

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
18 December 2003 (18.12.2003)

PCT

(10) International Publication Number  
**WO 03/104392 A2**

(51) International Patent Classification<sup>7</sup>: C12N

(21) International Application Number: PCT/US02/38423

(22) International Filing Date: 2 December 2002 (02.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/341,919 18 December 2001 (18.12.2001) US

(71) Applicant (for all designated States except US): **UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL** [US/US]; 308 Bynum Hall, Campus Box 4105, Chapel Hill, NC 27599-4105 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SAMULSKI, Richard, Jude** [US/US]; 102 Darlin Circle, Chapel Hill, NC 27514 (US). **RABINOWITZ, Joseph, E.** [US/US]; 401 NC Highway 54 Bypass, Carrboro, NC 27510 (US).

(74) Agent: **MYERS, BIGEL, SIBLEY & SAJOVEC**; P.O. Box 37428, Raleigh, NC 27627 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



**WO 03/104392 A2**

(54) Title: IMPROVED REAGENTS AND METHODS FOR PRODUCING PARVOVIRUSES

(57) Abstract: The present invention provides polynucleotides comprising chimeric parvovirus (e.g., AAV) rep coding sequences. The polynucleotides optionally encode parvovirus cap coding sequences. Also provided are vectors and cells comprising the inventive polynucleotides. Further provided are improved methods of making hybrid parvovirus stocks using the chimeric rep coding sequences of the invention.

## Improved Reagents and Methods for Producing Parvoviruses

5

### Related Application Information

This application claims the benefit of United States Provisional Application Serial No. 60/341,919, Filed 18 December 2001; the disclosure of which is incorporated herein by reference in its entirety.

10

### Statement of Federal Support

This invention was made, in part, with government support under grant numbers DK54419, HL51818 and GM59290 from the National Institutes of Health. The United States government has certain rights to this invention.

15

### Field of the Invention

This invention relates to improved reagents and methods for producing virus, and more particularly relates to improved reagents and methods for producing parvovirus stocks.

20

### Background of the Invention

The adeno-associated viruses (AAV) are members of the family *Parvoviridae* and the genera *Dependoviruses*. Serotypes 1 through 4 were originally identified as contaminants of adenovirus preparations (Carter and Laughlin (1984) *in, The Parvoviruses* p. 67-152 New York, N.Y.) whereas type 25 5 was isolated from a patient wart that was HPV positive. To date, seven molecular clones have been generated representing the serotypes of AAV (Bantel-Schaal et al. (1999) *J. Virol.* **73**: 939, Chiorini et al. (1999) *J. Virol.* **73**:1309, Muramatsu et al. (1996) *Virology* **221**:208, Rutledge et al. (1998) *J.* 30 *Virol.* **72**:309, Srivastava et al. (1983) *J. Virol.* **45**:555, Xiao et al. (1999) *J. Virol.* **73**:3994). These clones have provided valuable reagents for studying the molecular biology of serotype specific infection. Transduction of these viruses naturally results in latent infections, with completion of the life cycle

generally requiring helper functions not associated with AAV viral gene products. As a result, all of these serotypes are classified as non-pathogenic and are believed to share a safety profile similar to the more extensively studied AAV type 2 (Carter and Laughlin (1984) *in, The Parvoviruses* p. 67-  
5 152 New York, N.Y.).

The extensive development of AAV type 2 as a vector has been facilitated by 30 years of studying its biology *in vitro*. Recombinant AAV type 2 (rAAV2) has proven to be a suitable gene transfer vector in many different organisms (Monohan and Samulski (2000) *Gene Ther.* **7**:24, Rabinowitz and  
10 Samulski (1998) *Curr. Opin. Biotechnol.* **9**:470). As the number of applications evaluating gene transfer increases *in vitro* and *in vivo*, limitations to efficient rAAV type 2 transduction have become apparent (Bartlett et al. (2000) *J. Virol.* **74**:2777, Davidson et al. (2000) *Proc. Natl. Acad. Sci. USA* **97**:3428, Hansen et al. (2001) *J. Virol.* **75**:4080, Samulski et al. (1999) *in, Adeno-associated*  
15 *viral vectors* Cold Spring Harbor, N.Y., Walters et al. (2000) *J. Virol* **74**:535, Xiao et al. (1999) *J. Virol.* **73**:3994, Zabner et al. (2000) *J. Virol.* **74**:3852). The natural tropism of any virus, including rAAV type 2, is a fundamental limitation to efficient gene transfer. With the identification of the AAV type 2 receptor, the requirements for efficient entry in target cells have become a critical topic  
20 of study (Summerford and Samulski (1998) *J. Virol.* **72**:1438). Efforts have been made to overcome these restrictions by broadening the host range using either bispecific antibodies to the virion shell (Bartlett et al. (1999) *Nat. Biotechnol.* **17**:181) or through capsid insertional mutagenesis (International patent publication WO 00/28004; Rabinowitz et al. (1999) *Virology* **265**:274;  
25 Girod et al. (1999) *Nat. Med.* **5**:1052, Wu et al. (2000) *J. Virol.* **74**:8635). While these efforts are beginning to bear fruit, utilizing the other serotypes of AAV may yet provide additional resources for making safe and efficient gene transfer vectors. To this end, a number of studies have begun to show the utility of serotype specific vectors *in vitro* and *in vivo* (International patent  
30 publication WO 00/28004; Chao et al. (2000) *Mol. Ther.* **2**:619, Chiorini et al. (3999) *J. Virol.* **73**:1309, Chiorini et al. (1998) *Mol. Cell. Biol.* **18**:5921, Davidson et al. (2000) *Proc. Natl. Acad. Sci. USA* **97**:3428, Hildinger et al. (2001) *J. Virol.* **75**:6199, Xiao et al. (1999) *J. Virol.* **73**:3994, Zabner et al.

(2000) *J. Virol.* **74**:3852). In general, each of these studies uncovered broader cell type specificity with increased gene transfer *in vivo*.

International patent publication WO 00/28004 describes "hybrid" parvoviruses that result from cross-packaging of a recombinant parvovirus genome in a capsid from a different parvovirus. There is a need in the art to  
5 further characterize the virus-specific vector components (*e.g.*, the cis-acting terminal repeats [TRs] and the trans-acting virion shell and replication proteins) to produce a new generation of optimized hybrid parvovirus vectors and reagents, and methods for producing the same.

10

### **Summary of the Invention**

The present invention is based, in part, on the discovery of new trans-acting elements that mediate parvovirus replication and the production of new parvovirus particles. The parvovirus replication proteins contain functional  
15 domains that are implicated in different aspects of viral propagation, *i.e.*, genomic DNA replication and capsid assembly/packaging. Accordingly, based on an understanding of these elements, the present invention provides improved reagents (*e.g.*, polynucleotides encoding parvovirus replication and/or capsid proteins, and vectors and cells comprising the same) for  
20 producing parvoviruses. Also provided are improved methods for producing hybrid parvoviruses (*i.e.*, the viral terminal repeats and capsid are from different parvoviruses) using the inventive reagents.

Accordingly, as one aspect, the present invention provides a polynucleotide comprising parvovirus rep coding sequences and parvovirus  
25 cap coding sequences, the rep coding sequences encoding a DNA binding domain from a first parvovirus; the rep coding sequences further encoding a capsid interacting domain from a different parvovirus from the first parvovirus; and the cap coding sequence comprising sequences from the different parvovirus.

30 As another aspect, the invention provides a polynucleotide comprising adeno-associated virus (AAV) rep coding sequences and AAV cap coding sequences, the rep coding sequences having a 5' portion and a 3' portion; the 5' portion comprising rep coding sequences from a first AAV; the 3' portion

comprising rep coding sequences from a different AAV from the first AAV; and the cap coding sequences comprising sequences from the different AAV.

5 A cell comprising parvovirus rep coding sequences and parvovirus cap coding sequences, the rep coding sequences encoding a DNA binding domain from a first parvovirus; the rep coding sequences further encoding a capsid interacting domain from a different parvovirus from the first parvovirus; the cap coding sequences comprising sequences from said different parvovirus; and the rep coding sequences being stably integrated into the genome of the cell.

10 As a further aspect, the invention provides a cell comprising adeno-associated virus (AAV) rep coding sequences and AAV cap coding sequences, the rep coding sequences having a 5' portion and a 3' portion; the 5' portion comprising rep coding sequences from a first AAV; the 3' portion comprising rep coding sequences from a different AAV from the first AAV; the cap coding sequences comprising sequences from the different AAV; and the rep coding sequences being stably integrated into the genome of the cell.

15 Also provided is a method of producing a recombinant hybrid parvovirus particle, comprising providing to a cell permissive for parvovirus replication: (a) a recombinant parvovirus template comprising (i) a heterologous nucleotide sequence, and (ii) a parvovirus terminal repeat sequence; (b) parvovirus rep coding sequences and parvovirus cap coding sequences; the rep coding sequences encoding a DNA binding domain from a first parvovirus that interacts with the parvovirus terminal repeat to mediate replication of the recombinant parvovirus template; the rep coding sequences further encoding a capsid interacting domain from a different parvovirus from the first parvovirus; and the cap coding sequences comprising sequences from the different parvovirus; wherein the parvovirus terminal repeat sequence may be from the first parvovirus but not from the different parvovirus; under conditions sufficient for the replication and packaging of the recombinant parvovirus template; whereby recombinant hybrid parvovirus particles comprising the parvovirus capsid encoded by the cap coding sequences and packaging the recombinant parvovirus template are produced in the cell.

20 25 30 As still a further aspect, the present invention provides a method of producing a recombinant hybrid adeno-associated virus (rAAV) particle, comprising providing to a cell permissive for AAV replication: (a) a rAAV

template comprising (i) a heterologous nucleotide sequence, and (ii) an AAV terminal repeat sequence; and (b) AAV rep coding sequences and AAV cap coding sequences; the rep coding sequences having a 5' portion and a 3' portion; the 5' portion comprising rep coding sequences from a first AAV that  
5 interacts with the AAV terminal repeat to mediate replication of the rAAV template; the 3' portion comprising rep coding sequences from a different AAV from the first AAV; and the cap coding sequences comprising sequences from the different AAV; wherein the AAV terminal repeat sequence may be from the first AAV but not from the different AAV; under conditions sufficient for the  
10 replication and packaging of the rAAV template; whereby infectious recombinant hybrid AAV particles comprising the AAV capsid encoded by the cap coding sequences and packaging the rAAV template are produced in the cell.

These and other aspects of the invention are set forth in more detail in  
15 the description of the invention below.

### **Brief Description of the Drawings**

**Figure 1** illustrates the construction and characterization of AAV serotype clones. Panel A) The capsid domain of each AAV serotype,  
20 generated by PCR, was cloned into the pBS+AAV2rep plasmid. The serotype specific capsid insertions (gray rectangles) are listed in order from type 1 to 5. Restriction sites are shown in the AAV2 diagram. Additionally, modifications containing the coding region of the carboxy termini of the Rep coding domain (gray striped) for each serotype were cloned into the constructs as needed.  
25 Panel B) An acrylamide gel of AAV serotype constructs pXR1 through 5 digested with *Bst*NI. White arrowheads point to common bands in the backbone and replication gene. A 50 bp DNA ladder (Amersham Pharmacia Biotech) flanks the lanes on which the constructs were loaded.

30 **Figure 2** depicts Western analysis of lysates derived from serotype-specific transfections. Twenty-four hours post-transfection, 5 micrograms of total protein was loaded into each well. After transfer the blots were incubated with: Panel A) The anti-rep monoclonal antibody 1F11, (the sizes of the proteins listed on the right side); or Panel B) The anti-capsid monoclonal

antibody B1, with the capsid subunits listed on the right side. The serotype specific helpers used in the transfection are listed above each blot. Panel C) The B1 recognition site, as determined by Wobus et al. (2000) *J. Virol.* 74:9281, is shown at the bottom of the figure. The amino acid sequence from this region for all five serotypes is shown. Asterisks indicate amino acids identical to AAV type 2.

**Figure 3** illustrates a Hirt analysis of low molecular weight DNA isolated from 293 cells 24 hours after the three plasmid transfection with serotype specific plasmids. Equal amounts (2.5 micrograms) of undigested (lanes 1-5) and *DpnI* digested DNA (lanes 7-11) from each serotype sample was loaded onto a 1% agarose gel (lane 6 DNA ladder). The resulting blot was probed with a 735 bp fragment of the GFP gene. Input DNA digested with *DpnI* reveals the replicating monomer (**M**) and dimer transgene (**D**). The lower bands in lanes 7-11 are *DpnI* digestion products of input plasmids.

**Figure 4** shows the transduction efficiency of fractions collected from a heparin sepharose affinity column for rAAV serotypes 1 through 5. The elution conditions were optimized originally for AAV2 (Zolotukhin et al. (1999) *Gene Ther.* 6:973). Numbered fractions were collected in 0.5 ml volumes, W (waste) was collected in a single 10 ml fraction, and C (control) was an aliquot of the 1 ml volume of virus applied to the column. Infections were performed in reference cell lines, with between 1/100 to 20 microliters of each fraction. Each bar represents the average of three separate infections, with the standard deviation indicated by the error bar.

**Figure 5** depicts the transduction efficiency of rAAV serotype clones in CHO mutant and reference cell lines. Cells (cell types are specified in the legend on right) were transduced with approximately 0.3 transducing units (TU)/cell, as determined relative to the reference cell lines (HeLa cells for AAV serotypes 1, 2, 3 and 5, Cos1 cells for AAV4). The transducing titers are given in TU/ $\mu$ l for each serotype in each cell line. The particle numbers used in this experiment (per microliter) were as follows: rAAV1,  $1.2 \times 10^8$ ; rAAV2,  $5.6 \times 10^8$ ; rAAV3,  $9.1 \times 10^8$ ; rAAV4,  $2.3 \times 10^8$ ; and rAAV5,  $5.4 \times 10^8$ . Each bar

represents the average of three separate infections, with the standard deviation indicated by the error bar.

**Figure 6** illustrates GFP protein expression resulting from subretinal  
5 injections via *in vivo* fluorescence imaging. Subretinal injections of rAAV were  
performed via a transcleral transchoroidal approach on wild-type Wistar rats  
as previously described (Rolling et al. (1999) *Hum. Gene Ther.* **10**:641).  
Briefly, the sclera and the choroid were punctured, a 33-gauge needle was  
then inserted in a tangential direction under an operating microscope. Three  
10 microliters of each of the five hybrid rAAV serotypes ( $5 \times 10^{10}$  particles/ml) was  
delivered into the subretinal space ( $n = 3$ , for each serotype). A new method  
using fundus photography has been developed and performed in order to  
control the accuracy and reproducibility of subretinal injections (Rolling et al.  
in preparation). GFP protein expression in live rats was monitored by  
15 fluorescent retinal imaging using a Canon UVI retinal camera connected to a  
digital imaging system and Lhediph Win software at 12, 26 and 46 days  
post-injection.

**Figure 7** shows an illustrative genomic DNA sequence for AAV-2;  
20 GenBank Accession No. NC 001401; **SEQ ID NO: 19**.

**Figure 8** shows an illustrative genomic DNA sequence for AAV-1;  
GenBank Accession No. NC 002077; **SEQ ID NO: 20**.

25 **Figures 9** shows an illustrative genomic DNA sequence for AAV-3A;  
GenBank Accession No. NC 001729; **SEQ ID NO: 21**.

**Figure 10** shows an illustrative genomic DNA sequence for AAV-3B  
GenBank Accession No. NC 001863; **SEQ ID NO: 22**.

30

**Figure 11** shows an illustrative genomic DNA sequence for AAV-4;  
GenBank Accession No. NC 001829; **SEQ ID NO: 23**.



**Figure 12** shows an illustrative genomic DNA sequence for AAV-5  
GenBank Accession No. NC Y18065; **SEQ ID NO: 24**.

**Figure 13** shows an illustrative genomic DNA sequence for AAV-6;  
5 GenBank Accession No. NC 001862; **SEQ ID NO: 25**.

**Figure 14** shows an illustrative genomic DNA sequence for AAV-7;  
GenBank Accession No. AF513851; **SEQ ID NO: 26**.

10 **Figure 15** shows an illustrative genomic DNA sequence for AAV-8  
GenBank Accession No. AF513852; **SEQ ID NO: 27**.

**Figure 16** shows an illustrative genomic DNA sequence for B19  
parvovirus; GenBank Accession No. NC 000883; **SEQ ID NO: 28**.

15

**Figure 17** shows an illustrative genomic DNA sequence for Minute  
Virus from Mouse (MVM); GenBank Accession No. NC 001510; **SEQ ID NO:  
29**.

20 **Figure 18** shows an illustrative genomic DNA sequence for goose  
parvovirus; GenBank Accession No. NC 001510; **SEQ ID NO: 30**.

**Figure 19** shows an alignment of the amino acid sequence of  
exemplary Rep40 proteins from AAV1 (**SEQ ID NO:31**), AAV2 (**SEQ ID  
25 NO:32**), AAV3A (**SEQ ID NO:33**), AAV3B (**SEQ ID NO:34**), AAV4 (**SEQ ID  
NO:35**), AAV5 (**SEQ ID NO:36**), AAV6 (**SEQ ID NO:37**), AAV7 (**SEQ ID  
NO:38**) and AAV8 (**SEQ ID NO:39**), as well as a consensus sequence (**SEQ  
ID NO:40**). Dashes indicate gaps in the sequence and shading indicates  
positions of sequence homology.

30

**Figure 20** shows an alignment of the amino acid sequence of  
exemplary Rep52 proteins from AAV1 (**SEQ ID NO:41**), AAV2 (**SEQ ID  
NO:42**), AAV3A (**SEQ ID NO:43**), AAV3B (**SEQ ID NO:44**), AAV4 (**SEQ ID  
NO:45**), AAV5 (**SEQ ID NO:46**), AAV6 (**SEQ ID NO:47**), AAV7 (**SEQ ID**



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 this invention belongs. The terminology used in the  
5 description of the invention herein is for the purpose of describing particular  
embodiments only and is not intended to be limiting of the invention. As used  
in the description of the invention and the appended claims, the singular forms  
"a", "an" and "the" are intended to include the plural forms as well, unless the  
context clearly indicates otherwise. All publications, patent applications,  
10 patents, and other references mentioned herein are incorporated by reference  
in their entirety.

Nucleotide sequences are presented herein by single strand only, in  
the 5' to 3' direction, from left to right, unless specifically indicated otherwise.  
Nucleotides and amino acids are represented herein in the manner  
15 recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or  
(for amino acids) by either the one-letter code, or the three letter code, both in  
accordance with 37 CFR §1.822 and established usage. See, e.g., *PatentIn  
User Manual*, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office).

Except as otherwise indicated, standard methods known to those  
20 skilled in the art may be used for the construction of recombinant parvovirus  
and rAAV constructs, packaging vectors expressing the parvovirus rep and/or  
cap sequences, and transiently and stably transfected packaging cells. Such  
techniques are known to those skilled in the art. See, e.g., SAMBROOK *et al.*,  
MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor,  
25 NY, 1989); F. M. AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR  
BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc.,  
New York).

The following terms are used in the description herein and the  
appended claims:

30 The term "parvovirus" as used herein encompasses the family  
*Parvoviridae*, including autonomously-replicating parvoviruses and  
dependoviruses. The autonomous parvoviruses include members of the  
genera *Parvovirus*, *Erythrovirus*, *Densovirus*, *Iteravirus*, and *Contravirus*.  
Exemplary autonomous parvoviruses include, but are not limited to, minute

virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. See, e.g., BERNARD N. FIELDS *et al.*,

5 VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine  
10 AAV. See, e.g., BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

The parvovirus particles, capsids and genomes of the present invention are preferably from, but not limited to, AAV. The genomic sequences of the various different serotypes of AAV and the autonomous parvoviruses, as well  
15 as the sequences of the terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC 002077, NC 001401, NC 001729, NC 001863, NC 001829, NC 001862, NC 000883, NC 001701, NC 001510, AF063497, U89790,  
20 AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, NC 001358, NC 001540, AF513851, AF513852; the disclosures of which are incorporated herein in their entirety. See also, e.g., Srivistava *et al.*, (1983) *J. Virology* **45**:555; Chiorini *et al.*, (1998) *J. Virology* **71**:6823; Chiorini *et al.*, (1999) *J. Virology*  
25 **73**:1309; Bantel-Schaal *et al.*, (1999) *J. Virology* **73**:939; Xiao *et al.*, (1999) *J. Virology* **73**:3994; Muramatsu *et al.*, (1996) *Virology* **221**:208; Shade *et al.*, (1986) *J. Virol.* **58**:921; Gao *et al.*, (2002) *Proc. Nat. Acad. Sci. USA* **99**:11854; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; U.S. Patent No. 6,156,303; the disclosures of which are  
30 incorporated herein in their entirety. See also, **Figures 7 – 22**. An early description of the AAV1, AAV2 and AAV3 terminal repeat sequences is provided by Xiao, X., (1996), "Characterization of Adeno-associated virus (AAV) DNA replication and integration," Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein in its entirety).

The term "tropism" as used herein refers to entry of the virus into the cell, optionally and preferably followed by expression (*e.g.*, transcription and, optionally, translation) of sequences carried by the viral genome in the cell, *e.g.*, for a recombinant virus, expression of the heterologous nucleotide sequences(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.*, for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of AAV, gene expression from the viral genome may be from a stably integrated provirus, from a non-integrated episome, as well as any other form in which the virus may take within the cell.

As used herein, "transduction" or "infection" of a cell by a parvovirus or AAV means that the parvovirus/AAV enters the cell to establish an active (*i.e.*, lytic) infection. As used herein, "transduction" of a cell by AAV means that the AAV enters the cell to establish a latent infection. See, *e.g.*, BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (3d ed., Lippincott-Raven Publishers).

The terms "5' portion" and "3' portion" are relative terms to define a spatial relationship between two or more elements. Thus, for example, a "3' portion" of a polynucleotide indicates a segment of the polynucleotide that is downstream of another segment. The term "3' portion" is not intended to indicate that the segment is necessarily at the 3' end of the polynucleotide, or even that it is necessarily in the 3' half of the polynucleotide, although it may be. Likewise, a "5' portion" of a polynucleotide indicates a segment of the polynucleotide that is upstream of another segment. The term "5' portion" is not intended to indicate that the segment is necessarily at the 5' end of the polynucleotide, or even that it is necessarily in the 5' half of the polynucleotide, although it may be.

As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

A "polynucleotide" is a sequence of nucleotide bases, and may be RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotide), but are preferably either single or double stranded DNA sequences.

As used herein, an "isolated" polynucleotide (*e.g.*, an "isolated DNA" or an "isolated RNA") means a polynucleotide separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the  
5 polynucleotide.

Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral  
10 structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

A "therapeutic polypeptide" is a polypeptide that may alleviate or reduce symptoms that result from an absence or defect in a protein in a cell or subject. Alternatively, a "therapeutic polypeptide" is one that otherwise confers  
15 a benefit to a subject, *e.g.*, anti-cancer effects or improvement in transplant survivability.

A "heterologous nucleotide sequence" will typically be a sequence that is not naturally occurring in the virus. Alternatively, a heterologous nucleotide sequence may refer to a viral sequence that is placed into a non-naturally  
20 occurring environment (*e.g.*, by association with a promoter with which it is not naturally associated in the virus).

As used herein, the term "vector" or "gene delivery vector" may refer to a parvovirus (*e.g.*, AAV) particle that functions as a gene delivery vehicle, and which comprises viral DNA (*i.e.*, the vector genome) packaged within a  
25 parvovirus (*e.g.*, AAV) capsid. Alternatively, in some contexts, the term "vector" may be used to refer to the vector genome/vDNA alone.

As used herein, a "recombinant parvovirus vector genome" is a parvovirus genome (*i.e.*, vDNA) into which a heterologous (*e.g.*, foreign) nucleotide sequence (*e.g.*, transgene) has been inserted. A "recombinant  
30 parvovirus particle" comprises a recombinant parvovirus vector genome packaged within a parvovirus capsid.

Likewise, a "rAAV vector genome" is an AAV genome (*i.e.*, vDNA) that comprises a heterologous nucleotide sequence. rAAV vectors generally require only the 145 base terminal repeat(s) in *cis* to generate virus. All other

viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* **158**:97). Typically, the rAAV vector genome will only retain the minimal terminal repeat (TR) sequence(s) so as to maximize the size of the transgene that can be efficiently packaged by the vector. The structural and non-structural protein coding sequences may be provided in *trans* (e.g., from a vector, such as a plasmid, or by stably integrating the sequences into a packaging cell). The rAAV vector genome comprises at least one AAV terminal repeat, more typically two AAV terminal repeats, which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s).

The term "template" or "substrate" is used herein to refer to a polynucleotide sequence that may be replicated to produce the parvovirus viral DNA. For the purpose of vector production, the template will typically be embedded within a larger nucleotide sequence or construct, including but not limited to a plasmid, naked DNA vector, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC) or a viral vector (e.g., adenovirus, herpesvirus, Epstein-Barr Virus, AAV, baculoviral, retroviral vectors, and the like). Alternatively, the template may be stably incorporated into the chromosome of a packaging cell.

The methods and reagents herein may further be used to produce a duplexed parvovirus particle as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety).

A "rAAV particle" comprises a rAAV vector genome packaged within an AAV capsid.

A "parvovirus terminal repeat" may be from any parvovirus, including autonomous parvoviruses and AAV (all as defined above). An "AAV terminal repeat" may be from any AAV with serotypes 1, 2, 3, 4, 5, 6, 7 and 8 being preferred, and serotypes 1, 2 and 5 being more preferred. The term "terminal repeat" includes synthetic sequences that function as an AAV inverted terminal repeat, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski *et al.*, the disclosure of which is incorporated in its entirety herein by reference. The AAV terminal repeats need not have a wild-type terminal repeat sequence (e.g., a wild-type

sequence may be altered by insertion, deletion, truncation or missense mutations), as long as the terminal repeat mediates the desired functions, e.g., replication, virus packaging, integration, and/or provirus rescue, and the like.

5           As used herein, parvovirus or AAV "rep coding sequences" indicate the nucleic acid sequences that encode the parvoviral or AAV non-structural proteins that mediate viral replication and the production of new virus particles. The parvovirus and AAV replication genes and proteins have been described in, e.g., BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2,  
10 chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

The "rep coding sequences" need not encode all of the parvoviral or AAV Rep proteins. For example, with respect to AAV, the rep coding sequences do not need to encode all four AAV Rep proteins (Rep78, Rep 68, Rep52 and Rep40), in fact, it is believed that AAV5 only expresses the spliced  
15 Rep68 and Rep40 proteins. Preferably, the rep coding sequences encode at least those replication proteins that are necessary for viral genome replication and packaging into new virions. The rep coding sequences will generally encode at least one large Rep protein (*i.e.*, Rep78/68) and one small Rep protein (*i.e.*, Rep52/40). In particular embodiments, the rep coding sequences  
20 encode the AAV Rep78 protein and the AAV Rep52 and/or Rep40 proteins. In other embodiments, the rep coding sequences encode the Rep68 and the Rep52 and/or Rep40 proteins. In a still further embodiment, the rep coding sequences encode the Rep68 and Rep40 proteins.

Those skilled in the art will further appreciate that it is not necessary  
25 that the replication proteins be encoded by the same polynucleotide. For example, for MVM, the NS-1 and NS-2 proteins (which are splice variants) may be expressed independently of one another. Likewise, for AAV, the p19 promoter may be inactivated and the large Rep protein(s) expressed from one polynucleotide and the small Rep protein(s) expressed from a different  
30 polynucleotide. Typically, however, it will be more convenient to express the replication proteins from a single construct. In some systems, the viral promoters (e.g., AAV p19 promoter) may not be recognized by the cell, and it is therefore necessary to express the large and small Rep proteins from separate expression cassettes. In other instance, it may be desirable to



express the large Rep and small Rep proteins separately, *i.e.*, under the control of separate transcriptional and/or translational control elements. For example, it may be desirable to control expression of the large Rep proteins, so as to decrease the ratio of large to small Rep proteins. In the case of  
5 insect cells, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g.*, Rep78/68) to avoid toxicity to the cells (*see, e.g.*, Urabe et al., (2002) *Human Gene Therapy* **13**:1935).

A "chimeric rep coding sequence" is a rep coding sequence comprising segments from two or more parvoviruses and encoding replication proteins,  
10 or portions thereof, from two or more parvoviruses. According to the present invention, the chimeric rep coding sequences will generally encode a DNA binding domain from one parvovirus and a capsid interacting domain from another parvovirus. These two different functional domains may be expressed as a single polypeptide or as separate polypeptides (*e.g.*, due to different  
15 transcriptional and/or translational starts sites and/or differential splicing).

It is not necessary that all, or even any, of the proteins encoded by the chimeric rep coding sequences be chimeric proteins. For example, in the case of AAV, the rep coding sequences may encode a large Rep protein that is chimeric and a small Rep protein that is not. Neither protein may be  
20 chimeric if each protein is expressed from a separate polynucleotide.

As used herein, the parvovirus or AAV "cap coding sequences" encode the structural proteins that form a functional parvovirus or AAV capsid (*i.e.*, can package DNA and infect target cells). Typically, the cap coding sequences will encode all of the parvovirus or AAV capsid subunits, but less  
25 than all of the capsid subunits may be encoded as long as a functional capsid is produced. Typically, but not necessarily, the cap coding sequences will be present on a single nucleic acid molecule.

The capsid structure of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS *et al.*, VIROLOGY, volume  
30 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

The parvovirus particles of the invention are "hybrid" parvovirus particles in which the viral terminal repeats and viral capsid are from different parvoviruses. Hybrid parvoviruses are described in more detail in international patent publication WO 00/28004 and Chao et al., (2000) *Molecular Therapy*

2:619 (the disclosures of which are incorporated herein in their entireties). Preferably, the viral terminal repeats and capsid are from different serotypes of AAV (*i.e.*, a "hybrid AAV particle").

The parvovirus capsid may further be a "chimeric" capsid (*e.g.*,  
5 containing sequences from different parvoviruses, preferably different AAV serotypes) or a "targeted" capsid (*e.g.*, having a directed tropism) as described in international patent publication WO 00/28004.

A "DNA binding domain" is a replication protein or portion thereof that binds to and interacts with the parvovirus terminal repeats (*e.g.*, AAV terminal  
10 repeats). The DNA binding domain binds to the parvovirus terminal repeats and mediates replication of the AAV genome (*e.g.*, by nicking the hairpin loop in the parvovirus terminal repeats by site-specific endonuclease cleavage at the terminal resolution site). The AAV Rep78/68 proteins, the large Rep  
15 proteins from autonomous parvoviruses such as the MVM NS-1 protein, B19 NS-1 protein, the densovirus *Junonia coenia* NS-1 protein, and the goose parvovirus Rep1 protein (Smith et al., (1999) *J. Virology* **73**:2930) are illustrative examples of proteins comprising "DNA binding domains".

A "capsid interacting domain" is a replication protein or portion thereof that interacts with the parvovirus capsid to facilitate packaging of new virions  
20 (*e.g.*, by facilitating particle assembly or packaging of the genomic DNA into assembled particles). While not wishing to be held to any particular theory of the invention, the capsid interacting domain may be associated with helicase activity. The AAV Rep52/40 proteins, the small Rep proteins from  
25 autonomous parvoviruses such as the MVM NS-2 protein, B19 NS-2 protein, the densovirus *Junonia coenia* NS-2 protein, and the goose parvovirus Rep2 protein are illustrative examples of capsid interacting domains. Likewise, the large Rep proteins (*i.e.*, autonomous parvovirus NS-1 protein or AAV Rep78/68), which include the small Rep protein sequences also comprise  
30 capsid interacting domains.

### **Chimeric Parvovirus Rep Coding Sequences.**

The inventors have identified novel trans-acting elements that may be used to achieve more efficient (*e.g.*, higher titer) production of hybrid parvovirus stocks. In particular, the parvovirus replication proteins comprise a

DNA binding domain that interacts with the parvovirus terminal repeats to initiate DNA replication and a capsid interacting domain that mediates new particle assembly (e.g., by mediating capsid assembly and/or packaging of the viral DNA into the capsid).

5 International patent publication WO 00/28004 (Rabinowitz et al.) discloses hybrid parvovirus particles in which viral genomes comprising terminal repeat(s) from one parvovirus are packaged within a capsid from a different parvovirus. This publication did not disclose that production of such hybrid parvoviruses may be improved or optimized by modifying the viral  
10 replication proteins.

The linear, single-stranded DNA genome of AAV encodes two open reading frames (*rep* and *cap*) flanked by 145 bp inverted terminal repeats (TR) (Srivastava et al., (1983) *J. Virol.* **45**:555). Replication of the AAV genome uses two viral components, the terminal repeat that serves as the  
15 origin of replication (Hauswirth et al., (1977) *Virology* **78**:488; Straus et al., (1976) *Proc. Natl. Acad. Sci. USA* **73**:742; Samulski et al., (1983) *Cell* **33**:135; Senepathy et al., (1984) *J. Mol. Biol.* **179**:1) and the *rep* gene products (Senepathy et al., (1984) *J. Mol. Biol.* **179**:1, Hermonat et al., (1984) *J. Virology* **51**:329; Tratschin et al., (1984) *J. Virology* **51**:611). The *rep* gene  
20 encodes four multifunctional proteins (Hermonat et al., (1984) *J. Virology* **51**:329; Tratschin et al., (1984) *J. Virology* **51**:611; Mendelson et al., (1986) *J. Virology* **60**:823; Trempe et al., (1987) *Virology* **161**:18) that are expressed from two promoters at map units 5 (p5) and 19 (p19). The larger Rep proteins transcribed from the p5 promoter (Rep78 and Rep68), are essentially identical  
25 except for unique carboxy termini generated from unspliced (Rep78) and spliced (Rep68) transcripts, respectively (Srivastava et al., (1983) *J. Virol.* **45**:555). Two smaller rep proteins (Rep52, Rep40), transcribed from the p19 promoter are amino terminal truncations of Rep78 and Rep68, respectively.

Several biochemical activities of Rep78 and Rep68 have been  
30 implicated in the process of AAV replication. These include specific binding to the AAV terminal repeat (Ashktorab et al., (1989) *J. Virology* **63**:3034; Im et al., 1989) *J. Virology* **63**:3095; Snyder et al., (1993) *J. Virology* **67**:6096) and site-specific endonuclease cleavage at the terminal resolution site (*trs*) (Im et al., (1990) *J. Virology* **63**:447; Im et al., (1992) *J. Virology* **66**:1119; Snyder et

al., (1990) *Cell* **60**:105; Snyder et al., (1990) *J. Virology* **64**:6204). Rep78/68 also possess ATP dependent DNA-DNA helicase (Im et al., (1990) *J. Virology* **63**:447; Im et al., (1992) *J. Virology* **66**:1119) and DNA-RNA helicase as well as ATPase activities (Wonderling et al., (1995) *J. Virology* **69**:3542). In  
5 addition to these activities associated with replication, Rep78/68 also regulate transcription from the viral promoters (Beaton et al., (1989) *J. Virology* **63**:4450; Labow et al., (1986) *J. Virology* **60**:251; Tratschin et al., (1986) *Mol. Cellular Biol.* **6**:2884; Kyostio et al., (1994) *J. Virology* **68**:2947; Pereira et al., (1997) *J. Virology* **71**:1079), and have been shown to mediate viral targeted  
10 integration (Xiao, W., (1996), "Characterization of *cis* and *trans* elements essential for the targeted integration of recombinant adeno-associated virus plasmid vectors", Ph.D. Dissertation, University of North Carolina-Chapel Hill; Balague et al., (1997) *J. Virology* **71**:3299; LaMartina et al., (1998) *J. Virology* **72**:7653; Pieroni et al., (1998) *Virology* **249**:249).

15 Mutant studies of the AAV Rep proteins have indicated that the activities of Rep can be divided into partially distinct functional domains that are spread throughout the protein (Chejanovsky et al., (1989) *Virology* **173**:120; McCarty et al., (1992) *J. Virology* **66**:4050; Yang et al., (1992) *J. Virology* **66**:6058; Owens et al., (1993) *J. Virology* **67**:997; Weitzman et al.,  
20 (1996) *J. Virology* **70**:2440; Walker et al., (1997) *J. Virology* **71**:2722; Walker et al., (1997) *J. Virology* **71**:6996; Davis et al., (1999) *J. Virology* **73**:2084; Urabe et al., (1999) *J. Virology* **73**:2682). These include regions required for binding to the terminal repeat; a putative NTP-binding/ATPase domain, nuclear localization domain, dimerization domain, and residues putatively  
25 required for nicking and helicase functions (see, e.g., Chiorini et al., (1999) *J. Virology* **73**:1309). Several mutations within the NTP-binding/ATPase domain that lacked *trs* endonuclease and viral replication were also defective for trans-activation functions suggesting a need for further mutant analysis (McCarty et al., (1992) *J. Virology* **66**:4050).

30 Likewise, the replication genes and proteins of a number of autonomous parvoviruses have been described. For example, minute virus of mouse (MVM) and H1 virus each produce two non-structural proteins, NS-1 and NS-2, which share similar activities to the AAV Rep proteins (see, e.g., BERNARD N. FIELDS *et al.*, *VIROLOGY*, volume 2, chapters 69 & 70 (4th

ed., Lippincott-Raven Publishers). See also, Ding et al., (2002) *J. Virology* **76**:338, characterizing the *Junonia coenia* densovirus NS-1 protein.

The present investigations have determined that the parvovirus replication proteins comprise a capsid interacting domain(s) that is involved in capsid assembly and/or packaging. Accordingly, in a hybrid parvovirus production system, more efficient viral production may be achieved by providing a capsid interacting domain that is compatible with or optimized for the particular parvovirus capsid. Thus, for example, the present investigations have determined that the packaging of a recombinant AAV2 genome (*i.e.*, with AAV2 terminal repeats) in an AAV5 capsid may be improved by providing AAV Rep proteins that comprise an AAV5, as opposed to an AAV2, capsid interacting domain.

The small AAV Rep proteins (Rep52 and Rep40) and the homologous regions that are found in the carboxyl portion of the large AAV Rep proteins (Rep78 and Rep68; see, *e.g.*, Chiorini et al., (1999) *J. Virology* **73**:1309, **Figures 19-22**) comprise a capsid interacting domain. In embodiments of the invention, the rep coding sequences encode the AAV Rep52 and/or Rep40 protein or the homologous regions of the large Rep proteins (*i.e.*, the rep coding sequences comprise the sequences 3' of the AAV p19 promoter, with or without the intron region). Alternatively, the rep coding sequences encode a functional portion (*i.e.*, functions as a capsid interacting domain, as defined above) of the AAV small Rep proteins (*e.g.*, a carboxy terminal portion). In other particular embodiments, the rep coding sequences encode the zinc finger domain, nuclear localization signal, dimerization domain(s), NTP binding pocket, the Rep splice domain (*i.e.*, the intron domain), and/or the carboxyl terminal regions encoded by the rep sequences 3' of the p40 promoter. In still further embodiments, the capsid interacting domain comprises approximately the carboxy terminal 50, 75, 100, 125, 150 or 200 amino acids of the Rep42 or Rep50 proteins.

In other embodiments, the rep coding sequences comprise the coding sequences 3' of the p40 promoter. Alternatively, the rep coding sequences comprise sequences encoding from amino acid 225 or 380 using AAV2 Rep78/68 numbering (or the analogous position of other AAV) through to the carboxyl end of the Rep78 or Rep68 protein (see, *e.g.*, Chiorini et al., (1999)

*J. Virology* 73:1309; **Figures 19-22**). In still other embodiments, the rep coding sequences comprise sequences encoding the amino acids from the p19 promoter, p40 promoter or Accl site through to about amino acid 225 or amino acid 380 using AAV2 numbering of the Rep78 protein or the analogous position on the Rep78 proteins of other AAV. In further embodiments, the Rep coding sequences comprise sequences encoding the amino acid sequence from the p19 promoter, the p40 promoter, or the Accl site through to the beginning of the intron region (*i.e.*, about amino acid 528 of AAV2 using Rep78/68 numbering, or the analogous position of other AAV). In yet other illustrative embodiments, the rep coding sequences comprise sequences encoding the amino acid sequence from about amino acid 225 or 380 using AAV2 numbering of the Rep78/68 proteins (or the analogous position on the Rep78/68 proteins of other AAV) through to beginning of the intron region.

In other embodiments of the invention, the rep coding sequences encode a capsid interacting domain from an autonomous parvovirus. The autonomous parvovirus replication proteins have structural and functional homology with the AAV Rep proteins (*see, e.g.*, Yoon et al., (2001) *J. Virology* 75:3230; Smith et al. (1999) *J. Virology* 73:2930; Zadori et al., (1995) *Virology* 212: 562; BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). For example, the MVM NS-2 protein, NS-2 protein of B19 and Rep2 protein of goose parvovirus may comprise a capsid interacting domain. In embodiments of the invention, the capsid interacting domain is a functional portion (*i.e.*, functions as a capsid interacting domain, as defined hereinabove) of the NS-2/Rep2 protein (*i.e.*, the "small" replication protein) of an autonomous parvovirus (*e.g.*, a carboxy terminal portion, a portion encoding zinc finger domains, a nuclear localization domain, an NTP binding pocket, a dimerization domain and/or a portion having helicase activity). In particular embodiments, the capsid interacting domain comprises approximately the carboxy terminal 50, 75, 100, 125, 150 or 200 amino acids of the NS-2 protein.

The capsid interacting domain may be provided by the small Rep proteins and/or the homologous regions located in the carboxyl terminal portion of the large Rep proteins.

The rep coding sequences encoding the capsid interacting domain are selected so that the capsid interacting domain is compatible with the parvovirus capsid to be packaged. The viral capsid and capsid interacting domain will typically be from the same parvovirus (e.g., AAV5 capsid and AAV5 capsid interacting domain or B19 capsid and B19 capsid interacting domain). Alternatively, there may be compatibility with respect to these elements among some of the parvoviruses. For example, the AAV1 and AAV2 capsid interacting domains may be compatible with both the AAV1 and AAV2 capsids. Likewise, the feline panleukemia virus and canine parvovirus elements may be able to interact with each other. Compatibility may be routinely determined by those skilled in the art by evaluation of sequence homology and by use of the techniques described herein.

One or more of the parvovirus replication proteins comprise a DNA binding domain(s) that interacts with the parvovirus terminal repeats. This functional element in the replication proteins interacts with the viral DNA to mediate replication thereof. Thus, production of hybrid parvoviruses may be improved (e.g., higher titers achieved) by selecting a DNA binding domain that is compatible with or optimized for the parvovirus terminal repeat(s). In the example of an AAV5 capsid with a rAAV2 genome, production titers may be improved by providing a DNA binding domain that is compatible with the AAV2 terminal repeat(s) and a capsid interacting domain that is compatible with the AAV5 capsid.

The large AAV Rep proteins (Rep78/68) comprise a DNA binding domain. Thus, in embodiments of the invention, rep coding sequences are provided that encode one or both of the AAV large Rep proteins, where the Rep protein(s) is selected so as to be compatible with the AAV terminal repeats to be packaged. Alternatively, the rep coding sequences may encode a functional portion of the AAV large Rep proteins (e.g., an amino-terminal portion). In other embodiments of the invention, the rep coding sequences encode the AAV large Rep protein specific amino acid sequences, i.e., the amino-terminal amino acid sequences that are not present in the AAV Rep52/40 proteins (i.e., encoded by the rep coding sequences 5' of the AAV p19 promoter) (see, e.g., Chiorini et al., (1999) *J. Virology* **73**:1309; **Figures 19-22**). As a further alternative, the rep coding sequences encode

approximately the amino-terminal 50, 75, 100, 150, 190, 200, 210, 215, 220, 225, 230, 240, 250, or 270 amino acids of the AAV large Rep78/68 proteins. In other alternative embodiments, the rep coding sequences encode approximately the amino-terminal 50, 75, 100, 150, 190, 200, 210, 215, 220, 225, 230, 240, 250, or 270 amino acids of the AAV2 large Rep78/68 proteins or the analogous sequences in other AAV serotypes (*see, e.g.*, Chiorini et al., (1999) *J. Virology* 73:1309; **Figures 7 - 22**).

The autonomous parvovirus large replication proteins also have DNA binding domains. As illustrative examples, the MVM and H1 NS-1 proteins, the goose parvovirus Rep1 protein, the B19 NS-1 protein, and the *Junonia coenia* densovirus NS-1 protein may comprise DNA binding domains that mediate replication of the viral DNA. In some embodiments, the DNA binding domain is a functional portion of an autonomous parvovirus NS-1/Rep1 protein (*i.e.*, the "large" replication protein). For example, the functional portion may be an amino terminal portion of the large Rep protein. In embodiments of the invention, the rep coding sequences encode approximately the amino terminal 50, 75, 100, 150, 190, 200, 210, 215, 220, 225, 230, 240, 250, or 270 amino acids of the autonomous parvovirus NS-1/Rep1 protein (*i.e.*, large Rep protein).

In embodiments of the invention, the replication protein DNA binding domain is selected so as to be compatible with the viral terminal repeats to be packaged. The viral terminal repeat and DNA binding domain may be from the same parvovirus (*e.g.*, AAV5 terminal repeats and AAV5 DNA binding domain or B19 terminal repeats and B19 DNA binding domain). Alternatively, there may be compatibility among some of the parvovirus DNA binding domains and the terminal repeats. For example, the AAV2 and AAV3 terminal repeats are highly homologous; thus, AAV2 terminal repeats can interact with DNA binding domains from AAV2 or AAV3 Rep protein, and vice versa. There may also be some compatibility of AAV2 and AAV3 with AAV1 and AAV4, although higher titers may be achieved with the serotype-specific DNA binding domain and terminal repeat. In general, it is preferred to use an AAV5 DNA binding domain to package AAV5 terminal repeats. Likewise, there may be compatibility between closely related autonomous parvoviruses, such as feline panleukemia virus and canine parvovirus, or MVM and H1 virus.



Generally, an AAV DNA binding domain will be used with an AAV terminal repeat, and an autonomous parvovirus DNA binding domain will be used with autonomous parvovirus terminal repeats. Compatibility between terminal repeats and DNA binding domains from different parvoviruses may be  
5 routinely determined by those skilled in the art by evaluation of sequence homology and by use of the methodologies described herein.

Accordingly, the present investigations have elucidated important functional domains in the parvovirus replication machinery. On the basis of this understanding, improved reagents and methods may be employed for  
10 more efficient production of hybrid parvoviruses.

While not wishing to be bound by any particular theory of the invention, in one model, a chimeric large Rep protein comprises a DNA binding domain from one parvovirus and a capsid interacting domain from a different parvovirus. The DNA binding domain interacts with the TRs and the capsid  
15 interacting domain interacts with the capsid. In an alternative model, these functions are provided by different Rep proteins; a large Rep protein comprises a DNA binding domain that interacts with the TRs, and a capsid interacting domain of a small Rep protein interfaces with the capsid.

As one aspect, the present invention provides polynucleotide  
20 sequences comprising chimeric parvovirus rep coding sequences (e.g., chimeric AAV rep coding sequences) and chimeric replication proteins encoded thereby. The chimeric parvovirus rep coding sequences encode a DNA binding domain from one parvovirus (preferably, an AAV) and a capsid interacting domain from another parvovirus.

The inventive rep coding sequences may further be associated with  
25 the parvovirus cap coding sequences in a single polynucleotide. In one embodiment, the invention provides a polynucleotide comprising parvovirus rep coding sequences and parvovirus cap coding sequences, the rep coding sequences encoding a DNA binding domain from a first parvovirus  
30 (preferably, the first parvovirus is an AAV), where the rep coding sequences further encode a capsid interacting domain from a different parvovirus from the first parvovirus; and the cap coding sequences comprise sequences that encode a parvovirus capsid that is compatible with and interacts with the capsid interacting domain to facilitate capsid assembly and/or packaging (as

described above). This rep/cap polynucleotide sequence may be used to produce hybrid parvoviruses, *i.e.*, a parvovirus having terminal repeats from one parvovirus and a capsid from another parvovirus, where the terminal repeats are compatible with the DNA binding domain encoded by the rep coding sequences and the capsid is compatible with the capsid interacting domain encoded by the rep coding sequences.

In another embodiment, the invention provides a polynucleotide comprising parvovirus rep coding sequences and parvovirus cap coding sequences, the rep coding sequences encoding a DNA binding domain from a first parvovirus (preferably, the first parvovirus is an AAV), where the rep coding sequences further encode a capsid interacting domain from a different parvovirus from the first parvovirus; and the cap coding sequences comprising sequences from the different parvovirus.

According to the previous embodiments, it is preferred that the first parvovirus is an AAV. In other embodiments, the different parvovirus is an AAV. In still further embodiments, both the first and different parvoviruses are AAV.

In particular embodiments of the invention, the rep coding sequences comprise a 5' and 3' portion, the 5' portion encoding the DNA binding domain from the first parvovirus; and the 3' portion encoding the capsid interacting domain from the different parvovirus.

In a further embodiment, the invention provides a polynucleotide comprising AAV rep coding sequences and AAV cap coding sequences, the rep coding sequences having a 5' and a 3' portion, the 5' portion comprising rep coding sequences from a first AAV and the 3' portion comprising rep coding sequences from a different AAV from the first AAV; and the cap coding sequences comprising sequences from the different AAV.

According to the foregoing embodiments, the capsid interacting domain and DNA binding domain encoded by the polynucleotides are as described above. In embodiments of the invention, the capsid interacting domain is from an autonomous parvovirus (*e.g.*, B19 virus, H1 virus, canine parvovirus, feline panleukemia virus, muskovy duck parvovirus, goose parvovirus, and MVM). In other embodiments, the capsid interacting domain is from an AAV (*e.g.*, AAV1, AAV2 or AAV5). Likewise, the DNA binding

domain may be from an autonomous parvovirus (*e.g.*, B19 virus, H1 virus, canine parvovirus, feline panleukemia virus, muskovy duck parvovirus, goose parvovirus, and MVM) or from an AAV (*e.g.*, AAV1, AAV2 or AAV5).

In certain embodiments, both the rep coding sequences and cap coding sequences are AAV sequences (*i.e.*, the AAV rep and cap sequences).

In particular embodiments of the invention, the rep coding sequences encode an AAV1, AAV2, AAV4 or AAV5 DNA binding domain. In other preferred embodiments, the rep coding sequences encode an AAV1, AAV2 or AAV5 capsid interacting domain. According to this embodiment, it is further preferred that the cap coding sequences encode a capsid of the same serotype as the capsid interacting domain.

In other embodiments, the rep coding sequences encode an AAV5 DNA binding domain and an AAV1, AAV2, AAV3, AAV4, AAV6, AAV7 or AAV8 capsid interacting domain. According to this embodiment, it is also preferred that the cap coding sequences encode a capsid of the same serotype as the capsid interacting domain.

In other embodiments, the rep coding sequences encode an AAV5 capsid interacting domain and an AAV1, AAV2, AAV3, AAV4, AAV6, AAV7 or AAV8 DNA binding domain. According to this embodiment, it is preferred that the cap coding sequences encode an AAV5 capsid.

In other embodiments of the invention, the capsid interacting domain and capsid are from an autonomous parvovirus (*e.g.*, B19 virus, goose parvovirus, H1 virus, MVM virus, and the like) and the DNA binding domain is from an AAV.

The rep coding sequences and cap coding sequences may be operatively associated with an expression control sequence, for example, a promoter. In embodiments of the invention, the rep coding sequences and cap coding sequences are each operatively associated with separate expression control sequences (*e.g.*, promoters). Promoters and other transcriptional and translational control elements are described in more detail hereinbelow in connection with the discussion of transgenes that may be encoded by a recombinant parvovirus genome. Preferably, the rep coding

sequences are operatively associated with a parvovirus promoter, more preferably, the AAV p5 or p19 promoters.

As described above, it may be desirable to associate the coding sequences for the large and small Rep proteins with separate transcriptional and/or translational control elements so that expression of these proteins may be differentially regulated. For example, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g.*, Rep78/68) as compared with the small Rep proteins (*e.g.*, Rep52/40).

Those skilled in the art will appreciate that the rep coding sequences encoding the DNA binding domain and the capsid interacting domain may be operatively associated with different promoters. In particular embodiments, the rep coding sequences encoding the capsid interacting domain are operatively associated with an AAV p19 promoter.

Chimeric AAV rep coding sequences may encode two, three or all four of the AAV Rep proteins. Typically, at least one of the large AAV Rep proteins and one of the small Rep proteins will be encoded. Further, it is not necessary that all of the Rep proteins encoded by the rep coding sequences be chimeric. For example, the large Rep protein may be chimeric and the small Rep protein need not be chimeric. As a further alternative, neither protein may be chimeric if each protein is expressed from a separate polynucleotide.

The present invention also encompasses vectors comprising the polynucleotides comprising the inventive chimeric rep coding sequences, optionally in conjunction with the cap coding sequences. The vector may be any vector known in the art. Illustrative vectors include, but are not limited to, plasmids, naked DNA vectors, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), cosmids, and viral vectors.

Any suitable viral vector may be employed, as known in the art, including single and double-stranded RNA and DNA viral vectors, with DNA being preferred. Exemplary viral vectors may derived be from Poxviridae (*e.g.*, pox virus or vaccinia virus), Papoviridae (*e.g.*, BKV, JCV, or SV40), Adenoviridae (*e.g.*, adenovirus), Herpesviridae (*e.g.*, Herpes Simplex Virus), Hepadnaviridae (*e.g.*, HBV), Retroviridae (*e.g.*, HIV, SIV, MoMLV, RSV, HTLV), Picornaviridae (*e.g.*, poliovirus, rhinovirus, coxsackieviruses, Caliciviridae, Togaviridae (*e.g.*, alphaviruses, rubella) Flaviviridae (*e.g.*, yellow

fever virus), Parvoviridae (*e.g.*, AAV), Coronaviridae (*e.g.*, HDV, TGEV, IBV, MHV, BCV), Rhabdoviridae, Filoviridae, Paramyxoviridae (*e.g.*, parainfluenza virus, mumps virus, measles virus, and respiratory syncytial virus), Orthomyxoviridae (*e.g.*, influenza virus), Bunyaviridae, Arenaviridae, hepatitis delta virus, Astroviruses, Epstein Barr Virus (EBV) and non-mammalian viruses, such as baculoviruses.

Preferred viral vectors include AAV, adenovirus, herpesvirus, EBV, baculovirus, and retroviral (*e.g.*, lentiviral) vectors.

The present invention also encompasses cells containing the inventive nucleotide sequences, vectors and chimeric replication proteins described above. The cell may be any cell known in the art, including bacterial, protozoan, yeast, fungal, plant and animal cells. Animal cells (*e.g.*, insect and mammalian cells) are preferred. Mammalian cells (*e.g.*, human) and insect cells are more preferred.

In some embodiments, the cell contains both chimeric rep coding sequences and the appropriate cap coding sequences, but the cap coding sequences and rep coding sequences are on separate polynucleotide molecules. Further, the rep coding sequences and/or the cap coding sequences may be stably integrated into the cellular chromosomes. Cells for use in methods of virus production and methods of gene delivery are described in more detail hereinbelow.

#### **Methods of Parvovirus Production.**

The present invention also encompasses methods of producing hybrid parvovirus particles using the inventive polynucleotide sequences, vectors and cells. In general, methods known to those skilled in the art of producing AAV and parvovirus vectors may be used to produce hybrid parvovirus vectors using the inventive reagents disclosed herein (*see, e.g.*, WO 00/28004, the disclosure of which is incorporated herein in its entirety by reference). Hybrid parvovirus particles may be produced by introducing a rAAV template to be replicated and packaged into a permissive or packaging cell, as those terms are understood in the art (*e.g.*, a "permissive" cell can be infected or transduced by the virus; a "packaging" cell is a stably transformed cell that expresses the replication and/or capsid proteins).

In one embodiment, the invention provides a method of producing a recombinant parvovirus particle, comprising providing to a cell (a) a recombinant parvovirus template comprising (i) a heterologous nucleotide sequence, and (ii) at least one parvovirus terminal repeat sequence; and (b) 5 chimeric parvovirus rep coding sequences and parvovirus cap coding sequences; the chimeric rep coding sequences encoding a DNA binding domain from a first parvovirus that interacts with the parvovirus terminal repeat to mediate replication of the recombinant parvovirus template; the rep coding sequences further encoding a capsid interacting domain from a 10 different parvovirus from the first parvovirus; and the cap coding sequences comprising sequences that encode a parvovirus capsid that is compatible with and interacts with the capsid interacting domain to facilitate capsid assembly and/or packaging. The cap coding sequences are from a different parvovirus than the parvovirus terminal repeat sequence, such that the resulting 15 parvovirus particle is "hybrid".

The recombinant parvovirus template and parvovirus rep coding sequences and parvovirus cap coding sequences are provided under conditions so that hybrid parvovirus particles comprising the recombinant parvovirus template are produced in the cell. Preferably, the hybrid parvovirus 20 particles will be infectious.

In another embodiment, the invention provides a method of producing a recombinant parvovirus particle, comprising providing to a cell (a) a recombinant parvovirus template comprising (i) a heterologous nucleotide sequence, and (ii) at least one parvovirus terminal repeat sequence; and (b) 25 chimeric parvovirus rep coding sequences and parvovirus cap coding sequences; the chimeric rep coding sequences encoding a DNA binding domain from a first parvovirus (preferably, an AAV) that interacts with the parvovirus terminal repeat to mediate replication of the recombinant parvovirus template; the rep coding sequences further encoding a capsid 30 interacting domain from a different parvovirus from the first parvovirus; and the cap coding sequences comprising sequences from the different parvovirus. The cap coding sequences are further from a different parvovirus than the parvovirus terminal repeat sequence, such that the resulting parvovirus particle is "hybrid".

The recombinant parvovirus template and parvovirus rep coding sequences and parvovirus cap coding sequences are provided under conditions so that infectious parvovirus particles comprising the rAAV template are produced in the cell.

5           According to the previous embodiments, it is preferred that the recombinant parvovirus template is a rAAV template and the parvovirus terminal repeat is an AAV terminal repeat sequence. In other embodiments, it is preferred that the cap coding sequences and the rep coding sequences encoding the capsid interacting domain are AAV sequences. It is further  
10 preferred that the recombinant parvovirus template (*e.g.*, rAAV template) has two terminal repeat sequences at the 5' and 3' ends of the heterologous nucleotide sequence. In other embodiments, the parvovirus terminal repeat is from the first parvovirus (*e.g.*, the same parvovirus as the DNA binding domain).

15           In particular embodiments, the chimeric rep coding sequences comprise a 5' portion and a 3' portion; the 5' portion encoding the DNA binding domain from the first parvovirus and the 3' portion encoding the capsid interacting domain from the different parvovirus.

          In a further embodiment, the invention provides a method of producing  
20 a rAAV particle, comprising providing to a cell: (a) a rAAV template comprising (i) a heterologous nucleotide sequence, and (ii) at least one AAV terminal repeat sequence (preferably, two AAV two terminal repeat sequences at the 5' and 3' ends of the heterologous nucleotide sequence); and (b) chimeric AAV rep coding sequences and AAV cap coding sequences; the chimeric rep  
25 coding sequences having a 5' portion and a 3' portion; the 5' portion comprising rep coding sequences from a first AAV that interacts with the AAV terminal repeat to mediate replication of the rAAV template; the 3' portion comprising rep coding sequences from a different AAV from the first AAV; and the cap coding sequences comprising sequences from the different AAV. The  
30 cap coding sequences are from a different AAV than the AAV terminal repeat sequence, such that the resulting AAV particle is "hybrid."

          The rAAV template, AAV rep coding sequences and AAV cap coding sequences are provided under conditions so that rAAV particles comprising the rAAV template are produced in the cell.

Preferably, the recombinant parvovirus template comprises a segment (e.g., a terminal repeat) that permits packaging within the parvovirus capsid. Conversely, it is preferred that the rep coding sequences and cap coding sequences are not associated with parvovirus terminal repeats to prevent packaging of these sequences into the parvovirus capsid. Other methods of preventing the packaging of AAV rep/cap sequences are known in the art (see, e.g., U.S. Patent 6,329,181 to Xiao et al.).

In embodiments of the invention, the method further comprises the step of collecting the infectious recombinant parvovirus particles. The method may further comprise the step of lysing the cell prior to collecting the recombinant parvovirus particles.

Particular combinations of the capsid interacting domain and DNA binding domain are as described in the previous section. It is further preferred that the parvovirus template comprises an AAV terminal repeat sequence (preferably two AAV terminal repeat sequences at the 5' and 3' ends of the vDNA). It is further preferred that the AAV terminal repeat(s) is from AAV1, AAV2, AAV4 or AAV5. Alternatively, the parvovirus template comprises an autonomous parvovirus terminal repeat (e.g. B19).

In particular embodiments of the invention, the rep coding sequences encode an AAV1, AAV2, AAV4 or AAV5 DNA binding domain. According to this embodiment, the parvovirus terminal repeat is an AAV terminal repeat and is compatible with the DNA binding domain, and is preferably from the same AAV serotype as the DNA binding domain. In the case of a DNA binding domain from AAV5, the parvovirus terminal repeat will also preferably be from AAV5.

Any suitable permissive or packaging cell known in the art may be employed to produce parvovirus vectors utilizing the invention, including bacterial, protozoan, yeast, fungal, plant and animal cells. Mammalian and insect cells are preferred. Preferred insect cells are those that are infected by baculovirus, e.g., Sf9 cells. Also preferred are trans-complementing packaging cell lines that provide functions deleted from a replication-defective helper virus, e.g., 293 cells or other E1a trans-complementing cells.

In embodiments of the invention, a baculovirus system is used to produce parvovirus particles in mammalian (e.g., human) or insect cells. For



example, the baculovirus may be used to provide the parvovirus template, rep coding sequences and/or cap coding sequences to the cell.

The inventive parvovirus rep coding sequences and cap coding sequences may be provided by any method known in the art. Current protocols typically provide the parvovirus rep and cap genes on a single plasmid. The parvovirus rep coding sequences and cap coding sequences need not be provided by a single construct, although it may be convenient to do so. The parvovirus rep and/or cap sequences may be provided by any viral or non-viral vector. For example, the rep/cap sequences may be provided by a hybrid adenovirus, baculovirus or herpesvirus vector, as described in the previous section (e.g., inserted into the E1a or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the parvovirus cap coding sequences and/or rep coding sequences. One advantage of this method is that EBV vectors are episomal, yet will maintain a high copy number throughout successive cell divisions (i.e., are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome", see Margolski, (1992) *Curr. Top. Microbiol. Immun.* **158**:67). As a further alternative, the rep coding sequences and/or cap coding sequences may be stably integrated into chromosome of the cell.

Typically, and preferably, the AAV rep and cap sequences described above will not be flanked by the AAV packaging sequences (e.g., AAV terminal repeats), to prevent rescue and/or packaging of these sequences. Other methods of preventing the packaging of AAV rep/cap sequences are known in the art (see, e.g., U.S. Patent 6,329,181 to Xiao et al.)

The recombinant parvovirus template (preferably, a rAAV template) is as described herein, and may be provided to the cell using any method known in the art. For example, the recombinant parvovirus template may be supplied by a non-viral (e.g., plasmid) or viral vector (as described above). In particular preferred embodiments, the recombinant parvovirus template is supplied by a herpesvirus or adenovirus vector (e.g., inserted into the E1a or E3 regions of a deleted adenovirus). As another illustration, Palombo et al., (1998) *J. Virology* **72**:5025, describe a baculovirus vector carrying a reporter gene flanked by the AAV terminal repeats. EBV vectors may also be employed to

deliver a rAAV template, as described above with respect to the *rep/cap* genes.

In another preferred embodiment, the AAV template is provided by a replicating rAAV virus. In still other embodiments, an AAV provirus is stably  
5 integrated into the chromosome of the cell.

Production of new AAV particles typically involves helper virus functions to complete the viral life cycle. Both adenovirus and herpes simplex virus may serve as helper viruses for AAV. See, e.g., BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).  
10 Exemplary helper viruses include, but are not limited to, Herpes simplex (HSV) varicella zoster, cytomegalovirus, and Epstein-Barr virus. The multiplicity of infection (MOI) and the duration of the infection will depend on the type of virus used and the packaging cell line employed. Any suitable helper vector may be employed.

15 Helper virus sequences, necessary for AAV replication are known in the art. In general, the helper functions are provided by the adenovirus early genes, more particularly, the E1a, E2a, E4orf6 and VA RNA adenovirus sequences. Typically, these sequences will be provided by a helper adenovirus or herpesvirus (preferably, adenovirus) vector. Alternatively, the  
20 adenovirus or herpesvirus sequences may be provided by another non-viral or viral vector, e.g., as a non-infectious adenovirus miniplasmid that carries all of the helper genes required for efficient AAV production as described by Ferrari *et al.*, (1997) *Nature Med.* **3**:1295, Xiao *et al.*, (1998) *J. Virology* **72**:2224, and U.S. Patent Nos. 6,040,183 and 6,093,570. The vector can be introduced into  
25 the packaging cell by any suitable method known in the art, as described above.

Other methods of producing rAAV stocks have been described, including but not limited to, methods that split the *rep* and *cap* genes onto separate expression cassettes to prevent the generation of replication-  
30 competent AAV (see, e.g., Allen *et al.*, (1997) *J. Virol.* **71**:6816), methods employing packaging cell lines (see, e.g., Gao *et al.*, (1998) *Human Gene Therapy* **9**:2353; Inoue *et al.*, (1998) *J. Virol.* **72**:7024; U.S. Patent No. 5,837,484; WO 98/27207; U.S. Patent No. 5,658,785; WO 96/17947), and

other helper virus free systems (*see, e.g.*, U.S. Patent No. 5,945,335 to Colosi).

Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously facilitate more scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 *rep* and *cap* genes has been described (Conway et al., (1999) *Gene Therapy* 6:986 and WO 00/17377, the disclosures of which are incorporated herein in their entireties).

Further, the helper virus functions may be provided by a packaging cell with the helper genes embedded in the chromosome or maintained as a stable extrachromosomal element. It is preferred that these helper virus sequences cannot be packaged into AAV virions, *e.g.*, are not flanked by AAV terminal repeats.

Those skilled in the art will appreciate that it may be advantageous to provide the AAV *rep* coding sequences and *cap* coding sequences and the helper virus sequences (*e.g.*, adenovirus sequences) on a single helper construct. This helper construct may be a non-viral or viral construct, but is preferably a hybrid adenovirus or hybrid herpesvirus comprising the AAV *rep/cap* genes as described above.

In one particular embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. This vector further contains the rAAV template. The AAV *rep/cap* sequences and/or the rAAV template may be inserted into a deleted region (*e.g.*, the E1a or E3 regions) of the adenovirus.

In a further embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. The rAAV template is provided as a plasmid template.

In another embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper vector, and the rAAV template is integrated into the cell as a provirus. Alternatively, the rAAV template is provided by an EBV vector that is maintained within the cell as an extrachromosomal element (*e.g.*, as an EBV based nuclear episome).

In a further exemplary embodiment, the AAV rep/cap sequences and adenovirus helper sequences are provided by a single adenovirus helper. The rAAV template is provided as a separate replicating viral vector. For example, the rAAV template may be provided by a rAAV particles or a second  
5 recombinant adenovirus particle.

According to the foregoing methods, the hybrid adenovirus vector typically comprises the adenovirus 5' and 3' cis sequences sufficient for adenovirus replication and packaging (*i.e.*, the adenovirus terminal repeats and PAC sequence). The AAV rep/cap sequences and, if present, the rAAV  
10 template are embedded in the adenovirus backbone and are flanked by the 5' and 3' cis sequences, so that these sequences may be packaged into adenovirus capsids. As described above, it is preferred that the adenovirus helper sequences and the AAV rep/cap sequences are not flanked by the AAV packaging sequences (*e.g.*, the AAV terminal repeats), so that these  
15 sequences are not packaged into the AAV virions.

Generally, the adenovirus is grown for a time sufficient to produce an adenovirus stock, optionally in the presence of a suitable helper virus or in a suitable packaging cell as known in the art (*i.e.*, to complement any genes deleted or inactivated in the adenovirus vector).

AAV vector stocks free of contaminating helper virus may be obtained  
20 by any method known in the art. For example, AAV and helper virus may be readily differentiated based on size. AAV may also be separated away from helper virus based on affinity for a heparin substrate (Zolotukhin et al. (1999) *Gene Therapy* 6:973). Preferably, deleted replication-defective helper viruses  
25 are used so that any contaminating helper virus is not replication competent. As a further alternative, an adenovirus helper lacking late gene expression may be employed, as only adenovirus early gene expression is required to mediate packaging of AAV virus. Adenovirus mutants defective for late gene expression are known in the art (*e.g.*, ts100K and ts149 adenovirus mutants).

The reagents and methods disclosed herein may be employed to  
30 produce high-titer stocks of the inventive parvovirus vectors, preferably at essentially wild-type titers. It is also preferred that the parvovirus stock has a titer of at least about  $10^5$  transducing units (tu)/ml, more preferably at least about  $10^6$  tu/ml, more preferably at least about  $10^7$  tu/ml, yet more preferably

at least about  $10^8$  tu/ml, yet more preferably at least about  $10^9$  tu/ml, still yet more preferably at least about  $10^{10}$  tu/ml, still more preferably at least about  $10^{11}$  tu/ml, or more.

Alternatively stated, the parvovirus stock preferably has a titer of at  
5 least about 1, 5, 10, 20, 50, 100, 250, 500, 1000, 2500 tu/cell or more.

The virus titers achieved with the inventive methods and chimeric rep coding sequences (or Rep proteins) may advantageously be greater than the titers achieved using a non-chimeric rep coding sequence as a control, *e.g.*, at least about 2-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold  
10 or 1000-fold greater or more. For example, as demonstrated in the working examples, virus titers of a hybrid AAV particle comprising a recombinant AAV2 genome (*i.e.*, with AAV2 TRs) packaged within a different AAV serotype capsid may be higher using a chimeric rep coding sequence of the invention that is compatible with both the AAV2 TRs and the capsid as compared with  
15 the titers achieved using a non-chimeric AAV2 rep coding sequence.

### **Recombinant Parvovirus Vectors.**

The parvovirus vectors produced according to the present invention are useful for the delivery of nucleic acids to cells *in vitro*, *ex vivo*, and *in vivo*. In  
20 particular, the parvovirus vectors can be advantageously employed to deliver or transfer nucleic acids to animal, more preferably mammalian, cells. Nucleic acids of interest include nucleic acids encoding polypeptides, preferably therapeutic (*e.g.*, for medical or veterinary uses) or immunogenic (*e.g.*, for vaccines) polypeptides.

Typically, the recombinant parvovirus genome will only retain the minimal terminal repeat (TR) sequence(s) so as to maximize the size of the transgene that can be efficiently packaged by the vector. The structural and non-structural protein coding sequences may be provided *in trans* (*e.g.*, from  
25 a vector, such as a plasmid, or by stably integrating the sequences into a packaging cell). The recombinant parvovirus vector genome comprises at  
30 least one terminal repeat, more typically two terminal repeats, which generally flank the 5' and 3' ends of the heterologous nucleotide sequence(s).

Any heterologous nucleotide sequence(s) (as defined above) may be delivered in a parvovirus particle produced according to the present invention.

Nucleic acids of interest include nucleic acids encoding polypeptides, preferably therapeutic (e.g., for medical or veterinary uses) or immunogenic (e.g., for vaccines) polypeptides.

The recombinant vector genome is preferably approximately the size of the wild-type parvovirus genome (e.g., the AAV genome) corresponding to the parvovirus capsid into which it will be packaged (e.g., from about 80% to about 105% of wt) and comprises an appropriate packaging signal. In the case of AAV, it is well-known in the art that the AAV capsid disfavors packaging of vDNA that substantially deviate in size from the wt AAV genome. In the case of an AAV capsid, the genome is preferably approximately 5.2 kb in size or less. In other embodiments, the genome is preferably greater than about 3.6, 3.8, 4.0, 4.2, or 4.4 kb in length and/or less than about 5.4, 5.2, 5.0 or 4.8 kb in length. Alternatively stated, the heterologous nucleotide sequence(s) will typically be less than about 5 kb in length (more preferably less than about 4.8 kb, still more preferably less than about 4.4 kb in length, yet more preferably less than about 4.2 kb in length) to facilitate packaging of the duplexed template by the parvovirus (e.g., AAV) capsid.

The present invention may be further used to produce a parvovirus vector to deliver a therapeutic polypeptide. Therapeutic polypeptides include, but are not limited to, cystic fibrosis transmembrane regulator protein (CFTR), dystrophin (including the protein product of dystrophin mini-genes, see, e.g., Vincent *et al.*, (1993) *Nature Genetics* 5:130), utrophin (Tinsley *et al.*, (1996) *Nature* 384:349), clotting factors (e.g., Factor XIII, Factor IX, Factor X, *etc.*), erythropoietin, angiostatin, endostatin, catalase, tyrosine hydroxylase, superoxide dismutase, leptin, the LDL receptor, lipoprotein lipase, ornithine transcarbamylase,  $\beta$ -globin,  $\alpha$ -globin, spectrin,  $\alpha_1$ -antitrypsin, adenosine deaminase, hypoxanthine guanine phosphoribosyl transferase,  $\beta$ -glucocerebrosidase, sphingomyelinase, lysosomal hexosaminidase, branched-chain keto acid dehydrogenase, RP65 protein, cytokines (e.g.,  $\alpha$ -interferon,  $\beta$ -interferon, interferon- $\gamma$ , interleukin-2, interleukin-4, granulocyte-macrophage colony stimulating factor, lymphotoxin, and the like), peptide growth factors and hormones (e.g., somatotropin, insulin, insulin-like growth factors 1 and 2, platelet derived growth factor, epidermal growth factor,

fibroblast growth factor, nerve growth factor, neurotrophic factor –3 and –4, brain-derived neurotrophic factor, glial derived growth factor, transforming growth factor – $\alpha$  and – $\beta$ , and the like), receptors (*e.g.*, the tumor necrosis growth factor receptor), monoclonal antibodies (including single chain  
5 monoclonal antibodies; an exemplary Mab is the herceptin Mab). Other illustrative heterologous nucleotide sequences encode suicide gene products (*e.g.*, thymidine kinase, cytosine deaminase, diphtheria toxin, and tumor necrosis factor), proteins conferring resistance to a drug used in cancer therapy, tumor suppressor gene products (*e.g.*, p53, Rb, Wt-1), and any other  
10 polypeptide that has a therapeutic effect in a subject in need thereof.

Heterologous nucleotide sequences encoding polypeptides include those encoding reporter polypeptides (*e.g.*, an enzyme). Reporter polypeptides are known in the art and include, but are not limited to, Green Fluorescent Protein,  $\beta$ -galactosidase, alkaline phosphatase, and  
15 chloramphenicol acetyltransferase gene.

Alternatively, the nucleic acid of interest may encode an antisense nucleic acid, a ribozyme (*e.g.*, as described in U.S. Patent No. 5,877,022), RNAs that effect spliceosome-mediated *trans*-splicing (see, Puttaraju *et al.*, (1999) *Nature Biotech.* **17**:246; U.S. Patent No. 6,013,487; U.S. Patent No.  
20 6,083,702); interfering RNAs (RNAi) that mediate gene silencing (see, Sharp *et al.*, (2000) *Science* **287**:2431) or other non-translated RNAs, such as “guide” RNAs (Gorman *et al.*, (1998) *Proc. Nat. Acad. Sci. USA* **95**:4929; U.S. Patent No. 5,869,248 to Yuan *et al.*), and the like.

The parvovirus vector may also encode a heterologous nucleotide  
25 sequence that shares homology with and recombines with a locus on the host chromosome. This approach may be utilized to correct a genetic defect in the host cell.

The present invention may be used to produce a parvovirus vector to express an immunogenic polypeptide in a subject, *e.g.*, for vaccination. The  
30 nucleic acid may encode any immunogen of interest known in the art including, but are not limited to, immunogens from human immunodeficiency virus, influenza virus, gag proteins, tumor antigens, cancer antigens, bacterial antigens, viral antigens, and the like.

The use of parvoviruses as vaccines is known in the art (see, e.g., Miyamura *et al.*, (1994) *Proc. Nat. Acad. Sci USA* **91**:8507; U.S. Patent No. 5,916,563 to Young *et al.*, 5,905,040 to Mazzara *et al.*, U.S. Patent No. 5,882,652, U.S. Patent No. 5,863,541 to Samulski *et al.*; the disclosures of which are incorporated herein in their entirety by reference). The antigen may be presented in the parvovirus capsid. Alternatively, the antigen may be expressed from a heterologous nucleic acid introduced into a recombinant vector genome. Any immunogen of interest may be provided by the parvovirus vector. Immunogens of interest are well-known in the art and include, but are not limited to, immunogens from human immunodeficiency virus, influenza virus, gag proteins, tumor antigens, cancer antigens, bacterial antigens, viral antigens, and the like.

An immunogenic polypeptide, or immunogen, may be any polypeptide suitable for protecting the subject against a disease, including but not limited to microbial, bacterial, protozoal, parasitic, and viral diseases. For example, the immunogen may be an orthomyxovirus immunogen (e.g., an influenza virus immunogen, such as the influenza virus hemagglutinin (HA) surface protein or the influenza virus nucleoprotein gene, or an equine influenza virus immunogen), or a lentivirus immunogen (e.g., an equine infectious anemia virus immunogen, a Simian Immunodeficiency Virus (SIV) immunogen, or a Human Immunodeficiency Virus (HIV) immunogen, such as the HIV or SIV envelope GP160 protein, the HIV or SIV matrix/capsid proteins, and the HIV or SIV gag, pol and env genes products). The immunogen may also be an arenavirus immunogen (e.g., Lassa fever virus immunogen, such as the Lassa fever virus nucleocapsid protein gene and the Lassa fever envelope glycoprotein gene), a poxvirus immunogen (e.g., vaccinia, such as the vaccinia L1 or L8 genes), a flavivirus immunogen (e.g., a yellow fever virus immunogen or a Japanese encephalitis virus immunogen), a filovirus immunogen (e.g., an Ebola virus immunogen, or a Marburg virus immunogen, such as NP and GP genes), a bunyavirus immunogen (e.g., RVFV, CCHF, and SFS viruses), or a coronavirus immunogen (e.g., an infectious human coronavirus immunogen, such as the human coronavirus envelope glycoprotein gene, or a porcine transmissible gastroenteritis virus immunogen, or an avian infectious bronchitis virus immunogen). The immunogen may



further be a polio immunogen, herpes antigen (e.g., CMV, EBV, HSV immunogens) mumps immunogen, measles immunogen, rubella immunogen, diphtheria toxin or other diphtheria immunogen, pertussis antigen, hepatitis (e.g., hepatitis A or hepatitis B) immunogen, or any other vaccine immunogen  
5 known in the art.

Alternatively, the immunogen may be any tumor or cancer cell antigen. Preferably, the tumor or cancer antigen is expressed on the surface of the cancer cell. Exemplary cancer and tumor cell antigens are described in S.A. Rosenberg, (1999) *Immunity* **10**:281). Other illustrative cancer and tumor  
10 antigens include, but are not limited to: BRCA1 gene product, BRCA2 gene product, gp100, tyrosinase, GAGE-1/2, BAGE, RAGE, NY-ESO-1, CDK-4,  $\beta$ -catenin, MUM-1, Caspase-8, KIAA0205, HPVE, SART-1, PRAME, p15, melanoma tumor antigens (Kawakami et al., (1994) *Proc. Natl. Acad. Sci. USA* **91**:3515); Kawakami et al., (1994) *J. Exp. Med.*, **180**:347); Kawakami et al., (1994) *Cancer Res.* **54**:3124), including MART-1 (Coulie et al., (1991) *J. Exp. Med.* **180**:35), gp100 (Wick et al., (1988) *J. Cutan. Pathol.* **4**:201) and  
15 MAGE antigen, MAGE-1, MAGE-2 and MAGE-3 (Van der Bruggen et al., (1991) *Science*, **254**:1643); CEA, TRP-1, TRP-2, P-15 and tyrosinase (Brichard et al., (1993) *J. Exp. Med.* **178**:489); HER-2/neu gene product (U.S. Pat. No. 4,968,603), CA 125, LK26, FB5 (endosialin), TAG 72, AFP, CA19-9, NSE, DU-PAN-2, CA50, SPan-1, CA72-4, HCG, STN (sialyl Tn antigen), c-erbB-2 proteins, PSA, L-CanAg, estrogen receptor, milk fat globulin, p53 tumor suppressor protein (Levine, (1993) *Ann. Rev. Biochem.* **62**:623); mucin antigens (international patent publication WO 90/05142); telomerases; nuclear  
25 matrix proteins; prostatic acid phosphatase; papilloma virus antigens; and antigens associated with the following cancers: melanomas, metastases, adenocarcinoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, colon cancer, non-Hodgkins lymphoma, Hodgkins lymphoma, leukemias, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical  
30 cancer, bladder cancer, kidney cancer, pancreatic cancer and others (see, e.g., Rosenberg, (1996) *Ann. Rev. Med.* **47**:481-91).

Alternatively, the heterologous nucleotide sequence may encode any polypeptide that is desirably produced in a cell *in vitro*, *ex vivo*, or *in vivo*. For

example, the parvovirus vectors may be introduced into cultured cells and the expressed gene product isolated therefrom.

It will be understood by those skilled in the art that the heterologous nucleotide sequence(s) of interest may be operably associated with appropriate control sequences. For example, the heterologous nucleic acid may be operably associated with expression control elements, such as transcription/translation control signals, origins of replication, polyadenylation signals, and internal ribosome entry sites (IRES), promoters, enhancers, and the like.

Those skilled in the art will appreciate that a variety of promoter/enhancer elements may be used depending on the level and tissue-specific expression desired. The promoter/enhancer may be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer may be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

Promoter/enhancer elements that are native to the target cell or subject to be treated are most preferred. Also preferred are promoters/enhancer elements that are native to the heterologous nucleic acid sequence. The promoter/enhancer element is chosen so that it will function in the target cell(s) of interest. Mammalian promoter/enhancer elements are also preferred. The promoter/enhance element may be constitutive or inducible.

Inducible expression control elements are preferred in those applications in which it is desirable to provide regulation over expression of the heterologous nucleic acid sequence(s). Inducible promoters/enhancer elements for gene delivery are preferably tissue-specific promoter/enhancer elements, and include muscle specific (including cardiac, skeletal and/or smooth muscle), neural tissue specific (including brain-specific), eye (including retina-specific and cornea-specific), liver specific, bone marrow specific, pancreatic specific, spleen specific, and lung specific promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible promoters/enhancer elements include, but are not limited to, a Tet on/off

element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

In embodiments wherein which the heterologous nucleic acid sequence(s) will be transcribed and then translated in the target cells, specific initiation signals are generally required for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which may include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

### 10 **Gene Transfer Technology.**

The parvovirus vectors produced according to the present invention also provide a means for delivering heterologous nucleotide sequences into a broad range of cells, including dividing and non-dividing cells. The parvovirus vectors may be employed to deliver a nucleotide sequence of interest to a cell *in vitro*, e.g., to produce a polypeptide *in vitro* or for *ex vivo* gene therapy. The vectors are additionally useful in a method of delivering a nucleotide sequence to a subject in need thereof, e.g., to express an immunogenic or therapeutic polypeptide. In this manner, the polypeptide may thus be produced *in vivo* in the subject. The subject may be in need of the polypeptide because the subject has a deficiency of the polypeptide, or because the production of the polypeptide in the subject may impart some therapeutic effect, as a method of treatment or otherwise, and as explained further below.

In general, the parvovirus vectors produced according to the present invention may be employed to deliver any foreign nucleic acid with a biological effect to treat or ameliorate the symptoms associated with any disorder related to gene expression. Illustrative disease states include, but are not limited to: cystic fibrosis (and other diseases of the lung), hemophilia A, hemophilia B, thalassemia, anemia and other blood disorders, AIDs, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, epilepsy, and other neurological disorders, cancer, diabetes mellitus, muscular dystrophies (e.g., Duchenne, Becker), Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases and other metabolic defects, retinal degenerative diseases (and

other diseases of the eye), diseases of solid organs (e.g., brain, liver, kidney, heart), and the like.

Alternatively, a gene transfer vector may be administered that encodes any therapeutic polypeptide.

5           Gene transfer has substantial potential use in understanding and providing therapy for disease states. There are a number of inherited diseases in which defective genes are known and have been cloned. In general, the above disease states fall into two classes: deficiency states, usually of enzymes, which are generally inherited in a recessive manner, and  
10           unbalanced states, which may involve regulatory or structural proteins, and which are typically inherited in a dominant manner. For deficiency state diseases, gene transfer could be used to bring a normal gene into affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For unbalanced disease states, gene  
15           transfer could be used to create a disease state in a model system, which could then be used in efforts to counteract the disease state. Thus parvovirus vectors produced according to the methods of the present invention permit the treatment of genetic diseases. As used herein, a disease state is treated by partially or wholly remedying the deficiency or imbalance that causes the  
20           disease or makes it more severe. The use of site-specific recombination of nucleic sequences to cause mutations or to correct defects is also possible.

          The parvovirus vectors produced according to the present invention may also be employed to provide an antisense nucleic acid to a cell *in vitro* or *in vivo*. Expression of the antisense nucleic acid in the target cell diminishes  
25           expression of a particular protein by the cell. Accordingly, antisense nucleic acids may be administered to decrease expression of a particular protein in a subject in need thereof. Antisense nucleic acids may also be administered to cells *in vitro* to regulate cell physiology, e.g., to optimize cell or tissue culture systems. Alternatively, the parvovirus vector may encode any other non-  
30           translated RNA, as described in more detail hereinabove.

          Finally, the parvovirus vectors produced according to the instant invention find further use in diagnostic and screening methods, whereby a gene of interest is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model.

### Delivery of Immunogenic Polypeptides.

As a further aspect, parvovirus vectors produced according to the present invention may be used to produce an immune response in a subject.

5 According to this embodiment, a parvovirus vector comprising a nucleotide sequence encoding an immunogen may be administered to a subject, and an active immune response is mounted by the subject against the immunogen. Immunogens are as described hereinabove. Preferably, a protective immune response is elicited.

10 Alternatively, the parvovirus vector may be administered to a cell *ex vivo* and the altered cell is administered to the subject. The heterologous nucleotide sequence is permitted to be introduced into the cell, and the cell is administered to the subject, where the heterologous nucleotide sequence encoding the immunogen is preferably expressed and induces an immune  
15 response in the subject against the immunogen. Preferably, the cell is an antigen presenting cell (*e.g.*, a dendritic cell) or a cancer.

An "active immune response" or "active immunity" is characterized by "participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in  
20 lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both." Herbert B. Herscovitz, *Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation*, in IMMUNOLOGY: BASIC PROCESSES 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the  
25 host after exposure to immunogens by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the "transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host." *Id.*

A "protective" immune response or "protective" immunity as used  
30 herein indicates that the immune response confers some benefit to the subject in that it prevents or reduces the incidence of disease. Alternatively, a protective immune response or protective immunity may be useful in the treatment of disease, in particular cancer or tumors (*e.g.*, by causing regression of a cancer or tumor and/or by preventing metastasis and/or by

preventing growth of metastatic nodules). The protective effects may be complete or partial, as long as the benefits of the treatment outweigh any disadvantages thereof.

5 According to the foregoing methods of inducing an immune response in a subject, it is preferred that the parvovirus vector carrying the heterologous nucleotide sequence is administered in an immunogenically effective amount, as described below.

10 The parvovirus vectors produced according to the present invention may also be administered for cancer immunotherapy by administration of a parvovirus vector expressing cancer cell antigens or any other immunogen that produces an immune response against a cancer cell. To illustrate, an immune response may be produced against a cancer cell antigen in a subject by administering a parvovirus vector comprising a heterologous nucleotide sequence encoding the cancer cell antigen, for example to treat a patient with  
15 cancer. The parvovirus vector may be administered to a subject *in vivo* or by using *ex vivo* methods, as described herein.

As used herein, the term "cancer" encompasses tumor-forming cancers. Likewise, the term "cancerous tissue" encompasses tumors. A "cancer cell antigen" encompasses tumor antigens.

20 The term "cancer" has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Exemplary cancers include, but are not limited to, leukemias, lymphomas, colon cancer, renal cancer, liver cancer, breast cancer, lung cancer, prostate cancer, ovarian cancer, melanoma, and  
25 the like. Preferred are methods of treating and preventing tumor-forming cancers.

The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign. Preferably, the methods disclosed herein  
30 are used to prevent and treat malignant tumors.

Cancer cell antigens according to the present invention have been described hereinabove. By the terms "treating cancer" or "treatment of cancer", it is intended that the severity of the cancer is reduced or the cancer is at least partially eliminated. Preferably, these terms indicate that metastasis

of the cancer is reduced or at least partially eliminated. It is further preferred that these terms indicate that growth of metastatic nodules (e.g., after surgical removal of a primary tumor) is reduced or at least partially eliminated. By the terms "prevention of cancer" or "preventing cancer" it is intended that the methods at least partially eliminate or reduce the incidence or onset of cancer. 5 Alternatively stated, the onset of cancer in the subject may be slowed, controlled, decreased in likelihood or probability, or delayed.

In particular embodiments, cells may be removed from a subject with cancer and contacted with parvovirus particles produced according to the instant invention. The modified cell is then administered to the subject, whereby an immune response against the cancer cell antigen is elicited. This method is particularly advantageously employed with immunocompromised subjects that cannot mount a sufficient immune response *in vivo* (i.e., cannot produce enhancing antibodies in sufficient quantities). 10

It is known in the art that immune responses may be enhanced by immunomodulatory cytokines (e.g.,  $\alpha$ -interferon,  $\beta$ -interferon,  $\gamma$ -interferon,  $\omega$ -interferon,  $\tau$ -interferon, interleukin-1 $\alpha$ , interleukin-1 $\beta$ , interleukin-2, interleukin-3, interleukin-4, interleukin 5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin 12, interleukin-13, 20 interleukin-14, interleukin-18, B cell Growth factor, CD40 Ligand, tumor necrosis factor- $\alpha$ , tumor necrosis factor- $\beta$ , monocyte chemoattractant protein-1, granulocyte-macrophage colony stimulating factor, and lymphotoxin). Accordingly, immunomodulatory cytokines (preferably, CTL inductive cytokines) may be administered to a subject in conjunction with the parvovirus vectors. 25

Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleotide sequence encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*.

In particular embodiments, the parvovirus vector may be administered as part of a method of treating cancer by administering anti-cancer agents (e.g., cytokines, tumor suppressor gene products, as described above) The 30

parvovirus particle may be administered to a cell *in vitro* or to a subject *in vivo* or by using *ex vivo* methods, as described herein and known in the art.

**Subjects, Pharmaceutical Formulations, and Modes of Administration.**

5 Parvovirus vectors produced according to the present invention find use in both veterinary and medical applications. Suitable subjects for *ex vivo* gene delivery methods as described above include both avians and mammals, with mammals being preferred. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys and  
10 pheasants. The term "mammal" as used herein includes, but is not limited to, humans, bovines, ovines, caprines, equines, felines, canines, lagomorphs, *etc.* Human subjects are most preferred. Human subjects include neonates, infants, juveniles, and adults.

In particular embodiments, the present invention provides a  
15 pharmaceutical composition comprising a virus particle of the invention in a pharmaceutically acceptable carrier and/or other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, *etc.* For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be  
20 respirable, and will preferably be in solid or liquid particulate form. As an injection medium, it is preferred to use water that contains the additives usual for injection solutions, such as stabilizing agents, salts or saline, and/or buffers.

In general, a "physiologically acceptable carrier" is one that is not toxic  
25 or unduly detrimental to cells. Exemplary physiologically acceptable carriers include sterile, pyrogen-free water and sterile, pyrogen-free, phosphate buffered saline. Physiologically acceptable carriers include pharmaceutically acceptable carriers.

By "pharmaceutically acceptable" it is meant a material that is not  
30 biologically or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects. Thus, such a pharmaceutical composition may be used, for example, in transfection of a cell *ex vivo* or in administering a viral particle or cell directly to a subject.



One aspect of the present invention is a method of transferring a nucleotide sequence to a cell *in vitro*. The virus particles may be added to the cells at the appropriate multiplicity of infection according to standard transduction methods appropriate for the particular target cells. Titers of virus to administer can vary, depending upon the target cell type and number, and the particular virus vector, and can be determined by those of skill in the art without undue experimentation. Preferably, at least about  $10^3$  infectious units, more preferably at least about  $10^5$  infectious units, are administered to the cell.

The cell(s) to be administered the parvovirus vector may be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons and oligodendrocytes), lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), epithelial cells (e.g., gut and respiratory epithelial cells), muscle cells, dendritic cells, pancreatic cells (including islet cells), hepatic cells, myocardial cells, bone cells (e.g., bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. Alternatively, the cell may be any progenitor cell. As a further alternative, the cell can be a stem cell (e.g., neural stem cell, liver stem cell). As still a further alternative, the cell may be a cancer or tumor cell. Moreover, the cells can be from any species of origin, as indicated above.

The parvovirus vectors may be administered to cells *in vitro* for the purpose of administering the modified cell to a subject. In particular embodiments, the cells have been removed from a subject, the parvovirus vector is introduced therein, and the cells are then replaced back into the subject. Methods of removing cells from subject for treatment *ex vivo*, followed by introduction back into the subject are known in the art (see, e.g., U.S. patent No. 5,399,346; the disclosure of which is incorporated herein in its entirety). Alternatively, the recombinant parvovirus vector is introduced into cells from another subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof.

Suitable cells for *ex vivo* gene therapy are as described above. Dosages of the cells to administer to a subject will vary upon the age,

condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about  $10^2$  to about  $10^8$ , preferably about  $10^3$  to about  $10^6$  cells, will be administered per dose in a pharmaceutically acceptable carrier. The cells  
5 transduced with the parvovirus vector are preferably administered to the subject in a therapeutically effective amount in combination with a pharmaceutical carrier.

A "therapeutically effective" amount as used herein is an amount that provides sufficient expression of the heterologous nucleotide sequence  
10 delivered by the vector to provide some improvement or benefit to the subject. Alternatively stated, a "therapeutically effective" amount is an amount that will provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit  
15 is provided to the subject.

In some embodiments, cells that have been transduced with a parvovirus vector may be administered to elicit an immunogenic response against the delivered polypeptide (e.g., expressed as a transgene or in the capsid). Typically, a quantity of cells expressing an immunogenic amount of  
20 the polypeptide in combination with a pharmaceutically acceptable carrier is administered. An "immunogenic amount" is an amount of the expressed polypeptide that is sufficient to evoke an active immune response in the subject to which the pharmaceutical formulation is administered. Preferably, the dosage is sufficient to produce a protective immune response (as defined  
25 above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof.

A further aspect of the invention is a method of treating subjects *in vivo* with the parvovirus particles. Administration of the parvovirus particles produced  
30 according to the present invention to a human subject or an animal in need thereof can be by any means known in the art for administering virus vectors. Preferably, the parvovirus vector is delivered in a therapeutically effective dose in a pharmaceutically acceptable carrier.

The parvovirus vectors of the invention may be administered to elicit an immunogenic response (e.g., as a vaccine). Typically, vaccines of the present invention comprise an immunogenic amount of infectious virus particles as disclosed herein in combination with a pharmaceutically acceptable carrier.

5 Preferably, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof. Subjects and immunogens are as described above.

10 Dosages of the parvovirus particles to be administered to a subject will depend upon the mode of administration, the disease or condition to be treated, the individual subject's condition, the particular virus vector, and the nucleic acid to be delivered, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are virus titers of at least  
15 about  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$ ,  $10^{11}$ ,  $10^{12}$ ,  $10^3$ ,  $10^{14}$ ,  $10^{15}$  transducing units or more, preferably about  $10^8$  –  $10^{13}$  transducing units, yet more preferably  $10^{12}$  transducing units.

In particular embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired  
20 level of gene expression.

Exemplary modes of administration include oral, rectal, transmucosal, topical, transdermal, *in utero* (or *in ovo*), inhalation, parenteral (e.g., intravenous, subcutaneous, intradermal, intramuscular, and intraarticular) administration, and the like, as well as direct tissue or organ injection,  
25 alternatively, intrathecal, direct intramuscular, intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus in a local rather than  
30 systemic manner, for example, in a depot or sustained-release formulation.

The parvovirus vector administered to the subject may transduce any permissive cell or tissue. Suitable cells for transduction by the parvovirus vectors are as described above.

In particularly preferred embodiments of the invention, the nucleotide sequence of interest is delivered to the liver of the subject. Administration to the liver may be achieved by any method known in the art, including, but not limited to intravenous administration, intraportal administration, intrabiliary  
5 administration, intra-arterial administration, and direct injection into the liver parenchyma.

In other preferred embodiments, the parvovirus particles are administered intramuscularly, more preferably by intramuscular injection or by local administration (as defined above). Delivery to the brain is also preferred.  
10 In other preferred embodiments, the parvovirus particles of the present invention are administered to the lungs.

The parvovirus vectors disclosed herein may be administered to the lungs of a subject by any suitable means, but are preferably administered by administering an aerosol suspension of respirable particles comprised of the  
15 parvovirus vectors, which the subject inhales. The respirable particles may be liquid or solid. Aerosols of liquid particles comprising the parvovirus vectors may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. See, e.g., U.S. Patent No. 4,501,729. Aerosols of solid particles  
20 comprising the parvovirus vectors may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

Having described the present invention, the same will be explained in  
25 greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

### Example 1

#### 30 Construction of Plasmids

The plasmid pBS+ (Stratagene) was used as the backbone for cloning AAV serotypes 1 to 5 capsid genes. The AAV2 replication gene was subcloned into the plasmid pAAV2Cap (Rabinowitz et al. (1999), *Virology*, 265:274) by using a *Xba*I (blunt ended) *Swa*I digestion of pACG2 (Li et al.

(1997) *J. Virol.* **71**:5236) and the *Sma*I/*Swa*I digestion of pAAV2Cap. The new plasmid, pAAV2rep, was digested with *Nae*I, blunt ended with mung bean nuclease, and digested with *Swa*I, removing the capsid genes and leaving only the replication gene from AAV2. Viral sequences from AAV1, -2, -3, -4, -5  
5 were cloned from ATCC stocks. Primers were designed for each of these serotypes, such that the *Swa*I was present before the coding region of Vp1 and a unique *Not*I site was present after the polyadenylation site. The forward primers used were AAV1 and -2 (5'-AATCAGGTATGGCTGCCGAT-3' **SEQ ID NO:1**), AAV3 (5'-AAATCAGGTATGGCTGCTGAT-3' **SEQ ID NO:2**), AAV4 (5'-  
10 AAATCAGGTATGGCTGCTGACGGTTAC-3' **SEQ ID NO:3**), and AAV5 (5'-AAATCAGGTATGGCTTTTGTGATCAC-3' **SEQ ID NO:4**). The reverse primers used were AAV1, -2, -3, and -4 (5'-GCGGCCGCGAGACCAAAGTTCAACTGA-3' **SEQ ID NO:5**) and AAV5 (5'-CGGCCGCAAGAGGCAGTATTTTACTGA-3' **SEQ ID NO:6**). *Pfu* polymerase  
15 (Stratagene) was used in the PCR to generate the serotype-specific clones with blunt ends. These serotype-specific capsid coding fragments were then cloned into pAAV2rep. A *Bst*NI digestion was used to confirm the presence and orientation of the positive clones (**Fig. 1**, panel B). In clones containing the serotype 3, 4, or 5 capsid gene, a portion of the serotype-specific rep  
20 gene was substituted for that of the AAV2 rep gene. The AAV3 serotype clone was digested with *Acc*I, as was the original plasmid (nucleotide 1424 to 4355 of the AAV3 sequence, **Fig. 1**, panel A). The AAV4 serotype clone was digested with *Acc*I and *Age*I, as was the original plasmid (nucleotides 1479 to 4488 of the AAV4 sequence, **Fig. 1**, panel A). The AAV5 serotype clone was  
25 digested with *Bam*HI, as was the original plasmid (nucleotides 1071 to 2276 of the AAV5 sequence, **Fig. 1**, panel A). These clones were designated pXR1, -2, -3, -4, and -5 respectively. An additional AAV5 serotype clone and the sixth construct shown in **Fig. 1**, panel A, was constructed by digestion of the serotype specific clone with *Nco*I and *Bam*HI (nucleotides 670 to 1076 of the  
30 AAV5 sequence), as well as the original plasmid. The vector plasmid pTR/CMV/GFP (enhanced green fluorescent protein [EGFP] transgene) contains AAV2 terminal repeats flanking a cytomegalovirus (CMV) immediate-early promoter driven transgene (Halbert et al. (2000) *J. Virol.* **74**:1524). The helper plasmid pXX6-80 containing adenovirus genes required for AAV

production, was a gift from Xiao Xiao, and the plasmid pCB-AAT was provided by Terry Flotte.

## Example 2

5

### Cell Culture

All cell lines (293 human embryonic kidney, HeLa, Cos 7, Cos 1, CHO K1 and CHO K1 mutant p) were originally obtained from ATCC, and maintained in 5% CO<sub>2</sub> saturation at 37°C. 293T, HeLa, Cos1 and Cos7 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (Sigma), and CHO K1, where as CHO K1and were cultured in F12 Nutrient Mixture (HAM) with 10% fetal bovine serum.

## Example 3

### Production of Serotype-Specific Recombinant AAV Vectors

15

Production of all recombinant AAV vectors used in these investigations utilized a three-plasmid transfection scheme outlined in Rabinowitz et al. (2002) *J. Virol.* **76**:(In press) and Xiao et al. (1998), *J. Virol.* **72**:2224. Cells were transfected using Superfect (Qiagen) according to manufacturer's specifications, in which equimolar amounts of each plasmid—75micrograms of pTR/CMV/GFP (vector), 150 micrograms of pXX6-80 (Ad helper), and 75 micrograms of serotype-specific plasmids pXR1, -2, -3, -4, or -5—was used. Cells were harvested 48 h post-transfection and recombinant virus was isolated as described in Rabinowitz et al. (1999), *Virology*, **265**:274.

Recombinant viruses were purified with two rounds of cesium chloride isopycnic centrifugation. To each milliliter of viral supernatant, 0.59 g of CsCl was added, and 1.38 g of CsCl/ml was added to a final volume of 12 ml. The solution was centrifuged at 37,000 rpm for 36 to 48 h (Sorvall Ultra 80 rotor). Peak fractions from the first cesium gradient were determined by infection of HeLa cells except for AAV4, which was tested on Cos1 cells. A second CsCl gradient was incorporated for further purity at 65,000 rpm for 4 h (Beckman NVT-65 rotor). Peak fractions from the second cesium gradient were determined by dot blot hybridization described below, then subjected to heparin-Sepharose column chromatography as described in Rabinowitz et al. (2002) *J. Virol.* **76**:(In press) and Rabinowitz et al. (1999), *Virology*, **265**:274.

30

#### Example 4

##### 5        **SDS Polyacrylamide Gel Electrophoresis and Western Blots**

Cell-free lysates were derived from transfected human embryonic kidney cells 293 as described in Rabinowitz et al. (1999), *Virology*, **265**:274 and subject to Western blot analysis. Briefly, an aliquot was removed, to which PMSF at 100  $\mu$ g/ml (Sigma), pepstatin A at 1  $\mu$ g/ml (Sigma), and leupeptine at 2  $\mu$ g/ml (Sigma) were added. Protein concentrations were  
10        estimated with a BCA Protein Assay kit (Pierce). Equivalent amounts of protein (5  $\mu$ g/lane) from lysates of each serotype were fractionated on a 10% denaturing polyacrylamide gel and then transferred to Hybond ECL nitrocellulose membranes (Amersham Pharmacia Biotech). Primary  
15        monoclonal antibodies 1F11, (Hunter and Samulski (1992) *J. Virol.* **66**:317), and B1 (a gift from Jurgen Kleinschmidt) were used to detect AAV Rep and capsid proteins, respectively, as previously described by using SuperSignal West Pico (Pierce) for the detection of the secondary antibody conjugate.

20

#### Example 5

##### Hirt Low Molecular Weight DNA

##### **Analysis from AAV Serotype Transfections**

DNA from AAV serotype transfected 293 cells was isolated 24 h post-transfection using a method described in Hirt (1967) *J. Mol. Biol.* **26**:365.  
25        Approximately half of each sample was digested with *DpnI*, and 2.5 micrograms of digested and undigested DNA was fractionated on a 0.8% agarose gel. After transferring the DNA onto GeneScreen Plus charged nylon membranes (NEN), the membranes were probed with a 735 basepair *NotI* fragment from pTR/CMV/GFP that carries the coding sequence for the  
30        packaged transgene (GFP). The positive signal was visualized by phosphorescence autoradiography using a Storm PhosphorImager and quantitated using ImageQuant software (Molecular Dynamics).

### Example 6

#### Heparin Column Chromatography

After recombinant virus from each of the five serotypes was purified by two rounds of CsCl isopycnic centrifugation and dialyzed against three  
5 changes of 1X PBS (Gibco-BRL) plus 10% sorbitol, an equal volume of virus was injected into a 1 ml HiTrap heparin column (Amersham Pharmacia Biotech) by using Amersham Pharmacia Biotech AKTA fast-performance liquid chromatography. A preset program (12 ml of buffer at 137 mM NaCl, 2 mM MgCl<sub>2</sub>, and 2 mM KCl run over the column at a flow rate 0.5 ml/min for  
10 the flowthrough and wash, followed by a waste wash of 10 ml of the same buffer at 1 ml/min) was implemented before elution. The elution step used a continuous salt gradient from 137 mM NaCl to 1 M NaCl at a flow rate of 0.5 ml/min. For the flowthrough, wash, and elution steps, 0.5 ml fractions were collected, with the waste step collected in a single fraction.

15 Transducing units (TU)/microliter were determined for each fraction by transducing either HeLa (AAV1, -2, -3, and -5) or Cos1 cells (AAV4) in triplicate. The titer of the input virus was also determined so that a percentage of the recovered TU to the total number could be calculated.

20

### Example 7

#### Dot Blot Analysis

Dot blots were performed as described previously in Rabinowitz et al. (1999), *Virology*, **265**:274. Two to 10 microliters from fractions off the cesium gradient, after dialysis, or off the heparin column were blotted onto  
25 GeneScreen Plus charged nylon membranes (NEN) using a dot blot manifold (Schleicher and Schuell) and the DNA was UV cross-linked to the membranes at 60 mJ with a Stratalinker 1800 UV crosslinker (Stratagene). The blots were probed for positive signal with the GFP DNA probe described in **Example 4**.

30

### Example 8

#### In vitro Transduction Assay

Cell lines were infected with rAAV serotypes by using a volume of virus equivalent to 10<sup>5</sup> TU. For each datum point at least three experiments were performed, and the average of these experiments is presented. The titers of



the AAV serotypes 1, 2, 3, and 5 were originally determined in HeLa cells, and that of AAV4 was determined in Cos1 cells. Between  $1 \times 10^5$  and  $1.75 \times 10^5$  cells were plated into 24 well plates 24 h prior to infection. The volume of each serotype equivalent to  $10^5$  TU was added to 100 microliters of DMEM that included adenovirus, and this was added to each well dropwise. After 24 h the number of GFP-positive cells was counted. Eight fields/well were counted, and the average number of GFP-positive cells/well was determined; this number was multiplied by the dilution in order to obtain the number of TU/microliter.

10

### Example 9

#### ELISA Analysis of Factor IX and $\alpha$ 1-Antitrypsin

NOD/Scid, BALB/c, and C57/BL mice (Jackson Laboratories) were maintained and treated in accordance with the guidelines of the Animal Care and Use Committee of University of North Carolina at Chapel Hill. Portal vein and muscular injection were performed and Canine factor IX antigen was detected by ELISA as previously described in Chao et al. (2000) *Mol. Ther.* **2**:619. A modified double antibody sandwich enzyme-linked immunosorbent assay was used for measurement of alpha-1-antitrypsin in biologic fluids as described in Michalski et al. (1985) *J. Immunol. Methods* **83**:101.

20

### Example 10

#### Subretinal Injection and *in vivo* Fluorescence Imaging

Subretinal injections of rAAV were performed via a transcleral transchoroidal approach on wild-type Wistar rats, as previously described in Rolling et al. (1999) *Hum. Gene Ther.* **10**:641. Briefly, the sclera and the choroid were punctured, a 33-gauge needle was then inserted in a tangential direction under an operating microscope. Three microliters of each of the five hybrid rAAV serotypes ( $5 \times 10^{10}$  particles/ml) was delivered into the subretinal space (n = 3, for each serotype). A new method using fundus photography has been developed and performed in order to control the accuracy and reproducibility of subretinal injections (Rolling et al. in preparation). GFP protein expression in live rats was monitored by fluorescent retinal imaging

30

using a Canon UVI retinal camera connected to a digital imaging system and Lhediph Win software. Retinas were examined at 12, 26, and 46 days post-injection.

5

## Example 11

### AAV Hybrid Helper Plasmids

The generation of AAV serotype specific hybrid helper plasmids utilized a common AAV2 rep gene pACG2 described in Li et al. (1997) *J. Virol.* **71**:5236, and the respective capsid coding sequences from each of the 5 serotypes (**Fig. 1** panel A). The ACG mutation of the p5 start site was chosen because this mutation has been shown to improve vector production by reducing rep78/68 while increasing AAV cap expression (Li et al. (1997) *J. Virol.* **71**:5236). pACG2 rep sequences were cloned into pAAV2Cap (Stratagene pBS+ backbone previously described (Rolling et al. (1999) *Hum. Gene Ther.* **10**:641), and the capsid gene was removed, by a *SwaI/NsiI* digestion. This intermediate plasmid was used for cloning each of the serotype specific capsid coding sequences (see **Fig. 1** panel A and methods for details). The capsid genes of each serotype were PCR amplified from ATCC viral stocks, and the product cloned into this *SwaI* blunted ended *NsiI*-digested intermediate. The new hybrid plasmids containing the common AAV2 ACG replication gene and the serotype specific capsid sequences were called pXR1-5. *BstNI* digestion (**Fig. 1** panel B) and DNA sequencing determined correct orientation and nucleotide sequence of the serotype specific capsid gene. When a number of independent isolates for each seroytpe specific helper were compared to traditional type 2 helpers, vector yields varied significantly among these constructs (**Table1**). Surprisingly, additions of serotype specific non-capsid coding sequences (5' to the VP1 start site) reduced this variation (see **Fig. 1, Table 1**). Additional analysis indicates that a Rep-specific capsid interacting domain is required for efficient serotype-specific encapsidation (Rolling et al., manuscript in preparation). Since these modified serotype specific helper plasmids (as described in **Fig. 1**) produced vector yields within the range of type 2 (**Table 1**), they were further analyzed in detail for AAV Rep and capsid production as well as ability to replicate AAV type 2 transgenes as described below.

**TABLE 1. Comparison of AAV helper constructs containing AAV2-only replication gene of portions of the serotype specific replication gene**

| AAV-2 only replication gene | AAV-2/serotype chimeric Rep |                           |
|-----------------------------|-----------------------------|---------------------------|
|                             |                             |                           |
| Serotype                    | Particles/ml (n)            | Particles/ml (n)          |
| rAAV1                       | $1.27 \times 10^{11}$ (10)  | not done                  |
| rAAV2                       | $9.79 \times 10^{12}$ (2)   | not applicable            |
| rAAV3                       | $9.00 \times 10^8$ (1)      | $7.16 \times 10^{11}$ (3) |
| rAAV4                       | $2.04 \times 10^{10}$ (4)   | $2.63 \times 10^{11}$ (2) |
| rAAV5                       | $7.64 \times 10^9$ (1)      | $4.60 \times 10^{11}$ (5) |
| rAAV5 to NcoI site          | 0                           | 0                         |
| rAAV5 with AAV5 ITR         | 0                           | $3.70 \times 10^7$ (3)    |

5

### Example 12

#### AAV Hybrid Helper Functions

A series of experiments were carried out on XR 1-5 helper plasmids to determine levels of type 2 Rep and serotype-specific capsid protein expression after transfection in 293 cells. Western blot analysis was used to detect the AAV rep gene products 78/68, 52/40 (**Fig. 2** panel A) and the three capsid sub-units Vp1, 2, and 3 (**Fig. 2** panel B) at 24 h post-transfection. A comparison of AAV2 Rep proteins in the context of different serotype helper plasmids was carried out by using monoclonal antibody 1F11 which recognizes each of the four AAV2 replication proteins (Hunter et al. (1992) *J. Virol.* **66**:317). Previous studies demonstrated that the p5 mutation in the context of the helper plasmid pACG-2 down regulated the expression of Rep 78/68, without affecting Rep 52/40 (Li et al. (1997) *J. Virol.* **71**:5236). The

results, shown in **Fig. 2** panel A, demonstrate that in each of the serotype specific helper constructs all 4 Rep proteins were made at levels equivalent to those described for the original AAV2 helper construct pACG-2 (Li et al. (1997) *J. Virol.* **71**:5236). The B1 monoclonal antibody (Wistuba et al. (1997) *J. Virol.* **71**:1341), which recognizes the amino acid recognition sequence IGTRYLTR (**SEQ ID NO:13**) in AAV type 2 structural proteins (Wobus et al. (2000) *J. Virol.* **74**:9281), was used to identify the serotype specific capsid subunits. This motif is conserved in all serotypes except AAV4 (**Fig. 2** panel C), which is evident by the lack of positive signal after Western analysis (**Fig. 2** panel B, lane 4). For the other helper plasmids, all three capsid proteins were detected (**Fig. 2** panel B). Although the data provided is a representative example, higher amounts of the structural proteins were consistently observed from serotype 1 compared with the other helper constructs (repeated 10X). Taken together, the results for replication and capsid protein expression for the five helper plasmids is within the range of AAV helper plasmids currently available for production (Haberman et al. (1999) *in, Current Protocols in Neuroscience, vol. 1*, New York, N.Y.).

### Example 13

#### 20            Replication and Cross-Packaging of Type 2 Vectors

Functional activity of these helper proteins were determined by replication and encapsidation of rAAV type 2 GFP template. The five serotype helper plasmids, the rAAV type 2 transgene pTRUFR, and the adenovirus helper plasmid (XX6-80) were triple transfected into 293T cells, and Hirt analysis carried out 24 hr post-transfection. *DpnI* digestion followed by Southern blot analysis using GFP gene specific probe determined the ratio of input plasmid to newly replicated vector DNA. All serotype specific helpers replicated the vector template (**Fig. 3** *DpnI* digested lanes). Although nearly equivalent amounts of input DNA were loaded in all lanes of the undigested samples, the highest level of transgene replication was observed from XR2 (**Fig. 3** lane 2). Repeated replication analysis demonstrated that all other helpers were essentially equal with XR1 generating 40% of XR2 levels (Fig. 3 lanes 3-5 and 1 respectively). Packaging efficiencies were determined by dot blot hybridization (**Table 1**). All helper constructs generated high vector yields

within  $10^{11}$  to  $10^{12}$  particles/ml. In general, serotypes 1, 4, and 5 were observed to have yields within 4 fold of each other after numerous production runs (5x), with serotypes 2 and 3 demonstrating the highest yields ( $9.8 \times 10^{12}$ ,  $7.2 \times 10^{11}$  respectively, **Table 1**). The serotype 1 helper produced the least amount of virus ( $1.27 \times 10^{11}$  and **Table 1**), consistent with the Hirt replication analysis.

#### Example 14

##### Heparin Column Binding Profiles of AAV Hybrid Serotypes

The use of iodixanol gradient and/or heparin column binding has allowed for the rapid purification of rAAV type 2 (Auricchio et al. (2001) *Hum. Gene Ther.* **12**:71, Zolotukhin et al. (1999) *Gene Ther.* **6**:973). In addition, these methods allow for purification of vector with better ratio of transducing units to particle numbers. This purification scheme was applied to the hybrid serotypes and elution profiles determined by GFP transduction as described in **Example 8**

Recombinant AAV2 demonstrated elution profiles as previously published (Zolotukhin et al. (1999) *Gene Ther.* **6**:973), with less than 1% of starting material recovered in the flow-through and the majority of the TU being recovered in the elution step (**Fig. 4**, AAV2). rAAV 3 displayed more efficient binding to the column and elution profiles near identical to type 2. These results indicate that the elution conditions used for rAAV2 purification can be applied to hybrid rAAV3. Recombinant AAV1 and -5 displayed similar profiles to one another with 60% of type 1 and 80% of type 5 TU recovered in the flow-through (**Fig. 4**, AAV1 and 5). The virus eluted from the salt gradient represent only a small portion of the total applied to the column (**Fig. 4**, AAV1 and 5).

The elution profile of rAAV4 was unique. Although the majority of the TU applied to the column was recovered in the elution steps, a significant fraction of the TU was recovered in the flow-through (**Fig. 4**, AAV4). These results suggest that if the salt conditions were altered virus might be recovered in the elution step, or that another ion exchange column might improve the recovery of AAV serotype 4.

## Example 15

### Hybrid Vector Transduction on Rodent, Monkey, and Human Cell Lines

Various cell lines were infected with the hybrid vectors in an effort to  
5 determine transducing titer *in vitro* (**Fig. 5**). Parental and mutant cell lines  
defected in heparan sulfate (HS) proteoglycan biosynthesis were analyzed for  
serotype specific vector transduction since previous studies have  
demonstrated a role for this cell surface protein in AAV type 2 infection. For  
rAAV1, a decrease in transduction on CHO pgs D cells that are heparin  
10 sulfate deficient was not observed. In fact, transduction efficiency for rAAV1  
was near identical for all cell lines tested (**Fig. 5**, AAV1). The lack of specific  
binding to heparin columns and ability to transduce mutant HS cell lines  
indicate that type 1 entry is distinct from type 2 and has yet to be identified.  
The transduction efficiency for rAAV2 and 3 were similar to what has been  
15 previously published (Handa et al. (2000) *J. Gen. Virol.* **81**:2077, Summerford  
and Samulski (1998) *J. Virol.* **72**:1438), with each showing a dependence on  
cell surface heparin sulfate (**Fig. 5**, AAV2 and 3) and efficient binding to HS  
columns (**Fig. 4**, AAV2 and 3). Hybrid AAV type 5 transduction paralleled that  
of AAV type 1 for all cell lines tested. After the completion of these studies,  
20 evidence that identifies sialic acid as a cell surface molecule involved in AAV  
type 5 transduction has been described in Kaludov et al. (2001) *J. Virol.*  
**75**:6884. The presence of sialic acid on the battery of cell lines tested here  
was not determined although efficient transduction was observed. Hybrid  
vector type 4 also transduced all cell lines tested. However the level of  
25 transduction never approached that observed on monkey derived cell lines  
(**Fig. 5**, AAV4). The restriction in the *in vitro* host range that has been  
observed with both the hybrid and traditional type 4 vectors indicates efficient  
transduction require a monkey specific factor. Overall, these results indicate  
that unlike AAV2 and -3, AAV1, -4 and -5 do not require heparin sulfate for  
30 infection. These observations are in agreement with other studies evaluating  
the transduction requirements of non AAV type 2 serotypes (Chiorini et al.  
(1999) *J. Virol.* **73**:1309, Chiorini et al. (1997) *J. Virol.* **71**:6823, Xiao et al.  
(1999) *J. Virol.* **73**:3994), and demonstrate the influence of the serotype  
specific virion shell to that of the type 2 vector sequences in transduction.

**Example 16**

**Hierarchy of Gene Expression for AAV Hybrid Vectors *in vivo***

The efficiency of gene expression of both therapeutic and marker transgenes were tested when delivered by each of the five hybrid vectors *in vivo*. The expression of human alpha-1-antitrypsin (hAAT) and factor IX was tested in outbred and inbred mice, as well as immunodeficient mouse lines (**Table 2**). The hAAT expression was also examined with respect to the route of injection (i.e. portal vein, intramuscular, and intravenous) (**Table 2**). ELISA was used to monitored expression of the therapeutic transgenes which consistently demonstrated that AAV type 1 was superior to the other hybrid vectors tested. This observation was true for all strains of mice routes of administration or transgenes tested (**Table 2**). However, small differences in the efficiency of expression for the remaining serotypes with respect to each variable was observed. For example, the expression of hAAT from the 5 hybrid vectors demonstrated AAV type 4 as least efficient, a feature ascribed to AAV type 2 when factor IX was tested.

**TABLE 2. Animal experiments with different serotypes of rAAV**

| Gene <sup>a</sup> | Mouse strain<br>(route of injection) <sup>b</sup> | Score with Serotype <sup>c</sup> |      |      |      |      |
|-------------------|---|----------------------------------|------|------|------|------|
|                   |   | AAV1                             | AAV2 | AAV3 | AAV4 | AAV5 |
| hAAT              | C57BL (i.m.)                                      | +++++                            | +++  | ++   | ++   | ++++ |
|                   | C57BL (p.v.)                                      | +++++                            | ++   | +++  | +++  | ++++ |
|                   | C57BL (i.v.)                                      | +++++                            | ++   | +++  | +++  | ++++ |
|                   | BALB/c (i.v.)                                     | +++++                            | ++++ | ++   | ++   | ++++ |
| dF9               | Scid (i.m.)                                       | +++++                            | +    | ++++ | ++++ | ++++ |
|                   | BALB/c (i.m.)                                     | +++++                            | +    | ++   | ++   | +++  |

<sup>a</sup> The transgene were chicken  $\beta$ -actin promoter with the CMV enhancer-driven hAAT (both C57/BL and BALB/c mice were injected with  $5 \times 10^{10}$  particles/mouse) and CMV immediate-early promoter-driven dog factor IX (dF9) (SCID mice were injected with  $2 \times 10^{11}$  particles/mouse, and BALB/c mice were injected with  $10^{10}$  particles/mouse.

<sup>b</sup> For each group of animals, serotype, and route of injection, a minimum of three animals were used. Abbreviations for route of injection: i.m., intramuscular injection; p.v., portal vein injection; i.v., intravenous injection.

<sup>c</sup> Scores for protein expression ranged from the maximum level of protein observed for each set of animals (+++++) to the lowest level of expression in the group (+).

### Example 17

#### Evaluation of AAV Serotypes Gene Transfer Efficiency in the Rat Retina

Previous studies have shown that AAV type 5 transduction is more  
5 efficient in brain when compared to type 4 or 2 (Davidson et al. (2000) *Proc. Natl. Acad. Sci. USA* **97**:3428). In our study, a type 1 vector appeared superior to all others when tested in non-neuronal targets. The hybrid vector analysis was extended to neuronal targets using rat retina as a substrate and green fluorescent protein (GFP) as a reporter. Each of the five serotype vectors  
10 was determined for efficient *in vivo* transduction using fundus color photography after subretinal injections. At twelve days post-injection, GFP expression could be detected in rAAV5, -4 and -1 injected animals, with type 5 and 4 hybrids displaying the most intense GFP signal. No signal was detected in animals injected with rAAV2 or -3 at this concentration and time point (**Fig. 6**).  
15 **6**). At 26 days post-injection, GFP expression increased proportionally for rAAV5, -4 and -1, with types 2 and -3 eventually displaying a small but positive signal (**Fig. 6**, 26 days). Finally, this trend continued for the duration of the experiment (*i.e.*, 48 days; **Fig. 6**).

Based on these observations, a different hierarchy of serotype specific  
20 transgene expression was observed in retina when compared with non-neuronal tissues. In this setting serotypes 5 and 4 were superior followed by types 1, 2 and 3 (**Fig. 6**). Low to moderate levels of expression from AAV type 1 was in sharp contrast with data obtained in non-neuronal tissue. Taken together, the expression of therapeutic and marker genes from the five hybrid  
25 serotypes demonstrates that AAV1 is the superior vehicle for non-neuronal delivery, while in the retina and in the brain (Davidson et al. (2000) *Proc. Natl. Acad. Sci. USA* **97**:3428) AAV5 produces the highest levels of marker gene expression. All in all, these data demonstrate the importance of determining serotype specific transduction *in vivo* when considering AAV as the delivery  
30 system of choice.



### Example 18

#### AAV-Serotype Chimeric Rep

##### Requirements for Cross-Packaging of Type 2 Vectors

As described above, the packaging efficiencies of type 2 rAAV vectors  
5 utilizing serotype chimeric Rep proteins were determined by dot blot  
hybridization and depicted in **Table 1**. These results indicate that chimeric  
Rep proteins with a serotype specific amino acids from the C-terminus are  
sufficient to increase the cross packaging efficiency of rAAV2 vectors 10 -  
1000-fold when compared with cross packaging with type 2 Rep proteins  
10 alone. Further, chimeric Rep proteins that possess the first 243 amino acids of  
the N-terminus of type 2 Rep protein are capable of efficient cross packaging  
of rAAV2 vectors, whereas those chimeric Rep proteins that possess only the  
first 103 amino acids from the N-terminus of type 2 Rep protein are not  
capable of efficient packaging of rAAV2 vectors.

15

### Example 19

#### Chimeric AAV-B19 Rep Proteins and Packaging of Type 2 Vectors in B19 Capsids

Two B19 helper plasmids carrying the B19 VP1 and VP2 coding  
20 sequences and a chimeric non-structural protein containing N-terminal  
elements from the AAV2 Rep 78/68 protein and C-terminal elements from the  
B19 NS-1 protein is constructed in a similar manner to the AAV hybrid helper  
plasmids described in Examples 1 and 9 by cloning the appropriate B19 viral  
sequences into the pXR2 construct described in **Example 1**. These include  
25 both the complete B19 capsid (VP1 and VP2) protein coding sequences, and  
the C-terminal portion of the B19 NS-1 protein. The two helper plasmids are  
constructed by the ligation of the PCR amplification products of: the B19  
sequences described in Shade et al. (1986) *J. Virol.* **58**:921, and the  
backbone of pXR2 containing the N-terminal portion of the AAV-2 rep coding  
30 sequence. The chimeric Rep proteins that are coded for in these constructs  
contain: (1) the portion of the N-terminal amino acids of AAV Rep78/68 up to  
the p19 promoter, and (2) up to the p40 promoter, and the appropriate C-  
terminal amino acids from B19 NS-1. The PCR primers that are used to  
prepare (1) are as follows: 5'-ATGGTAAACTGGTTGTGTGAAAAC-3' (**SEQ ID**

**NO:7)** and 5'-GCAACAACATAATTTTTTAACCAC-3' (**SEQ ID NO:8**), which amplify B19 sequences as described in Shade et al. (1986) *J. Virol.* **58**:921, and 5'-GTACCTGGCTGAAGTTTTTGATCT-3' (**SEQ ID NO:9**) and 5'-GGCCGCTCGATAAGCTTTTTGTTCC-3' (**SEQ ID NO:10**), which amplify the pXR2 backbone carrying the AAV2 rep coding region up to the P19 promoter. The PCR primers used to prepare (2) are as follows: 5'-AGCACGAGTGGTGGTGAAAGCTCT-3' (**SEQ ID NO:11**) and 5'-GCAACAACATAATTTTTTAACCAC-3' (**SEQ ID NO: 8**), which amplify B19 sequences as described in Shade et al. (1986) *J. Virol.* **58**:921, and 5'-TGGCTGGCGAACTGACTCGCGCAC-3' (**SEQ ID NO:12**) 5'-GGCCGCTCGATAAGCTTTTTGTTCC-3' (**SEQ ID NO:10**), which amplify the pXR2 backbone carrying the AAV2 rep coding region up to the P40 promoter. In both cases, the resulting PCR products are then blunt end ligated together to generate the desired construct, and whose structure is confirmed by restriction digestion.

The resulting AAV helper plasmids are used in conjunction with the Ad helper plasmid pXX6-80 to package the rAAV-2 transgene pTRUF as described in Example 2. The resulting virus preparations from this AAV helper construct are analyzed for particle yield as described in **Example 11**.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

25

**THAT WHICH IS CLAIMED IS:**

1. A polynucleotide comprising parvovirus rep coding sequences  
5 and parvovirus cap coding sequences,  
said rep coding sequences encoding a DNA binding domain from a first  
parvovirus;  
said rep coding sequences further encoding a capsid interacting domain  
from a different parvovirus from said first parvovirus; and  
10 said cap coding sequence comprising sequences from said different  
parvovirus.
2. The polynucleotide of Claim 1, said rep coding sequences  
comprising a 5' portion and a 3' portion;  
15 said 5' portion encoding said DNA binding domain from said first  
parvovirus;  
said 3' portion encoding said capsid interacting domain from said  
different parvovirus.
- 20 3. The polynucleotide of Claim 1 or Claim 2, wherein said first  
parvovirus is an autonomous parvovirus.
4. The polynucleotide of Claim 1 or Claim 2, wherein said first  
parvovirus is an AAV.  
25
5. The polynucleotide of Claim 4, wherein said first parvovirus is  
selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5m  
AAV6, AAV7 and AAV8.
- 30 6. The polynucleotide of Claim 5, wherein said first parvovirus is  
AAV2 or AAV5.

7. The polynucleotide of any of Claims 4-6, wherein said DNA binding domain comprises about the first 220 amino acids of the large Rep proteins from said first parvovirus.

5 8. The polynucleotide of any of Claims 4-6, wherein said DNA binding domain comprises the Rep 78/68-specific amino acid sequences from said first parvovirus.

10 9. The polynucleotide of any of Claims 1-8, wherein said different parvovirus is an autonomous parvovirus.

15 10. The polynucleotide of Claim 9, wherein said different parvovirus is selected from the group consisting of B19 virus, canine parvovirus, feline panleukemia virus, muskovy duck parvovirus, goose parvovirus, minute virus of mouse, and H1 virus.

20 11. The polynucleotide of Claim 9 or Claim 10, wherein said capsid interacting domain comprises an autonomous parvovirus small Rep protein or a functional portion thereof.

25 12. The polynucleotide of Claim 11, wherein said capsid interacting domain comprises a functional carboxy-terminal portion of said autonomous parvovirus small Rep protein.

30 13. The polynucleotide of any of Claims 1-8, wherein said different parvovirus is an AAV.

14. The polynucleotide of Claim 13, wherein said capsid interacting domain comprises the AAV Rep52 or Rep40 amino acid sequences or a functional portion thereof.

15. The polynucleotide of Claim 13 or Claim 14, wherein:  
said first parvovirus is selected from the group consisting of  
AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8; and

said different parvovirus is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

16. The polynucleotide of Claim 15, wherein said different parvovirus  
5 is selected from the group consisting of AAV1, AAV4 and AAV5.

17. The polynucleotide of any of Claims 1-16, wherein said polynucleotide is a DNA sequence.

10 18. The polynucleotide of any of Claims 1-17, wherein said rep coding sequences encoding said capsid interacting domain are operatively associated with a promoter such that said capsid interacting domain can be expressed independently of said DNA binding domain.

15 19. The polynucleotide of Claim 19, wherein said promoter is the AAV p19 promoter.

20. A polynucleotide comprising adeno-associated virus (AAV) rep coding sequences and AAV cap coding sequences,  
20 said rep coding sequences having a 5' portion and a 3' portion;  
said 5' portion comprising rep coding sequences from a first AAV;  
said 3' portion comprising rep coding sequences from a different AAV from said first AAV; and  
said cap coding sequences comprising sequences from said different  
25 AAV.

21. The polynucleotide of Claim 20, wherein said first AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

30 22. The polynucleotide of Claim 20 or Claim 21, wherein said first AAV is AAV2.

23. The polynucleotide of Claim 20 or Claim 21, wherein said first AAV is AAV5.

24. The polynucleotide of any of Claims 20-23, wherein said 5' portion comprises the rep coding sequences 5' of the AAV p19 promoter.

25. The polynucleotide of any of Claims 20-23 wherein said 5' portion encodes at least the first 220 amino acids of the Rep 78/68 proteins.

26. The polynucleotide of any of Claims 20-25, wherein said 3' portion encodes the Rep52 or Rep40 proteins or a functional portion thereof.

27. The polynucleotide of any of Claims 20-26, wherein:  
said first AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8; and  
said different AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

28. The polynucleotide of Claim 27, wherein said different AAV is selected from the group consisting of AAV1, AAV4 and AAV5.

29. The polynucleotide of Claim 27, wherein said first AAV is AAV2 and said different AAV is AAV5.

30. The polynucleotide of Claim 27, wherein said first AAV is AAV5 and said different AAV is AAV2.

31. The polynucleotide of any of Claims 20-30, wherein said polynucleotide is a DNA sequence.

32. The polynucleotide of any of Claims 20-31, wherein said 3' portion is operatively associated with a promoter such that said 3' portion can be expressed independently of said 5' portion.

33. The polynucleotide of Claim 32, wherein said promoter is the AAV p19 promoter.
34. A vector comprising the polynucleotide of any of Claims 1-33.
35. The vector of Claim 34, wherein said vector is a plasmid vector.
36. The vector of Claim 34, wherein said vector is a viral vector.
37. The vector of Claim 36, wherein said vector is selected from the group consisting of an adenovirus vector, a baculovirus vector, an AAV vector, a herpesvirus vector, and an Epstein-Barr virus vector.
38. A cell comprising the polynucleotide of any of Claims 1-33.
39. The cell of Claim 38, wherein said polynucleotide is stably integrated into the genome of said cell.
40. A cell comprising the vector of any of Claims 34-39.
41. A cell comprising parvovirus rep coding sequences and parvovirus cap coding sequences,  
said rep coding sequences encoding a DNA binding domain from a first parvovirus;  
said rep coding sequences further encoding a capsid interacting domain from a different parvovirus from said first parvovirus;  
said cap coding sequences comprising sequences from said different parvovirus; and  
said rep coding sequences being stably integrated into the genome of said cell.
42. The cell of Claim 41, wherein said cap coding sequences are stably integrated into the genome of said cell.

43. A cell comprising adeno-associated virus (AAV) rep coding sequences and AAV cap coding sequences,  
said rep coding sequences having a 5' portion and a 3' portion;  
said 5' portion comprising rep coding sequences from a first AAV;  
5 said 3' portion comprising rep coding sequences from a different AAV  
from said first AAV;  
said cap coding sequences comprising sequences from said different  
AAV; and  
said rep coding sequences being stably integrated into the genome of  
10 said cell.
44. The cell of Claim 43, wherein said cap coding sequences are  
stably integrated into the genome of said cell.
- 15 45. A method of producing a recombinant hybrid parvovirus particle,  
comprising providing to a cell permissive for parvovirus replication:
- (a) a recombinant parvovirus template comprising (i) a heterologous  
nucleotide sequence, and (ii) a parvovirus terminal repeat  
sequence;
- 20 (b) parvovirus rep coding sequences and parvovirus cap coding  
sequences;  
the rep coding sequences encoding a DNA binding domain from  
a first parvovirus that interacts with the parvovirus terminal repeat to  
mediate replication of the recombinant parvovirus template;
- 25 the rep coding sequences further encoding a capsid interacting  
domain from a different parvovirus from the first parvovirus; and  
the cap coding sequences comprising sequences from the  
different parvovirus;
- wherein the parvovirus terminal repeat sequence may be from the first  
30 parvovirus but not from the different parvovirus;  
under conditions sufficient for the replication and packaging of the  
recombinant parvovirus template;



whereby recombinant hybrid parvovirus particles comprising the parvovirus capsid encoded by the cap coding sequences and packaging the recombinant parvovirus template are produced in the cell.

5           46.    The method of Claim 45, further comprising the step of collecting the recombinant parvovirus particles.

          47.    The method of Claim 46, further comprising the step of lysing the cell prior to collecting the recombinant parvovirus particles.

10

          48.    The method of any of Claims 45-47, wherein the rep coding sequences and cap coding sequences cannot be packaged into the recombinant parvovirus particles.

15           49.    The method of any of Claims 45-48, wherein the recombinant parvovirus template comprises the heterologous nucleotide sequence flanked by 5' and 3' parvovirus terminal repeats.

          50.    The method of any of Claims 45-49, wherein the rep coding sequences and/or cap coding sequences are provided by a plasmid.

20

          51.    The method of any of Claims 45-50, wherein the rep coding sequences and/or cap coding sequences are provided by a viral vector.

25           52.    The method of Claim 51, wherein the viral vector is selected from the group consisting of an adenovirus vector, herpesvirus vector, Epstein-Barr virus vector, and baculovirus vector.

          53.    The method of any of Claims 45-52, wherein the rep coding sequences are stably integrated into the cell.

30

          54.    The method of any of Claims 45-53, wherein the cap coding sequences are stably integrated into the cell.

55. The method of any of Claims 45-54, wherein the recombinant parvovirus template is provided by a plasmid or a viral vector or is stably integrated into the cell as a provirus.

5 56. The method of any of Claims 45-55, wherein the rep coding sequences comprise a 5' portion and a 3' portion;  
the 5' portion encoding the DNA binding domain from the first parvovirus;  
the 3' portion encoding the capsid interacting domain from the different  
10 parvovirus.

57. The method of any of Claims 45-56, wherein the parvovirus terminal repeat sequence is an autonomous parvovirus terminal repeat sequence and the first parvovirus is an autonomous parvovirus.  
15

58. The method of Claim 57, wherein the terminal repeat sequence is selected from the group consisting of a B19 virus, canine parvovirus, feline panleukemia virus, muskovy duck parvovirus, goose parvovirus, minute virus of mouse, and H1 virus terminal repeat sequence; and  
20 the first parvovirus is selected from the group consisting of B19 virus, canine parvovirus, feline panleukemia virus, muskovy duck parvovirus, goose parvovirus, minute virus of mouse, and H1 virus.

59. The method of Claim 57 or Claim 58, wherein the parvovirus terminal repeat sequence is from the first parvovirus.  
25

60. The method of any of Claims 45-56, wherein the parvovirus terminal repeat sequence is an adeno-associated virus (AAV) terminal repeat sequence and the first parvovirus is an AAV.  
30

61. The method of Claim 60, wherein the AAV terminal repeat sequence is selected from the group consisting of an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8 terminal repeat sequence.

62. The method of Claim 60 or Claim 61, wherein the first parvovirus is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

5 63. The method of any of Claims 60-62, wherein the AAV terminal repeat sequence is from the first AAV.

64. The method of any of Claims 45-63, wherein the different parvovirus is an autonomous parvovirus.

10

65. The method of Claim 64, wherein the different parvovirus is selected from the group consisting of B19 virus, canine parvovirus, feline panleukemia virus, muskovy duck parvovirus, goose parvovirus, minute virus of mouse, and H1 virus.

15

66. The method of any of Claims 45-65, wherein the different parvovirus is an AAV.

20

67. The method of Claim 66, wherein:  
the first parvovirus is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8; and  
the different parvovirus is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

25

68. The method of Claim 66 or Claim 67, wherein:  
the parvovirus terminal repeat sequence is selected from the group consisting of a AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8 terminal repeat sequence; and  
the different parvovirus is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

30

69. The method of any of Claims 66-68, wherein the different parvovirus is selected from the group consisting of AAV1, AAV4 and AAV5.

70. The method of any of Claims 45-69, wherein the rep coding sequences encoding the capsid interacting domain are operatively associated with a promoter such that the capsid interacting domain may be expressed independently of the DNA binding domain.

5

71. The method of Claim 70, wherein the promoter is the AAV p19 promoter.

72. A method of producing a recombinant hybrid adeno-associated virus (rAAV) particle, comprising providing to a cell permissive for AAV replication:

(a) a rAAV template comprising (i) a heterologous nucleotide sequence, and (ii) an AAV terminal repeat sequence; and  
(b) AAV rep coding sequences and AAV cap coding sequences;  
the rep coding sequences having a 5' portion and a 3' portion;  
the 5' portion comprising rep coding sequences from a first AAV that interacts with the AAV terminal repeat to mediate replication of the rAAV template;

the 3' portion comprising rep coding sequences from a different AAV from the first AAV; and

the cap coding sequences comprising sequences from the different AAV;

wherein the AAV terminal repeat sequence may be from the first AAV but not from the different AAV;

under conditions sufficient for the replication and packaging of the rAAV template;

whereby infectious recombinant hybrid AAV particles comprising the AAV capsid encoded by the cap coding sequences and packaging the rAAV template are produced in the cell.

30

73. The method of Claim 72, further comprising the step of collecting the infectious rAAV particles.

74. The method of Claim 73, further comprising the step of lysing the cell prior to collecting the infectious rAAV particles.

75. The method of any of Claims 72-74, further comprising providing  
5 helper virus sequences which encode the helper virus functions essential for a productive AAV infection, wherein the helper virus sequences cannot be packaged into the rAAV particles.

76. The method of any of Claims 72-75, wherein the rep coding  
10 sequences and cap coding sequences cannot be packaged into the rAAV particles.

77. The method of any of Claims 72-76, wherein the AAV template  
15 comprises the heterologous nucleotide sequence flanked by 5' and 3' AAV inverted terminal repeats.

78. The method of any of Claims 72-77, wherein the rep coding sequences and/or cap coding sequences are provided by a plasmid.

79. The method of any of Claims 72-78, wherein the rep coding  
20 sequences and/or cap coding sequences are provided by a viral vector.

80. The method of Claim 79, wherein the viral vector is selected from  
25 the group consisting of an adenovirus vector, herpesvirus vector, Epstein-Barr virus vector, and baculovirus vector.

81. The method of any of Claims 72-80, wherein the rep coding sequences are stably integrated into the cell.

82. The method of any of Claims 72-81, wherein the cap coding  
30 sequences are stably integrated into the cell.

83. The method of any of Claims 72-82, wherein the rAAV template is provided by a plasmid or a viral vector or is stably integrated into the cell as a provirus.

5 84. The method of any of Claims 72-83, wherein the first AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

10 85. The method of any of Claims 72-84, wherein the AAV terminal repeat sequence is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8 terminal repeat sequence.

86. The method of Claim 84 or Claim 85, wherein the first AAV is AAV2 or AAV5.

15

87. The method of any of Claims 84-86, wherein the AAV terminal repeat sequence is an AAV2 or AAV5 terminal repeat sequence.

20 88. The method of any of Claims 70-87, wherein the AAV terminal repeat sequence is from the first AAV.

89. The method of any of Claims 72-88, wherein:  
the first AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8; and  
25 the different AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

90. The method of any of Claims 72-89, wherein:  
the AAV terminal repeat sequence is selected from the group  
30 consisting of an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8 terminal repeat sequence; and  
the different AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

91. The method of any of Claims 72-90, wherein the different AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 and AAV8.

5 92. The method of Claim 91, wherein the first AAV is AAV2 and the different AAV is AAV5.

93. The method of Claim 91, wherein the first AAV is AAV5 and the different AAV is AAV2.

10

94. The method of any of Claims 72-93, wherein the 3' portion is operatively associated with a promoter such that the 3' portion can be expressed independently of the 5' portion.

15 95. The method of Claim 94, wherein the promoter is the AAV p19 promoter.

20

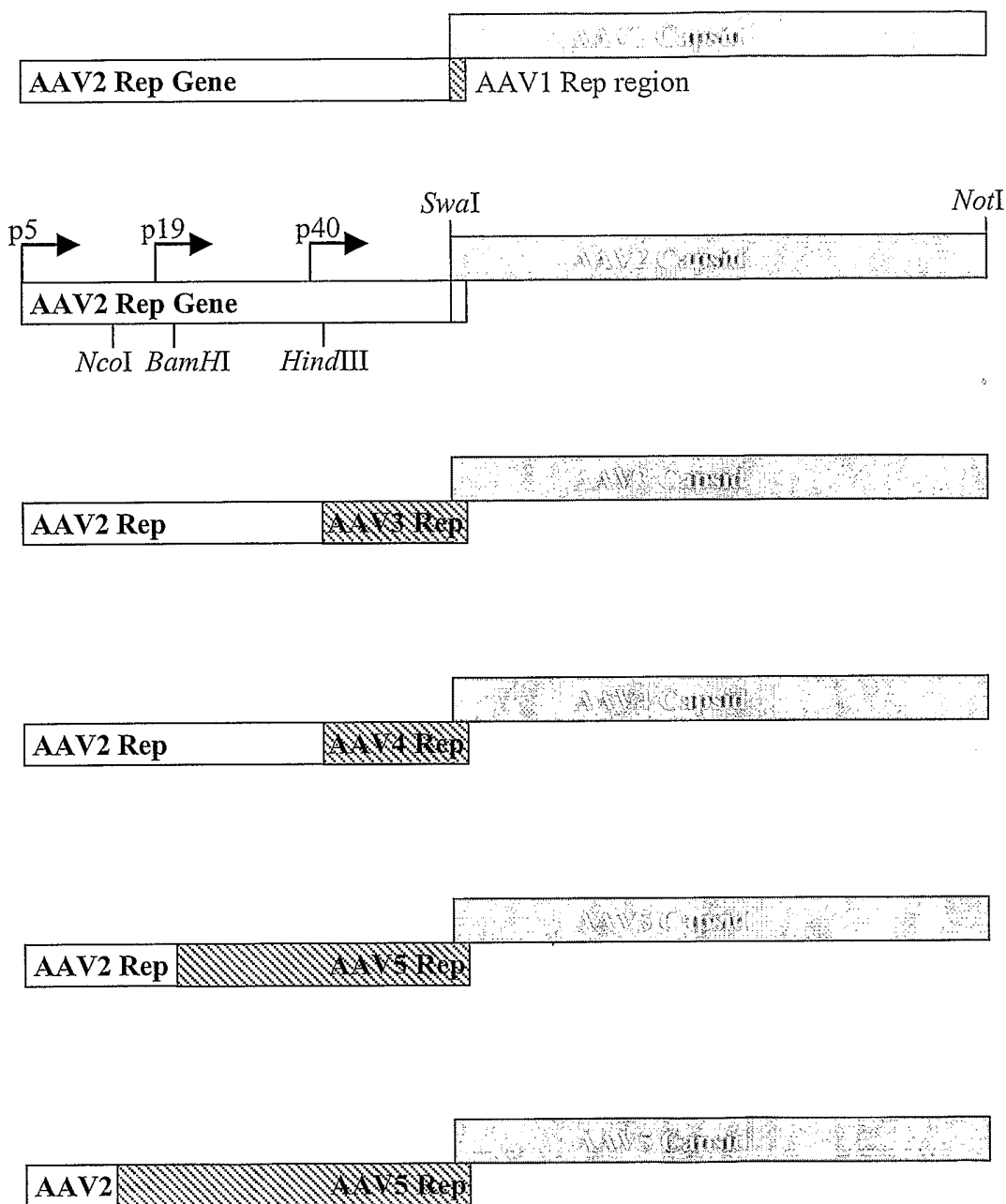


FIG. 1A



# *Bst* NI digestion of plasmids pXR1-5

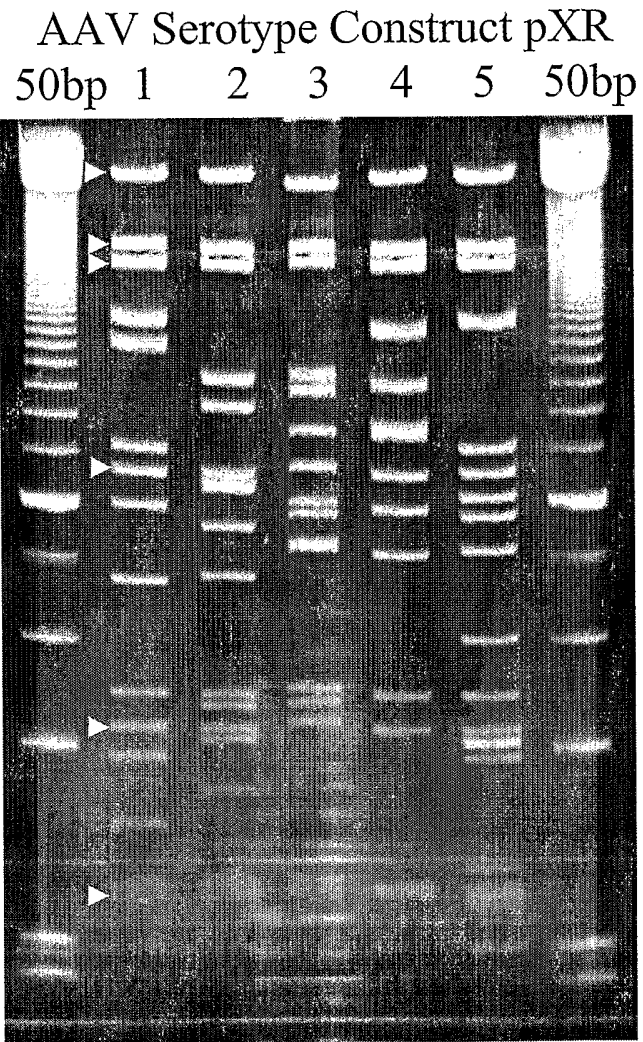
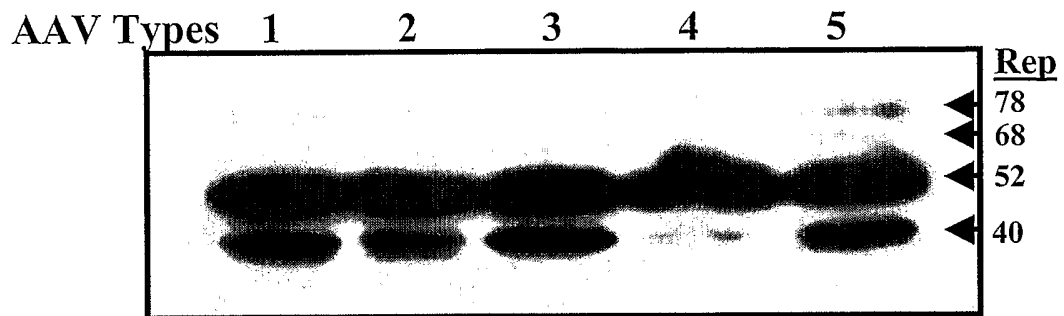
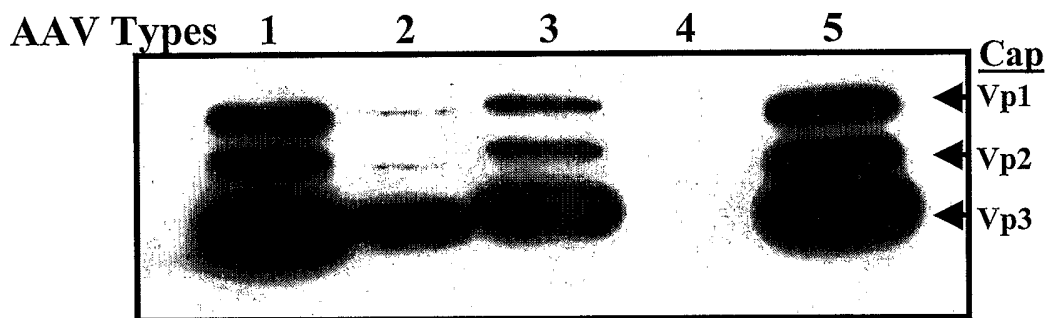


FIG. 1B

**A. Anti-Rep monoclonal 1F11<sup>2</sup>**



**B. Anti-Capsid monoclonal B1<sup>1</sup>**



**C. Monoclonal Antibody B1 Recognition site**

AAV1 T\*\*\*\*\*P\* (SEQIDNO:14)  
 AAV2 SEPRPIGTRYLTRNL (SEQIDNO:15)  
 AAV3 \*\*\*\*\*  
 AAV4 T\*\*\*A\*\*\*\*\*HH\* (SEQIDNO:16)  
 AAV5 RTT\*\*\*\*\*P\* (SEQIDNO:17)  
           IGTRYLTR (SEQIDNO:18)

FIG. 2

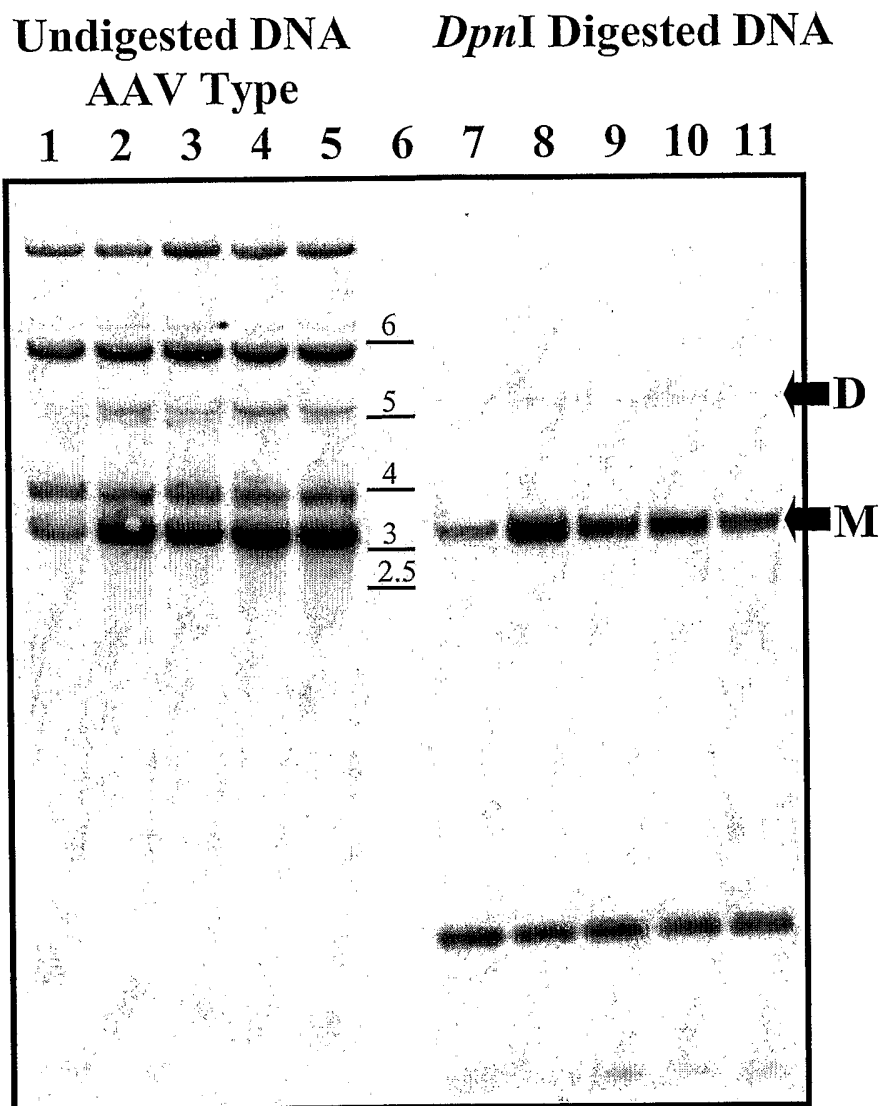


FIG. 3



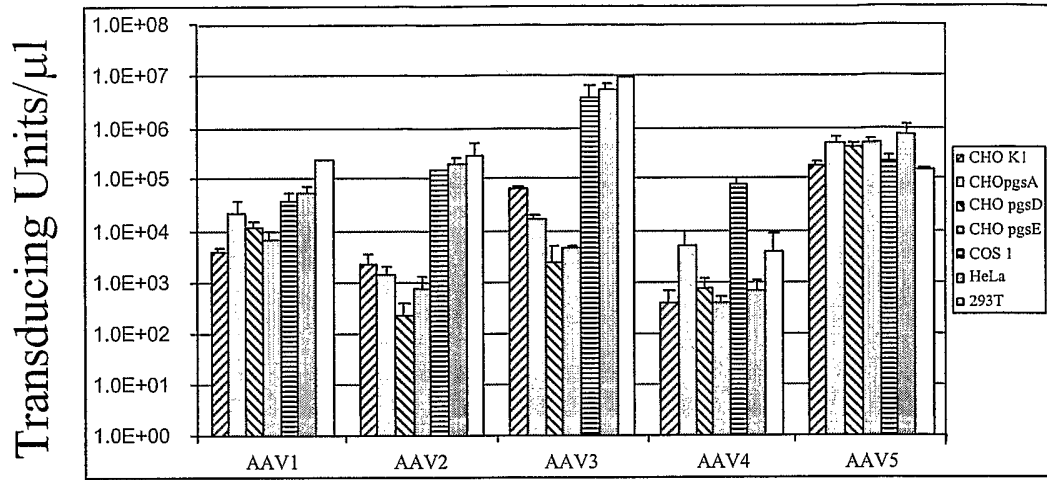


FIG. 5

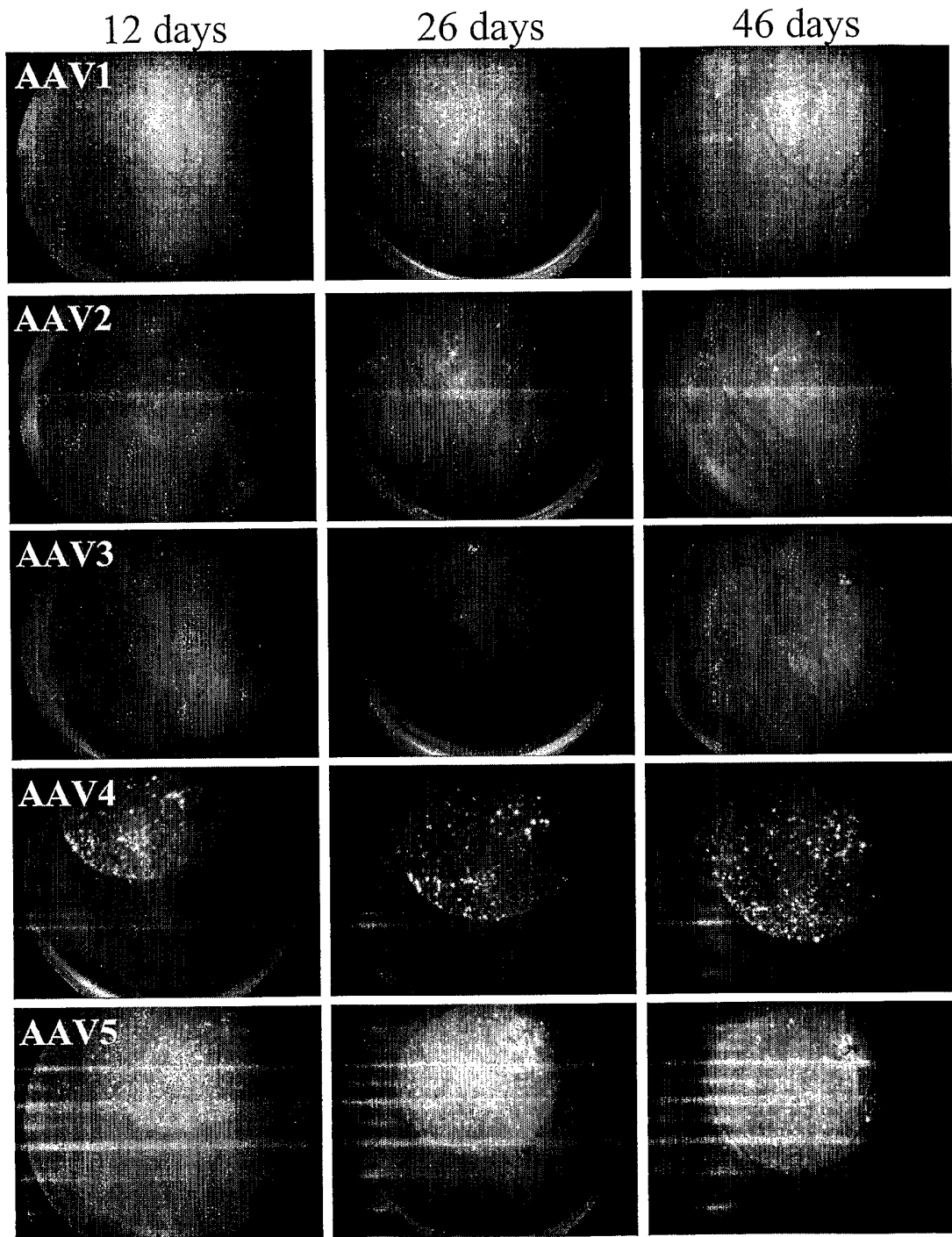


FIG. 6

8/36  
FIG. 7

AAV-2, complete sequence, GenBank Accession No. NC\_001401 and Chiorini et al., (1999) *J. Virol.* 73:1309

```

1  ttggccactc cctctctgcg cgctcgctcg ctactgagg cggggcgacc aaaggtcgcc
61  cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc gagcgcgag agagggagtg
121 gccaactcca tcaactagggg ttcttgaggg ggtggagtcg tgacgtgaat tacgtcatag
181 ggttagggag gtctgttatt agaggtcacg tgagtgtttt gcgacatttt gcgacacat
241 gtggtcacgc tgggtattta agcccagtg agcacgcagg gtctccattt tgaagcggga
301 ggtttgaacg cgcagccgcc atgccggggt tttacgagat tgtgattaag gtccccagcg
361 accttgacgg gcatctgccc ggcatttctg acagctttgt gaactgggtg gccgagaagg
421 aatgggagtt gccgccagat tctgacatgg atctgaatct gattgagcag gcaccctga
481 ccgtggccga gaagctgcag cgcgactttc tgacggaatg gcgccgtgtg agtaaggccc
541 cggaggccct tttctttgtg caatttgaga agggagagag ctacttccac atgcacgtgc
601 tcgtgaaaac caccggggtg aaatccatgg ttttgggacg tttcctgagt cagattcgcg
661 aaaaactgat tcagagaatt taccgcgggg tcgagccgac tttgccaaac tggttcgcg
721 tcacaaagac cagaaatggc gccggaggcg ggaacaaggt ggtggatgag tgctacatcc
781 ccaattactt gctcccaaaa acccagcctg agctccagtg ggcgtggact aatatggaac
841 agtatttaag cgctgtttg aatctcacgg agcgtaaacg gttgggtggcg cagcatctga
901 cgcacgtgtc gcagacgcag gacgagaaca aagagaatca gaatcccaat tctgatgctc
961 cggatgacag atcaaaaact tcagccaggt acatggagct ggtcgggtgg ctcgtggaca
1021 aggggattac ctccggagaag cagtggatcc agggaggacca ggcctcatac atctccttca
1081 atcgccctc caactcgcg gtcacccaaa tcccaaatca aggctgcctt ggacaatgcy ggaagatta
1141 tgagcctgac taaaaccgcc cccgactacc tgggtggcca gcagcccgty gaggacattt
1201 ccagcaatcg gatttataaa attttggaa taaacgggta cgatcccaa tatcggtctt
1261 ccgtctttct gggatgggcc acgaaaagt tggcaagag gaacaccatc tggctgtttg
1321 ggctgcaac taccgggaag accaacatcg cggaggccat agcccacact gtgcccttct
1381 acgggtgcyt aaactggacc aatgagaact ttcccttcaa cgaactgtct gacaagatgg
1441 tgatctggtg ggaggagggg aagatgaccg ccaaggctct ggagtcggcc aaagccatc
1501 tcggaggaag caaggtgcyt gtggaccaga aatgcaagtc ctcgcccgag atagaccga
1561 ctcccgtgat cgtcacctcc aacaccaaca tgtgcyccgt gattgacggg aactcaacga
1621 ccttgaaca ccagcagccg ttgcaagacc ggatgttcaa atttgaactc acccgccgtc
1681 tggatcatga ctttgggaag gtcaccaagc aggaagtcaa agactttttc cgggtggcaa
1741 aggatcacgt ggttgaggtg gagcatgaat tctacgtcaa aaagggtgga gccaaagaaa
1801 gaccgcccc cagtgcgca gatataagt agcccaaacy ggtgcygag tcagttgcyt
1861 agccatcgac gtcagcgcg gaagcttoga tcaactacy agacaggtac caaaacaat
1921 gttctgctca cgtgggcatg aatctgatgc tgtttccctg cagacaatgc gagagaatga
1981 atcagaattc aaatatctgc ttaactacy gacagaaaga ctgtttagag tgctttcccg
2041 tgtcagaatc tcaaccggt tctgtctca aaaaggcgt tcaaaaactg tgctacatc
2101 atcatatcat gggaaaggtg ccagacgctt gcactgctc cgatctggtc aatgtggatt
2161 tggatgactg catctttgaa caataaatga tttaaatcag gtatggctgc cgatggttat
2221 cttccagatt ggctcgagga cactctctct gaaggaataa gacagtggty gaagctcaa
2281 cttggccac caccacaaa gcccgagag cggcataagg acgacagcag gggctctgtg
2341 cttctgggt acaagtacct cggaccttc aacggactcy acaagggaga gccggtcaac
2401 gagcagacy cgcggccct cgagcacgac aaagcctacy accggcagct cgacagcgg
2461 gacaaccgt acctcaagta caaccagcc gacgcygagt ttcaggacy ccttaagaa
2521 gatactctt ttgggggcaa cctcggacy gcygtcttc aggcgaaaaa gagggtctt
2581 gaaactctg gcctggttga ggaacctgtt aagacygct cgggaaaaaa gaggccggt
2641 gagactctc ctgtggagcc agactcctcc tgggaaccg gaaaggcgg ccagcgcct
2701 gcaagaaaaa gattgaattt tggctcagact ggagacygag actcagatc tgaccccg
2761 cctctcgga agccaccag agccccctc ggtctggga ctaatacga ggctacagg
2821 agtggcgcac caatggcaga caataacyg ggcgcygag gagtgggtaa ttctccgga
2881 aatggcatt gcyattccac atggatgggc gacagagtc taccaccag caccgaaac
2941 tgggcccctg ccacctaca caaccacct tacaacaaa tttccagca atcaggagc
3001 tcgaacyca atcaactact tggctacag accccttgg ggtattttga cttcaacga
3061 ttccactgcc acttttcacc acgtgactgg caaagactca tcaacaaca ctgggattc
3121 cgaccaaga gactcaact caagctctt aacattcaag tcaagaggt cagcagaat
3181 gacggtacy cgcgattgc caataacct accagcagcy ttcaggtgt tactgactc
3241 gactaccag tcccgtact cctcggctcy gcyatcaag gatgcctcc gccgttcca
3301 gcagacytct tcatggtgcc acagatgga tacctcacc tgaacaacy gactcaggca

```

3361 gtaggacgct cttcatttta ctgcctggag tactttcctt ctcagatgct gcgtaccgga  
3421 aacaacttta ccttcagcta cacttttgag gacgttcctt tccacagcag ctacgctcac  
3481 agccagagtc tggaccgtct catgaatcct ctcacgacc agtacctgta ttacttgagc  
3541 agaacaaca ctccaagtgg aaccaccacg cagtcaaggc ttcagttttc tcaggccgga  
3601 gcgagtgaca ttcgggacca gtctaggaac tggcttcctg gaccctgtta ccgccagcag  
3661 cgagtatcaa agacatctgc ggataacaac aacagtgaat actcgtggac tggagctacc  
3721 aagtaccacc tcaatggcag agactctctg gtgaatccgg gcccgccat ggcaagccac  
3781 aaggacgatg aagaaaagtt ttttctcag agcggggttc tcatctttgg gaagcaaggc  
3841 tcagagaaaa caaatgtgaa cattgaaaag gtcatgatta cagacgaaga ggaaatcggg  
3901 acaaccaatc ccgtggctac ggagcagtat ggttctgtat ctaccaacct ccagagaggc  
3961 aacagacaag cagctaccgc agatgtcaac acacaaggcg ttcttccagg catggtctgg  
4021 caggacagag atgtgtacct tcagggggccc atctgggcaa agattccaca cacggacgga  
4081 cttttcacc cctctcccct catgggtgga ttcggactta aacaccctcc tccacagatt  
4141 ctcatcaaga acaccccggt acctgcgaat ccttcgacca cttcagtgcc ggcaaagttt  
4201 gcttccttca tcacacagta ctccacggga caggtcagcg tggagatcga gtgggagctg  
4261 cagaaggaaa acagcaaacg ctggaatccc gaaattcagt acaacttcaa ctacaacaag  
4321 tctgttaatc gtggacttac cgtggatact aatggcgtgt attcagagcc tcgccccatt  
4381 ggcaccagat acctgactcg taatctgtaa ttgcttgta atcaataaac cgtttaattc  
4441 gtttcagttg aactttggtc tctgcgtatt tctttcttat ctagtttcca tggctacgta  
4501 gataagtagc atggcgggtt aatcattaac tacaaggaac ccctagtgat ggagttggcc  
4561 actccctctc tgcgcgctcg ctccgtcaact gaggcggggc gaccaaaggt cgcccgacgc  
4621 ccgggctttg cccgggcggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaa

**FIG. 7 (Continued)**



10/36  
FIG. 8

AAV-1, complete sequence, GenBank Accession No. NC\_002077

```

1 ttgcccactc cctctctgcg cgctcgctcg ctcggtgggg cctgcgacc aaaggtcccg
61 agacggcaga gctctgctct gccggcccca ccgagcgagc gagcgcgag agagggagtg
121 ggcaactcca tcactagggg taatcgcgaa gcgcctccca cgctgccgcy tcagcgctga
181 cgtaaattac gtcatagggg agtggctctg tattagctgt cacgtgagtg cttttgcyac
241 attttgcyac accacgtggc catttagggg atatattggc gagtgagcga gcaggatctc
301 cttttgcyac gcgaaatttg aacgagcagc agccatgccg ggcttctacg agatcgtgat
361 caaggtgccg agcgacctgg acgagcacct gccgggcatt tctgactcgt ttgtgagctg
421 ggtggccgag aaggaatggg agctgcccc ggattctgac atggatctga atctgattga
481 gcaggcacc ctgacctggg ccgagaagct gcagcgcyac ttcttggctc aatggcgccg
541 cgtgagtaag gccccggagg cctcttctt tgttcagttc gagaagggcg agtcctactt
601 ccacctccat attctgggtg agaccacggg ggtcaaatcc atggtgctgg gccgcttctc
661 gagtcagatt agggacaagc tggtgcyac catctaccgc gggatcgagc cgacctgccc
721 caactggttc gcggtgacca agacgcgtaa tggcgccgga ggggggaaca aggtgggtga
781 cgagtgcctc atccccaaat acctctgccc caagactcag cccgagctgc agtgggcytg
841 gactaacatg gaggagtata taagcgctg tttgaacctg gccgagcyga aacggctcgt
901 ggcygcyac ctgacctcag tcagccagc ccaggagcyg aacaaggaga atctgaacct
961 caattctgac gcgctgtca tccggtcaaa aacctccgcy cgctacatgg agctggctcg
1021 gtggctggtg gaccggggca tcacctcga gaagcagtg atccaggagg accaggcctc
1081 gtacatctcc ttcaacggc cttccaaact gcggtcccag atcaaggccg ctctggcyaa
1141 tgccggcaag atcatggcgc tgaccaaact ccgccccgac tacctggtag gccccgctcc
1201 gcccgcyac attaaaacca accgcatcta ccgcatcctg gagctgaacg gctacgaacc
1261 tgctacgccc ggctccgtct ttctcggtg gccccagaaa aggttcggga agcgcaaac
1321 catctggctg tttggggcgg ccaccacggg caagaccaac atcgcygaag ccatcgccca
1381 cgccgtgccc ttctacggct gcgtcaactg gaccaatgag aactttccct tcaatgattg
1441 cgtcgcyaa atggtgatct ggtgggagga gggcaagatg acggccaagg tcgtggagtc
1501 cgccaaggcc attctcggcg gcagcaaggt gcgcytgac caaaagtgc agtcgtccgc
1561 ccagatcgac cccacccccg tgatcgtcac ctccaacacc aacatgtgcg ccgtgattga
1621 cgggaacagc accaccttcg agcaccagca gccgttgcyg gaccgatgt tcaaattga
1681 actcaccgc cgtctggagc atgactttgg caagtgaca aagcaggaag tcaaagagtt
1741 cttccgctgg gcgcygagc acgtgaccga ggtggcyat gattctacg tcgaaaggg
1801 tggagccaac aaaagaccgc cccccgatga ccgcyataaa agcyagccca agcgggcctg
1861 cccctcagtc gcggtccat cgacgtcaga ccggaagga gctccggtg actttgcccga
1921 caggtacca aacaaatggt ctgctcagc gggcatgctt cagatgctt ttccctgcaa
1981 gacatgcgag agaatgaatc agaatttcaa ctttgctt acgcacggga cgagagactg
2041 ttcagagtg ttccccggcg tgtcagaatc tcaaccggtc gtcagaaaga ggacgtatcg
2101 gaaactctgt gccattcatc atctgctggg gcgggctccc gagattgctt gctcgccctg
2161 cgatctggtc aacgtggacc tggatgactg tgtttctgag caataaatga cttaaaccag
2221 gtatggctgc cgatggttat cttccagatt ggctcgagga caacctctt gagggcattc
2281 gcgagtggtg ggacttgaaa cctggagccc cgaagcccaa agccaaccag caaaagcagg
2341 acgagggccg gggctctggtg ctctcctggct acaagtacct cggaccttc aacggactcg
2401 acaaggggga gcccgctcaac gcggcggagc cagcgccctc cgagcagcy aaggcctacg
2461 accagcyct caaagcgggt gacaatccgt acctgcggt taaccacgc gacgcyagt
2521 ttcagagcy tctgcaagaa gatacgtctt ttgggggcaa cctcgggcga gcagcttcc
2581 agccaagaa gcgggtctc gaacctctc gctgggtga ggaaggcgt aagacggctc
2641 ctggaagaa acgtccggtg gagcagtcgc cacaagagcc agactcctc tcgggcatcg
2701 gcaagcagc ccagcagccc gctaaaaaga gactcaattt tggtcagact ggcgactcag
2761 agtcagctcc cgatccacaa cctctcggag aacctccagc aacccccgct gctgtgggac
2821 ctactacaat ggcttcaggc ggtggcgcac caatggcaga caataacgaa ggcgcyagc
2881 gagtgggtaa tgctcagga aattggcatt gcgattccac atggctgggc gacagagtc
2941 tcaccaccag caccgcacc tgggccttgc ccacctaca taaccacctc tacaagcaaa
3001 tctccagtc ttcaacgggg gccagcaacg acaaccacta cttcggtac agcaccctc
3061 ggggtatct tgatttcaac agattccact gccactttc accacgtgac tggcagcyac
3121 tcatcaacaa caattgggga ttccggccca agagactcaa cttcaaacctc ttcaacatcc
3181 aagtcaagga ggtcacgagc aatgatggcg tcacaacct cgctaataac cttaccagca
3241 cggttcaagt cttctcggac tcggagtacc agcttccgta cgtcctcggc tctcgcyacc
3301 agggctgct cctccgctc ccggcggagc tgttcatgat tccgcaatac ggtcagctga
3361 cgctcaacaa tggcagccaa gccgtgggac gttcatcctt ttactgcctg gaatatttc

```

3421 cttctcagat gctgagaacg ggcaacaact ttaccttcag ctacaccttt gaggaagtgc  
 3481 ctttccacag cagctacgcg cacagccaga gctggaccg gctgatgaat cctctcatcg  
 3541 accaatacct gtattacctg aacagaactc aaaatcagtc cggagtgcc caaaacaagg  
 3601 acttgctggt tagccgtggg tctccagctg gcatgtctgt tcagccaaa aactggctac  
 3661 ctggaccctg ttatcggcag cagcgcgttt ctaaaacaaa aacagacaac aacaacagca  
 3721 attttacctg gactgggtgct tcaaaatata acctcaatgg gcgtgaatcc atcatcaacc  
 3781 ctggcactgc tatggcctca cacaaagacg acgaagacaa gttctttccc atgagcgggtg  
 3841 tcatgatttt tggaaaagag agcgcggag cttcaaacac tgcattggac aatgtcatga  
 3901 ttacagacga agaggaaatt aaagccacta acctgtggc caccgaaaga tttgggaccg  
 3961 tggcagtcaa tttccagagc agcagcacag acctgcgac cggagatgbg catgctatgg  
 4021 gagcattacc tggcatggtg tggcaagata gagacgtgta cctgcagggt cccatttggg  
 4081 ccaaaattcc tcacacagat ggacactttc acccgtctcc tcttatgggc ggctttggac  
 4141 tcaagaacct gcctcctcag atcctcatca aaaacacgcc tgttcctgcg aatcctccgg  
 4201 cggagttttc agctacaaag tttgcttcat tcatcaccca atactccaca ggacaagtga  
 4261 gtgtggaaat tgaatgggag ctgcagaaaag aaaacagcaa gcgctggaat cccgaagtgc  
 4321 agtacacatc caattatgca aaatctgcc aagttgattt tactgtggac aacaatggac  
 4381 tttatactga gcctcgcccc attggcaccg gttaccttac ccgccccctg taattacgtg  
 4441 ttaatcaata aaccggttga ttogtttcag ttgaactttg gtctcctgtc cttcttatct  
 4501 tatcggttac catgggtata gtttacacat taactgcttg gttgcgcttc gcgataaaag  
 4561 acttacgtca tcgggttacc cctagtgatg gagttgcca ctccctctct gcgcgctcgc  
 4621 tcgctcggtg gggcctgcgg accaaaggtc cgcagacggc agagctctgc tctgccggcc  
 4681 ccaccgagcg agcagagcgcg cagagagggg gtgggcaa

FIG. 8 (Continued)

12/36  
FIG. 9

AAV-3A, complete sequence, GenBank Accession No. NC\_001729

```

1  ttggccactc cctctatgcg cactcgcctcg ctccggggggg cctgggcgacc aaaggtcgcc
61  agacggacgt gctttgcacg tccggcccca cccgagcgcgc gagtgcgcat agaggggagtg
121 gccaactcca tctactagagg tatggcagtg acgtaacgcg aagcgcgcgga agcgagacca
181 cgcctaccag ctgctcagc agtcagggtga cccttttgcg acagtttgcg acaccacgtg
241 gccgctgagg gtatatattc tcgagtgagc gaaccaggag ctccattttg acccgaaat
301 ttgaacgagc agcagccatg cccgggttct acgagattgt cctgaaggtc ccgagtgacc
361 tggacgagcg cctgccgggc atttctaact cgtttgttaa ctgggtggcc gagaaggaat
421 gggacgtgcc gccggattct gacatggatc cgaatctgat tgagcaggca cccctgaccg
481 tggccgaaaa gcttcagcgc gagttcctgg tggagtgccg ccgcgtgagt aaggccccgg
541 aggccctctt tttgtccag ttcgaaaagg gggagacctt cttccacctg cacgtgctga
601 ttgagaccat cggggtcaaa tccatggtgg tcggccgcta cgtgagccag attaaagaga
661 agctggtgac ccgcatctac cgcgggggct agccgcagct tccgaactgg ttcgcggtga
721 ccaaacgcgc aatggcgcc gggggcgggg acaagggtgg ggacgactgc tacatccca
781 actacctgct cccaagacc cagcccgagc tccagtgggc gtggactaac atggaccagt
841 atttaagcgc ctgtttgaat ctgcggagc gtaaacggct ggtggcgcag catctgacgc
901 acgtgtcgca gacgaggag cagaacaaag agaatacaga cccaattctt gacgcgccgg
961 tcatcaggtc aaaaacctca gccaggtaaa tggagctggc cgggtggctg gtggaccgcg
1021 ggatcacgtc agaaaagcaa tggattcagg aggaccaggc ctctacatc tccttcaacg
1081 ccgctccaa ctgcggtcc cagatcaagg ccgcgctgga caatgcctcc aagatcatga
1141 gcctgacaaa gacggctccg gactacctgg tgggcagcaa cccgcggag gacattacca
1201 aaaatcggat ctaccaaatc ctggagctga acgggtacga tccgcagtac gccgctccg
1261 tcttctggg ctgggcgcaa aagaagtctg ggaagaggaa caccatctgg ctctttgggc
1321 cggccacgac gggtaaac aacatcgcgc aagccatcgc ccacgccgtg cccttctacg
1381 gctgctaaa ctggaccaat gagaactttc cttcaacga ttgcgtcgac aagatggtga
1441 tctggtggga ggaggcaag atgacggcca aggtcgtgga gagcgccaag gccattctgg
1501 gcggaagcaa ggtgcgcgtg gacaaaagt gcaagtcac ggccagatc gaaccactc
1561 ccgtgatcgt cacctccaac accaacaatg gcgccgtgat tgacgggaac agcaccact
1621 tcgagcatca gcagccgctg caggaccgga tgtttgaatt tgaacttacc cgccgtttgg
1681 accatgactt tgggaaggtc accaaacagg aagtaaaagg cttttccggt tgggttccg
1741 atcacgtgac tgacgtggct catgagtctt acgtcagaaa ggggtggagc aagaacgcc
1801 ccgctccaa tgacgcggat gtaagcgcgc caaacggga gtgcacgtc cttgcgcgc
1861 cgacaacgtc agacgcggaa gcaccggcg actacgcgga caggtacca aacaatggt
1921 ctctgacgt gggcatgaat ctgatgcttt tccctgtaa aacatgcgag agaatgaatc
1981 aaatttcaa tgtctgtttt acgcatggtc aaagagactg tggggaatgc tccctggaa
2041 tgtcagaatc tcaaccggt tctgtctgca aaaagaagac ttatcagaaa ctgtgtcaa
2101 ttcacatata cctgggaagg gcacccgaga ttgcctgttc ggccctgcgat ttggccaatg
2161 tggacttgya tgactgtgtt tctgagcaat aatgactta aaccaggat ggctgctgac
2221 ggttatcttc cagattggct cgaggacaac ctttctgaag gcattcgtga gtggtgggt
2281 ctgaaacctg gactccctca accaaagcg aaccaacaac accaggaca cgtcggggg
2341 cttgtgcttc cgggttacia atacctcgga cccggtaacg gactcgaca aggagagccg
2401 gtcaacgagg cggacgcggc agccctcgaa cacgacaaag cttacgacca gcagctcaag
2461 gccggtgaca acccgtacct caagtacaac cacgcgcagc ccgagtttca ggagctctt
2521 caagaagata cgtcttttg gggcaacctt ggcagagcag tcttccaggc caaaaagagg
2581 atccttgagc ctcttggctc ggttgaggaa gcagctaaaa cggctcctgg aaagaagggg
2641 gctgtagatc agtctcctca ggaaccggac tcatcatctg gtgttgcaa atcgggcaa
2701 cagcctgcca gaaaaagact aaatttcggt cagactggag actcagagtc agtcccagac
2761 cctcaacctc tcggagaacc accagcagcc cccacaagtt tgggatctaa tacaatggct
2821 tcaggcggtg gcgcaccaat ggcagacaat aacgagggtg ccgatggagt gggtaattcc
2881 tcaggaaatt ggcattgcga tcccaatgg ctgggcgaca gagtcatcac caccagcacc
2941 agaactggyg cctgcccac ttacaacaac catctctaca agcaaactc cagccaatca
3001 ggagcttcaa acgacaacca ctactttggc tacagcacc cttgggggta tttgacttt
3061 aacagattcc actgccactt ctcaccacgt gactggcagc gactcattaa caaactgg
3121 ggattccggc ccaagaaact cagcttcaag ctcttcaaca tccaagttag aggggtcacg
3181 cagaacgatg gcacgacgac tattgccaat aacctacca gcacggttca agtgtttacg
3241 gactcggagt atcagctccc gtacgtgctc gggtcggcgc accaaggctg tctcccgccg
3301 ttcacgcgg acgtcttcat ggtccctcag tatggatacc tcacctgaa caacggaagt
3361 caagcggtyg gacgctcatc cttttactgc ctggagtact tcccttcgca gatgctaagg

```

13/36

3421 actggaaata acttccaatt cagctatacc ttcgaggatg taccttttca cagcagctac  
 3481 gctcacagcc agagtttggg tgccttgatg aatcctctta ttgatcagta tctgtactac  
 3541 ctgaacagaa cgcaaggaac aacctctgga acaaccaacc aatcacggct gcttttttagc  
 3601 caggctgggc ctcagtctat gtctttgcag gccagaaatt ggctacctgg gccctgctac  
 3661 cggcaacaga gactttcaaa gactgctaac gacaacaaca acagtaactt tccttggaaca  
 3721 gggccagca aatatcatct caatggccgc gactcgctgg tgaatccagg accagctatg  
 3781 gccagtcaca aggacgatga agaaaaatth tccctatgc acggcaatct aatatttggc  
 3841 aaagaaggga caacggcaag taacgcagaa ttagataatg taatgattac ggatgaagaa  
 3901 gagattcgta ccaccaatcc tgtggcaaca gagcagtatg gaactgtggc aaataacttg  
 3961 cagagctcaa atacagctcc cagcactgga actgtcaatc atcagggggc cttacctggc  
 4021 atgggtgtggc aagatcgtga cgtgtacctt caaggaccta tctgggcaaa gattcctcac  
 4081 acggatggac actttcatcc ttctcctctg atgggaggct ttggactgaa acatccgctt  
 4141 cctcaaatca tgatcaaaaa tactccggta cggcaaatc ctccgacgac tttcagcccg  
 4201 gccagtttg cttcatttat cactcagtac tccactggac aggtcagcgt ggaaattgag  
 4261 tgggagctac agaaagaaaa cagcaaactg tggaaatccag agattcagta cacttccaac  
 4321 tacaacaagt ctgttaatgt ggactttact gtagacacta atgggtgtta tagtgaacct  
 4381 cgccctattg gaaccgggta tctcacacga aacttgtgaa tcctgggtaa tcaataaacc  
 4441 gtttaattcg tttcagttga actttggctc ttgtgcactt ctttatcttt atcttgtttc  
 4501 catggctact gcgtagataa gcagcggcct gcggcgcttg cgcttcgagg ttacaactg  
 4561 ctgggttaata tttaactctc gccatacctc tagtgatgga gttggccact ccctctatgc  
 4621 gcaactcgctc gctcgggtggg gcctggcgac caaaggctgc cagacggagc tgctttgcac  
 4681 gtccggcccc accgagcgag cgagtgcga tagagggagt ggccaa

FIG. 9 (Continued)

14/36  
FIG. 10

AAV-3B, complete sequence, GenBank Accession No. NC\_001863

```

1  tggccactcc ctctatgcgc actcgcctcgc tcggtggggc ctggcgacca aaggctcgcca
61  gacggacgtg ctttgcacgt cgggccccac cgagcgagcg agtgcgcata gagggagtgg
121 ccaactccat cactagaggt atggcagtga cgtaacgcga agcgcgcgaa gcgagaccac
181 gcctaccagc tgcgtcagca gtcaggtgac ccttttgca cagtttgca caccacgtgg
241 ccgctgaggg tatatattct cgagtgcgcg aaccaggagc tccattttga ccgcgaaatt
301 tgaacgagca gcagccatgc cggggttcta cgagattgtc ctgaaggctc cgagtgaact
361 ggacgagcac ctgccgggca tttctaactc gtttgtaac tgggtggccg agaaggaatg
421 ggagctgccg cgggattctg acatggatcc gaatctgatt gagcaggcac ccctgaccgt
481 ggccgaaaag cttcagcgcg agttcctggt ggagtggcgc cgcgtgagta aggcccccga
541 ggccctcttt tttgtccagt tcgaaaaggg ggagacctac ttccacctgc acgtgctgat
601 tgagaccatc ggggtcaaat ccatggtggt cggccgctac gtgagccaga ttaaagagaa
661 gctggtgacc cgcactacc gcggggtcga gccgcagctt ccgaactggt tcgcggtgac
721 caaacgcga aatggcgccg ggggcgggaa caagtggtg gacgactgct acatccccaa
781 ctacctgctc cccaagacc agcccagct ccagtggcg tggactaaca tggaccagta
841 tttaaagcgc tgtttgaatc tcgcgagcg taaacggctg gtggcgagc atctgacgca
901 cgtgtcgcag acgcaggagc agaacaaaga gaatcagaac cccaattctg agcgcgccgt
961 catcaggtca aaaacctcag ccaggtacat ggagtggctc ggggtggctc tggaccgctg
1021 gatcacgtca gaaaagcaat ggattcagga ggaccaggcc tcgtacatct ccttcaacgc
1081 cgcctccaac tcgcggtccc agatcaaggc cgcgtggac aatgcctcca agatcatgag
1141 cctgacaaag acggctccgg actacctggt gggcagcaac ccgcccagg acattacca
1201 aaatcggatc taccaaatcc tggagctgaa cgggtacgat ccgcagtacg cggcctcgt
1261 ctctcctggc tgggcgcaaa agaagtccgg gaagaggaac accatctggc tctttgggcc
1321 ggccacgacg ggtaaaacca acatcgcgga agccatcgcc cacgcccgtc ccttctacgg
1381 ctgcgtaaac tggaccaatg agaactttcc cttcaacgat tgcgtcgaca agatggtgat
1441 ctggtgggag gagggcaaga tgacggccaa ggtcgtggag agcgcgaagg ccattctggg
1501 cggaagcaag gtgcgcgtgg accaaaagtg caagtcatcg gccagatcg aaccactcc
1561 cgtgatcgtc acctccaaca ccaacatgtg cgcctgatt gacgggaaca gcaccactt
1621 cgagcatcag cagccgctgc aggaccggat gtttaaattt gaacttacc cccgtttgga
1681 ccatgacttt ggggaaggtc ccaaacagga agtaaaggac tttttccgtt gggcttccga
1741 tcacgtgact gacgtggctc atgagttcta cgtcagaaag ggtggagcta agaaacgcc
1801 cgcctccaat gacgcggatg taagcagacc aaaacggcag tgcacgtcac ttgcgcagcc
1861 gacaacgtca gacgcggaag caccggcgga ctacgcggac aggtaccaa acaaatgttc
1921 tcgtcacgtg ggcattgaatc tgatgctttt tcctgtaaa acatgcgaga gaatgaatca
1981 aatttccaat gtctgtttta cgcattgtca aagagactgt ggggaatgct tccctggaat
2041 gtcagaatct caaccgcttt ctgtcgtcaa aaagaagact tatcagaaac tgtgtccaat
2101 tcatcatatc ctgggaaggg caccgagat tgctgttcg gctgcgatt tggccaatgt
2161 ggacttggat gactgtgttt ctgagcaata aatgacttaa accaggtatg gctgctgacg
2221 gttatcttcc agattggctc gaggacaacc tttctgaagg cattcgtgag tgggtggctc
2281 tgaaacctgg agtccctcaa ccaaagcga accaacaaca ccaggacaac cgtcggggtc
2341 ttgtgcttcc gggttacaaa tacctcggac ccggtaacgg actcgacaaa ggagagccgg
2401 tcaacgaggg ggacgcggca gccctcgaac acgacaaagc ttacgaccag cagctcaagg
2461 ccggtgacaa cccgtacctc aagtacaacc acgccgacgc cgagtttcag gagctcttc
2521 aagaagatac gtcttttggg ggcaaccttg gcagagcagt cttccaggcc aaaaagagga
2581 tccttgagcc tcttggctg gttgaggaag cagctaaaac ggctcctgga aagaagaggc
2641 ctgtagatca gtctctcag gaaccggact catcatctgg tgttgcaaa tcgggcaaac
2701 agcctgccag aaaaagacta aatttcggtc agactggcga ctcagagtca gtcccagacc
2761 ctcaacctct cggagaacca ccagcagccc ccacaagttt gggatctaat acaatggctt
2821 caggcgtggt cgcaccaatg gcagacaata acgaggggtc cgatggagtg ggtaattcct
2881 caggaaattg gcattgcgat toccaatggc tgggcgacag agtcatcacc accagacca
2941 gaacctgggc cctgcccact tacaacaacc atctctaaa gcaaatctcc agccaatcag
3001 gagcttcaaa cgacaaccac tactttggct acagcaccac ttgggggtat tttgacttta
3061 acagattcca ctgccacttc toaccacgtg actggcagcg actcattaac acaactggg
3121 gattccggcc caagaaactc agcttcaagc tcttcaacat ccaagttaaa gaggtcacgc
3181 agaacgatgg cacgacgact attgccaata acctaccag cacggttcaa gtgtttacgg
3241 actcggagta tcagctcccg tacgtgctcg ggtcggcgca ccaaggctgt ctcccgcgt
3301 ttccagcggc cgtcttcatg gtccctcagt atggatacct caccetgaac aacggaagt
3361 aagcggtggt acgctcatcc ttttactgcc tggagtactt cccttcgcag atgctaagg

```

15/36

3421 ctggaaataa cttccaattc agctatacct tcgaggatgt accttttcac agcagctacg  
 3481 ctcacagcca gagtttggat cgcttgatga atcctcttat tgatcagtat ctgtactacc  
 3541 tgaacagaac gcaaggaaca acctctggaa caaccaacca atcacggctg ctttttagcc  
 3601 aggctgggccc tcagtctatg tctttgcagg ccagaaattg gctacctggg ccctgctacc  
 3661 ggcaacagag actttcaaag actgctaacg acaacaacaa cagtaacttt ccttggacag  
 3721 cggccagcaa atatcatctc aatggccgcg actcgctggt gaatccagga ccagctatgg  
 3781 ccagtcacaa ggacgatgaa gaaaaatfff tccctatgca cggcaatcta atatttggca  
 3841 aagaagggac aacggcaagt aacgcagaat tagataatgt aatgattacg gatgaagaag  
 3901 agattcgtac caccaatcct gtggcaacag agcagtatgg aactgtggca aataacttgc  
 3961 agagctcaaa tacagctccc acgactagaa ctgtcaatga tcagggggccc ttacctggca  
 4021 tgggtgtggca agatcgtgac gtgtaccttc aaggacctat ctggggcaaag attcctcaca  
 4081 cggatggaca ctttcatcct tctcctctga tgggaggctt tggactgaaa catccgcctc  
 4141 ctcaaatcat gatcaaaaat actccggtac cggcaaatcc tccgacgact ttcagcccgg  
 4201 ccaagtttgc ttcattttatc actcagtaact ccaactggaca ggtcagcgtg gaaattgagt  
 4261 gggagctaca gaaagaaaac agcaaacggt ggaatccaga gattcagtac acttccaact  
 4321 acaacaagtc tgtaaatgtg gactttactg tagacactaa tgggtgtttat agtgaacctc  
 4381 gccctattgg aaccgggtat ctcacacgaa acttgtaatc ctgggttaatc aataaacctg  
 4441 ttaattcgtt tcagttgaac tttggctctt gtgcacttct tatcttatct tgtttccatg  
 4501 gctactgcgt agataagcag cggcctgagg cgcttgcgt tcgagggtta caactgctgg  
 4561 ttaatattta actctcgcca tacctctagt gatggagttg gccactccct ctatgcgcac  
 4621 tcgctcgctc ggtggggccg gacgtgcaaa gcacgtccgt ctggcgacct ttggtcgcca  
 4681 ggccccaccg agcagcagag tgcgcataga gggagtggcc aa

FIG. 10 (Continued)

16/36  
FIG. 11

AAV-4, complete sequence, GenBank Accession No. NC\_001829

```

1  ttggcactc cctctatgcg cgctcgtca ctcactcggc cctggagacc aaaggtctcc
61 agactgccg cctctggccg gcagggccga gtgagtgagc gagcgcgcat agagggagtg
121 gccactcca tcatctaggt ttgcccactg acgtcaatgt gacgtcctag ggtagggag
181 gtccctgtat tagcagtcac gtgagtgtcg tatttcgctg agcgtagcgg agcgcatacc
241 aagctgccac gtcacagcca cgtgggtccgt ttgcgacagt ttgcgacacc atgtggtcag
301 gagggtatat aaccgcgagt gagccagcga ggagctccat ttgcccgcg aattttgaac
361 gagcagcagc catgccgggg ttctacgaga tcgtgctgaa ggtgcccgagc gacctggagc
421 agcacctgcc cggcattttct gactcttttg tgagctgggt ggccgagaag gaatgggagc
481 tgccgccgga ttctgacatg gacttgaatc tgattgagca ggcacccttg accgtggccg
541 aaaagctgca acgcgagttc ctggctcagat ggcgccgcgt gagtaaggcc ccggaggccc
601 tcttctttgt ccagttcgag aagggggaca gctacttcca cctgcacatc ctggtggaga
661 ccgtgggctg caaatccatg gtgggtggcc gctacgtgag ccagattaaa gagaagctgg
721 tgaccgcgat ctaccgcggg gtccgagccg agcttccgaa ctggttcgct gtgaccaaga
781 cgcgtaatgg cgccggaggc gggaacaagg tgggtggacga ctgctacatc cccaactacc
841 tgctcccca gaccagccc gagctccagt gggcgtggac taacatggac cagtataata
901 gcgctgttt gaatctcgcg gagcgtaaac ggtgggtggc gcagcatctg acgcagctgt
961 cgcagcgaac ggagcagaac aaggaaaacc agaaccctaa ttctgacgct ccggtcatca
1021 ggtcaaaaa ctccgccagg tacatggagc tggctcgggtg gctggtggac cgcgggatca
1081 cgtcagaaaa gcaatggatc caggaggacc aggcgtccta catctccttc aacgccgcct
1141 ccaactcgcg gt,cacaaatc aaggccgcgc tggacaatgc ctccaaaatc atgagcctga
1201 caaagacggc tccggactac ctgggtggcc agaaccgcgc ggaggacatt tccagcaacc
1261 gcatctaccg aatcctcgag atgaacgggt acgatccgca gtacgcggcc tccgtcttcc
1321 tgggctgggc gcaaaagaag ttccgggaaga ggaacacccat ctggctcttt gggccggcca
1381 cgacgggtaa aaccaacatc gcggaagcca tcgcccacgc cgtgcccttc tacggctgctg
1441 tgaactggac caatgagaac tttccgttca acgattgctg cgacaagatg gtgatctggt
1501 gggaggaggc caagatgacg gccaaaggct tagagagcgc caaggccatc ctgggaggaa
1561 gcaaggtgct cgtggaccaa aagtgcaagt catcggccca gatcgacca actcccgtga
1621 tcgtcacctc caacaccaac atgtgcgcgg tcatcgacgg aaactcgacc accttcgagc
1681 accaacaacc actccaggac cggatgttca agttcgagct caccaagcgc ctagagcagc
1741 actttggcaa ggtcaccaag caggaagtca aagacttttt ccggtgggctg ctgatcacg
1801 tgactcaggt gactcacgag ttttacgtca gaaaggggtg agctagaaag aggccgccc
1861 ccaatgacgc agatataagt gagcccaagc gggcctgtcc gtcagttgct cagccatcga
1921 cgtcagacgc ggaagctccg gtggactacg cggacaggta ccaaaacaaa tgttctcgct
1981 acgtgggtat gaatctgatg ctttttccct gccggcaatg cgagagaatg aatcagaatg
2041 tggacatttg cttcacgcac ggggtcatgg actgtgccga gtgcttcccc gtgtcagaat
2101 ctcaaccctg gtctgtcgtc agaaagcggc cgtatcagaa actgtgtccg attcatcaca
2161 tcatggggag ggcgcccgag gtggcctgct cggcctgcga actggccaat gtggacttgg
2221 atgactgtga catggaacaa taaatgactc aaaccagata tgactgacgg ttaccttcca
2281 gattggctag aggacaacct ctctgaaggc gttcgagagt ggtgggcgct gcaacctgga
2341 gccctaaac ccaaggcaaa tcaacaacat caggacaacg ctcggggtct tgtgcttccg
2401 ggttacaat acctcggacc cggcaacgga ctcgacaagg gggaaccctg caacgcagcg
2461 gacgcggcag ccctcgagca cgacaaggcc tacgaccagc agctcaaggc cggtgacaac
2521 ccctacctca agtacaacca cgccgacgct gagttccagc agcggcttca ggcgcacaca
2581 tcgtttgggg gcaacctcgg cagagcagtc ttccaggcca aaaagaggggt tcttgaacct
2641 cttggtctgg ttgagcaagc ggtgagacg gctcctggaa agaagagacc gttgattgaa
2701 tcccccgagc agcccgactc ctccacgggt atcggcaaaa aaggcaagca gccggctaaa
2761 aagaagctcg ttttcgaaga cgaaactgga gcaggcgacg gacccctga gggatcaact
2821 tccggagcca tgtctgatga cagtgagatg cgtgcagcag ctggcggagc tgcagtcgag
2881 ggccgacaag gtgccgatgg agtgggtaat gcctcgggtg attggcattg cgattccacc
2941 tggctctgag gccacgtcac gaccaccagc accagaacct gggcttggcc cacctacaac
3001 aaccacctct acaagcgact cggagagagc ctgcagtcca acacctaaa cggattctcc
3061 acccctggg gatactttga ctcaaccgc ttocactgcc acttctcacc acgtgactgg
3121 cagcgactca tcaacaacaa ctggggcatg cgacccaaag ccatgcgggt caaatcttc
3181 aacatccagg tcaaggaggt cacgacgtcg aacggcgaga caacgggtggc taataacctt
3241 accagcagcg ttcagatctt tgccgactcg tcgtacgaac tgccgtacgt gatggatcgg
3301 ggtcaagagg gcagcctgcc tcttttccc aacgacgtct ttatgggtgcc ccagtagcgg
3361 tactgtggac tggtagccgg caacacttcc cagcaacaga ctgacagaaa tgccttctac

```

3421 tgcctggagt actttccttc gcagatgctg cggactggca acaactttga aattacgtac  
 3481 agttttgaga aggtgccttt ccactcgatg tacgcgcaca gccagagcct ggaccggctg  
 3541 atgaacccctc tcatcgacca gtacctgtgg ggactgcaat cgaccaccac cggaaaccacc  
 3601 ctgaatgccg ggactgccac caccaacttt accaagctgc ggctaccaa cttttccaac  
 3661 tttaaaaaga actggctgcc cgggccttca atcaagcagc agggcttctc aaagactgcc  
 3721 aatcaaaact acaagatccc tgccaccggg tcagacagtc tcatcaaata cgagacgcac  
 3781 agcactctgg acggaagatg gaggccctg acccccggac ctccaatggc cacggctgga  
 3841 cctgctggaca gcaagttcag caacagccag ctcatctttg cggggcctaa acagaacggc  
 3901 aacacggcca ccgtaccggg gactctgatc ttcacctctg aggaggagct ggcagccacc  
 3961 aacgccaccg atacggacat gtggggcaac ctacctggcg gtgaccagag caacagcaac  
 4021 ctgccgaccg tggacagact gacagccttg ggagccgtgc ctggaatggt ctggcaaaac  
 4081 agagacatth actaccaggg tcccatttgg gccaagattc ctcataccga tggacacttt  
 4141 caccctctac cgctgattgg tgggtttggg ctgaaacacc cgcctcctca aatthttatc  
 4201 aagaacaccc cggtacctgc gaatcctgca acgacctca gctctactcc ggtaaactcc  
 4261 ttcattactc agtacagcac tggccagggt tcggtgcaga ttgactggga gatccagaag  
 4321 gagcgggtcca aacgctggaa ccccgaggtc cagtttacct ccaactacgg acagcaaaac  
 4381 tctctgttgt gggctcccga tgccgctggg aaatacactg agcctagggc tatcgggtacc  
 4441 cgctacctca cccaccacct gtaataacct gttaatcaat aaaccggttt attcgtttca  
 4501 gttgaacttt ggtctccgtg tccttcttat cttatctcgt ttccatggct actgcgtaca  
 4561 taagcagcgg cctgcggcgc ttgcgcttcg cggtttacia ctgccggtta atcagtaact  
 4621 tctggcaaac cagatgatgg agttggccac attagctatg cgcgctcgt cactcactcg  
 4681 gccctggaga ccaaaggtct ccagactgcc ggctctggc cggcagggcc gactgactga  
 4741 gcgagcgcgc atagagggag tggccaa

FIG. 11 (Continued)



18/36  
FIG. 12

AAV-5, complete sequence, GenBank Accession No. Y18065 and Ciorini et al., (1999) *J. Virol.* 73:1309

```

1 ctctccccc tgtcgcgttc gctcgcctcgc tggctcgttt ggggggggtgg cagctcaaag
61 agctgccaga cgacggccct ctggccgtcg ccccccaaa cgagccagcg agcgagcgaa
121 cgcgacaggg gggagagtgc cacactctca agcaaggagg ttttgaagc agtgatgtca
181 taatgatgta atgcttattg tcacgcgata gttaatgatt aacagtcatg tgatgtgttt
241 tatccaatag gaagaaagcg cgcgtatgag ttctcgcgag acttccgggg tataaaagac
301 cgagtgaacg agcccgccgc cattctttgc tctggactgc tagaggaccc tcgctgccat
361 ggctaccttc tatgaagtca ttgttcgcgt cccatttgac gtggaggaac atctgcctgg
421 aatttctgac agctttgtgg actgggtaac tgggtcaaatt tgggagctgc ctccagagtc
481 agattttaa attgactctgg ttgaacagcc tcagttgacg gtggctgata gaattcgccg
541 cgtgttctctg tacgagtgga acaaattttc caagcaggag tccaaattct ttgtgcagtt
601 tgaaaagggg tctgaatatt ttcactctga cacgcttggt gagacctccg gcatctcttc
661 catggtcctc ggccgcctacg tgagtcagat tcgcgccag ctggtgaaag tgggtctcca
721 ggggaattgaa ccccagatca acgactgggt cgccatcacc aaggtaaaga agggcgggagc
781 caataagggtg gtggattctg ggtatattcc cgcctacctg ctgccgaagg tccaaccgga
841 gcttcagtggt gcggtggacaa acctggacga gtataaattg gccgccctga atctggagga
901 gcgcaaaccgg ctcgctcgcgc agtttctggc agaactctcg cagcgtctgc aggaggcggc
961 ttcgcagcgt gagttctcgg ctgaccgggt catcaaaagc aagacttccc agaaatacat
1021 ggcgctcgtc aactggctcg tggagcacgg catcacttcc gagaagcagg gatccagga
1081 aaatcaggag agctacctct cttcaactc caccggcaac tctcggagcc agatcaaggc
1141 cgcgctcgac aacgcgacca aaattatgag tctgacaaaa agcgcgggtg actacctcgt
1201 ggggagctcc gttcccagag acatttcaaa aacagaatc tggcaaattt ttgagatgaa
1261 tggctacgac ccggcctacg cgggatccat cctctacggc tgggtgcagc gctccttcaa
1321 caagaggaac accgtctggc tctacggacc cgccacgacc ggcaagacca acatcgcgga
1381 ggccatcgcc cacactgtgc ctttttacgg ctgctgtaac tggaccaatg aaaactttcc
1441 ctttaatgac tgtgtggaca aaatgctcat ttggtgggag gagggaaaga tgaccaacaa
1501 ggtggttgaa tccgccaagg ccatcctggg gggctcaaag gtgctgggtcg atcagaaatg
1561 taaatcctct gttcaaattg attctacccc tgtcattgta acttcaata caaacatgtg
1621 tgtggtggtg gatgggaatt ccacgacctt tgaacaccag cagccgctgg aggaccgat
1681 gttcaaattt gaactgacta agcggctccc gccagatttt ggcaagatta ctaagcagga
1741 agtcaaggac ttttttgctt gggcaaaggt caatcaggtg ccggtgactc acgagtttaa
1801 agttcccagg gaattggcgg gaactaaagg ggcggagaaa tctctaaaac gccactggg
1861 tgagctcacc aatactagct ataaaagtct ggagaagcgg gccaggtctc ctttgttcc
1921 cgagagcctt cgcagttcag acgtgactgt tgatcccgtt cctctcgcac cgctcaattg
1981 gaattcaagg tatgattgca aatgtgacta tcatgctcaa ttgacaaca tttctaaca
2041 atgtgatgaa tgtgaatatt tgaatcgggg caaaaatgga tgtatctgtc acaatgtaac
2101 tcaactgtca atttgtcatg ggattcccc ctgggaaaag gaaaacttgt cagattttgg
2161 ggattttgac gatgccata aagaacagta aataaagcga gtagtcatgt cttttgttga
2221 tcaccctcca gattggttgg aagaagttgg tgaaggtctt cgcgagttt tgggccttga
2281 agcgggcccc ccgaaaccaa aaccatca gcagcatcaa gatcaagccc gtggtcttgt
2341 gctgcctggt tataactatc tcggaccggg aaacggtctc gatcgaggag agcctgtcaa
2401 cagggcagac gaggtcgcgc gagagcacga catctcgtac aacgagcagc ttgaggcggg
2461 agacaacccc tacctcaagt acaaccacgc ggacgccgag tttcaggaga agctcgccga
2521 cgacacatcc ttcgggggaa acctcggaaa ggcagtcttt caggccaaga aaagggttct
2581 cgaacctttt ggctggttg aagaggggtgc taagacggcc cctaccgaa agcggataga
2641 cgaccctttt ccaaaaagaa agaaggctcg gaccgaagag gactccaagc ctccacctc
2701 gtcagacgcc gaagctggac ccagcggatc ccagcagctg caaatcccag cccaaccagc
2761 ctcaagtttg ggagctgata caatgtctgc gggaggtggc ggcccattgg gcgacaataa
2821 ccaaggtgcc gatggagtgg gcaatgcctc gggagattgg cattgcgatt ccacgtggat
2881 gggggacaga gtcgtacca agtccaccgg aacctgggtg ctgccagct acaacaacca
2941 ccagtaccga gagatcaaaa gcggtccgt cgacggaagc aacgccaacg cctactttgg
3001 atacagcacc cctggggggt actttgactt taaccgcttc cacagccact ggagcccccg
3061 agactggcaa agactcatca acaactactg gggcttcaga ccccggtccc tcagagtcaa
3121 aatcttcaac attcaagtca aagaggtcac ggtgcaggac tccaccacca ccatcgccaa
3181 caacctcacc tccaccgtcc aagtgtttac ggacgacgac taccagctgc cctacgtcgt
3241 cggcaacggg accgagggat gcctgcccgc cttccctccg caggtcttta cgctgccgca
3301 gtacggttac gcgacgctga accgcgacaa cacagaaaat cccaccgaga ggagcagctt

```

19/36

3361 cttctgccta gagtactttc ccagcaagat gctgagaacg ggcaacaact ttgagtttac  
 3421 ctacaacttt gaggagggtgc ctttccactc cagcttcgct cccagtcaga acctcttcaa  
 3481 gctggccaac ccgctggtgg accagtactt gtaccgcttc gtgagcacia ataactctgg  
 3541 cggagtccag ttcaacaaga acctggccgg gagatacgcc aacacctaca aaaactgggt  
 3601 cccggggccc atggggccgaa cccagggctg gaacctgggc tccgggggtca accgcgccag  
 3661 tgtcagcgcc ttcgccacga ccaataggat ggagctcgag ggcgaggtt accaggtgcc  
 3721 cccgcagccg aacggcatga ccaacaacct ccagggcagc aacacctatg ccctggagaa  
 3781 cactatgatc ttcaacagcc agccggcgaa cccgggcacc accgccacgt acctcgaggg  
 3841 caacatgctc atcaccagcg agagcgagac gcagccgggtg aaccgctgtg cgtacaacgt  
 3901 cggcgggagc atggccacca acaaccagag ctccaccact gccccgcga ccggcacgta  
 3961 caacctccag gaaatcgtgc ccggcagcgt gtggatggag agggacgtgt acctccaagg  
 4021 acccatctgg gcccaagatcc cagagacggg ggcgcacttt caccctctc cggccatggg  
 4081 cggattcggc ctcaaacacc caccgcccac gatgctcatc aagaacacgc ctgtgcccgg  
 4141 aaatatcacc agcttctcgg acgtgcccgt cagcagcttc atcaccagt acagcaccgg  
 4201 gcaggtcacc gtggagatgg agtgggagct caagaaggaa aactccaaga ggtggaacc  
 4261 agagatccag tacacaaaca actacaacga ccccagttt gtggactttg ccccgacag  
 4321 caccggggaa tacagaacca ccagacctat cggaaaccga taccttacc gacccttta  
 4381 acccattcat gtcgcatacc ctcaataaac cgtgtattcg tgtcagtaaa atactgcctc  
 4441 ttgtggtcat tcaatgaata acagcttaca acatttaca aacctcctt cttgagagt  
 4501 tggcactctc cccctgtcg cgttcgctcg ctcgctggct cgtttggggg ggcgacggcc  
 4561 agaggccgt cgtctggcag ctctttgagc tgccaccccc ccaaacgagc cagcgagcga  
 4621 gcgaacgcga caggggggag ag

FIG. 12 (Continued)

20/36  
FIG. 13

AAV-6, complete sequence, GenBank Accession No. NC\_001862

```

1  ttggccactc cctctctgcg cgctcgetcg ctcaactgagg ccgggcgacc aaaggtcgcc
61  cgacgcccgg gctttgcccg ggcgccctca gtgagcgcag gagcgcgcag agagggagtg
121  gccaaactcca tcaactagggg ttcctggagg ggtggagtcg tgacgtgaat tacgtcatag
181  ggtttagggg gtctctgtatt agaggtcacg tgagtgtttt gcgacatttt gcgacaccat
241  gtggtcacgc tgggtattta agcccagtg agcacgcagg gtctccattt tgaagcggga
301  ggtttgaacg cgcagcgcca tgcgggggt ttacgagatt gtgattaagg tccccagcga
361  ccttgacgag catctgcccg gcatttctga cagctttgtg aactgggtgg ccgagaagga
421  atgggagttg ccgccagatt ctgacatgga tctgaatctg attgagcagg caccctgac
481  cgtggccgag aagctgcagc gcgacttctt ggtccagtgg cgccgcgtga gtaaggcccc
541  ggaggccctc ttctttgttc agttcgagaa gggcgagtcc tacttccacc tccatattct
601  ggtggagacc acgggggtca aatccatggt gctgggcccg ttcttgagtc agattagggg
661  caagctgggtg cagaccatct accgcgggat cgagccgacc ctgcccaact ggttcgcggg
721  gaccaagacg cgtaatggcg ccggaggggg gaacaagggt gtggacgagt gctacatccc
781  caactacctc ctgcccaaga ctacgcccga gctgcagtgg gcgtggacta acatggagga
841  gtatataagc gcgtgtttaa acctggccga gcgcaaacgg ctctgggccc acgacctgac
901  ccacgtcagc cagaccagg agcagaacaa ggagaatctg aacccaatt ctgacgcgcc
961  tgtcatccgg tcaaaaacct ccgcacgcta catggagctg gtcgggtggc tggaggaccg
1021  gggcatcacc tccgagaagc agtggatcca ggaggaccag gcctcgtaca tctccttcaa
1081  cgccgcctcc aactcgcggg cccagatcaa ggccgctctg gacaatgccg gcaagatcat
1141  ggcgctgacc aaatccgcgc ccgactacct ggtaggcccc gctccgcccc ccgacattaa
1201  aaccaaccgc atttaccgca tcctggagct gaacggctac gaccctgcct acgccggctc
1261  cgtctttctc ggctgggccc agaaaagggt cggaaaacgc aacaccatct ggctgtttgg
1321  gccggccacc acgggcaaga ccaacatcgc ggaagccatc gccacgccc tgcccttcta
1381  cggctgcgtc aactggacca atgagaactt tcccttcaac gattgcgctg acaagatggt
1441  gatctggtgg gaggagggca agatgacggc caaggtegtg gagtccgcca aggccattct
1501  cggcggcagc aaggtgcgcg tggacaaaa gtgcaagtctg tccgcccaga tcgatcccac
1561  ccccgatgat gtcacctcca acaccaacat gtgcgcccgtg attgacggga acagcaccac
1621  ctctcagcac cagcagccgt tgcaggaccg gatgttcaaa tttgaactca cccgccgtct
1681  ggagcatgac tttggcaagg tgacaaagca ggaagtcaaa gaggttctcc gctgggcgca
1741  ggatcacgtg accgaggtgg cgcattgatt ctacgtcaga aagggtggag ccaacaagag
1801  acccgcccc gatgacgcgg ataaaagcga cccaagcgg gcctgcccc cagtcgcgga
1861  tccatccccc tcagacgcgg aaggagctcc ggtggacttt gccgacaggt accaaaacaa
1921  atgttctcgt ttttggtctg cagcgggcca tgcttcagat gctgtttccc tgcaaacat gcgagagaat
1981  gaatcagaat ttcaacattt gcttcacgca cgggaccaga gactgttcag aatgtttccc
2041  cggcgtgtca gaatctcaac cggctcgtcag aaagaggacg tatcggaac tctgtgccat
2101  tcatcatctg ctggggcggg ctcccagat tgcttgctcg gcctgcgatc tggccaacgt
2161  ggatctggat gactgtgttt ctgagcaata aatgacttaa accaggtatg gctgccgatg
2221  gttatcttcc agattggctc gaggacaacc tctctgaggg cattcgcgag tgggtggact
2281  tgaaacctgg agccccgaaa cccaaagcca accagcaaaa gcaggacgac ggccgggggtc
2341  tgggtgcttc tggctacaag tacctcggac ccttcaacgg actcgacaag ggggagcccc
2401  tcaacgcggc ggatgcagcg gccctcgagc acgacaaggc ctacgaccag cagctcaaag
2461  cgggtgacaa tccgtacctg cggataaacc acgcccagcgc cgagtttcag gagcgtctgc
2521  aagaagatac gtcttttggg ggcaacctcg ggcgagcagt cttccaggcc aagaagaggg
2581  ttctcgaacc ttttggtctg gttgaggaag gtgctaagac ggctcctgga aagaaacgtc
2641  cggtagagca gtcgccacaa gagccagact cctcctcggg cattggcaag acaggccagc
2701  agcccgctaa aaagagactc aattttggtc agactggcga ctcagagtca gtccccgacc
2761  cacaacctct cgggagaacct ccagcaaccc ccgctgctgt gggacctact acaatggctt
2821  caggcgggtg cgcaccaatg gcagacaata acgaaggcgc cgacggagtg ggtaatgcct
2881  caggaaattg gcattgcgat tccacatggc tgggogacag agtcatcac accagcacc
2941  gaacatgggc cttgccacc tataacaacc acctctaca gcaaatctcc agtgcttcaa
3001  cgggggcccag caacgacaac cactacttcg gctacagcac cccctggggg tattttgatt
3061  tcaacagatt ccaactgcat ttctcaccac gtgactggca gcgactcatc aacaacaatt
3121  ggggattccg gcccaagaga ctcaacttca agctcttcaa catccaagtc aaggaggtca
3181  cgacgaatga tggcgtcacg accatcgcta ataaccttac cagcacgggt caagtcttct
3241  cggactcggg gtaccagttg ccgtacgtcc tcggtctctg gcaccagggc tgccctcctc
3301  cgttcccggc ggacgtgttc atgattccgc agtacggcta cctaacgctc aacaatggca
3361  gccaggcagt gggacgggtca tccttttact gcctggaata tttcccatcg cagatgctga

```

3421 gaacgggcaa taactttacc ttcagctaca ctttcgagga cgtgcctttc cacagcagct  
 3481 acgcgcacag ccagagcctg gaccggctga tgaatcctct catcgaccag tacctgtatt  
 3541 acctgaacag aactcagaat cagtccggaa gtgccccaaa caaggacttg ctgttttagcc  
 3601 ggggggtctcc agctggcatg tctgttcagc ccaaaaactg gctacctgga ccctggtacc  
 3661 ggcagcagcg cgtttctaaa acaaaaacag acaacaacaa cagcaacttt acctggactg  
 3721 gtgcttcaaa atataacctt aatgggctg aatctataat caaccctggc actgctatgg  
 3781 cctcacacaa agacgacaaa gacaagttct ttcccatgag cgggtgcatg atttttggaa  
 3841 aggagagcgc cggagcttca aacactgcat tggacaatgt catgatcaca gacgaagagg  
 3901 aaatcaaagc cactaacccc gtggccaccg aaagatttgg gactgtggca gtcaatctcc  
 3961 agagcagcag cacagaccct gcgaccggag atgtgcatgt tatgggagcc ttacctggaa  
 4021 tgggtgtggca agacagagac gtatacctgc aggtcctat ttgggcaaaa attcctcaca  
 4081 cggatggaca ctttcacccg tctcctctca tgggctgctt tggacttaag caccgcctc  
 4141 ctcagatcct catcaaaaac acgcctgttc ctgcgaatcc tccggcagag ttttcggcta  
 4201 caaagtttgc ttcattcatc acccagtatt ccacaggaca agtgagcgtg gagattgaat  
 4261 gggagctgca gaaagaaaac agcaaacgct ggaatcccga agtgagat acatctaact  
 4321 atgcaaaatc tgccaacggt gatttcaact tggacaacaa tggactttat actgagcctc  
 4381 gccccattgg caccggttac ctcaccgctc ccctgtaatt gtgtgttaat caataaaccg  
 4441 gtttaattcgt gtcagttgaa ctttgggtctc atgtcgttat tatcttatct ggtcaccata  
 4501 gcaaccgggt acacattaac tgcttagttg cgcttcgca atacccttag tgatggagtt  
 4561 gccactccc tctatgcgcg ctgctcgtc cggtggggcc ggcagagcag agctctgccg  
 4621 tctgctgacc tttggtccgc agggcccacc gagcgagcga gcgcgcatag agggagtggg  
 4681 caa

FIG. 13 (Continued)

22/36  
FIG. 14

AAV-7, complete sequence, GenBank Accession No. AF513851

1 ttgccactc cctctatgcg cgctcgctcg ctcggtgggg cctgcgacc aaaggtccg  
61 agacggcaga gctctgctct gccggcccca cggagcgagc gagcgcgcat agagggagtg  
121 gccaaactcca tcaactagggg taccgcgaag cgctccac gctgcccgt cagcgtgac  
181 gtaaatacag tcatagggga gtggtcctgt attagctgtc acgtgagtc tttgcgaca  
241 tttgcgaca ccacgtggcc atttgagga tatatggccg agtgagcgag caggatctcc  
301 atttgaccg cgaatttga acgagcagca gccatgccgg gtttctacga gatcgtgatc  
361 aaggtgccga gcgacctgga cgagcacctg ccggcattt ctgactcgtt tgtgaactgg  
421 gtggccgaga aggaatggga gctgccccg gattctgaca tggatctgaa tctgatcgag  
481 caggcaccac tgacctggc cgagaagctg cagcgcgact tctggtcca atggcggccg  
541 gtgagtaagg ccccgaggc cctgttctt gttcagttc agaagggcga gagctactc  
601 cacctcagc ttctggtgga gaccacggg gtcaagtcca tgggtctagg ccgcttctg  
661 agtcagattc gggagaagct ggtccagacc atctaccgcg gggctgagcc cagctgccc  
721 aactggttcg cggtagccaa gacgcgtaat ggcgccggcg gggggaacaa ggtggtggac  
781 gagtgctaca tcccaacta cctcctgcc aagaccagc ccgagctgca gtggcgtgg  
841 actaacatgg aggagtatat aagcgcgtgt tgaacctgg ccgaacgcaa acggctcgtg  
901 ggcagcacc tgaccacgt cagccagacg caggagcaga acaaggagaa tctgaacccc  
961 aattctgacg cggcgtgat caggcaaaa acctccgcg gctacatgga gctggtcggg  
1021 tggctggtg accggggcat cacctccgag aagcagtgga tccaggagga ccaggcctc  
1081 tacatctct tcaacggcg ctccaactc cggctccaga tcaaggccg gctggacaat  
1141 gccggcaaga tcatggcgt gaccaatcc gcgcccact acctggtgg gccctcgtc  
1201 cccgaggaca taaaaccaa ccgcatctac cgcatcctg agctgaacg gtacgatct  
1261 gcctacggc gctccgtct tctcggtgg gccagaaaa agttcgggaa gcgcaacacc  
1321 atctggtgt ttggcccg caccaccggc aagaccaaca ttgcggaagc catgcccac  
1381 gccgtgccct tctacggctg ctcaactgg accaatgaga actttocct caacgattg  
1441 gtcgacaaga tggatgctg gtggaggag ggcaagatga cggccaaggt cgtggagtcc  
1501 gccaaaggcca ttctggcg cagcaaggtg cgcgtggacc aaaagtcaa gtcgtccgc  
1561 cagatcgacc ccaccccgat gatcgtcacc tcaacacca acatgtcgc cgtgattgac  
1621 gggaacagca ccacctcga gcaccagcag ccgttcgagg accggtgtt caaattgaa  
1681 ctaccggcc gtctggagca cgacttggc aaggtgacga agcaggaagt caaagagttc  
1741 ttccgctgg ccagtgatca cgtgaccgag gtggcgtatg agttctact cagaaagggc  
1801 ggagccagca aaagaccgc cccgatgac gcggatataa gcgagccca ggggctcgc  
1861 cctcagtcg cggatccat gacgtcagc gcggaaggag ctccggtgga cttgcccac  
1921 aggtacaaa acaaatgtc tcgtcacgc ggcatgattc agatgctgt tccctgcaa

1981 acgtgcgaga gaatgaatca gaattcaac attgctca cacacgggt cagagactgt  
2041 ttagagtgt tccccggcgt gtcagaatct caaccggtcg tcagaaaaaa gacgtatcgg  
2101 aaactctcgc cgattcatca tctgctgggg cgggcgccc agattgcttg ctggcctgc  
2161 gacctgtca acgtggacct ggacgactgc gttctgagc aataaatgac taaaccagg  
2221 tatggctgcc gatggtatc ttccagattg gctcaggac aacctctcg agggcattcg  
2281 cgagtgttg gacctgaaac ctggagcccc gaaacccaaa gccaaccagc aaaagcagga  
2341 caacggccgg ggtctggtgc ttctggcta caagtacctc ggaccctca acggactcga  
2401 caagggggag cccgtcaacg cggcggacgc agcggccctc gagcacgaca aggcctacga  
2461 ccagcagctc aaagcgggtg acaatccgta cctgcggtat aaccacgccc acgcccagtt  
2521 tcaggagcgt ctgcaagaag atacgtcatt tgggggcaac ctgggagcag cagtctcca  
2581 ggccaagaag cgggtctcg aacctctcg tctggtgag gaaggccta agacggctcc  
2641 tgcaagaag agaccggtag agccgtacc tcagcgtcc cccgactcct ccacgggcat  
2701 cggcaagaaa ggccagcagc ccgccagaaa gagactcaat ttcggtcaga ctggcgactc  
2761 agagtacgtc cccgaccctc aacctctcg agaacctcca gcagcgcctc ctagtgtgg  
2821 atctgttaca gtggctcag gcggtggcgc accaatggca gacaataacg aaggtgccga  
2881 cggagtgggt aatgcctcag gaaattggca ttgcgattcc acatggctgg ggcacagagt  
2941 cattaccacc agcaccgaa cctgggccct gccacctac aacaaccacc tctacaagca  
3001 aatctccagt gaaactgag gtatgaccaa cgacaacacc tacttcggct acagcacc  
3061 ctgggggtat ttgactta acagattcca ctgccactc tcaccacgtg actggcagc  
3121 actatcaac aacaactggg gattccggcc caagaagctg cggttcaagc tottcaacat  
3181 ccaggtcaag gaggtcacga cgaatgacgg cgttacgacc atcgtaata acctaccag  
3241 cacgattcag gtattctcg actcggata ccagctgccg tacgtcctcg gctctcgcga  
3301 ccagggctgc ctgctccgt tcccggcga cgtctcatg attcctcagt acggctacct  
3361 gacttcaac aatggcagtc agtctgtgg acgttctcc ttctactgcc tggagtactt  
3421 cccctcag atgtgagaa cgggcaacaa cttgagttc agctacagct tcgaggacgt  
3481 gcctttcac agcagctacg cacacagcca gagcctggac cggctgatga atccccat  
3541 cgaccagtac ttgtactacc tggccagaac acagagtaac ccaggaggca cagctggcaa  
3601 tcgggaactg cagtttacc agggcgggcc ttcaactatg gccgaacaag ccaagaattg  
3661 gttacctgga cttgcttc ggcaacaaag agtctcaaa acgctggatc aaaacaaca  
3721 cagcaacttt gcttgactg gtgccacaa atatcacctg aacggcagaa actcgttgg  
3781 taatccggc gtcgcatgg caactcaca ggacgacgag gaccgcttt tccatccag  
3841 cggagtctg attttgaa aaactggagc aactacaaa actacattg aaaatgtgt  
3901 aatgacaaat gaagaagaaa ttcgtctac taatctgta gccacggaag aatacgggat  
3961 agtcagcagc aactacaag cggtaatac tgcagcccag acacaagtg tcaacaacca  
4021 gggagcctta cctggcatg tctggcagaa cgggacgtg tacctgcagg gtccatctg  
4081 ggccaagatt cctcacagc atggcaact tcaccgtct ctttgatgg gcggcttgg  
4141 acttaaacat ccgcctctc agatctgat caagaacact cccgtccc ctaatcctc  
4201 ggaggtgtt actcctcca agttgcttc gttcatcaca cagtacagca cggacaagt

FIG. 14 (Continued)

4261 cagcgtggaa atcgagtggg agctgcagaa ggaaaacagc aagcgtgga acccgagat  
4321 tcagtacacc tccaacttg aaaagcagac tgggtggac ttgccgtg acagccagg  
4381 tgttactct gagcctgcc ctattggcac tcgtacctc accgtaatc tgaattgca  
4441 tgtaatcaa taaaccggtt gattcgttc agtgaactt tggctcctg tgcttctat  
4501 cttatcggtt tccatagcaa ctggttacac attaactgct tgggtgcgct tcacgataag  
4561 aacctgacg tcaccgCGGT acccctagt atggagtgg cactccctc tatgcgcgt  
4621 cgctcgtcgt gtggggcctg cggaccaaag gtccgcagac ggcagagctc tgctctgccg  
4681 gccccaccga gcgagcgagc gcgcatagag ggagtggcca a

**FIG. 14 (Continued)**

25/36  
FIG. 15

AAV-8, complete sequence, GenBank Accession No. AF513852

1 cagagagggga gtggccaact ccatcactag gggtagcgcg aagcgctcc cacgctgccg  
61 cgtcagcgct gacgtaaatt acgtcatagg ggagtgtcc tgtattagct gtcacgtgag  
121 tgcttttgcg gcattttgcg acaccacgtg gccatttgag gtatatatgg ccgagtgagc  
181 gagcaggatc tccattttga ccgcgaaatt tgaacgagca gcagccatgc cgggcttcta  
241 cgagatcggt atcaagggtc cgagcgacct ggacgagcac ctgccgggca tttctgactc  
301 gtttgtgaac tgggtggccg agaaggaatg ggagctgccc ccgattctg acatggatcg  
361 gaatctgac gagcaggcac ccctgaccgt ggccgagaag ctgcagcgcg acttctgtgt  
421 ccaatggcgc cgcgtgagta aggccccgga ggccctcttc tttgtcagt tcgagaaggg  
481 cgagagctac tttcacctgc acgttctgtt cgagaccacg ggggtcaagt ccatggtgct  
541 aggccgcttc ctgagtgcga ttccggaaaa gcttggcca gaccatctac ccgcggggtc  
601 gagccccacc ttgccaact ggttcgcggt gaccaaagac gcgtaaatgg cgccggcggg  
661 ggggaacaag gtggtggacg agtgctacat cccaactac ctctgccca agactcagcc  
721 cgagctgcag tgggctgga ctaacatgga ggagtatata agcgcgtgct tgaacctggc  
781 cgagcgcaaa cggctcgtgg cgcagcacct gaccacgctc agccagacgc aggagcagaa  
841 caaggagaat ctgaacccca attctgacgc gcccgatc aggtcaaaaa cctccgcgcg  
901 ctatatggag ctggtcgggt ggctgtgga ccggggcacc acctccgaga agcagtggat  
961 ccaggaggac caggcctcgt acatctcctt caacgccgcc tccaactcgc ggtcccagat  
1021 caaggccgcg ctggacaatg ccggcaagat catggcgctg accaaatccg cgcccgacta  
1081 cctggtgggg ccctcgtgc ccgcggacat taccagaac cgcactacc gcatctcgc  
1141 tctcaacggc tacgacctg cctacgccg ctccgtctt ctcggtggg ctcaaaaa  
1201 gttcgggaaa cgcaacacca tctggctgtt tggaccgcc accaccggca agaccaacat  
1261 tgcggaagcc atgccccacg ccgtgccctt ctacggctgc gtcaactgga ccaatgagaa  
1321 ctttccctc aatgattgcg tcgacaagat ggtgatctg tgggaggagg gcaagatgac  
1381 ggccaagtc gtggagtcc ccaaggccat tctcggcggc agcaaggtgc gcgtggacca  
1441 aaagtgaag tcgtccgcc agatcgacc caccctcgt atcgtcacct ccaacaccaa  
1501 catgtgcgc gtgattgac ggaacagcac cacctcagc caccagcagc ctctccagga  
1561 ccggatggtt aagtgcgaac tcaccgccg tctggagcac gactttggca aggtgacaaa  
1621 gcaggaagtc aaagagtct tccgctgggc cagtgatcac gtgaccgagg tggcgcata  
1681 gttttacgc agaaagggcg gagccagcaa aagaccgcc ccgatgacg cggataaaag  
1741 cgagccaag cgggcctgcc cctcagtcgc ggatccatcg acgtcagacg cggaaggagc  
1801 tccggtggac tttccgaca ggtacaaaa caaatgtct cgtcacgcg gcatgctca  
1861 gatgctgtt ccctcaaaa cgtgcgagag aatgaatcag aattcaaca tttgcttac  
1921 acacggggtc agagactgct cagagtgtt ccccgcgctg tcagaatctc aaccggtcgt  
1981 cagaaagagg acgtatcga aactctgtc gattcatcat ctgctggggc gggctccga  
2041 gattgctgc tcggcctgc atctgtcaa cgtggacctg gatgactgtg tttctgagca  
2101 ataatgact taaaccaggt atggctgcc atggttatct tccagattgg ctgaggaca



2161 acctctctga gggcattcgc gagtgggtggg cgctgaaacc tggagccccg aagcccaaag  
2221 ccaaccagca aaagcaggac gacggccggg gtctgggtct tcttggctac aagtacctcg  
2281 gacccttcaa cggactcgc aaggggggagc ccgtcaacgc ggccggacgca gcggccctcg  
2341 agcatgacaa ggcttacgac cagcagctgc aggcgggtga caatccgtac ctgcggtata  
2401 accacgccga cgccgagttt caggagcgtc tgcaagaaga tacgtctttt gggggcaacc  
2461 tggggcgagc agtctccag gccaaagaagc gggttctoga acctctcggg ctggttgagg  
2521 aaggcgctaa gacggctcct ggaaagaaga gaccggtaga gccatcacc cagcgttctc  
2581 cagactcctc tacgggcatc ggcaagaagc gccaacagcc cgccagaaaa agactcaatt  
2641 ttggtcagac tggcgactca gagtcagttc cagaccctca acctctcggg gaacctccag  
2701 cagcgcctc ttgtgtggga cctaatacaa tggctgcagg cgggtggcgca ccaatggcag  
2761 acaataacga aggcgccgac ggagtgggta gttctcggg aaattggcat tgcgattcca  
2821 catggctggg cgacagagtc atcaccacca gcacccgaac ctgggccctg cccacctaca  
2881 acaaccacct ctacaagcaa atctcaacg ggacatcggg aggagccacc aacgacaaca  
2941 cctactcgg ctacagcacc cctgggggt atttgactt taacagattc cactgccact  
3001 ttcaccacg tgactggcag cgactcatca acaacaactg gggattccgg cccaagagac  
3061 tcagcttcaa gctcttaac atccaggta aggaggtcac gcagaatgaa ggcaccaaga  
3121 ccatcgccaa taacctcacc agcaccatcc aggtgtttac ggactcggag taccagctgc  
3181 cgtacgttct cgctctgcc caccagggct gctgcctcc gttccggcg gacgtgtca  
3241 tgattccca gtacggctac ctaactca acaacgtag tcaggccgtg ggacgtcct  
3301 ccttctactg cctggaatac ttcctcgc agatgctgag aaccggcaac aacttccagt  
3361 ttacttacac ctccaggac gtgccttcc acagcagcta cgcccacagc cagagcttg  
3421 accggctgat gaatctctg attgaccagt acctgtacta ctgtctcgg actcaaaaa  
3481 caggaggcac ggcaatacag cagactctgg gcttcagcca aggtgggct aatacaatg  
3541 ccaatcaggc aaagaactgg ctgccaggac cctgttaccg ccaacaacgc gtctcaacga  
3601 caaccgggca aaacaacaat agcaacttg cctggactgc tgggacaaa taccatctga  
3661 atggaagaaa ttcattggct aatctggca tcgctatggc aacacacaaa gacgacgagg  
3721 agcgttttt tccagtaac gggatcctga ttttggcaa acaaatgtct gccagagaca  
3781 atcgggatta cagcgatgc atgctacca gcgaggaaga aatcaaaaacc actaacctg  
3841 tggctacaga ggaatacgg atcgtggcag ataacttca gcagcaaac acggctcctc  
3901 aaattggaac tgtcaacagc cagggggcct taccgggtat ggtctggcag aaccgggacg  
3961 tgtacctga gggctccatc tggccaaga ttctcacac ggacggcaac ttccacctg  
4021 ctccgctgat gggcggcttt ggctgaaac atctccgcc tcagatctg atcaagaaca  
4081 cgctgtacc tgcggatcct ccgaccacct tcaaccagtc aaagctgaac tcttcatca  
4141 cgcaatacag caccggacag gtcagcgtgg aaattgaatg ggagctgcag aaggaaaaca  
4201 gcaagcgtg gaaccccgag atccagtaca cctccaacta ctacaaatct acaagtgtg  
4261 acttctgt taatacagaa ggcgtgtact ctgaaccccg cccattggc acccgttacc  
4321 tcaccgtaa tctgtaattg cctgtaatc aataaacgg ttgattcgtt tcagttgaac  
4381 tttgtctct gcg

FIG. 15 (Continued)

27/36  
FIG. 16

B19 Parvovirus, complete sequence, GenBank Accession No. NC\_000883 and Shade et al., (1986) *J. Virol.* 58:921

```

1  ccaaatcaga  tgccgcccgt  cgccgcccgt  aggggggact  tccggtacaa  gatggcggac
61  aattacgtca  tttcctgtga  cgtcatttcc  tgtgacgtca  cttccgggtg  gcgggacttc
121  cggaattagg  gttggctctg  ggccagcttg  cttggggttg  ccttgacact  aagacaagcg
181  gcgcgccgct  tgtcttagtg  gcacgtcaac  cccaagcgct  ggcccagagc  caaccctaat
241  tccggaagtc  ccgcccaccg  gaagtgcagt  cacaggaaat  gacgtcacag  gaaatgacgt
301  aattgtccgc  catcttgtag  cggaagtccc  gcctaccggc  ggcgaccggc  ggcattctgat
361  ttgggtgctt  cttttaaatt  ttagcgggct  tttttcccgc  cttatgcaaa  tgggcagcca
421  ttttaagtgt  ttcactataa  ttttattggg  cagttttgta  acggttaaaa  tgggcgggagc
481  gtaggcgggg  actacagtat  atatagcacg  gcactgccgc  agctctttct  ttctgggctg
541  cttttcctg  gactttcttg  ctgttttttg  tgagctaact  aacaggattt  tatactactt
601  gttaacatac  taacatggag  ctatttagag  ggggtgcttc  agtttcttct  aatgttctgg
661  actgtgctaa  cgataactgg  tgggtgctct  tactggattt  agacacttct  gactgggaac
721  cactaactca  tactaacaga  ctaatggcaa  tatacttaag  cagtgtggct  tctaagcttg
781  actttaccgg  ggggccacta  gcgggggtgt  tgtacttttt  tcaagtagaa  tgtaacaaat
841  ttgaagaagg  ctatcatatt  catgtgggta  ttggggggcc  aggggtaaac  cccagaacc
901  tcacagtgtg  tgtagagggg  ttatttaata  atgtacttta  tcaccttgta  actgaaaatg
961  taaagctaaa  atttttgcca  ggaatgacta  caaaaggcaa  atactttaga  gatggagagc
1021  agtttataga  aaactattta  atgaaaaaaaa  tacctttaaa  tgttgtagtg  tgtgttacta
1081  atattgatgg  atatatagat  acctgtattt  ctgctacttt  tagaagggga  gcttgccatg
1141  ccaagaaacc  ccgcattacc  acagccataa  atgacactag  tagtgatgct  ggggagtcta
1201  gcggcacagg  ggcagagggt  gtgccaatta  atgggaaggg  aactaaggct  agcataaagt
1261  tcaaacctat  ggtaaactgg  ttgtgtgaaa  acagagtgtt  tacagaggat  aagtggaaac
1321  tagttgactt  taaccagtac  actttactaa  gcagtagtca  cagtggaaat  tttcaaattc
1381  aaagtgcact  aaaactagca  atttataaag  caactaattt  agtgcctaca  agcacatttc
1441  tattgcatac  agactttgag  caggttatgt  gtattaaaga  caataaaaat  gttaaattgt
1501  tactttgtca  aaactatgac  cccctattag  tggggcagca  tgtgttaaag  tggattgata
1561  aaaaatgtgg  caagaaaaat  aactgtggtt  tttatgggcc  gccaaagtaca  ggaaaaacaa
1621  acttggcaat  ggccattgct  aaaagtgttc  cagtatatgg  catggttaac  tggaataatg
1681  aaaactttcc  atttaatgat  gtagcagggg  aaagcttggg  ggtctgggat  gaaggtatta
1741  ttaagtctac  aattgtagaa  gctgcaaaaag  ccattttagg  cgggcaacc  accagggtag
1801  atcaaaaaat  gcgtggaagt  gtagctgtgc  ctggagtacc  tgtggttata  accagcaatg
1861  gtgacattac  ttttgttgta  agcgggaaca  ctacaacaac  tgtacatgct  aaagccttaa
1921  aagagcgaat  ggtaaagtta  aactttactg  taagatgcag  ccctgacatg  gggttactaa
1981  cagaggctga  tgtacaacag  tggcttacat  ggtgtaatgc  acaaagctgg  gaccactatg
2041  aaaactgggc  aataaactac  acttttgatt  tccctggaat  taatgcagat  gccctccacc
2101  cagacctcca  aaccacccca  attgtcacag  acaccagtat  cagcagcagt  ggtggtgaaa
2161  gctctgaaga  actcagtgaa  agcagctttt  ttaacctcat  caccacaggc  gcctggaaca
2221  ctgaaacccc  gcgctctagt  acgccatcc  ccgggaccag  ttcaggagaa  tcatttgcg
2281  gaagctcagt  ttctccgaa  gttgtagctg  catcgtggga  agaagccttc  tacacacctt
2341  tggcagacca  gtttcgtgaa  ctgttagttg  gggttgatta  tgtgtgggac  ggtgtaaggg
2401  gtttacctgt  gtgtgtgtg  caacatatta  acaatagtgg  gggaggcttg  ggactttgtc
2461  cccattgcat  taatgtaggg  gcttgggtata  atggatggaa  atttcgagaa  ttaccaccag
2521  atttggtgcg  gtgtagctgc  catgtgggag  cttctaattc  cttttctgtg  ctaacctgca
2581  aaaaatgtgc  ttacctgtct  ggattgcaaa  gctttgtaga  ttatgagtaa  agaaagtggc
2641  aaatggtggg  aaagtgatga  taaatttgct  aaagctgtgt  atcagcaatt  tgtggaattt
2701  tatgaaaagg  ttactggaac  agacttagag  cttattcaaa  tattaaaaga  tcactataat
2761  atttcttag  ataatcccct  agaaaaccca  tcctctctgt  ttgacttagt  tgctcgtatt
2821  aaaaataacc  ttaaaaactc  tccagactta  tatagtcatc  attttcaaag  tcatggacag
2881  ttatctgacc  acccccatgc  cttatcatcc  agtagcagtc  atgcagaacc  tagaggagaa
2941  aatgcagtat  tatctagtga  agacttacac  aagcctgggc  aagttagcgt  acaactacc
3001  ggtactaact  atgttggggc  tggcaatgag  ctacaagctg  ggccccgcga  aagtgtgtt
3061  gacagtgtct  caaggattca  tgactttagg  tatagccaac  tggctaagtt  gggaaataat

```

3121 ccatatactc attggactgt agcagatgaa gagcttttaa aaaatataaa aaatgaaact  
 3181 gggtttcaag cacaagtagt aaaagactac tttactttta aagggtgcagc tgcccctgtg  
 3241 gcccattttc aaggaagttt gccggaagtt cccgcttaca acgcctcaga aaaatacca  
 3301 agcatgactt cagttaattc tgcaagaagcc agcactgggtg caggaggggg tggcagtaat  
 3361 cctgtcaaaa gcatgtggag tgagggggcc acttttagtg ccaactctgt aacttgtaca  
 3421 ttttccagac agtttttaat tccttatgac ccagagcacc attataaggt gttttctccc  
 3481 gcagcaagca gctgccacaa tgccagtggg aaggaggcaa aggtttgcac aattagttcc  
 3541 ataatgggat actcaacccc atggagatat ttagatttta atgctttaaa tttatttttt  
 3601 tcacctttag agtttcagca cttaattgaa aattatggaa gtatagctcc tgatgcttta  
 3661 actgtaacca tatcagaaat tgctgttaag gatgttacag acaaaaactgg agggggggta  
 3721 caggttactg acagcactac agggcgctta tccatgttag tagaccatga atacaagtac  
 3781 ccatatgtgt taggacaagg tcaggatact ttagccccag aacttcctat ttgggtatac  
 3841 tttccccctc aatatgctta cttaacagta ggagatgtta acacacaagg aatctctgga  
 3901 gacagcaaaa aattagcaag tgaagaatca gcattttatg ttttggaaaca cagttctttt  
 3961 cagcttttag gtacaggagg tacagcaact atgtcttata agtttctctc agtgcccca  
 4021 gaaaatttag agggctgcag tcaacacttt tatgaaatgt acaatccctt atacggatcc  
 4081 cgcttagggg ttcctgacac attaggagggt gacccaaaat ttagatcttt aacacatgaa  
 4141 gaccatgcaa ttcagcccca aaacttcatg ccagggccac tagtaaaact agtgtctaca  
 4201 aaggagggag acagctctaa tactggagct ggaaaagcct taacaggcct taacacaggc  
 4261 acctctcaaa acactagaat atccttacgc cctgggcccag tgtcacagcc ataccaccac  
 4321 tgggacacag ataaatatgt tccaggaata aatgccattt ctcatggcca gaccacttat  
 4381 ggtaacgctg aagacaaaga gtatcagcaa ggagtgggta gatttccaaa tgaaaaagaa  
 4441 cagctaaaac agttacaggg tttaaacatg cacacctatt tccccataa aggaaccag  
 4501 caatatacag atcaaattga gcgcccccta atgggtgggt ctgtatggaa cagaagagcc  
 4561 cttcactatg aaagccagct gtggagtaaa attccaaatt tagatgacag ttttaaaact  
 4621 cagtttgcag ccttaggagg atggggtttg catcagccac ctctcaaat attttataaa  
 4681 atattaccac aaagtgggcc aattggagggt attaaatcaa tgggaattac taccttagtt  
 4741 cagtatgccg tgggaattat gacagtaact atgacattta aattggggcc ccgtaaagct  
 4801 acgggacggg ggaatcctca acctggagta tatccccgc acgcagcagg tcatttacca  
 4861 tatgtactat atgaccacac agctacagat gcaaaacaac accacaggca tggatcga  
 4921 aagcctgaag aattgtggac agccaaaagc cgtgtgcacc cattgtaaac actccccacc  
 4981 gtgcctcag ccaggatgag taactaaacg cccaccagta ccaccagac tgtacctgcc  
 5041 cctcctgta cctataagac agcctaacac aaaagatata gacaatgtag aatttaagta  
 5101 cttaaccaga tatgaacaac atgttattag aatgttaaga ttgtgtaata tgtatcaaaa  
 5161 tttagaaaaa taaacatttg ttgtggttaa aaaattatgt tgttgcgctt taaaaatta  
 5221 aaagaagaca ccaaatcaga tgccgcccgt cgccgcccgt aggggggact tccggtacaa  
 5281 gatggcggac aattacgtca tttcctgtga cgtcatttcc tgtgacgtca cttccggtgg  
 5341 gcgggacttc cggaattagg gttggctctg ggccagcgc tggggttgac gtgccactaa  
 5401 gacaagcggc gcgcccgttg tcttagtgtc aaggcaacc caagcaagct ggcccagagc  
 5461 caaccctaat tccggaagtc ccgcccacc gaagtgacgt cacaggaaat gacgtcacag  
 5521 gaaatgacgt aattgtccgc catctgtac cgggaagtccc gcctaccggc ggcgaccggc  
 5581 ggcacatctgat ttgg

FIG. 16 (Continued)

29/36  
FIG. 17

Minute Virus from Mouse (MVM), complete sequence, GenBank Accession No. NC\_001510

```

1 attttttagaa ctgaccaacc atgttcacgt aagtgacgtg atgacgcgcg ctgcgcgcg
61 gccttcggac gtcacacgtc acttacgttt cacatggttg gtcagttcta aaaatgataa
121 gcggttcagg gagtttaaac caaggcgcga aaaggaagtg ggcgtggttt aaagtatata
181 agcaactact gaagtcagtt acttatcttt tctttcattc tgtgagtcga gacgcacaga
241 aagagagtaa ccaactaacc atggctggaa atgcttactc tgatgaagtt ttggggagcaa
301 ccaactgggtt aaaggaaaaa agtaaccagg aagtgttctc atttgttttt aaaaatgaaa
361 atgttcaact gaatggaaaa gatabcggat ggaatagtta caaaaaagag ctgcaggagg
421 acgagctgaa atctttacaa cgaggagcgg aaactacttg ggaccaaagc gaggacatgg
481 aatgggaaac cacagtggat gaaatgacca aaaagcaagt attcattttt gattctttgg
541 ttaaaaaatg tttatttgaa gtgcttaaca caaagaatat atttcctggg gatgttaatt
601 ggtttgtgca acatgaatgg ggaaaagacc aaggctggca ctgccatgta ctaattggag
661 gaaaggactt tagtcaagct caagggaaat ggtggagaag gcaactaaat gtttactgga
721 gcagatgggtt ggtaacagcc tgtaatgtgc aactaacacc agctgaaaga attaaactaa
781 gagaaatagc agaagacaat gagtgggtta ctctacttac ttataagcat aagcaaacca
841 aaaaagacta taccaagtgt gttctttttg gaaacatgat tgcttactat tttttaacta
901 aaaagaaaat aagcactagt ccaccaagag acggaggcta tttcttagc agtgactctg
961 gctggaaaaa taacttttta aaagaaggcg agcgccatct agtgagcaaa ctatacactg
1021 atgacatgcy gccagaaacg gttgaaacca cagtaaccac tgcgcaggaa actaagcgcg
1081 gcagaattca aactaaaaaa gaagtttcta ttaaaactac acttaaagag ctggtgcata
1141 aaagagtaac ctcaccagag gactggatga tgatgcagcc agacagttac attgaaatga
1201 tggctcaacc aggtggagaa aacctgctga aaaatagcct agagatttgt acactaactc
1261 tagccagaac caaaacagca tttgacttaa ttttagaaaa agctgaaacc agcaaaactaa
1321 ccaacttttc actgcctgac acaagaacct gcagaatttt tgcttttcat ggctggaact
1381 atgttaaagt ttgccatgct atttgctgtg ttttaaacag acaaggaggc aaaagaaata
1441 ctgttttatt tcatggacca gccagcacag gcaaatctat tattgcacaa gccatagcac
1501 aagcagttgg caatgttggg tgctataatg cagccaatgt aaactttcca tttaatgact
1561 gtaccaacaa gaacttgatt tgggtagaag aagctggtaa ctttgacag caagtaaacc
1621 agtttaaaag catttgctct ggtcaaacta ttgcattga tcaaaaagga aaaggcagca
1681 aacagattga accaacacca gtcatcatga ccacaaatga gaacattaca gtggtcagaa
1741 taggtgcga agaagacca gaacacactc aaccaatcag agacagaatg cttaacattc
1801 atctaacaca taccttgccct ggtgactttg gtttggttga caaaaatgaa tggcccatga
1861 tttgtgcttg gttggtaaag aatggttacc aatctacat ggcaagctac tgtgctaaat
1921 ggggcaaaag tcctgattgg tcagaaaact gggcggagcc aaagggtgcca actcctataa
1981 atttactagg ttcggcacgc tcaccattca cgacaccgaa aagtacgctc ctcagccaga
2041 actatgcact aactccactt gcacggatc tcgaggacct ggcttttagag ccttgagca
2101 caccaaatac tcctgttgcy ggcactgcag aaaccagaa cactggggaa gctggtcca
2161 aagcctgcca agatggtcaa ctgagcccaa cttggtcaga gatcgaggag gatttgagag
2221 cgtgcttcgy tgcggaaccg ttgaagaaag acttcagcga gccgctgaac ttggactaag
2281 gtacgatggc gcctccagct aaaagagcta aaagaggtaa gggtttaagg gatggttggg
2341 tgggtgggta ttaatgttta attacctgtt ttacaggcct gaaatcactt ggttttaggt
2401 tgggtgcctc ctggctacaa gtacctggga ccagggaaca gccttgacca aggagaacca
2461 accaatccat ctgacgcgcg tgccaaagag cagcagagg cctatgatca atacatcaa
2521 tctggaaaaa atccttaact gtacttctct gctgctgatc aacgctttat tgaccaaacc
2581 aaggcgcga aagactgggg aggcaagggt ggtcactact tttttagaac caagcgcgct
2641 ttgacaccta agcttgctac tgactctgaa cctggaactt ctggtgtaag cagagctggg
2701 aaacgcacta gaccacctgc ttacattttt attaaccaag ccagagctaa aaaaaactt
2761 acttctctct ctgcacagca aagcagtcaa accatgagtg atggcaccag ccaacctgac
2821 agcggaaacg ctgtccactc agctgcaaga gttgaacgag cagctgacgg ccctggaggc
2881 tctgggggtg ggggctctgg cgggggtggg gttggtggtt ctactgggtc ttatgataat
2941 caaacgcatt atagattcct gggtgacggc tgggtagaaa ttactgcact agcaactaga
3001 ctagtacatt taacatgcc taatcagaa aactattgca gaatcagagt tcacaataca
3061 acagacacat cagtcaaagg caacatggca aaagatgatg ctcatgagca aatttggaca
3121 ccatggagct tgggtgatgc taatgcttgg ggagtttggc tccagccaag tgactggcaa
3181 tacatttgca acacctgag ccagcttaac ttggtatcac ttgatcaaga aatattcaat
3241 gtagtgctga aaactgttac agagcaagac ttaggaggtc aagctataaa aatatacaac
3301 aatgacctta cagcttgcac gatggttgcg gttagactcaa acaacatttt gccatacaca

```

3361 cctgcagcaa actcaatgga aacacttggg ttctaccctt ggaaaccaac catagcatca  
 3421 ccatacaggt actatTTTTT cgttgacaga gatctttcag tgacctacga aaatcaagaa  
 3481 ggcacagttg aacataatgt gatgggaaca ccaaaaggaa tgaattctca atTTTTTacc  
 3541 attgagaaca cacacaatat cacattgctc agaacagggg acgaatttgc cacagggtact  
 3601 tactactttg acacaaattc agttaaactc acacacacgt ggcaaaccaa ccgtcaactt  
 3661 ggacagcctc cactgctgtc aacctttcct gaagctgaca ctgatgcagg tacacttact  
 3721 gctcaagga gcagacatgg aacaacacaa atgggggtta actgggtgag tgaagcaatc  
 3781 agaaccagac ctgctcaagt aggattttgt caaccacaca atgactttga agccagcaga  
 3841 gctggaccat ttgctgcccc aaaagtcca gcagatatta ctcaaggagt agacaaagaa  
 3901 gccaatggca gtgttagata cagttatggc aacagcatg gtgaaaattg ggcttcacat  
 3961 ggaccagcac cagagcgcta cacatgggat gaacaagct ttggttcagg tagagacacc  
 4021 aaagatgggt ttattcaatc agcaccacta gttgttccac caccactaaa tggcattcct  
 4081 acaaatgcaa accctattgg gactaaaaat gacattcatt tttcaaatgt ttttaacagc  
 4141 tatgggtccac taactgcatt ttcacacca agtctgtat acctcaagg acaaatatgg  
 4201 gacaaagaac tagatcttga acacaaacct agacttcaca taactgctcc atttgtttgt  
 4261 aaaaacaatg cacctggaca aatggttggt agattaggac caaacctaac tgaccaatat  
 4321 gatccaaacg gagccacact ttctagaatt gttacatagc gtacattttt ctggaaagga  
 4381 aaactaacca tgagagcaaa acttagagct aacaccactt ggaaccagt gtaccaagta  
 4441 agtgctgaag acaatggcaa ctcatatag agtgtaacta aatggttacc aactgctact  
 4501 ggaaacatgc agtctgtgcc gcttataaca agacctgttg ctagaaatac ttactaacta  
 4561 accatgcttt ttctttctgt acttcatata ttattaagac taataaagat acaacataga  
 4621 aatataatat tacgtataga ttttaagaaat agaataatat ggtacttagt aactgttaaa  
 4681 aataatagaa cctttggaat aacaagatag ttagtgggtt aatgttagat agaataagaa  
 4741 gatcatgtat aatgaataaa aggggtggaag ggtgggtggg aggttaatgt tagatagaat  
 4801 aagaagatca tgtataatga ataaaagggg ggaaggggtg ttggtaggta ttcccttaga  
 4861 cttgatgtta aggaccaaaa aaataataaa acttttttaa aactcaacca agactactgt  
 4921 ctattcagtg aaccaactga accattagta ttactatggt tttaggggtg gaggggtggg  
 4981 gatacatgtg ttcgctatga gcgaactggg actgggtggg tgctctgctc aaccaaccag  
 5041 accggcaaa cgggtctggg tgggtgagcg caaccaacca gtaccagttc gctcatagcg  
 5101 aacacatgta tctcccacc tcccacccta aaaacatagt aataactaat

FIG. 17 (Continued)

31/36  
FIG. 18

Goose Parvovirus, complete sequence, GenBank Accession No. NC\_001510

```

1  ctcatggag  ggttcgttcg  ttogaaccag  ccaatcaggg  gagggggaag  tgacgcaagt
61  tccggtcaca  tgcttccggg  gacgcacatc  cggtgacgta  gttccgggtca  cgtgcttcct
121  gtcacgtggt  tccgggtcacg  tgacttccgg  tcatgtgact  tccggtgacg  tgtttccggc
181  tgttaggttg  accacgcgca  tgccgcgcgg  tcagoccaat  agttaagccg  gaaacacgtc
241  accggaagtc  acatgaccgg  aagtcaagtg  accggaaaca  cgtgacagga  agcacgtgac
301  cggaactacg  tcaccggatg  tgcgtcaccg  gaagcatgtg  accggaactt  gcgtcacttc
361  cccctcccct  gattggctgg  ttogaacgaa  cgaaccctcc  aatgagactc  aaggacaaga
421  ggatattttg  cgcgccagga  agtgacgtgc  aatgccaccc  tatataagcc  aggaaacttc
481  cggtttagtt  cattcgttac  tctgctctca  gagagaacgg  acctcaggtc  ggagagatgg
541  cactttctag  gcctcttcag  atttcttctg  ataaattcta  tgaagttatt  attagattat
601  catcggatat  tgatcaagat  gtccccggtc  tgtctcttaa  cttttagtaa  tggctttcta
661  ccggagtttg  ggagcccacg  ggcactctgga  acatggagca  tegtgaatcta  ccgatgggta
721  ccttggcaga  gaagatcaag  aacattttca  tacaagatg  gaatcagttc  aaccaggacg
781  aaacggactt  cttctttcaa  ctggaagaag  gcagtgagta  cattcatctt  cattgctgta
841  ttgccagggg  caatgtacgg  tcttttgttc  tcgggagata  tatgtctcag  ataaaagact
901  ctatcataag  agatgtatat  gaagggaaac  aaatcaagat  ccccgattgg  tttgctatta
961  ctaaaaccaa  gaagggagga  cagaataaga  ccgtgactgc  agcatacata  ctgcattacc
1021  ttattcctaa  aaagcaacct  gaactgcaat  gggcctttac  caatatgctt  ttattcactg
1081  ctgctgctct  ttgtctgcaa  aagcggcaag  aattgctgga  tgcatttcaa  gaaagtgatt
1141  tggctgcccc  tttacctgat  cctcaagcat  caactgtggc  accgcttatt  tccaacagag
1201  cggcaaagaa  ctatagcaac  cttgttgatt  ggctcattga  aatggggata  acatctgaga
1261  agcaatggct  cactgagaac  cgagagagct  acagaagctt  tcaagcaact  tcttcaaata
1321  atagacaagt  gaaagctgca  ctggaaaatg  cccgtgctga  aatgttattg  acaaagactg
1381  caactgatta  cctgatagga  aaagaccctg  tcctggatat  aactaagaat  agggctatc
1441  aaattctgaa  aatgaataac  tacaaccctc  aatacatagg  aagtatcctg  tgcggctggg
1501  tgaagagaga  gttcaacaaa  agaaacgcca  tatggctcta  cggacctgcc  accaccggga
1561  agaccaacat  tgcagaagct  attgcccatg  ctgtaccctt  ctatggctgt  gttactgga
1621  ctaatgagaa  ctttcttttt  aatgattgtg  ttgataaaat  gctgatttgg  tgggaggagg
1681  gaaaaatgac  taataaggtt  gttgaactcg  caaaagcaat  tttgggaggg  tttgctgtcc
1741  gggtagacca  gaaatgtaaa  ggatctggtt  gtattgaacc  tactcctgta  attattacta
1801  gtaactga  tatgtgtatg  attgttgatg  gcaactctac  tacaatggaa  catagaatac
1861  cattagagga  gcgtatgttt  caaattgtcc  tatcacataa  attggagcct  tcttttgtaa
1921  aaatttctaa  aaaagaagtc  agagaatttt  tcaaatgggc  caatgacaat  ctagtctctg
1981  ttgtgtctga  gttcaaagtc  cgaactaatg  aacaaaccaa  cttgccagag  cccgttctctg
2041  aacgagcgaa  cgagccggag  gagcctccta  agatctgggc  tctcctact  agggaggagt
2101  tagaagagct  ttaagagcc  agcccagaat  tgttctcatc  agtcgctcca  attcctgtga
2161  ctctcagaa  ctcccctgag  cctaagagaa  gcaggaacaa  ttaccaggta  cgctgcgctt
2221  tgcatactta  tgacaattct  atggatgtat  ttgaatgtat  ggaatgtgag  aaagcaact
2281  ttctgaatt  tcaacctctg  ggagaaaatt  attgtgatga  acatgggtgg  tatgattgtg
2341  ctatagttaa  agagttgaaa  aatgaacttg  cagaaattga  gcatgtgttt  gagcttgatg
2401  atgctgaaaa  tgaacaataa  agatgactca  aagcagatat  gtctactttt  ttagattctt
2461  ttgaagagtg  gtatgagact  gcagccgctt  cgtggcgga  tctgaaagct  ggagcccctc
2521  agccaaaacc  aaaccagcag  tctcagtctg  tgtctccaga  cagagaacc  gaacgaaaag
2581  ataataatcg  gggctttgta  cttcctggct  ataagtatct  tgggcctggt  aacggcctgg
2641  ataaaggccc  acctgtcaat  aaggcggaca  gcgtcgcgct  tgaacacgac  aaggcctatg
2701  accagcagct  taaagcggga  gacaaccct  atataaaatt  caatcacgct  gaccaggact
2761  ttatagatag  cctccaagac  gaccagtcat  tcggaggtaa  tcttgaaag  gctgtatttc
2821  aggccaaaaa  acgtatctta  gagccatttg  gcctagtaga  agatcctgtc  aacacggcac
2881  ctgcaaaaaa  aaatacaggg  aagcttactg  accattacc  ggtagttaag  aagcctaac
2941  ttaccgagga  agtcagtgcg  ggaggtggta  gcagtgcctg  acaagacgga  ggagccaccg
3001  cggagggcac  cgaacctgtg  gcagcatctg  aatggcaga  gggaggaggc  ggagctatgg
3061  gcgactcttc  aggggtgccc  gatggagtgg  gtaatgcctc  gggaaattgg  cattgcgatt
3121  cccaatggat  gggaaacaca  gtcatcaca  agaccaccag  aacctgggtc  ctgccaagct
3181  acaacaacca  catctacaaa  gcaattacca  gcggaacctc  tcaagatgca  aatgtccagt
3241  atgcagata  cagtaccccc  tgggggtact  ttgatttcaa  ccgcttccac  tgccactctt
3301  cccctagaga  ctggcagaga  cttatcaaca  accttgggg  aatcagacc  aagtctctta
3361  aattcaagat  ctcaatgtc  caagtcaaac  aagtcacaac  gcaggatcag  acaaagacca

```

```

3421 ttgcaaacaa tctcacctca acaattcaag tctttacgga tgatgagcat caactcccgt
3481 atgtcctggg ctccggctacg gaaggcacca tgccgccggt cccgtcggat gtctatgccc
3541 tgccgcagta cgggtactgc acaatgcaca ccaaccagaa tggagcacgg ttcaatgacc
3601 gtagtgatt ctactgctta gactacttcc ctagtccgat gctaagaaca ggcaacaact
3661 ttgagttcac atttgacttt gaagaagtcc ctttccatag catgttcgct cattcacagg
3721 acttagacag gctgatgaac cccctagtgg atcaataacct ctggaatttc aatgaggtag
3781 acagcagcag aaatgctcaa tttaaaaagg ctgtgaaagg ggcttatggc accatgggcc
3841 gcaattggct gccaggacct aaattcctgg atcaaagagt tagggcctac acaggaggaa
3901 cagacaacta tgcaaaactgg aacatctgga gtaatgggaa caaggatgaat ttgaaagaca
3961 gacagtatct cctacaaccc ggacctgtgt cagctactta cacagaaggg gaggcttcca
4021 gccttccagc tcaaaatatt ttagggatag ctaaagatcc atacagatca ggcagcacta
4081 cagcaggaat aagtgacatt atggtcacgg aagaacaaga agtagcacct acaaatggag
4141 tagggtgtaa accatatggg aggactgtaa cgaatgaaca aaacactact acagctccta
4201 caagttcaga tctggatggt cttggagctt taccaggaat ggtttggcag aacagggata
4261 tatactctgca gggacctatt ggggcaaaaa taccgaagac tgatggtaaa ttccatcctt
4321 ctccgaatct cggaggattt ggctgcaca atccaccacc gcagggtgtc atcaagaata
4381 caccagtgcc tgcagaccct ccagtagaat acgtgcacca gaagtgggat tcctacataa
4441 cccagtactc tacgggccag tgtacagtag agatgggtgt ggagctgaga aaagagaatt
4501 caaagagatg gaaccagaa atccagttca ccagtaattt cagtaacaga acaagcataa
4561 tgtttgacc taatgaaact ggtggatag tagaagatag attgattgga accagatctc
4621 taactcaaaa tctgtaaatt ctgtgtaaaa attcaaataa agcacttccct ggcgcgcaaa
4681 atatectctt gtccttgagt ctattggag ggttcgttcg ttogaaccag ccaatcaggg
4741 gagggggaag tgacgcaagt tccggtcaca tgcttccggt gacgcacatc cggtgacgta
4801 gttccggtca cgtgcttccct gtcacgtgtt tccggtcacc tgacttccgg tcatgtgact
4861 tccggtgacg tgtttccggc ttaactattg ggtgaccgc gcgcatgccc gtggtcaacc
4921 taacagccgg aaacacgtca ccggaagtca catgaccgga agtcacgtga ccggaaacac
4981 gtgacaggaa gcacgtgacc ggaactacgt caccggatgt gcgtcaccgg aagcatgtga
5041 ccggaacttg cgtcacttcc cctcccctg attggctggt tcgaacgaac gaaccctcca
5101 atgaga

```

FIG. 18 (Continued)

|              |   |         |                |     |     |     |     |     |     |     |   |
|--------------|---|---------|----------------|-----|-----|-----|-----|-----|-----|-----|---|
|              | 1   | 10      | 20             | 30  | 40  | 50  | 60  | 70  | 80  | 92  | 1 |
| aav1 rep 40  | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav2 rep 40  | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav3a rep 40 | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav3b rep 40 | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav4 rep 40  | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav5 rep 40  | MALVNLVHEGITSEKOWIQENQESISFNSTGNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE        |         |                |     |     |     |     |     |     |     |   |
| aav6 rep 40  | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav7 rep 40  | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| aav8 rep 40  | MELVGLVDRGITSEKOWIQEDQASISFNAAASNSRSQIKAAALDNAGKIMSLTAPDYLVPAPPADIKNRIYRLELNGYDFAYAGSVFLGWAQKRE       |         |                |     |     |     |     |     |     |     |   |
| Consensus    | 00  | 110     | 120            | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 2 |
| aav1 rep 40  | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav2 rep 40  | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav3a rep 40 | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav3b rep 40 | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav4 rep 40  | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav5 rep 40  | NKRNTIWLFGPATGKTNIAEAAIAHVYPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav6 rep 40  | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav7 rep 40  | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| aav8 rep 40  | GKRNTIWLFGPATGKTNIAEAAIAHAVPFYGCVNNNTNENFPFNDVCDKMIWMEEGKMTAKVVEESAKALLGGSKVRVDQCKSSAQIDPTPVIIVTSNTNM |         |                |     |     |     |     |     |     |     |   |
| Consensus    | 00  | 210     | 220            | 230 | 240 | 250 | 260 | 270 | 280 | 290 | 3 |
| aav1 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav2 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav3a rep 40 | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav3b rep 40 | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav4 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav5 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav6 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav7 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| aav8 rep 40  | ICAVIDGNSTTFEHOQPLQDRMFKFELRRLEHDFGKVTQKQEVKDFRWAQDHVTEVAHEFYVR-----KGGAN--KRPAPDDADKSEP-----KRACPS   |         |                |     |     |     |     |     |     |     |   |
| Consensus    | 00  | 310     | 320            | 330 | 340 | 350 | 360 | 370 | 380 | 390 | 4 |
| aav1 rep 40  | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 31   |         |                |     |     |     |     |     |     |     |   |
| aav2 rep 40  | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 32   |         |                |     |     |     |     |     |     |     |   |
| aav3a rep 40 | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 33   |         |                |     |     |     |     |     |     |     |   |
| aav3b rep 40 | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 34   |         |                |     |     |     |     |     |     |     |   |
| aav4 rep 40  | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 35   |         |                |     |     |     |     |     |     |     |   |
| aav5 rep 40  | PETPRSSDYIVDPAPIRPLNNNSLYGPSW-----SEQ 10 NO: 36   |         |                |     |     |     |     |     |     |     |   |
| aav6 rep 40  | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 37   |         |                |     |     |     |     |     |     |     |   |
| aav7 rep 40  | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 38   |         |                |     |     |     |     |     |     |     |   |
| aav8 rep 40  | VADPSTSDAEGAPVDFADLARGOPL-----SEQ 10 NO: 39   |         |                |     |     |     |     |     |     |     |   |
| Consensus    | VA  | PSTSDAE | APVDFADLARGOPL |     |     |     |     |     |     |     |   |

FIG 19

SEQ 10 NO: 31  
 SEQ 10 NO: 32  
 SEQ 10 NO: 33  
 SEQ 10 NO: 34  
 SEQ 10 NO: 35  
 SEQ 10 NO: 36  
 SEQ 10 NO: 37  
 SEQ 10 NO: 38  
 SEQ 10 NO: 39







1 10 20 30 40 50 60 70 80 92 100 110 120 130 140 1

aav1 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav2 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav3a rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav3b rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav4 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav5 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav6 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav7 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

aav8 rep78 MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

Consensus MEGFEIVLIVKVPESDLDHLEFGISDSFVSNVAEKEMELFFSRMDLNLTEQAPITVAEKLODFLQVRKVSNAPEALFVQEKESYFHLLILVETGVKSVWLGRELSQIAKLVQIVYRG-IEPTLPNFAVITKRNAGAGG-NKVV

1 160 170 180 190 200 210 220 230 240 256 280 290 3

aav1 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav2 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav3a rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav3b rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav4 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav5 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav6 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav7 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

aav8 rep78 DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

Consensus DECTIENYLLPRTQPELQWAWTMEEYI SACLINAEKRVVAQHLHSHVSOEQEAKNENLNPNSDA PVRKTSARVWELVGHVDRITSEKVAIQEDDASYSFNAA SNRSQIKAA LDNAGKIMALKSA PVLVWGPAPADLQNRH

00 310 320 330 340 350 360 370 380 390 400 413 420 430 440 4

aav1 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav2 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav3a rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav3b rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav4 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav5 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav6 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav7 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

aav8 rep78 YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

Consensus YRILELNGYDPAVAGSVFLGWAQKKEKRNITLFGPATGKTHIAEALAHAVFYCYVWNTNENFPHDCVKKVIMWEGRHAKVESA KALLGSKYRVVQKCSAQIDPTEVIVTNTMCAVLDGNSITTEHQOPLQDRMEKF

50 460 470 480 494 500 510 520 530 540 550 560 573 580 590 6

aav1 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav2 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav3a rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav3b rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav4 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav5 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav6 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav7 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

aav8 rep78 ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

Consensus ELTRRLHDEGKVTQKGVKDFEFAQDHVTEVAHEFVYR-----KGGAK---KRPADPADKSEP-----KRA-----CFSVADPSTSDAEAPVADRYQNKSRHAGIMLFPCKTERRMNFNICTHGTDCSECFPGVBSQ

00 610 620 630 646

aav1 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:60

aav2 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:61

aav3a rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:62

aav3b rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:63

aav4 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:64

aav5 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:65

aav6 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:66

aav7 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:67

aav8 rep78 --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:68

Consensus --PVVRKTYRKLCAIHLHGRAPETACSACDLVNVLDLDCVSEQ-----SQR ID NO:69

91622

SQR ID NO:60  
 SQR ID NO:61  
 SQR ID NO:62  
 SQR ID NO:63  
 SQR ID NO:64  
 SQR ID NO:65  
 SQR ID NO:66  
 SQR ID NO:67  
 SQR ID NO:68  
 SQR ID NO:69