obtained from a desired culture following transfection of host cells with a rAAV containing the molecule encoding the desired product and culturing the cell culture under conditions which permit expression. The expressed product may then be purified and isolated, as desired. Suitable techniques for transfection, cell culturing, purification, and isolation are known to those of skill in the art.

The present invention also provides any one of the embodiments disclosed below and indicated with the numbers 1 to 23.

1. A method of detecting adeno-associated virus (AAV) sequences in a sample, said method comprising the steps of:

(a) subjecting a sample containing DNA to amplification via polymerase chain reaction (PCR) using a first set of primers which specifically amplify a first region of AAV nucleic acid sequences;

(b) optionally subjecting the DNA to further amplification using a second set of primers which specifically amplify a second region which comprises the first region of AAV sequences and sequences which are 5' to the first region, such that AAV 5' extension sequences which anneal to the 5' end of the AAV sequences amplified by the primers for the first region are obtained;

(c) optionally subjecting the DNA to further amplification using a third set of primers which specifically amplify a third region which comprises the first region of AAV sequences and sequences which are 3' to the first region, such that AAV 3' extension sequences which anneal to the 3' end of the AAV sequences amplified by the primers for the first region are obtained,

wherein each of said regions is predetermined based upon the alignment of the nucleic acid sequences of at least two AAV serotypes and each of said regions comprises nucleic acid sequences which are highly conserved over at least 18 base pairs at the 5' end, optionally variable sequences in the middle, and sequences which are highly conserved over at least 18 base pairs at the 3' end of the sequences of the region, relative to the sequences of the at least two aligned AAV serotypes; and

wherein each of the sets of primers consist of a 5' primer and a 3' primer;

wherein the presence of amplified sequences indicates the presence of an AAV in the sample.

2. A method according to claim 1, wherein steps (b) and (c) are performed, and further comprising the step of :

(d) using the amplified sequences to construct a sequence comprising a partial and/or complete AAV gene, thereby isolating an AAV gene sequence from the sample.

3. The method according to claim 2, wherein the first region is in the AAV capsid, the second region extends to the 3' end of the rep genes, and the third region extends to 5' of the AAV 3' ITR, such that step (d) permits the construction of AAV gene sequences comprising the complete AAV capsid gene.

4. The method according to claim 2, further comprising the step of subjecting the DNA to amplification with a fourth set of primers which specifically amplifies a fourth region which extend 5' to the third region.

5. The method according to claim 4, wherein the fourth region extends to 3' of the AAV 5' ITR, such that step (d) permits the construction of AAV gene sequences comprising the complete AAV rep and cap genes.

6. The method according to claim 1 or claim 2, wherein said sample comprises cellular or genomic DNA.

7. The method according to claim 1 or claim 2, wherein said DNA has been extracted from the group consisting of cells, cell culture, tissue, tissue culture, and biological fluids.

8. The method according to claim 1 or claim 2, wherein the first region is about 250 base pairs in length.

9. The method according to claim 1 or 2, wherein the first region is highly conserved over at least about 25 base pairs at the 5' and/or 3' end of the region.

10. The method according to claim 1or 2, wherein the first region is highly conserved over at least about 30 base pairs at the 5' and/or 3' end of the region.

11. The method according to claim 1 or claim 2, wherein the highly conserved sequences of the first region have at least 80% identity among the aligned AAV serotypes at the 5' and/or 3' end of the region.

12. The method according claim 11, wherein the highly conserved sequences of the first region have at least 90% identity among the aligned AAV serotypes at the 5' and/or 3' end of the region.

13. The method according to claim 1 or claim 2, wherein the variable sequences in the middle of the first region have less than 70% identity among the aligned AAV serotypes.

14. The method according to claim 1 or claim 2, wherein the first region spans about bp 2800 to about 3200 of AAV 1, SEQ ID NO:6, and corresponding base pairs in other AAV serotypes.

15. The method according to claim 14, wherein the first region is 257 bp spanning bp 2886 to about 3143 bp of AAV 1, SEQ ID NO:6, and corresponding base pairs in other AAV serotypes.

16. The method according to claim 1, wherein the primers are AV1ns and AV2cas.

17. A method of identifying the serotype of adeno-associated virus (AAV) sequences in a sample as known or unknown, said method comprising the steps of:

(a) obtaining an enzymatic digestion analysis of a sample containing DNA molecules comprising AAV sequences which span all or a portion of bp 2886 to 3143 of AAV1, SEQ ID NO:1, and corresponding regions of other AAV serotypes; and

(b) comparing the enzymatic digestion analysis from the sample to enzymatic digestion analysis for corresponding regions of one or more AAV serotypes,

thereby identifying the AAV sequences in the sample as being from one of the one or more AAV serotypes or from an unknown serotype.

18. The method according to claim 17, wherein the AAV sequences were obtained by the method of claim 1.

19. A diagnostic kit for detecting the presence of an unknown adeno-associated virus (AAV) in a sample, said kit comprising:

(a) a first set of primers which specifically amplify a first region of AAV nucleic acid sequences;

(b) optionally a second set of primers specific for a second region of the AAV nucleic acid sequences which comprises the first region of AAV sequences and sequences which are 5' to the first region, such that AAV 5' extension sequences which anneal to the 5' end of the AAV sequences amplified by the primers for the first region are obtained;

(c) optionally a third set of primers which specifically amplify a third region which comprises the first region of AAV sequences and sequences which are 3' to the first region, such that AAV 3' extension sequences which anneal to the 3' end of the AAV sequences amplified by the primers for the first region are obtained,

wherein each of said regions is predetermined based upon the alignment of the nucleic acid sequences of at least two AAV serotypes and each of said regions comprises nucleic acid sequences which are highly conserved over at least 18 base pairs at the 5' end, optionally variable sequences in the middle, and sequences which are highly conserved over at least 18 base pairs at the 3' end of the sequences of the region, relative to the sequences of the at least two aligned AAV serotypes

wherein each of the sets of primers consist of a 5' primer and a 3' primer.

20. A method for isolating novel adeno-associated viruses (AAV) from a cell, said method comprising the steps of: (a) infecting the cell with a virus which provides helper functions to said AAV; (b) isolating infectious clones containing AAV; (c) sequencing the isolated AAV; and (d) comparing the sequences of the isolated AAV to known AAV serotypes, whereby differences in the sequences of the isolated AAV and known AAV

serotypes indicates the presence of a novel AAV.

21. The method according to claim 20, wherein said virus is an adenovirus.

22. The method according to claim 21, wherein said adenovirus is of human or non-human primate origin.

23. A novel adeno-associated virus (AAV) serotype identified by a method according to claim 2 or claim 20.

The following examples illustrate several aspects and embodiments of the invention.

EXAMPLES

Example 1: PCR amplification, cloning and characterization of novel AAV sequences.

Tissues from nonhuman primates were screened for AAV sequences using a PCR method based on oligonucleotides to highly conserved regions of known AAVs. A stretch of AAV sequence spanning 2886 to 3143 bp of AAV1 [SEQ ID NO:6] was selected as a PCR amplicon in which a hypervariable region of the capsid protein (Cap) that is unique to each known AAV serotype, which is termed herein a "signature region," is flanked by conserved sequences. In later analysis, this signature region was shown to be located between conserved residues spanning hypervariable region 3.

An initial survey of peripheral blood of a number of nonhuman primate species revealed detectable AAV in a subset of animals from species such as rhesus macaques, cynomologous macaques, chimpanzees and baboons. However, there were no AAV sequences detected in some other species tested, including Japanese macaques, pig-tailed macaques and squirrel monkeys. A more extensive analysis of vector distribution was conducted in tissues of rhesus monkeys of the University of Pennsylvania and Tulane colonies recovered at necropsy. This revealed AAV sequence throughout a wide array of tissues.

A. *Amplification of an AAV signature region*

DNA sequences of AAV1-6 and AAVs isolated from Goose and Duck were aligned to each other using "Clustal W" at default settings. The alignment for AAV1-6, and

including the information for the novel AAV7, is provided in Fig. 1. Sequence similarities among AAVs were compared.

obtained from a desired culture following transfection of host cells with a rAAV containing the molecule encoding the desired product and culturing the cell culture under conditions which permit expression. The expressed product may then be purified and isolated, as desired. Suitable techniques for transfection, cell culturing, purification, and isolation are known to those of skill in the art.

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CLAIMS

1. An adeno-associated virus (AAV) comprising at least an AAV8 capsid having an amino acid sequence having the sequence of amino acids 1 to 738 of SEQ ID NO: 95 or a sequence having at least 95% identity therewith, wherein said AAV further comprises a minigene having AAV inverted terminal repeats (ITRs) and a heterologous gene operably linked to regulatory sequences which direct its expression in a host cell.

2. The AAV according to claim 1 wherein the ITRs are from AAV 2.

3. A recombinant capsid protein comprising an AAV8 protein selected from the group consisting of:

vp1 capsid protein, amino acids (aa) 1 to 738 of SEQ ID NO: 95
vp2 capsid protein, aa 138 to 738 of SEQ ID NO: 95;
vp3 capsid protein, aa 203 to 738 of SEQ ID NO: 95;
or a sequence having at least 95% identity with vp1, vp2 or vp3.

4. An adeno-associated virus (AAV) comprising at least an AAV8 vp3 capsid having an amino acid sequence having the sequence of amino acids 203 to 738 of SEQ ID NO: 95 or a sequence having at least 95% identity therewith, wherein said AAV further comprises a minigene having AAV inverted terminal repeats (ITRs) and a heterologous gene operably linked to regulatory sequences which direct its expression in a host cell.

5. An isolated or synthetic nucleic acid molecule encoding a protein according to claim 3.

6. An isolated or synthetic nucleic acid molecule encoding a novel adeno-associated virus (AAV) 8 capsid protein having an amino acid sequence 1 to 738 of SEQ ID NO: 95.

7. An isolated or synthetic nucleic acid molecule encoding a fragment of an

adeno-associated virus 8 capsid protein, said nucleic acid sequence selected from the group consisting of:

vpl, nt 2121 to 4335 of SEQ ID NO: 4;

vp2, nt 2532 to 4335 of SEQ ID NO: 4;

vp3, nt 2730 to 4335 of SEQ ID NO: 4;

and a sequence sharing at least 95% identity with vpl, vp2 or vp3.

8. A molecule according to any of claims 5 to 7, wherein said molecule is a plasmid.

9. A molecule according to any of claims 5 to 7, wherein said molecule further comprises a functional AAV *rep* gene.

10. A method of generating a recombinant adeno-associated virus (AAV) comprising an AAV serotype capsid comprising the steps of culturing a host cell containing: (a) a molecule according to any of claims 5 to 7 which encodes an adeno-associated virus capsid; (b) a functional rep gene; (c) a minigene comprising AAV inverted terminal repeats (ITRs) and a transgene; and (d) sufficient helper functions to permit packaging of the minigene into the AAV capsid protein.

11. An in vitro host cell transfected with an adeno-associated virus according to any of claims 1 to 2 or a molecule according to any of claims 5 to 9.

12. A composition comprising an AAV according to claim 1 or claim 4, and a physiologically compatible carrier.

13. A composition comprising a molecule according to any of claims 6 to 9 and a physiologically compatible carrier.

14. An adeno-associated virus (AAV) comprising an AAV8 vp2 capsid having an amino acid sequence having the sequence of amino acids 138 to 738 of SEQ ID NO: 95 or a sequence having at least 95% identity therewith, wherein said AAV further comprises a

minigene having AAV inverted terminal repeats (ITRs) and a heterologous gene operably linked to regulatory sequences which direct its expression in a host cell.

15. The AAV according to claim 1, 4 or 14, wherein the heterologous gene is an immunoglobulin.

16. The AAV according to claim 1, 4 or 14, wherein the heterologous gene is a Factor VIII.

17. The AAV according to claim 1, 4 or 14, wherein the heterologous gene is a Factor IX.