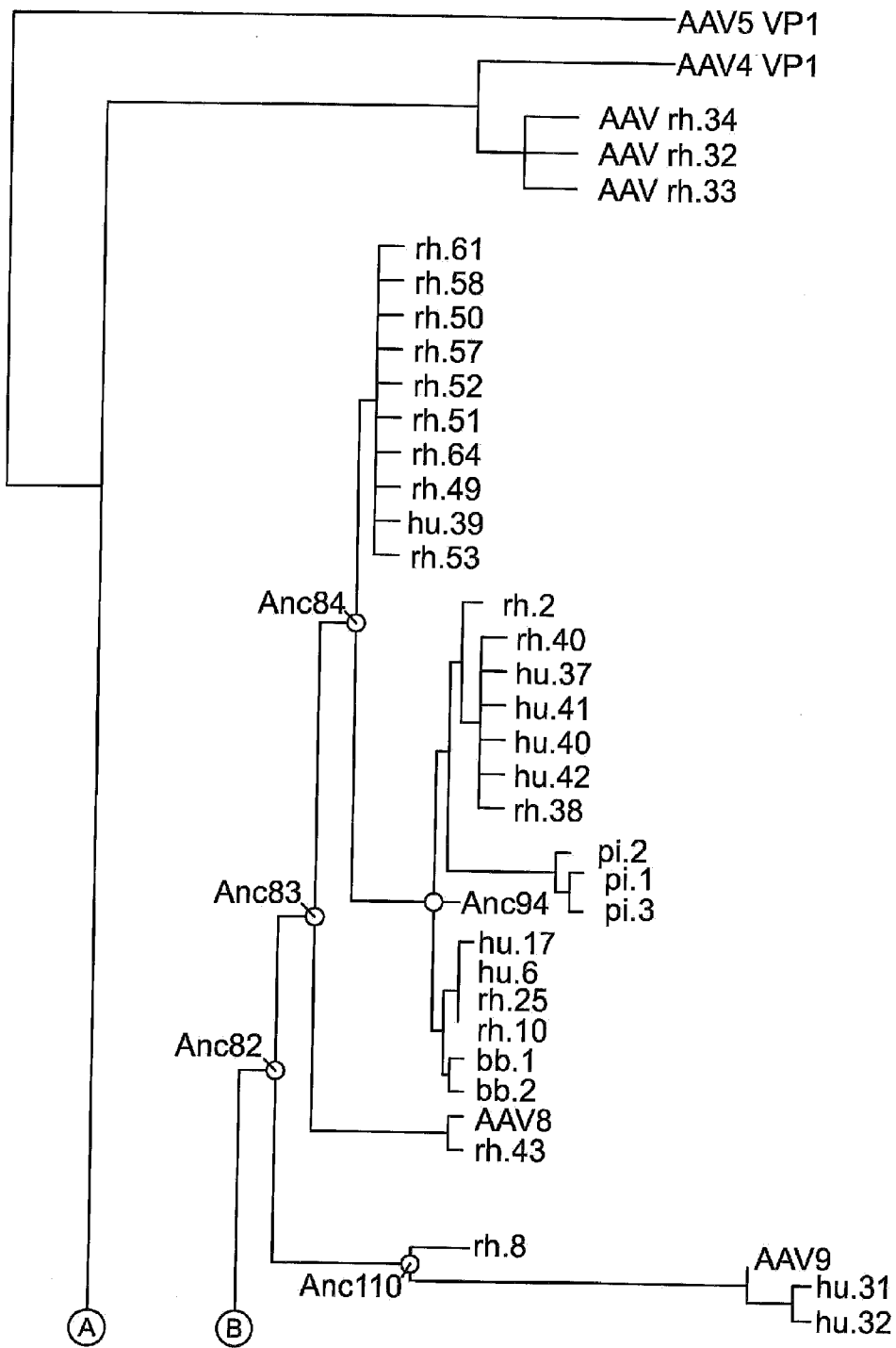


FIG. 3

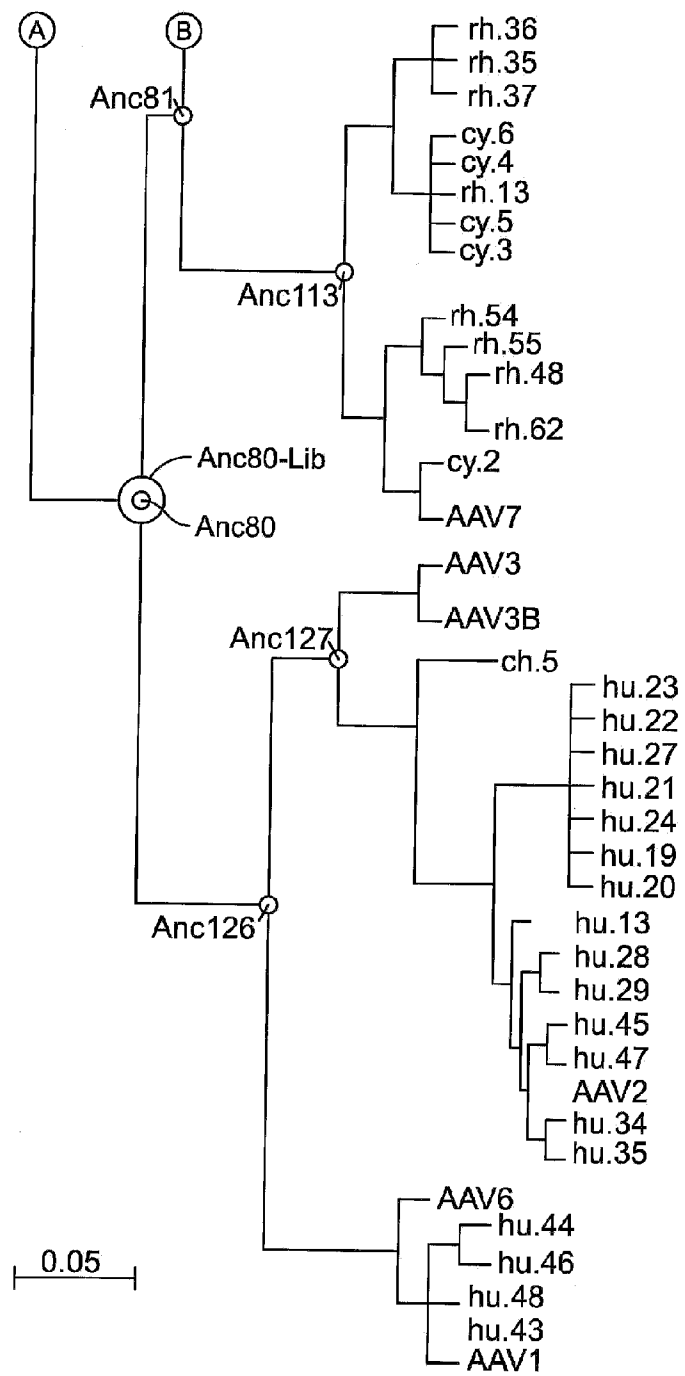


FIG. 3 (Cont.)

L0065	410	420	430	440	450	460	470	480	490	500
L0027										
L0033	SQMLRTGNNFQFSYTFEDVPHSSYAHQSLSLDRIMNPLIDQYLYLSRFTQTTSGTAGNRTLQFSQAGPSSMANQAKNWLPGFCYRQQRVSKTTNQNNNSN									
L0036		E.....								A.....
L0044		E.....								A.....
L0059		E.....				E.....				A.....
L0060		E.....				E.....				
L0062		E.....				E.....				
L0065	510	520	530	540	550	560	570	580	590	600
L0027										
L0033	FAWTGATKYHLNGRDSLVPQZAMATHKDEDEKFFPMSGVLIIFGQAGNSNVLDLDNVMLTNEEIIKTTNPVATEEYCTVAINLQSANAPATGTVNSQG									
L0036										
L0044						S.....				
L0059						S.....				
L0060										
L0062						S.....				
L0065	610	620	630	640	650	660	670	680	690	700
L0027										
L0033	ALPGVWQDRDVYLQGPWAKIPHTDGHFHPSPMLGCGFLKHPPPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ									
L0036										
L0044		N.....								
L0059		N.....								
L0060		N.....								
L0062		E.....								
L0065	710	720	730							
L0027										
L0033	YTSNYSKSTNVDFAVDITNGVYSEPRPIGTIRLTNL*									
L0036										
L0044										
L0059										
L0060										
L0062										

FIG. 4 (Cont.)

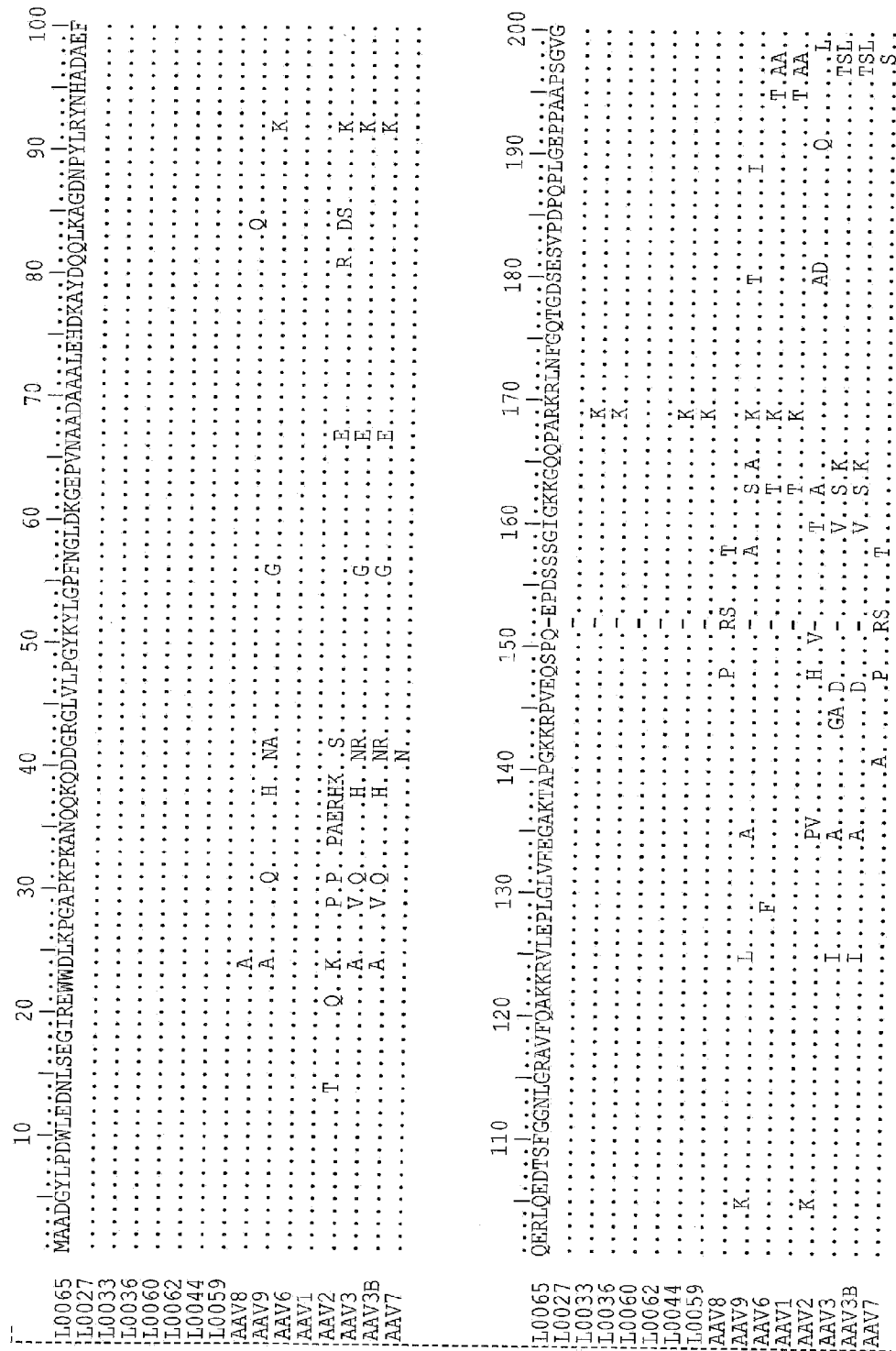


FIG. 5A

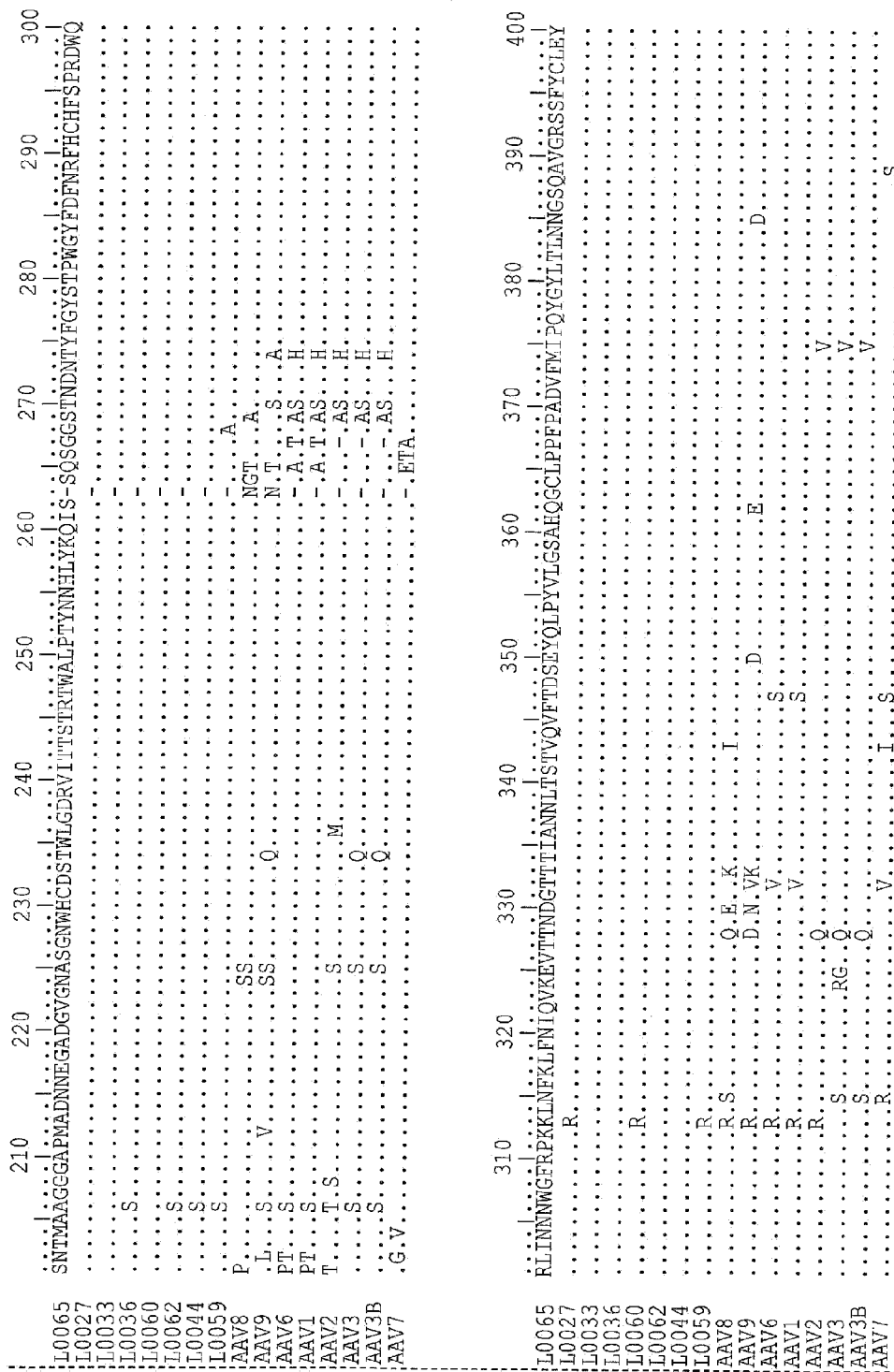


FIG. 5A (Cont.)

```

410 420 430 440 450 460 470 480 490 500
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0065 FFSQMLRTGNFQFSYTFEDVPFESSYAHSQSLDRIMNPLIDQYLYLSRTQ-TTSGTAGNRTLQFSQAGPSSMANQAKNWLPGPCYFQQRVSKTTNQNN
L0027 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0033 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0036 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0060 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0062 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0044 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0059 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV8 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV9 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV6 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV1 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV2 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV3 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV3B .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV7 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

```

```

510 520 530 540 550 560 570 580 590 600
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0065 NSNEFAWTGATKYHLNGRDSLVNPGPAMATHKDEDEKFFPMSGVLIFGKQAGNSNVDLDNMVITHEEIKTTNPVATEEYGTVAITLQSAANTAPATGTVN
L0027 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0033 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0036 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0060 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0062 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0044 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L0059 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV8 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV9 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV6 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV1 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV2 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV3 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV3B .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAV7 .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

```

FIG. 5B

	610	620	630	640	650	660	670	680	690	700
L0065										
L0027	SQALPGWVQDRDYLQGIWAKIPHTDGHFSPFLMGGFGLKHPFPQILIKNTVPANPPTTFSPAKFASFITQYSTGQVSVIEIWEIQKENSQRWNP									
L0033										
L0036										
L0060										
L0062										
L0044										
L0059										
AAV8										
AAV9	N..I.....		N.....			D.....	NQS.LN.			
AAV6	VM.....		N.....	M.....		D.....	A.NKD.LN.			
AAV1	AM.....						AE..AT.			
AAV2	T..V.....			N.....			AE..AT.			
AAV3	H.....				M.....		S...A.			
AAV3B	D.....				M.....					
AAV7	N.....		N.....				EV.T.			

	710	720	730
L0065			
L0027	EIQYTSNKNKSTNWDFAVDTNGVYSEPRPIGTRYLTRNL		
L0033			
L0036			
L0060			
L0062			
L0044			
L0059			
AAV8	Y..S.....	N.E.....	
AAV9	Y..N..E.....	N.E.....	
AAV6	V.....	A.A...T.N..I.T.	P.
AAV1	V.....	A.A...T.N..I.T.	P.
AAV2		V...T.....	
AAV3		V...T.....	

FIG. 5B (Cont.)

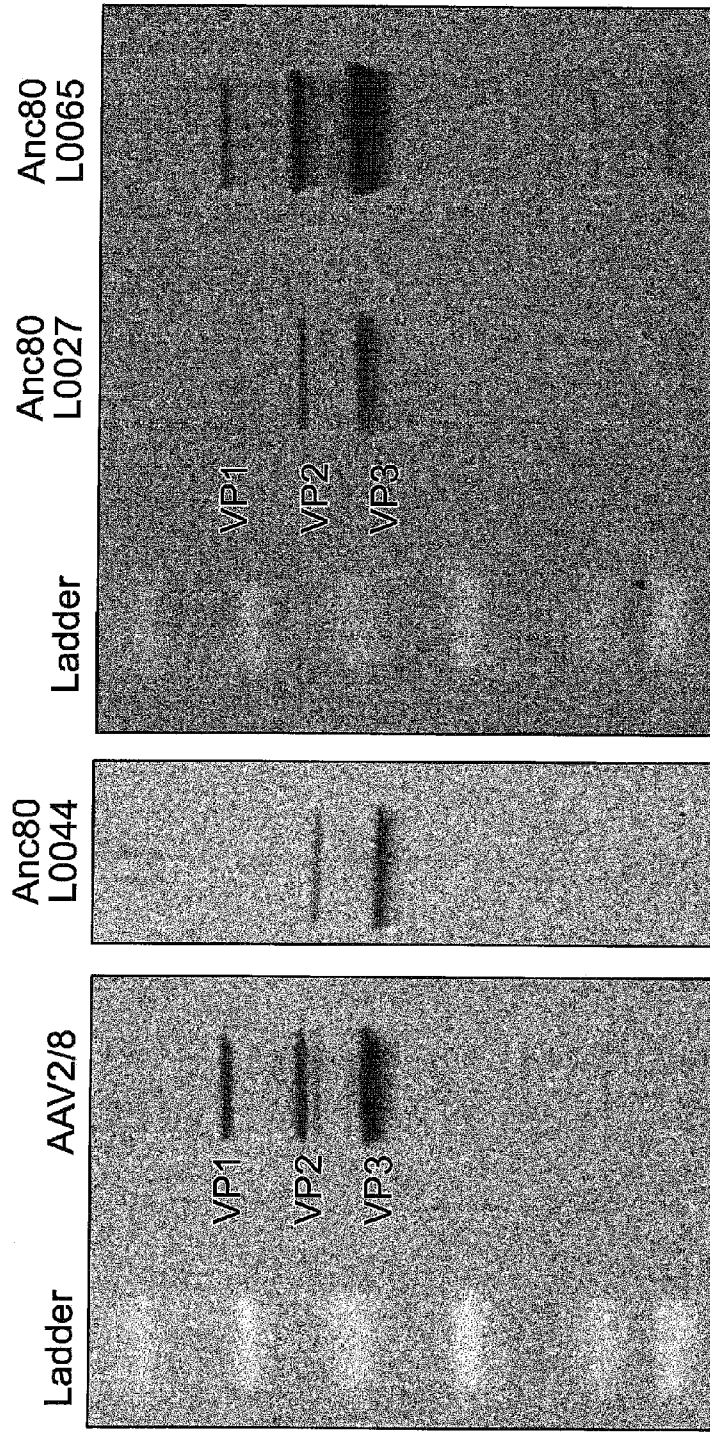


FIG. 6

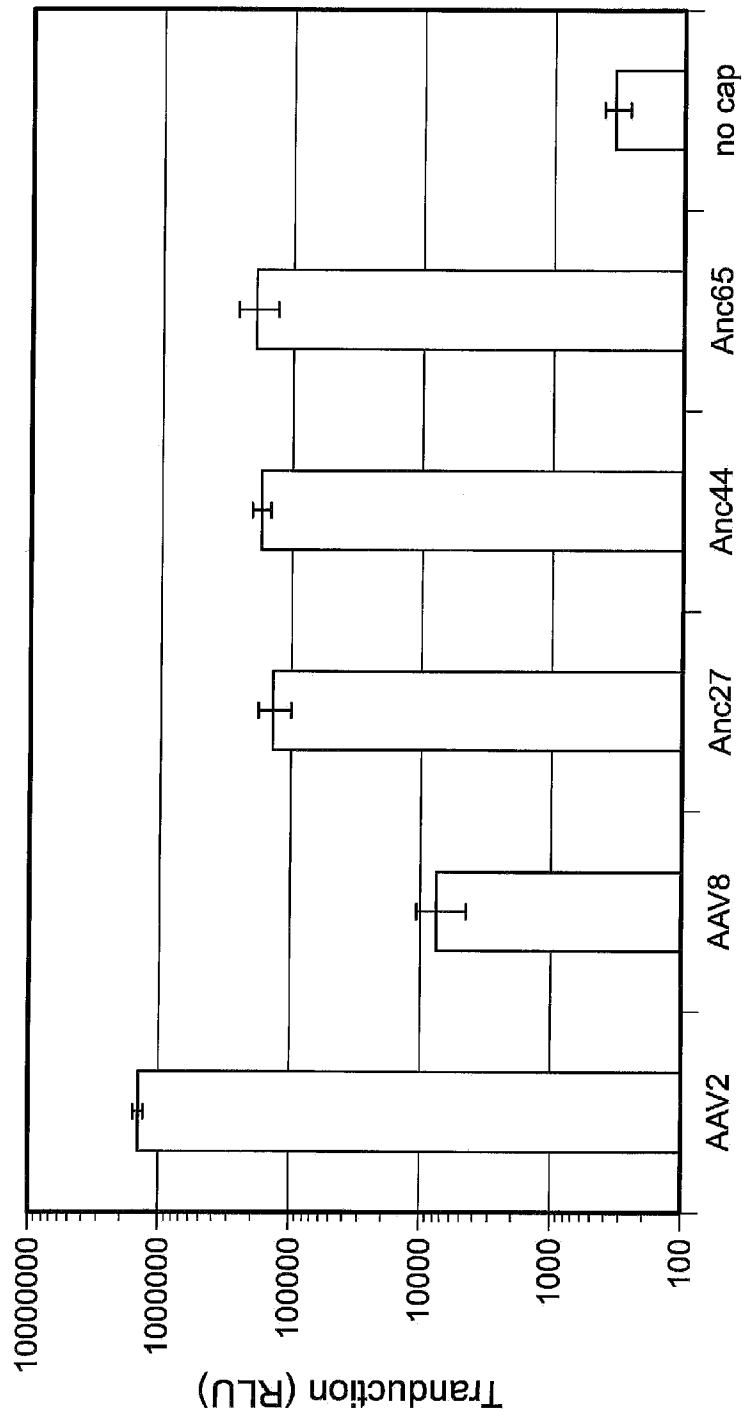


FIG. 7

VP1 seq. Δ	AAV5	AAV2	AAV8	rh10	Anc80L65	Anc80Lib
AAV5	ID	43.0%	42.6%	43.0%	40.5%	39.9-40.5%
AAV2	320	ID	17.1%	15.9%	12.2%	12.0-12.8%
AAV8	317	127	ID	6.5%	9.1%	8.7-10.1%
rh10	320	118	48	ID	8.6%	7.8-8.9%
L0065	301	91	68	64		0-1.5%
Anc80Lib	297-302	89-95	65-75	58-66	0-11	ID

FIG. 8

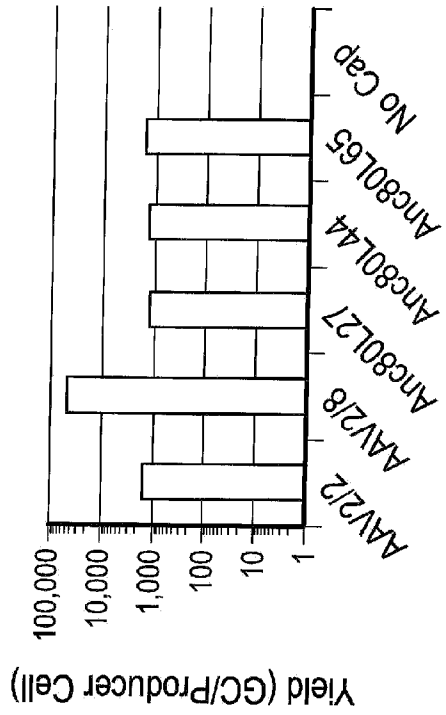


FIG. 9A

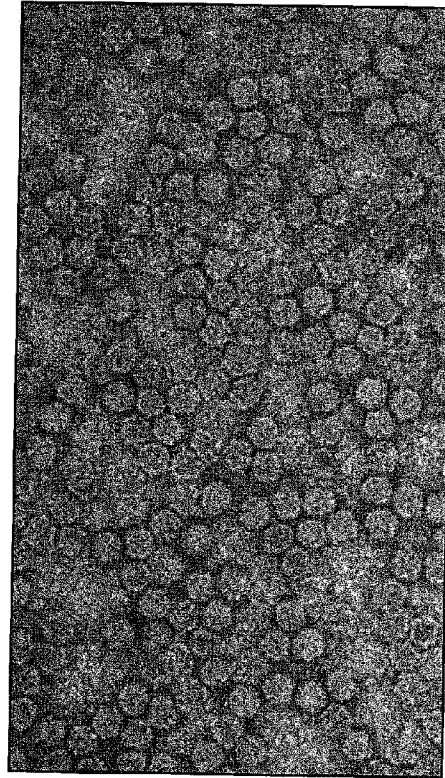


FIG. 9B

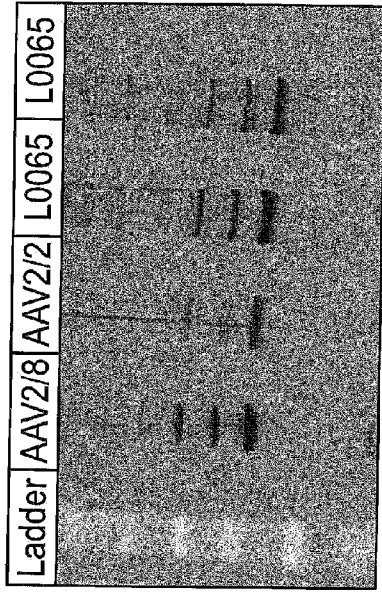


FIG. 9C

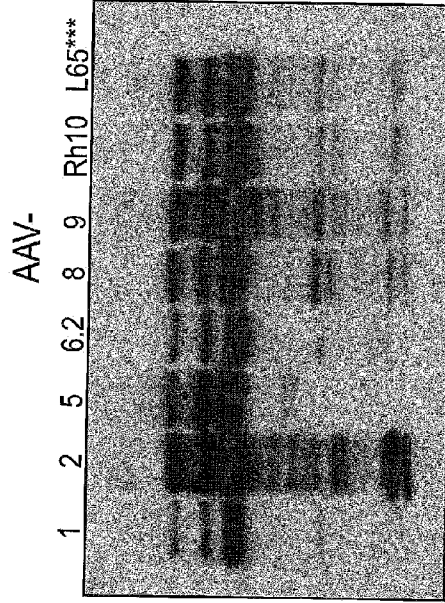
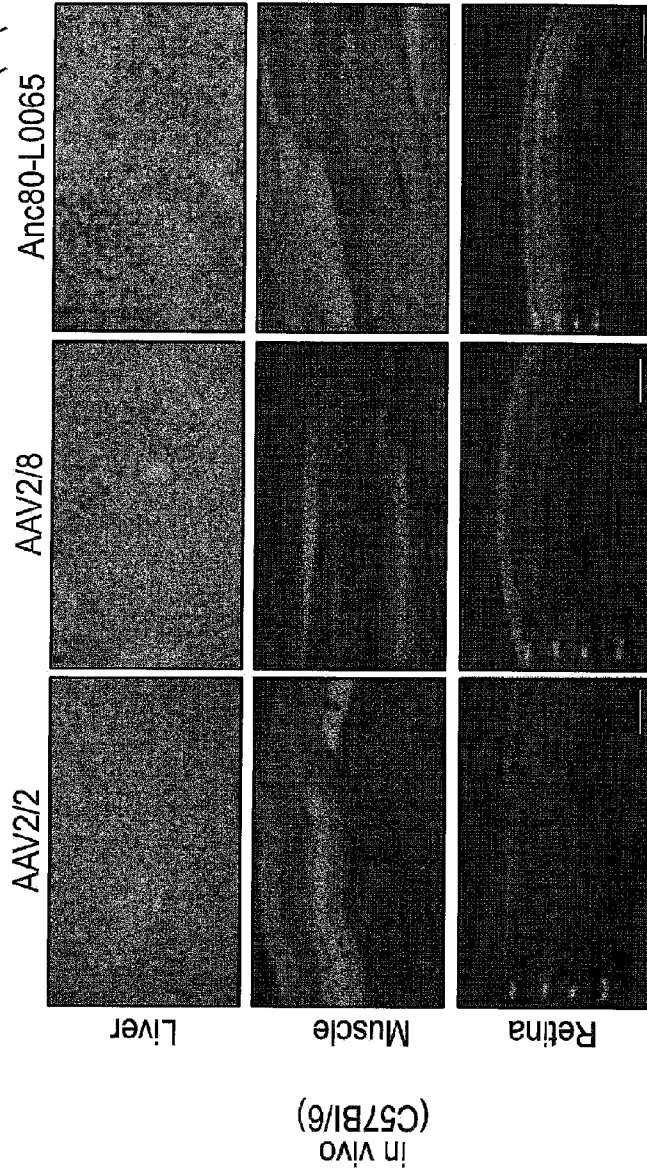
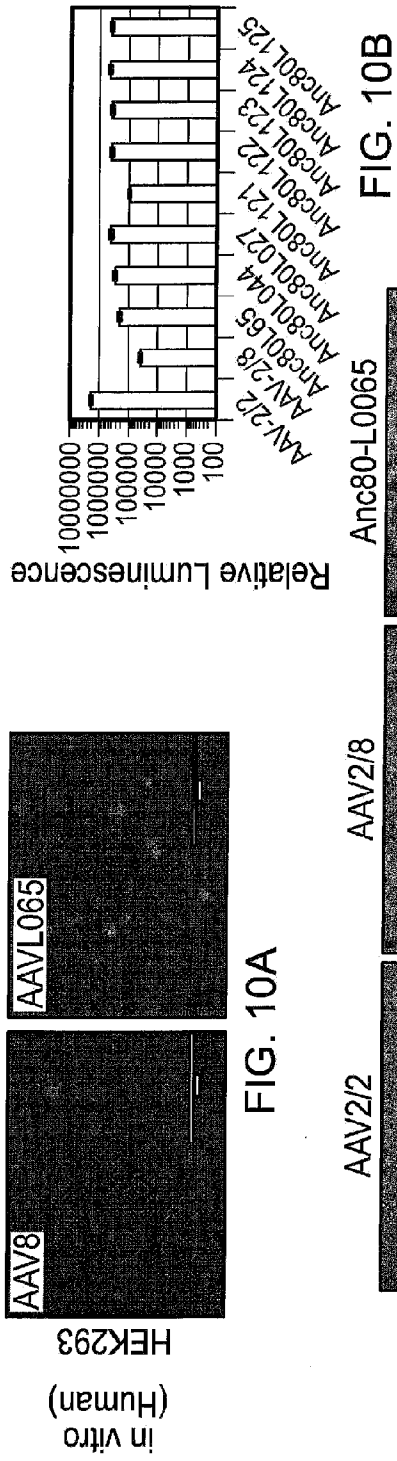


FIG. 9D



	Anc81	Anc127	Anc83	Anc110	Anc113	AAV3	AAV2	AAV9					
L0065	Anc126	Anc84	AAV8	AAV7	AAV1	AAV2	AAV9						
L0065	ID	97.8%	97.2%	95.5%	95.5%	94.0%	93.4%	90.7%	90.5%	89.5%	87.9%	86.1%	
Anc81	ID	95.9%	93.6%	97.4%	95.6%	93.9%	95.1%	92.1%	94.7%	91.4%	88.6%	86.7%	86.5%
Anc126	ID	97.4%	93.4%	92.0%	90.7%	89.1%	91.8%	88.8%	91.9%	91.5%	89.2%	85.8%	
Anc127	ID	91.7%	90.7%	89.7%	90.7%	88.3%	89.8%	87.3%	94.4%	89.4%	90.7%	86.0%	
Anc82	ID	98.2%	96.2%	97.4%	94.7%	92.8%	89.7%	86.8%	86.4%	85.2%	87.8%		
Anc83	ID	97.8%	95.6%	96.0%	91.7%	89.3%	86.1%	85.3%	84.8%	87.1%			
Anc84	ID	93.7%	94.5%	91.3%	89.1%	85.5%	85.0%	84.1%	86.3%				
Anc110	ID	92.5%	90.5%	88.2%	86.3%	86.5%	85.6%	89.0%					
AAV8	ID	89.7%	87.8%	85.3%	84.0%	82.9%	85.2%						
Anc113	ID	96.4%	85.9%	86.7%	83.8%	83.3%							
AAV7	ID	84.2%	85.0%	82.3%	81.8%								
AAV3	ID	86.4%	87.3%	83.7%									
AAV1	ID	83.2%	82.4%										
AAV2	ID	81.9%											
AAV9	ID												

FIG. 11A

	Anc81	Anc127	Anc83	Anc84	Anc110	AAV8	AAV3	Anc113	AAV7	AAV2	AAV1	AAV9			
L0065	ID	Anc126	Anc82	Anc84	Anc110	AAV8	AAV3	Anc113	AAV7	AAV2	AAV1	AAV9			
L0065	ID	97.3%	96.6%	94.9%	94.3%	92.1%	90.2%	92.1%	88.5%	90.6%	92.1%	88.7%	88.7%	86.8%	84.1%
Anc81	ID	94.7%	92.7%	96.6%	94.3%	92.7%	93.6%	90.4%	88.4%	88.4%	93.4%	89.7%	87.4%	85.2%	84.4%
Anc126	ID	97.5%	91.5%	89.7%	88.7%	89.9%	86.7%	92.6%	89.9%	86.5%	90.8%	89.5%	83.3%		
Anc127	ID	90.2%	88.7%	88.0%	88.5%	86.3%	94.9%	88.2%	85.2%	92.1%	87.6%	83.1%			
Anc82	ID	97.7%	95.7%	97.0%	93.8%	86.1%	90.6%	87.1%	85.6%	82.8%	86.3%				
Anc83	ID	97.7%	94.7%	95.5%	85.0%	89.3%	86.3%	84.8%	81.4%	85.6%					
Anc84	ID	92.8%	93.4%	88.4%	85.8%	84.4%	81.6%	84.6%							
Anc110	ID	91.4%	85.0%	88.0%	85.6%	82.4%	80.1%	83.5%							
AAV8	ID	84.5%	86.7%	84.5%	82.4%	80.1%	83.5%								
AAV3	ID	85.4%	82.8%	88.9%	85.7%	80.9%									
Anc113	ID	95.8%	84.1%	83.5%	80.5%										
AAV7	ID	82.4%	81.8%	78.9%											
AAV2	ID	83.3%	81.4%												
AAV1	ID	79.6%													
AAV9	ID														

FIG. 11B

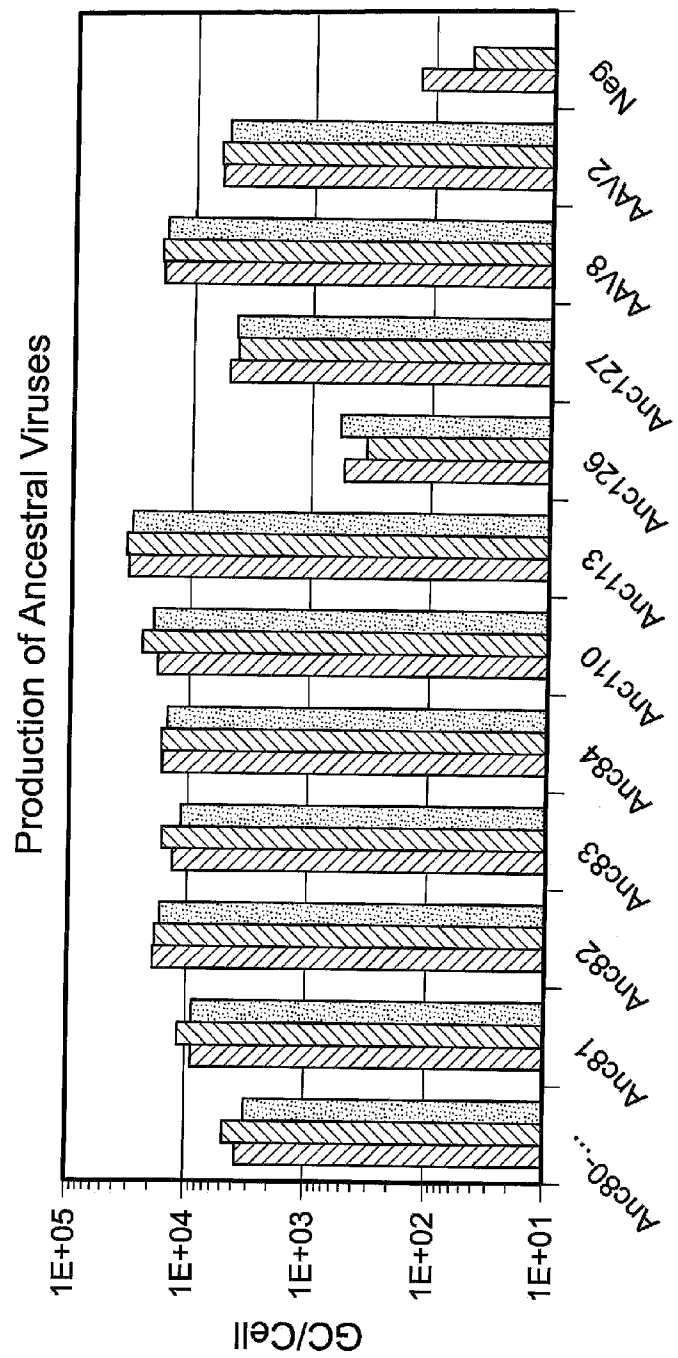


FIG. 12

Viral Production Relative to AAV8 (%)	
Anc80-L0065	21.75%
Anc81	54.14%
Anc82	100.99%
Anc83	76.86%
Anc84	91.20%
Anc110	118.97%
Anc113	183.10%
Anc126	2.71%
Anc127	25.34%
AAV2	32.56%
Neg	0.27%

FIG. 13

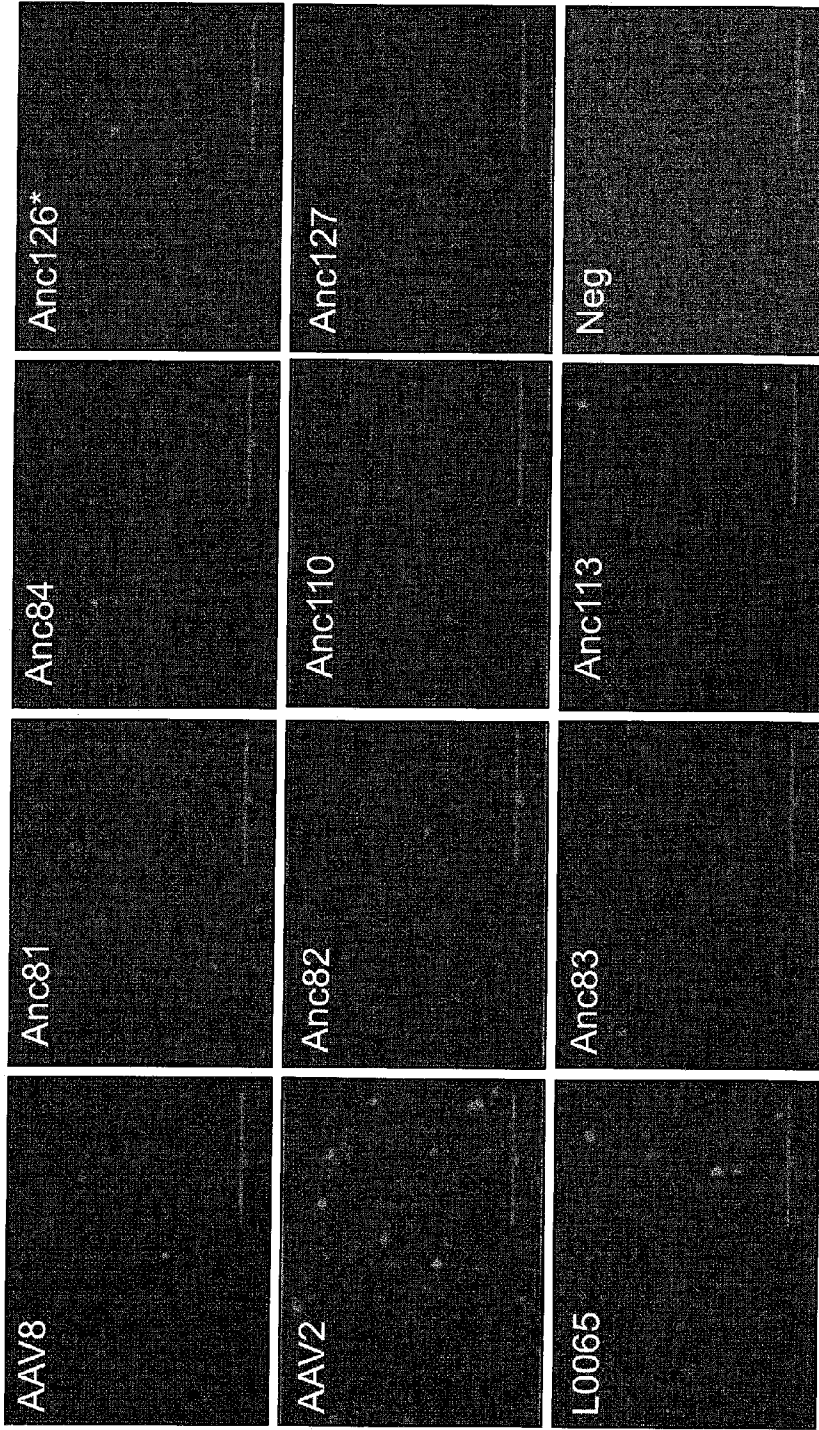


FIG. 14

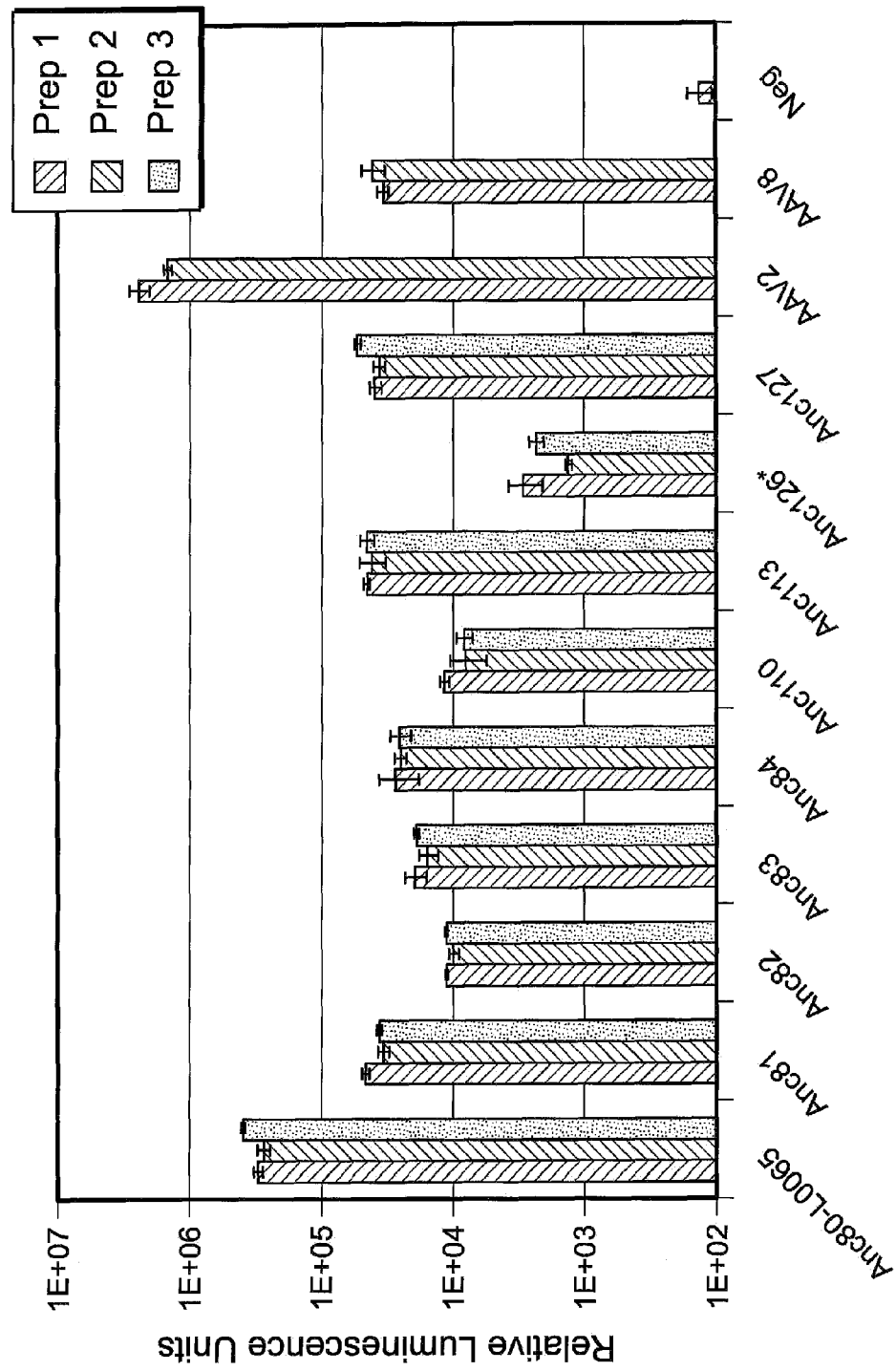


FIG. 15

Viral Infectivity Relative to AAV8 (%)	
Anc80-L0065	850.83%
Anc81	102.26%
Anc82	28.31%
Anc83	47.36%
Anc84	68.78%
Anc110	24.56%
Anc113	117.12%
Anc127	113.78%
AAV2	5225.61%
Neg	0.35%

FIG. 16

AAV8 AAV2		Anc80- L0065	Anc81	Anc82	Anc83	Anc84	Anc110	Anc113	Anc126	Anc127
Infectivity	=	+++	+++	=	-	-	-	=	ND	=
Production	=	-	--	-	=	=	=	++	--	-

Key

- +++ : > 225%
- ++ : 175% - 224.5%
- + : 125 - 174.9%
- = : 75% - 124.9%
- : 25-74.9%
- : 0-24.9%
- ND : Not Determined

FIG. 17

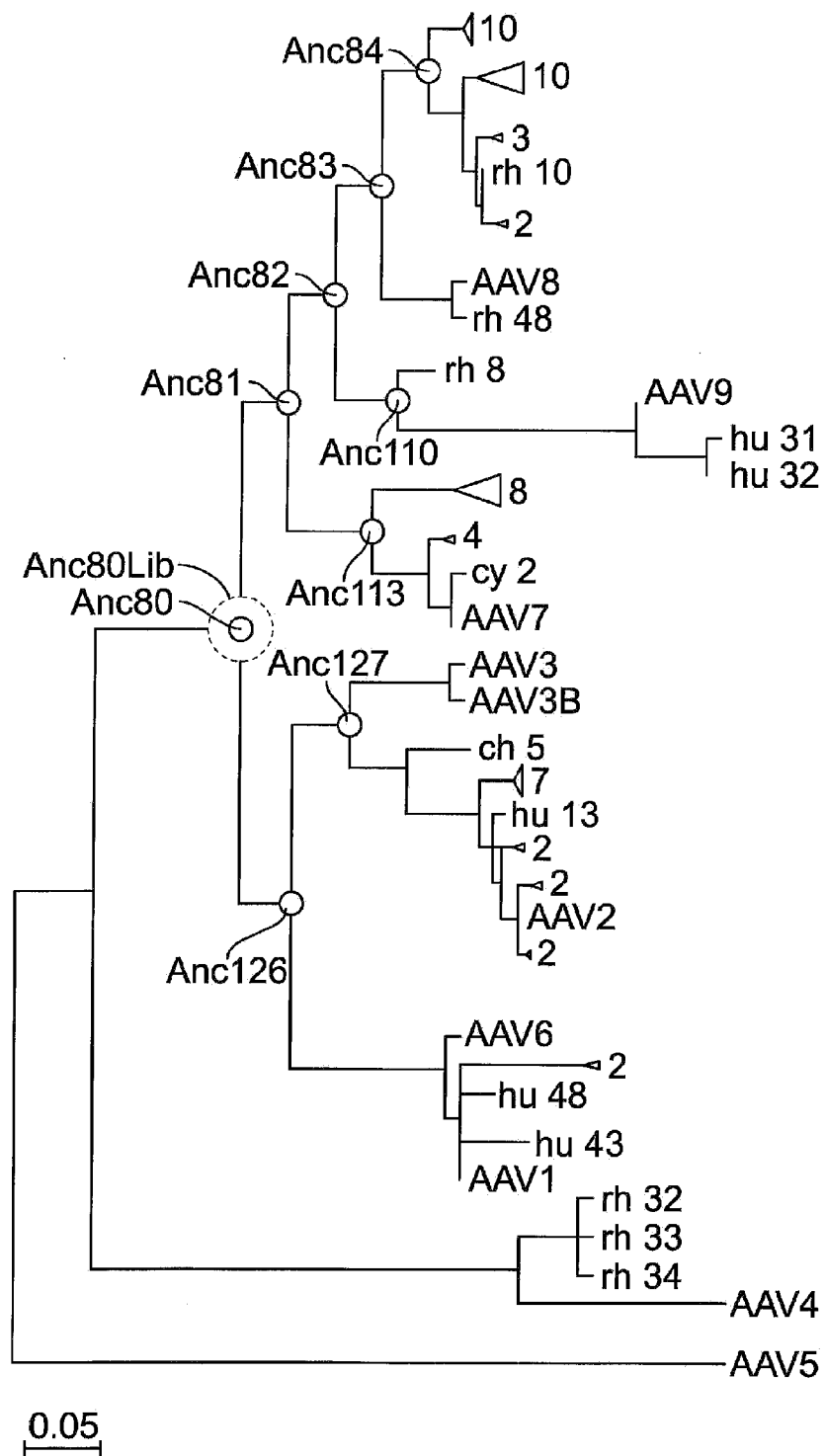


FIG. 18

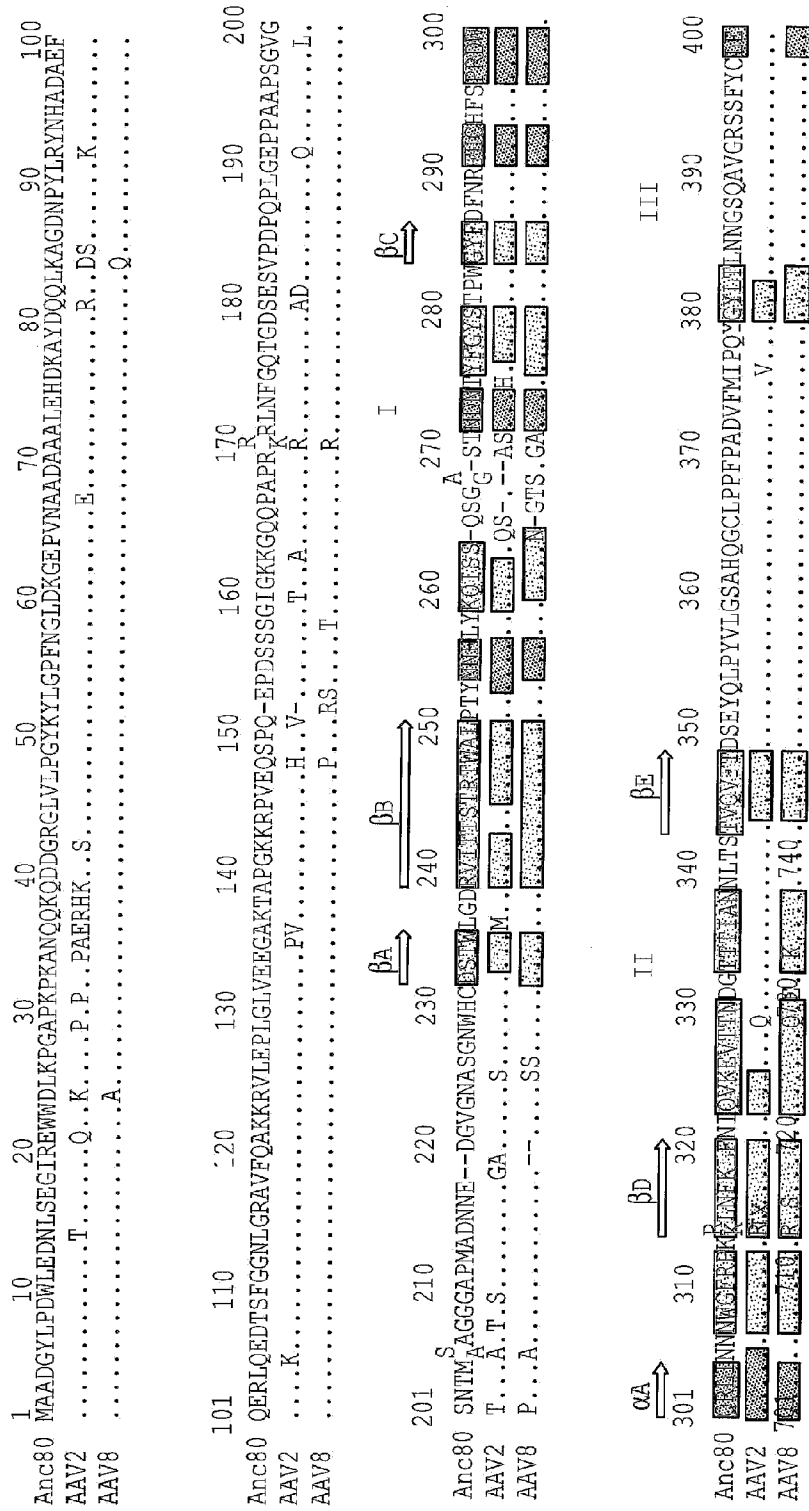


FIG. 19A

	AAV5	AAV2	AAV8	rh10	Anc80L65	Anc80Lib
AAV5		43.0%	42.6%	43.0%	40.5%	39.9-40.5%
AAV2	320		17.1%	15.9%	12.2%	12.0-12.8%
AAV8	317	127		6.5%	9.1%	8.7-10.1%
rh10	320	118	48		8.6%	7.8-8.9%
L0065	301	91	68	64		0-1.5%
Anc80Lib	297-302	89-95	65-75	58-66	0-11	

FIG. 19B

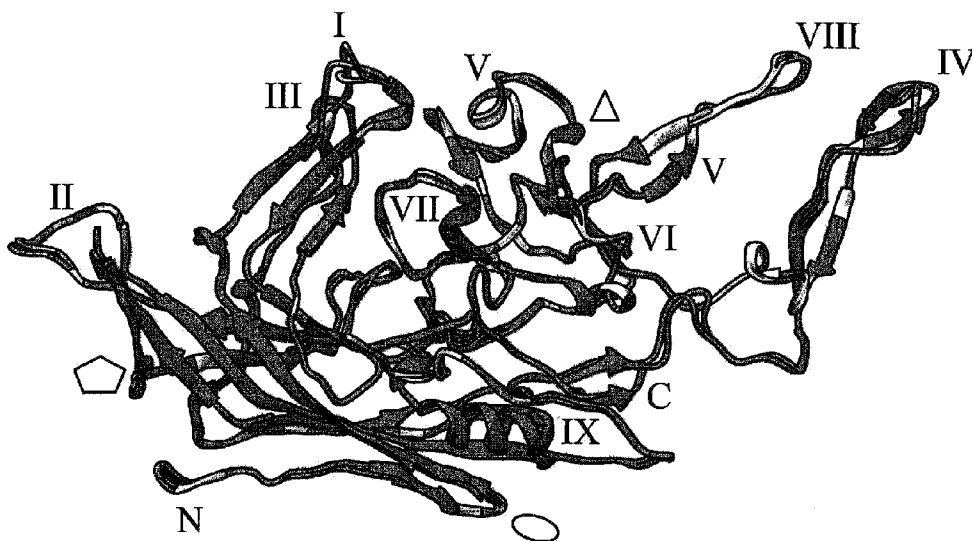


FIG. 19C

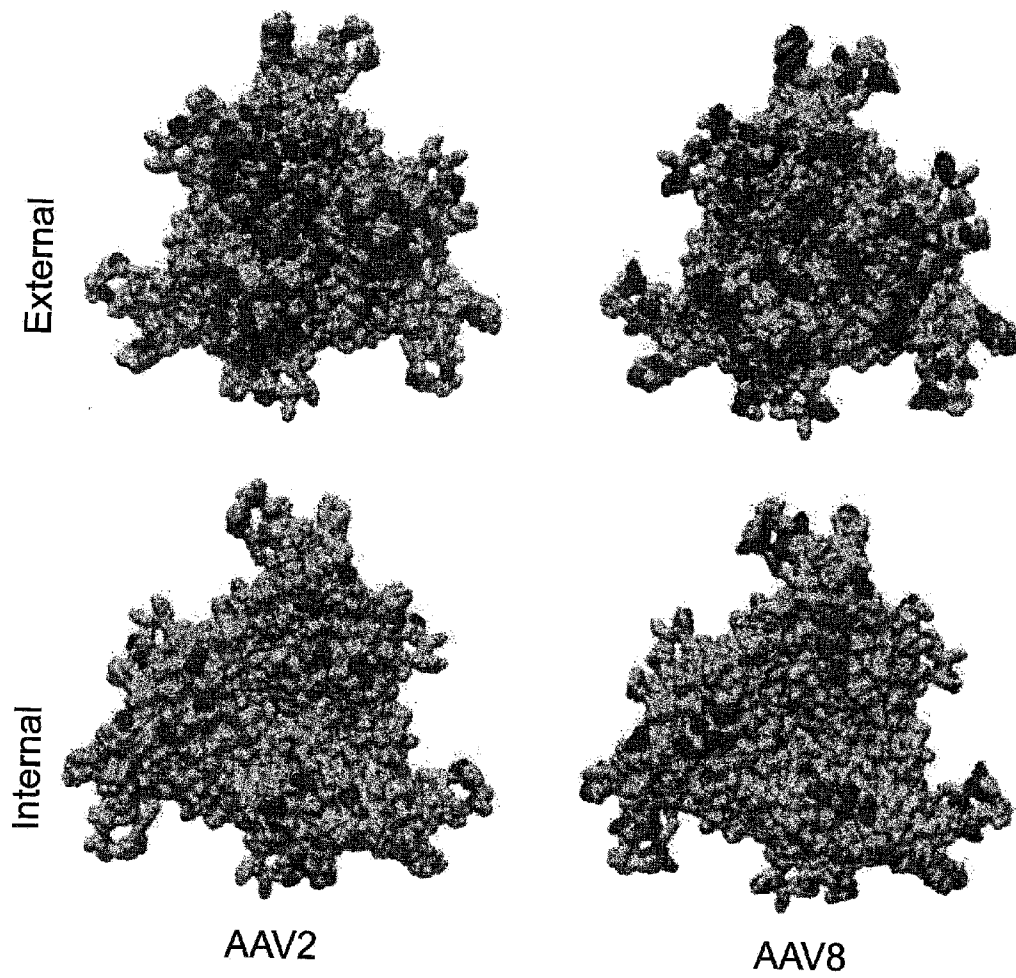


FIG. 19D

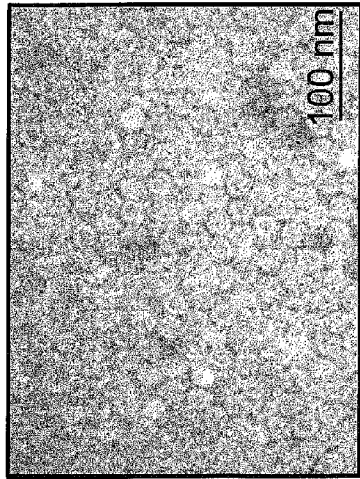
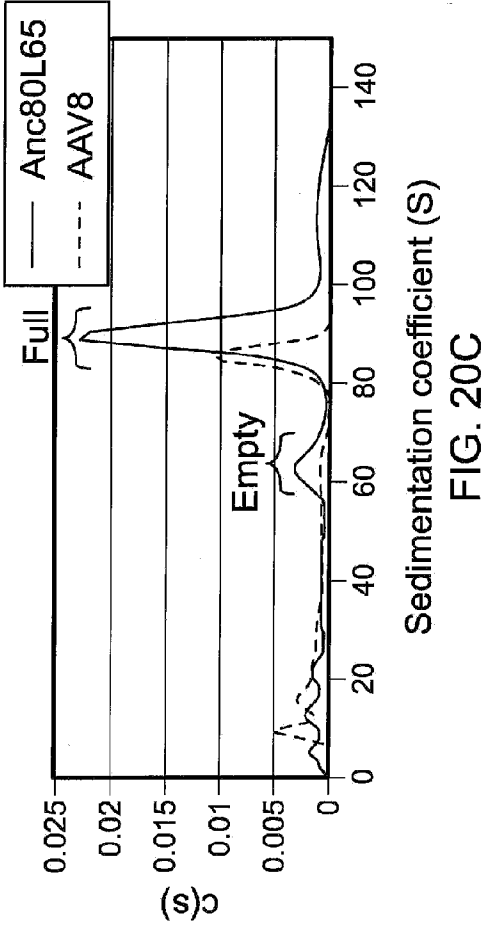
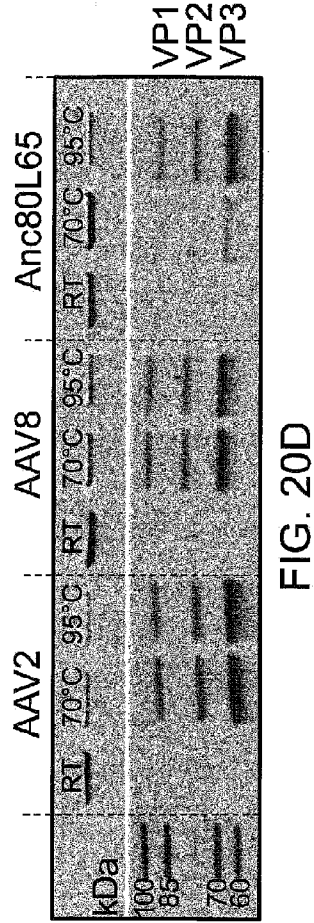
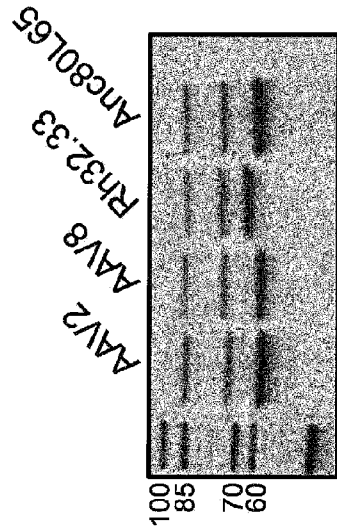


FIG. 20A



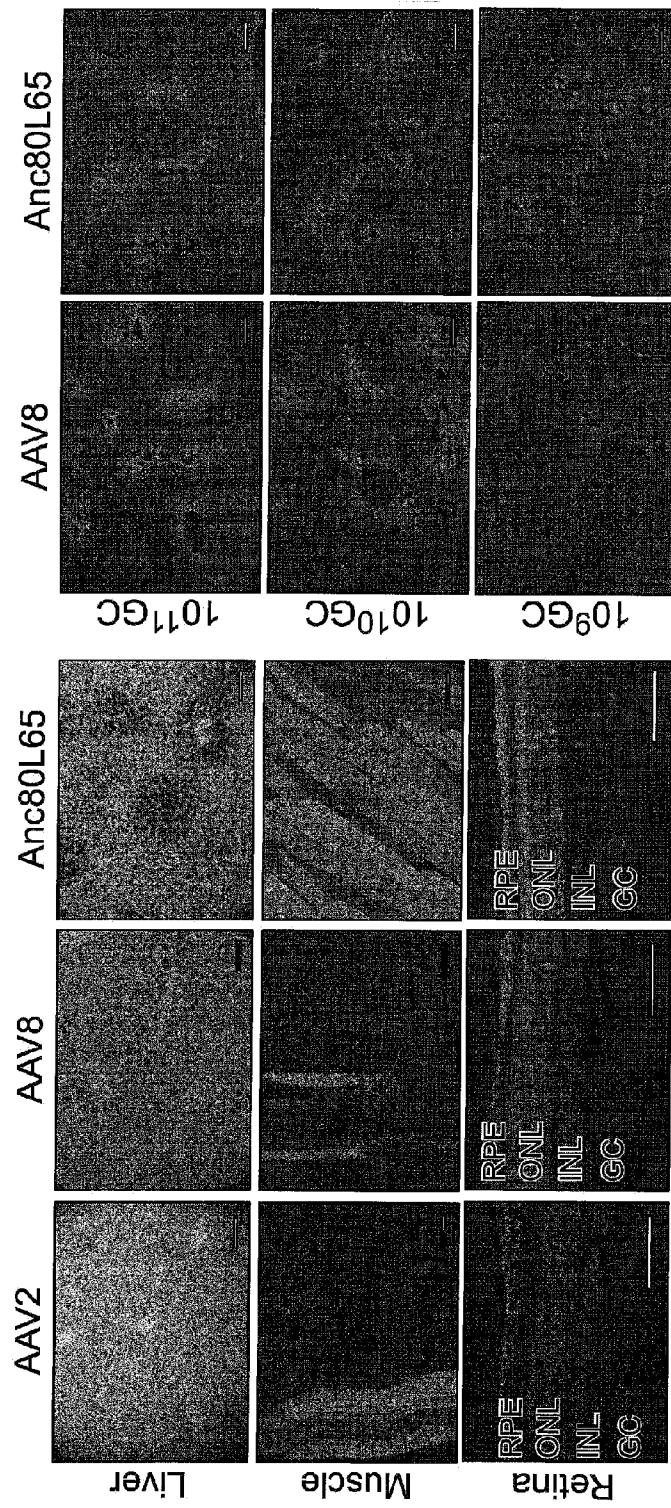


FIG. 21A

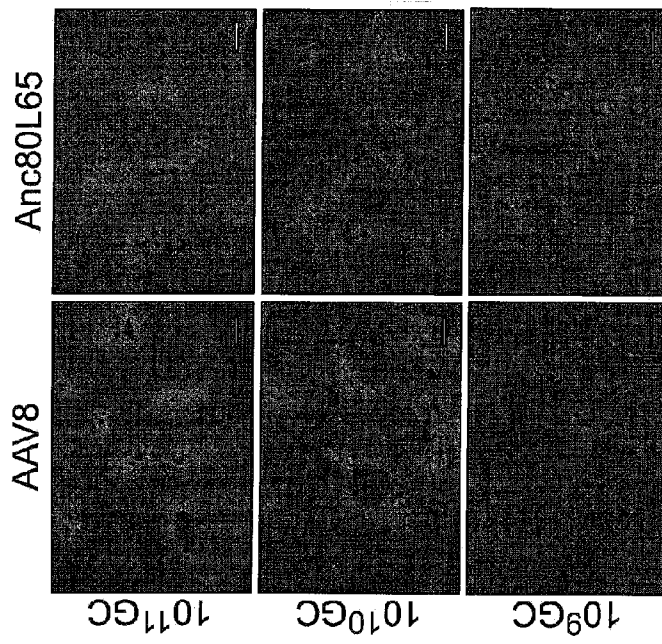


FIG. 21B

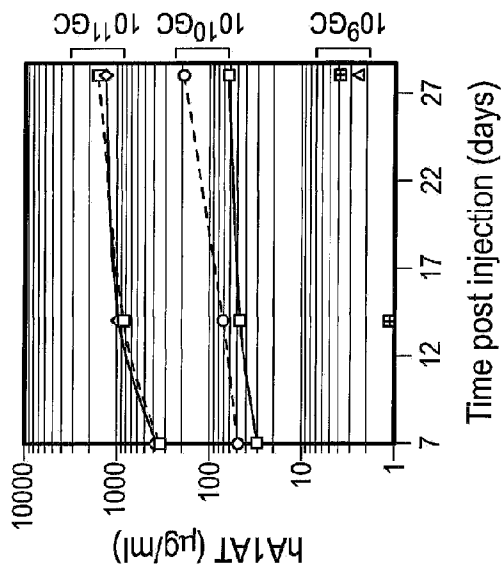


FIG. 21C

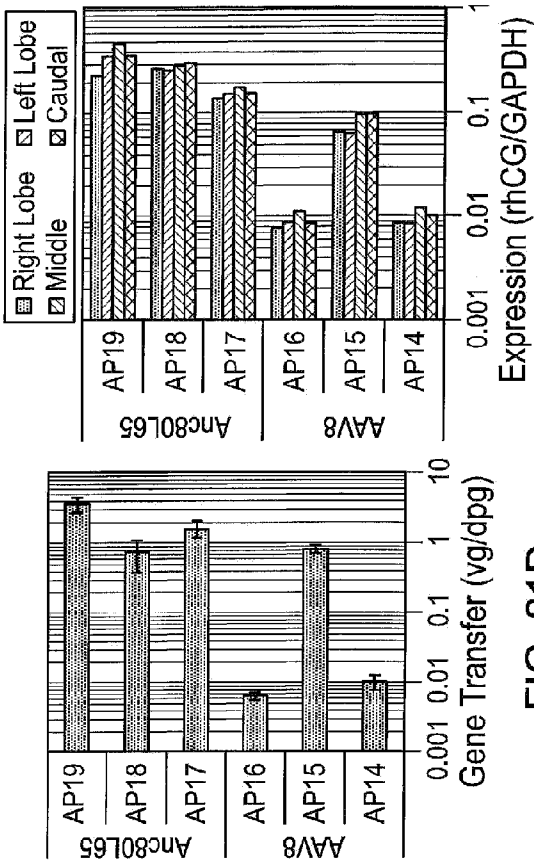


FIG. 21D

FIG. 21E

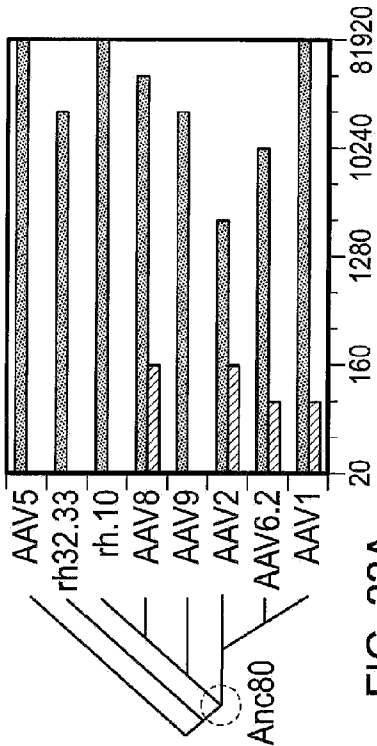


FIG. 22A

AAV8>AAV8		AAV8>Anc80	
% control	NAB8 NAB80	% control	NAB8 NAB80
<0.2%	64	116%	128
<0.2%	128	66%	64
<0.2%	512	<0.2%	512
<0.2%	512	<0.2%	512
<0.2%	256	117%	512

FIG. 22B

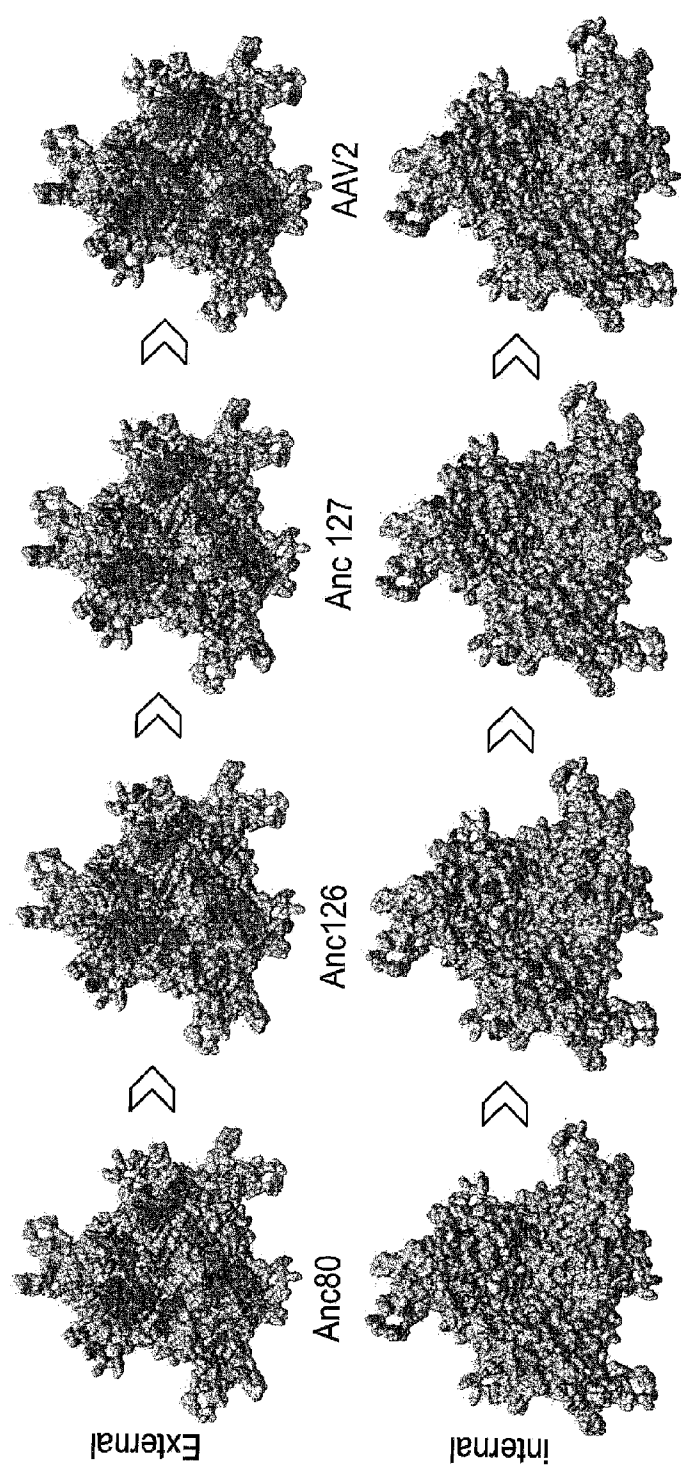


FIG. 22C

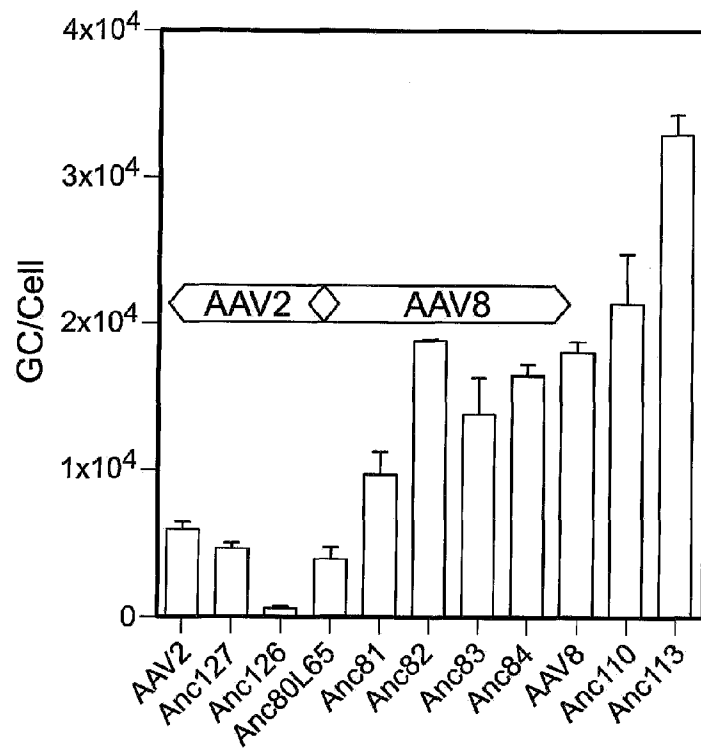


FIG. 23A

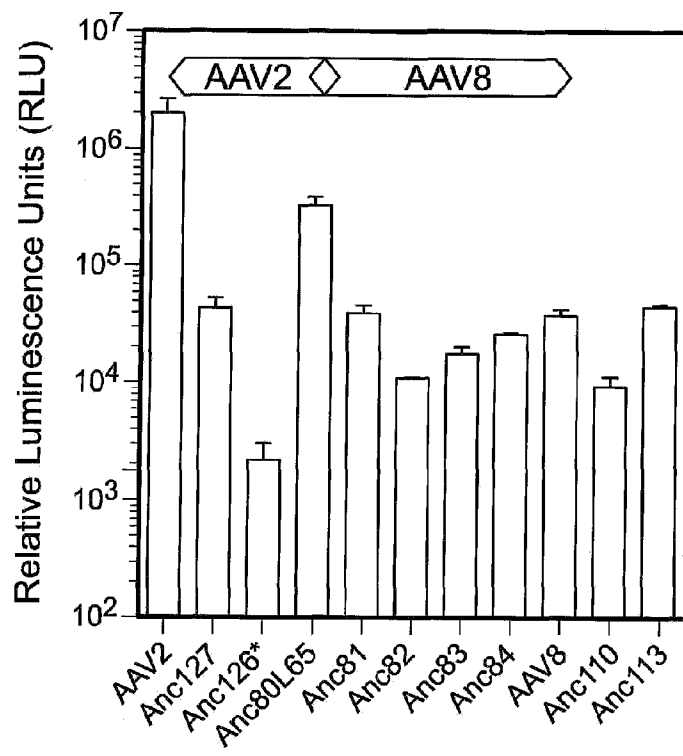


FIG. 23B

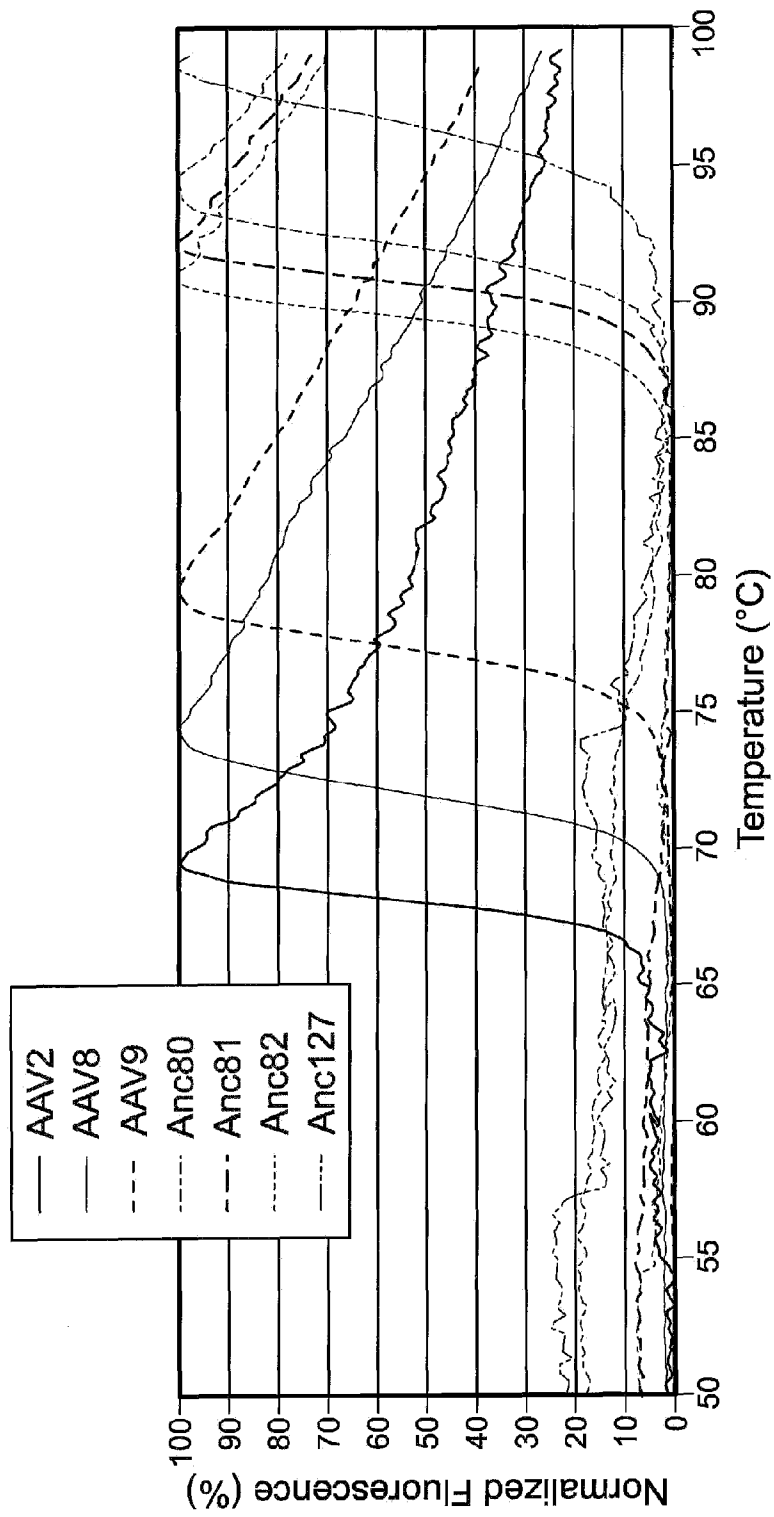


FIG. 23C

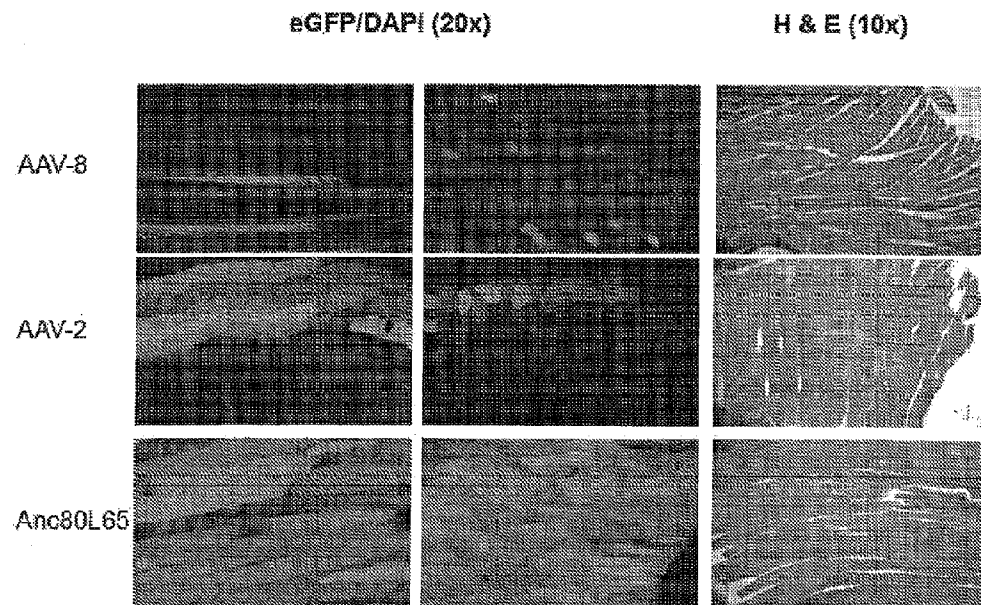


Figure 24

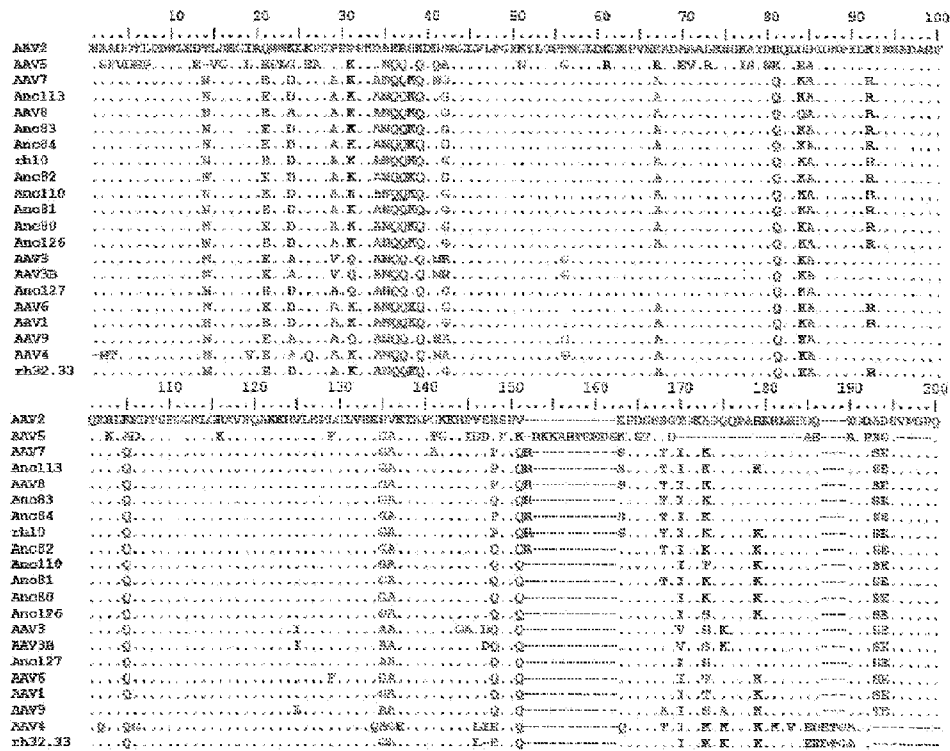


Figure 25

[illegible]

Figure 25 (Continued)

[illegible]

Figure 25 (Continued)

[illegible]

Figure 25 (Continued)

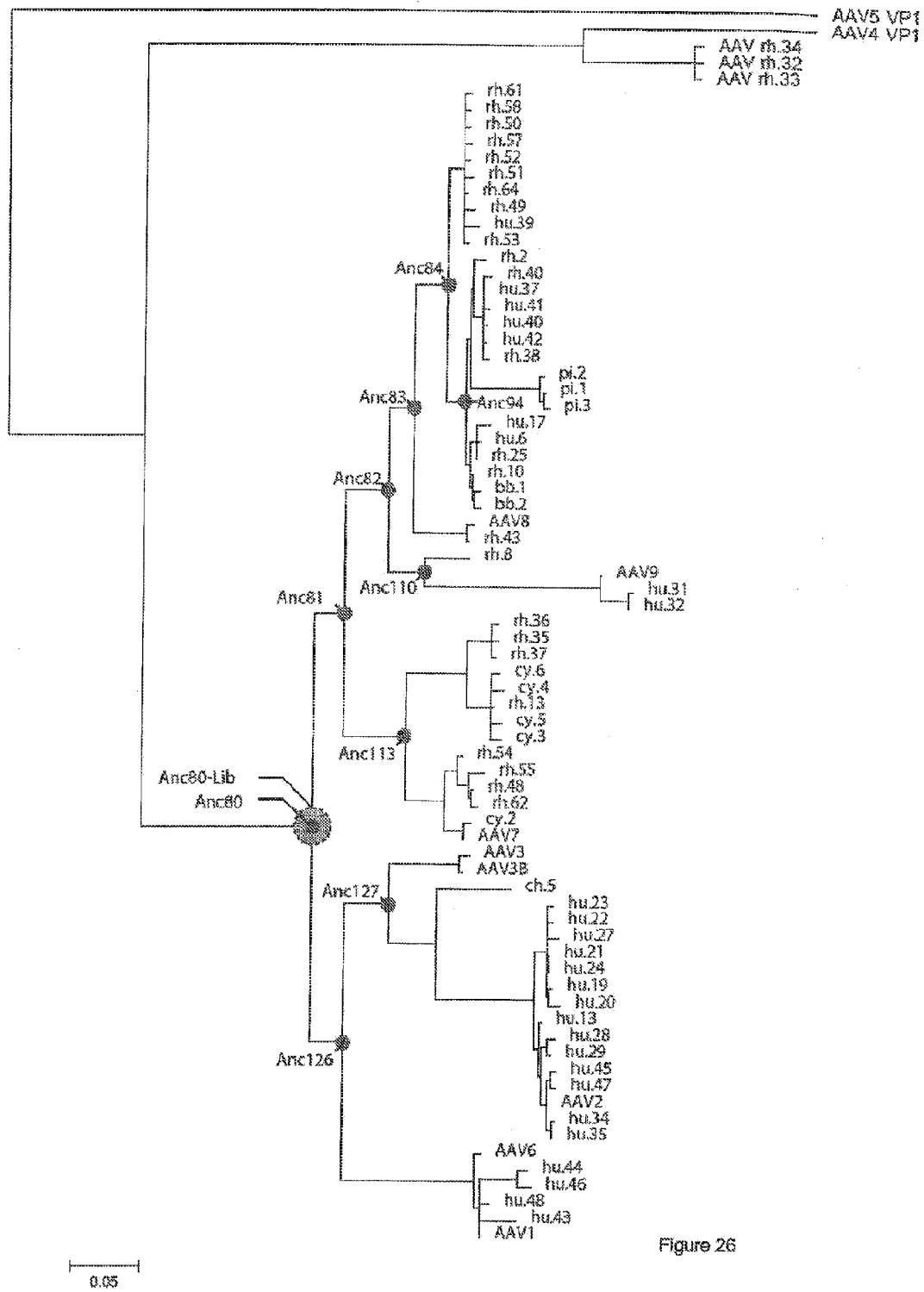


Figure 26

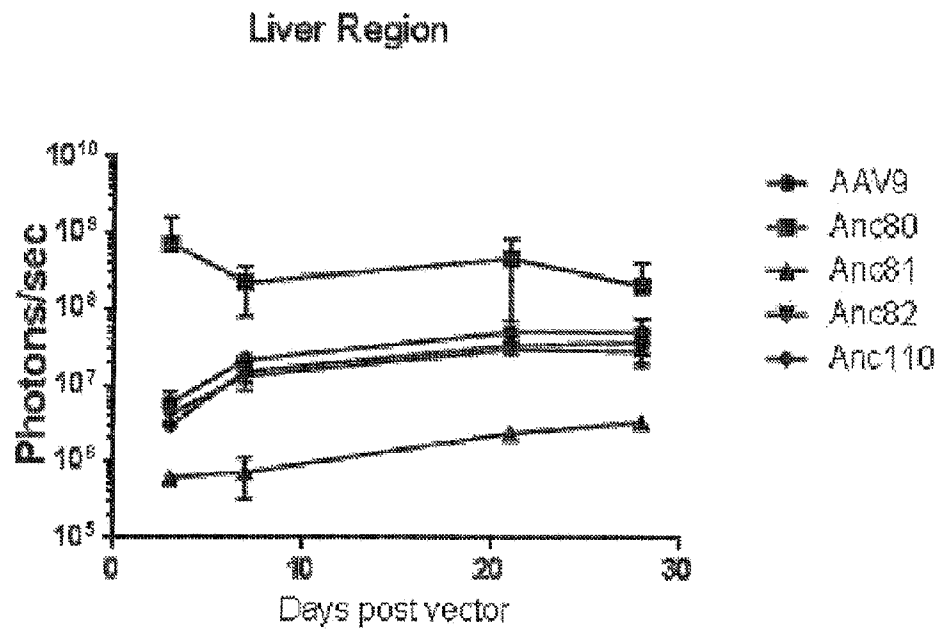


Figure 27

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

