

IN VITRO PARVOVIRUS CAPSID MANUFACTURING

Field of the Invention

The present disclosure relates to *in vitro* methods for assembling parvovirus capsids, *in vitro* methods for filling parvovirus capsids and *in vitro* methods for manufacturing filled parvovirus capsids. The present disclosure further relates to assembled parvovirus capsids, filled parvovirus capsids and compositions comprising such.

Background of the Invention

Research and clinical applications of recombinant parvoviruses, such as recombinant adeno-associated virus (rAAV), has significantly increased in recent years alongside regulatory approvals of rAAV gene therapy products. To date, most rAAV vectors, as well as AAV empty capsids, are produced in eukaryotic cells. However, during the manufacturing of AAV vectors, transgene packaging is usually imperfect. The capsids that do not incorporate a transgene payload are referred to as empty capsids, which highlights the necessity of improving the proportion of capsids filled with DNA for the process of scalable vector production.

Furthermore, the three distinct viral capsid proteins (e.g., viral protein 1 (VP1), VP2, and VP3) that compose an AAV capsid each play a unique role in cell-entry and transduction, though AAV capsid assembly is stochastic, leading to a highly heterogeneous populations of capsids of variable viral capsid protein composition. For example, it has been demonstrated that even the single-most abundant viral capsid protein stoichiometry represents only a small percentage of a given manufactured AAV capsid population. This heterogeneity highlights the need for improved methods of parvovirus capsid production strategies that can enable controlled, reproducible viral capsid protein stoichiometries at large scale and low cost.

Summary of the Invention

The present disclosure relates to *in vitro* methods for assembling parvovirus capsids, *in vitro* methods for filling parvovirus capsids and *in vitro* methods for manufacturing filled parvovirus capsids. The present disclosure further relates to assembled parvovirus capsids, filled parvovirus capsids and compositions comprising such.

In a first aspect, the present disclosure relates to a method of *in vitro* assembling parvovirus capsids, comprising the steps of:

- a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;

- b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
- c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids,
thereby assembling parvovirus capsids and providing assembled parvovirus capsids.

In another aspect, the present disclosure relates to a method of *in vitro* filling parvovirus capsids, comprising the steps of:

- 10 a. providing:
- i. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
 - ii. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*; and
 - 15 iii. a Rep protein *in vitro*;
- b. combining the one or more populations of assembled parvovirus capsids, the nucleic acid molecule, and the Rep protein *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein,
- thereby filling parvovirus capsids and providing filled parvovirus capsids.

In another aspect, the present disclosure relates to a method of *in vitro* filling parvovirus capsids, comprising the steps of:

- 25 a. providing:
- i. a population of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
 - ii. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*;
 - iii. a Rep proteins *in vitro*; and
 - iv. a reaction mixture *in vitro*;
- 30 b. combining the population of assembled parvovirus capsids, the nucleic acid molecule, the Rep protein and the reaction mixture *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids and providing filled parvovirus capsids.

35 In another aspect, the present disclosure relates to a method of *in vitro* manufacturing filled parvovirus capsids, comprising the steps of:

- 5 a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*,
wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an
assembly inhibitor;
- b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
- 5 c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b)
in vitro, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the
assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled
parvovirus capsids,
thereby providing one or more populations of assembled parvovirus capsids comprising
10 parvovirus viral capsid proteins *in vitro*
- d. providing:
- i. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain
in vitro; and
- 15 ii. a Rep protein *in vitro*;
- e. combining the population of assembled parvovirus capsids obtained in c., the nucleic acid
molecule and the Rep protein *in vitro* under filling conditions which allow filling of the
assembled capsids with the nucleic acid molecule by the Rep protein,
thereby filling parvovirus capsids,
thereby manufacturing filled parvovirus capsids.
- 20

In another aspect, the present disclosure relates to a method of *in vitro* manufacturing filled parvovirus capsids, comprising the steps of:

- 25 a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*,
wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an
assembly inhibitor;
- b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
- c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b)
in vitro, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the
assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled
30 parvovirus capsids,
thereby providing one or more populations of assembled parvovirus capsids comprising
parvovirus viral capsid proteins *in vitro*
- d. providing:
- i. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain
35 *in vitro*;
- ii. a Rep protein *in vitro*; and

- iii. a reaction mixture *in vitro*
- e. combining the population of assembled parvovirus capsids obtained in c., the nucleic acid molecule, the Rep protein and the reaction mixture *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein,
5 thereby filling parvovirus capsids,
thereby manufacturing filled parvovirus capsids.

In another aspect, the present disclosure relates to a method of *in vitro* controlling the stoichiometry of parvovirus viral capsid proteins in assembled parvovirus capsids, comprising the step of:

- 10 a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor, wherein the one or more populations of inhibited parvovirus viral capsid proteins are provided at a defined stoichiometry;
- b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
- 15 c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids,
thereby assembling parvovirus capsids and providing assembled parvovirus capsids with a
20 defined stoichiometry of parvovirus viral capsid proteins.

In another aspect, the present disclosure relates to an AAV Rep fusion protein comprising:

- a. An AAV Rep domain or a AAV Rep protein, and
- 25 b. A cleavable amino acid sequence, such as a SUMO tag.

In another aspect, the present disclosure relates to a vector encoding the AAV Rep fusion protein according to the present disclosure.

In another aspect, the present disclosure relates a method of producing the AAV Rep fusion protein according to the present disclosure, comprising the steps of:

- 30 a. Transforming a prokaryotic microbial system, such as *E. coli*, with the vector encoding the AAV Rep fusion protein according to the present disclosure; and
- b. Incubating the prokaryotic microbial system, such as *E. coli*,
thereby producing the AAV Rep fusion protein.

35

In another aspect, the present disclosure relates a method of producing the AAV Rep fusion protein according to the present disclosure, comprising the steps of:

- a. Transforming an eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*, with the vector encoding the AAV Rep fusion protein according to the present disclosure,
- 5 b. Incubating the eukaryotic microbial system, such as *P. pastoris*, or *S. cerevisiae*, thereby producing the AAV Rep fusion protein.

In another aspect, the present disclosure relates an AAV VP fusion protein comprising:

- a. An AAV VP domain or an AAV VP protein, and
- 10 b. A cleavable amino acid sequence, such as a SUMO tag.

In another aspect, the present disclosure relates a vector encoding the AAV VP fusion protein according to the present disclosure.

15 In another aspect, the present disclosure relates to a method of producing a method of producing the AAV VP fusion protein according to the present disclosure, comprising the steps of:

- a. Transforming a prokaryotic microbial system, such as *E. coli*, with the vector encoding the AAV VP fusion protein according to the present disclosure; and
- 20 b. Incubating the prokaryotic microbial system, such as *E. coli*, thereby producing the AAV VP fusion protein.

In another aspect, the present disclosure relates to a method of producing the AAV fusion protein according to the present disclosure, comprising the steps of:

- a. Transforming a eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*, with the vector encoding the AAV VP fusion protein according to the present disclosure
- 25 b. Incubating the eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*, thereby producing the AAV fusion protein.

30 In another aspect, the present disclosure relates to a fusion protein consisting of or comprising an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from SEQ ID NOs: 35-43, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity an amino acid sequence selected from SEQ ID NOs: 35-43.

35

In another aspect, the present disclosure relates to a fusion protein consisting of or comprising an amino acid sequence selected from SEQ ID NOs: 35-43.

Brief Description of the Drawings

5 **Figure 1**

Figure 1 shows a novel method of *in vitro* AAV capsid filling: (A) Expression of empty AAV2 capsids and rep proteins by suspension HEK293 cells in serum-free and antibiotic-free medium and using double plasmid transfection method. (B) Generation of plasmid containing backbone, ITR regions, CMV enhancer, CMV promoter, eGFP reporter gene, and poly-A signal. (C) Capsid quantification through
10 ELISA, (D) In vitro capsid filling; (E) Genome copy quantification using qPCR.

Figure 2

Figure 2 shows ELISA standard curve: Capsid concentration was quantified by generating a sigmoidal curve through four Parameter Logistic (4PL) fitting. A 100-fold sample dilution was found to be in the
15 linear range of this curve. As such, the original sample concentration was determined to be 1.86×10^{10} empty capsids/mL.

Figure 3

Figure 3 shows final genome copy concentrations, measured by qPCR, of processed samples and two
20 pure plasmid controls (one treated with DNase, the other without DNase).

Figure 4

Figure 4 shows effect of capsid addition on plasmid digestion by DNase. The x-axis shows the concentration of plasmid added to each condition/control (prior to capsid filling and/or DNase treatment).
25 This was determined by using untreated pGFP as a reference concentration and extrapolating sample concentrations based on dilution factor. The y-axis shows the final qPCR readout after DNase treatment.

Figure 5

Figure 5 shows plasmid packaging and capsid filling efficiencies. Plasmid packaging efficiency refers to the
30 relative number of genomes that have been encapsulated; capsid filling efficiency refers to the relative number of filled capsids compared to the total number of capsids.

Figure 6

Figure 6 shows qPCR results from the different tested capsid filling condition.
35

Figure 7

Figure 7 shows a novel method of *in vitro* AAV capsid filling: (A) Expression of empty AAV2 capsids and rep proteins by suspension HEK293 cells in serum-free and antibiotic-free medium and using double plasmid transfection method. (B) Generation of plasmid containing backbone, ITR regions, CMV enhancer, CMV promoter, eGFP reporter gene, and poly-A signal. (C) Capsid quantification through ELISA. (D) *In vitro* capsid filling. (E) Affinity chromatographic purification using AAVX. (F) Genome copy quantification using qPCR. (G) Infectious unit quantification using eGFP reporter fluorescent assay.

Figure 8

Figure 8 shows gating strategy for Hek293 cells transduced with AAV2 CMV-GFP vectors.

Figure 9

Figure 9 shows vector genome titer by qPCR.

Figure 10

Figure 10 A) shows capsid titer by AAV2 capsid ELISA. Figure 10 B) shows full capsid ratio as calculated by dividing vector genome titer (VG/mL) with capsids titer (Cap/mL).

Figure 11

Figure 11 shows a fluorescence microscopy picture. Cells transduced with Control and *in vitro* filled AAV2-GFP vectors and subsequently analyzed for GFP expression by fluorescence microscope.

Figure 12

Figure 12 shows infectivity. Cells transduced with Control and *in vitro* filled AAV2-GFP vectors and subsequently analyzed for GFP expression by flow cytometry.

Figure 13

Figure 13 shows infectivity as a function of the number of capsids (full and empty) added to the cells.

Figure 14

Figure 14 shows genome copy concentrations, measured by qPCR, for *in vitro* capsid filling for AAV5.

Figure 15

Figure 15 shows SDS PAGE of clarified *E. coli* lysate containing his-SUMO-Rep-EPEA fusion proteins.

Number indicates proposed bands for his-SUMO Rep proteins based on expected MW. 1. his-SUMO Rep40, MW ~58 kDa; 2. his-SUMO Rep52, MW ~70 kDa; 3. his-SUMO Rep68, MW ~84 kDa; 4. His-

SUMO Rep78, MW ~94 kDa. Expression of IPTG induced cultures, shown as '+IPTG' lanes, were compared to uninduced expression.

Figure 16

- 5 Figure 16 shows SDS PAGE of purified Rep proteins with his-SUMO fusion tag. Black arrows indicate Rep protein (Rep40, MW 40 kDa; Rep52, MW 52 kDa; Rep68, MW 68 kDa; Rep78, MW 78 kDa) + his-SUMO (MW ~18 kDa).

Figure 17

- 10 Figure 17 shows SDS PAGE showing successful cleavage of SUMO after incubation with SUMO protease for Rep40 and Rep52. Black arrows indicate Rep protein (Rep40, MW 40 kDa; Rep52, MW 52 kDa; Rep68, MW 68 kDa; Rep78, MW 78 kDa) + his-SUMO (MW ~18 kDa). White arrows indicate Rep40 and Rep 52 (Rep40, MW 40 kDa; Rep52, MW 52 kDa) after successful his-SUMO cleavage (SUMO band unmarked at ~18 kDa).

15

Figure 18

- Figure 18 shows SDS PAGE showing successful cleavage of his-SUMO after incubation with SUMO protease at 5°C for Rep68 and Rep78. Black arrows indicate Rep68 (Rep68, MW 68 kDa) + his-SUMO, and Rep78 (Rep78, MW 78 kDa) + his-SUMO illustrating unsuccessful cleavage, molecular weight references for uncleaved proteins shown in respective '-SUMO' lane. White arrows indicate examples of Rep68 (Rep68, MW 68 kDa, example lane 3), and Rep78 (Rep78, MW 78 kDa, example lane 10) after successful cleavage of his-SUMO (MW ~18 kDa). Incubation at room temperature (lanes RT) showed incomplete cleavage of his-SUMO.

Figure 19

- 25 Figure 19 shows genome copy concentrations, measured by qPCR, for chemically defined *in vitro* capsid filling for AAV5.

Figure 20

- 30 Figure 20 shows SDS PAGE (left panel) and western blot (right panel) of purified inclusion bodies solubilized with urea. Left panel, lane 1: PageRuler Plus, lane 2: AAV5 VP3, lane 3: AAV5 VP2, lane 4: AAV5 VP1. Right panel, lane 1: AAV5 VP3, lane 2: AAV5 VP2, lane 3: AAV5 VP1, lane 4. The proteins of interest are indicated with numbered arrows: 1, AAV5 VP3; 2, AAV5 VP2; 3, AAV5 VP1.

Figure 21

- 35 Figure 21 shows SDS PAGE of AAV5 VP3 after ion exchange purification. Lane 1: PageRuler Plus, lane

2: AAV5 VP3. The protein of interest is indicated with a numbered arrow (1).

Figure 22

Figure 22 shows ELISA results of AAV5 VP3 refolding, i.e., capsid formation, using buffer 1 to 17.

5

Figure 23

Figure 23 shows ELISA results of AAV5 VP3 refolding using buffer 11 (section 7). ON: overnight (16 hours), RT: room temperature (22°C).

10 **Figure 24**

Figure 24 shows SDS PAGE of AAV5 VP1, AAV5 VP2, and AAV5 VP3 after ion exchange and gel filtration. Lane 1: PageRuler Plus, lane 2: AAV5 VP1, lane 3: AAV5 VP2, lane 4: AAV5 VP3. The proteins of interest are indicated with numbered arrows: 1, AAV5 VP1; 2, AAV5 VP2; 3, AAV5 VP3.

15 **Figure 25**

Figure 25 shows Western blot of AAV5 capsid particles. Lane 1: PageRuler Plus, lane 2: 15 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 1:1:20; lane 2, 10 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 1:1:20; lane 4, 5 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 1:1:20; lane 5: 15 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 1:1:1; lane 6, 10 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 1:1:1; lane 7, 5 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 1:1:1; lane 8: 15 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 20:20:1; lane 9, 10 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 20:20:1; lane 10, 5 µL AAV5 VP1, AAV5 VP2, AAV5 VP3 ratio 20:20:1. The proteins of interest are indicated with numbered arrows: 1, AAV5 VP1; 2, AAV5 VP2; 3, AAV5 VP3.

25 **Figure 26**

Figure 26 shows Western blot of purified AAV2-VP3-SUMO produced in *P. pastoris* before (lane 1), after 1 hour (lane 2), and after 16 hours (lane 3) at room temperature (22°C) in the presence of SUMO and thrombin proteases. The left arrow indicates the protein of interest before cleavage, and the right arrow indicates the protease-cleaved protein of interest.

30

Figure 27

Figure 27 shows ELISA of AAV2 capsids. Samples were measured diluted 10x and 100x in ELISA assay buffer.

35 **Figure 28**

GFP primer-based qPCR (A) and fill rate result (B).

Figure 29

SDS PAGE of purified MBP-Rep78. Black arrows indicate Rep78 protein (MW 78 kDa) + MBP-linker (MW 42.7 kDa).

5

Figure 30

Western blot of purified MBP-Rep78. Black arrows indicate Rep78 protein (MW 78 kDa) + MBP-linker (MW 42.7 kDa). The primary antibody was an anti-AAV2 Replicase mouse mAb, while the secondary was a goat anti-mouse HRP conjugate.

10

Figure 31

qPCR (left panel) and fill rate result (right panel).

Figure 32

15 Gaussian fits through the main populations of the chemically defined *in vitro* filled AAVs ("Fuse") and HEK-filled AAVs representing the empty, partially filled, and fully filled adeno-associated viruses.

Definitions

As used herein, the terms "a" and "an" refer to at least one, i.e., one or more. Accordingly, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

20

As used herein, the term "about" refers to a value that is within 5% above and below the value being described. For example, "pH of about 1" as used herein includes quantities that are within 5% above and below pH 1. Additionally, when used in the context of a list of numerical quantities, it is to be understood that the term "about," when preceding a list of numerical quantities, applies to each individual quantity recited in the list. For example, "about pH 1, pH 2, or pH 3" is to be construed as equivalent to individually reciting "about pH 1," "about pH 2," and "about pH 3."

25

As used herein, the term "adeno-associated virus" (AAV), includes but is 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, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, or any other AAV now known and later discovered. See, e.g., Fields et al. Virology, 4th ed. Lippincott-Raven Publishers, Philadelphia, 1996. Additional AAV serotypes and clades have been identified recently. (See, e.g., Gao et al. *J. Virol.* 78:6381 (2004); Mori et al. *Virology* 33:375 (2004). The genomic sequences of various serotypes of AAV, as well as the sequences of the native ITRs, AAV Rep proteins, and capsid subunits are known in the art.

30

35

Such sequences may be found in the literature and 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, NC—006152, NC—006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, 5 AY028226, AY028223, AY631966, AX753250, EU285562, NC—001358, NC—001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Bantel-Schaal et al. *J. Virol.* 73:939 (1999); Chiorini et al. *J. Virol.* 71:6823 (1997); Chiorini et al. *J. Virol.* 73:1309 (1999); Gao et al. *Proc. Nat. Acad. Sci. USA* 99:11854 (2002); Mori et al. *Virol.* 33:375 (2004); Muramatsu et al. *Virol.* 221:208 (1996); 10 Ruffing et al. *J. Gen. Virol.* 75:3385 (1994); Rutledge et al. *J. Virol.* 72:309 (1998); Schmidt et al. *J. Virol.* 82:8911 (2008); Shade et al. *J. Virol.* 58:921 (1986); Srivastava et al. *J. Virol.* 45:555 (1983); Xiao et al. *J. Virol.* 73:3994 (1999); WO 00/28061, WO 99/61601, WO 98/11244; and US 6,156,303; the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences.

15 As used herein, the term “cell-free” refers to a system, a method or a process, such as the assembly of capsids and/or the filling of capsids, in cell free conditions.

As used herein, the term “*in vitro*” refers to a system, a method or a process, such as the assembly of capsids and/or the filling of capsids, taking place outside a living organism, such as in a test tube or a 20 culture dish.

The term “cell-free” and the term “*in vitro*” might be used interchangeable within the disclosure.

As used herein, the terms “fill” and “filling,” and the like refer to a series of enzymatically-driven events 25 involving one or more Rep proteins that result in the filling of a capsid with one or more nucleic acid molecules. Thus, when a suitable population of viral capsid proteins (e.g., VP1, VP2, and/or VP3), conditions, and one or more Rep proteins are combined with a nucleic acid molecule, the respective viral proteins are assembled into a capsid and the capsid can be filled with one or more nucleic acid molecules to form an assembled capsid particle packaged with one or more nucleic acid molecules. The filling 30 process occurs after capsid assembly is complete.

An “ITR” is a palindromic nucleic acid, e.g., an inverted terminal repeat, that is about 120 nucleotides to about 250 nucleotides in length and capable of forming a hairpin. The term “ITR” includes the site of the viral genome replication that can be recognized and bound by a parvoviral protein (e.g., an AAV Rep 35 protein e.g., Rep78/68). An ITR may be from any parvovirus (e.g., an AAV). An ITR includes a replication protein binding element (RBE) and a terminal resolution sequence (TRS). The term “ITR”

does not require a wild-type parvoviral ITR (e.g., a wild-type nucleic acid sequence may be altered by insertion, deletion, truncation, and missense mutations), as long as the ITR functions to mediate nucleic acid molecule packaging, replication, and/or integration, and the like. The “5’ ITR” is intended to mean the parvoviral ITR located at the 5’ boundary of the nucleic acid molecule; and the term “3’ ITR” is intended to mean the parvoviral ITR located at the 3’ boundary of the nucleic acid molecule.

“Nucleic acid molecule” and “polynucleotide,” as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA, which may include naturally occurring and/or synthetically-derived nucleic acids.

As used herein, the term “operably linked” refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For example, a promoter sequence is operably linked to a coding sequence if the promoter sequence promotes transcription of the coding sequence. As another example, an intron sequence is operably linked to a transcriptional unit if the intron contains splice donor and splice acceptor sites allowing for proper splicing of the transcription unit. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two coding regions, contiguous and in reading frame. However, since enhancers may function when separated from the promoter by several kilobases and intronic sequences may be of variable length, some nucleotide sequences may be operably linked but not contiguous.

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, snake parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. See, e.g., Fields et al. Virology, 4th ed. Lippincott-Raven Publishers, Philadelphia, 1996. The genus Dependovirus contains the 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, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, AAV type rh10, AAV type rh74, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, and ovine AAV.

“Percent (%) sequence identity” with respect to a reference polynucleotide and polypeptide sequence is defined as the percentage of nucleic acids and amino acids in a coordinate sequence that are identical to the nucleic acids and amino acids in the reference polynucleotide and polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence

identity. Alignment for purposes of determining percent nucleic acid and amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, and Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms
5 needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid and amino acid sequence, A, to, with, and against a given nucleic acid and amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid and amino acid sequence, A that has a certain percent
10 sequence identity to, with, and against a given nucleic acid and amino acid sequence, B) is calculated as follows:

$$100 \text{ multiplied by (the fraction } X/Y)$$

where X is the number of nucleotides and amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number
15 of nucleic acids in B. It will be appreciated that where the length of nucleic acid and amino acid sequence A is not equal to the length of nucleic acid and amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

As used herein, the term "pharmaceutical composition" is intended to also disclose that the capsid
20 included within a pharmaceutical composition can be used for the treatment of the human or animal body by therapy. It is thus meant to be equivalent to the "capsid for use in therapy".

As used herein, the term "plasmid" refers to a DNA molecule into which additional DNA segments may be ligated, such as an extrachromosomal circular double-stranded DNA molecule, doggybone DNA
25 (dbDNA), a single-stranded loop, or a capsomere. A plasmid is a type of vector, a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Certain plasmids are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial plasmids having a bacterial origin of replication and episomal mammalian plasmids). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell,
30 and thereby are replicated along with the host genome. Certain plasmids are capable of directing the expression of genes to which they are operably linked (e.g., a viral capsid protein and/or a Rep protein).

As used herein, the term "polynucleotide" refers to a molecule including one or more nucleic acid subunits, and nucleotides, and can be used interchangeably with "nucleic acid" and "oligonucleotide". A
35 polynucleotide can include one or more nucleotides selected from adenosine (A), cytosine (C), guanine

(G), thymine (T) and uracil (U), and variants thereof. A nucleotide can include a nucleoside and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and more phosphate (PO₃) groups. A nucleotide can include a nucleobase, a five- carbon sugar (either ribose and deoxyribose), and one or more phosphate groups. Ribonucleotides are nucleotides in which the sugar is ribose. Polyribonucleotides and ribonucleic acids, and RNA, can refer to macromolecules that include multiple ribonucleotides that are polymerized via phosphodiester bonds. Deoxyribonucleotides are nucleotides in which the sugar is deoxyribose.

As used herein, a "population" refers to a group of at least two (e.g., at least 2, 3, 4, 5, 10, 15, 20, 30, 40, or more) proteins of the specified kind (e.g., at least 2 viral capsid proteins or at least 2 VP1).

As used herein, the term "reaction mixture" refers to a mixture of required components for carrying out a given process, such as the encapsidation or filling of a capsid with a polynucleotide molecule by a capsid in cell free conditions. Such a reaction mixture may entail components such as adenosine triphosphate, a pH buffer, magnesium chloride, radiation, light, and ultraviolet or condition factors such as pH, ionic strength/conductivity, temperature, time, hydrophobicity, space, pressure, and conductivity. Such a reaction mixture may additionally require one or more Rep proteins (e.g., an AAV Rep protein) to enable encapsidation or filling of a capsid with a nucleic acid molecule.

"Rep proteins" in accordance with the present invention are proteins which fill capsids with a polynucleotide molecule (e.g., viral deoxyribonucleic acid (DNA)) into a capsid. In addition, a Rep protein may enable nucleic acid molecule replication, transcriptional regulation of a nucleic acid molecule, and/or site-specific integration (e.g., chromosomal integration) of a nucleic acid molecule. The Rep protein may be from any parvovirus. As one of skill in the art will appreciate, in some parvovirus family virus species, a Rep protein is referred to as a "non-structural (NS) protein". As used herein, a Rep protein may refer to a NS protein. As used herein, a Rep protein may refer to an AAV Rep protein, which have been found in all AAV serotypes examined to date, and a synthetic Rep protein. For example, in some embodiments, a AAV Rep protein (e.g., AAV Rep40, AAV Rep52, AAV Rep68, and AAV Rep78) is a capsid protein having an amino acid sequence derived from a particular AAV serotype, for example 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 6.2, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, and ovine AAV. Alternatively, for example, as used herein, a Rep protein may include a synthetic Rep protein. Any suitable Rep protein may be used.

As used herein, the term "subject" refers to any organism to which a composition in accordance with the invention may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic

purposes. Typical subjects include any animal (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans). A subject may seek and be in need of treatment, require treatment, be receiving treatment, be receiving treatment in the future, and be a human and animal who is under care by a trained professional for a particular disease and condition. In preferred embodiments, the subject is a human.

As used herein, the term "synthetic" may refer to a modified ribonucleotide, a synthetic nucleic acid sequence, or a synthetic protein. A synthetic nucleic acid herein may include an engineered nucleic acid, while a synthetic protein may include an engineered protein (e.g., non-native) and/or a protein including synthetic nucleic or amino acids, such as an engineered protein, nucleic acid, or amino acid not occurring in nature. For example, as used herein, a synthetic protein may include a synthetic viral protein (VP; e.g., VP1, VP2, and VP3) or a synthetic Rep protein.

As used herein, the term "transgene" refers to a recombinant nucleic acid (e.g., DNA) encoding a gene product, such as a peptide, protein, or RNA (e.g., a protein-encoding mRNA or an inhibitory RNA, such as a miRNA, shRNA, or the like). In addition to the coding region for the gene product, the transgene may include, or be operably linked to, one or more elements to facilitate or enhance expression, such as a promoter, enhancer(s), destabilizing domain(s), response element(s), reporter element(s), insulator element(s), polyadenylation signal(s), and/or other functional elements. Embodiments of the disclosure may utilize any known suitable promoter, enhancer(s), destabilizing domain(s), response element(s), reporter element(s), insulator element(s), polyadenylation signal(s), and/or other functional elements.

As used herein, the term "vector" includes a nucleic acid vector, e.g., a DNA vector, such as a plasmid, an RNA vector, virus, and other suitable replicon (e.g., viral vector). A variety of vectors have been developed for the delivery of polynucleotides encoding exogenous proteins into a prokaryotic and eukaryotic cell. Examples of such expression vectors are disclosed in, e.g., WO 1994/011026; incorporated herein by reference as it pertains to vectors suitable for the expression of a gene of interest (e.g., a viral capsid protein, a Rep protein, a nucleic acid molecule, or a reporter protein). Expression vectors suitable for use with the compositions and methods described herein contain a polynucleotide sequence as well as, e.g., additional sequence elements used for the expression of proteins and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Certain vectors that can be used for the expression of a viral capsid protein or a Rep protein as described herein include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of viral capsid proteins or Rep proteins contain polynucleotide sequences that enhance the rate of translation of these genes and improve the stability

and nuclear export of the mRNA that results from gene transcription. These sequence elements include, e.g., 5' and 3' untranslated regions, an IRES, and polyadenylation signal site in and to direct efficient transcription of the gene carried on the expression vector. The expression vectors suitable for use with the compositions and methods described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker are genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, nourseothricin, and zeocin.

As used herein, the term "viral capsid protein" refers to a capsid protein composing a proteinaceous shell. Such a proteinaceous shell is generally composed of one or more viral capsid proteins and when assembled is capable of being loaded with one or more polynucleotide molecules. A viral capsid protein described herein may, for example, be a viral protein (VP) 1, VP2, and VP3. Further, a viral capsid protein described herein may refer to a synthetic protein or a viral capsid protein from Parvoviridae (e.g., an AAV).

As used herein, the terms "viral protein 1" and "VP1" refer to any capsid protein that is a component of a capsid, for example, a parvovirus (e.g., AAV) capsid particle. As used herein, a VP1 may possess a surface binding site that interacts with one or more molecules on the surface of a cell to initiate the process of cell entry (e.g., endocytic entry and receptor-mediated fusion). As used herein, a VP1 may self-assemble into a structure consisting of VP1, VP2, and/or VP3 molecules. VP1 may exhibit self-binding properties and self-assemble around the exterior of a respective VP1-containing capsid. As used herein, a VP1 may be synthetic or a VP1 derived from Parvoviridae (e.g., an AAV). For example, a VP1 derived from an AAV may be a VP1 derived from AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, or any other AAV now known and later discovered. A VP1, as used herein, may include an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. Alternatively, and in addition, a VP1, as used herein, may have an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 2 or 4.

As used herein, the terms "viral protein 2" and "VP2" refer to any capsid protein that is a component of a capsid, for example, a parvovirus (e.g., AAV) capsid particle. As used herein, a VP2 may facilitate capsid entry into a host cell, for example, by mediating associations with and exit from the endoplasmic reticulum of a host cell and by facilitating the entry of a nucleic acid molecule into a host cell nucleus. As used

herein, a VP2 may self-assemble into a structure consisting of VP1, VP2, and/or VP3 molecules. VP2 may self-assemble within the interior of a respective VP2-containing capsid. As used herein, a VP2 may be synthetic or a VP2 derived from Parvoviridae (e.g., an AAV). For example, a VP2 derived from an AAV may be a VP2 derived from AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, or any other AAV now known and later discovered. A VP2, as used herein, may include an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. Alternatively, and in addition, a VP2, as used herein, may have an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 13, 16, or 17.

As used herein, the terms “viral protein 3” and “VP3” refer to any capsid protein that is a component of a capsid, for example, a parvovirus (e.g., AAV) capsid particle. As used herein, a VP3 may facilitate capsid entry into a host cell, for example, by mediating associations with and exit from the endoplasmic reticulum of a host cell and by facilitating the entry of a nucleic acid molecule into a host cell nucleus. As used herein, a VP3 may self-assemble into a structure consisting of VP1, VP2, and/or VP3 molecules. VP3 may self-assemble within the interior of a respective VP3-containing capsid. As used herein, a VP3 may be synthetic or a VP3 derived from Parvoviridae (e.g., an AAV). For example, a VP3 derived from an AAV may be a VP3 derived from AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, or any other AAV now known and later discovered. A VP3, as used herein, may include an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. Alternatively, and in addition, a VP3, as used herein, may have an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.

As used herein, the term “*P. pastoris*” refers to “*Pichia pastoris*”. As used herein, the term “*S. cerevisiae*” refers “*Saccharomyces cerevisiae*”. As used herein, the term “*E. coli*” refers to “*Escherichia coli*”.

Detailed Description

***In vitro* capsid assembly**

The present inventors have developed a method to enable *in vitro* AAV capsid assembly. Furthermore, the present inventors have identified several key factors for the *in vitro* capsid assembly method which
5 can be used to further improve the assembly method. Such *in vitro* assembly methods have many benefits over conventional methods, including control of viral protein (VP) stoichiometry.

Method development for sterically controlled *in vitro* AAV capsid assembly for high capsid titers.

- 10 In some embodiments, the present disclosure relates to a method of *in vitro* assembling parvovirus capsids, comprising the steps of:
- a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;
 - b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
 - 15 c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids,
thereby assembling parvovirus capsids and providing assembled parvovirus capsids.

20

Assembly inhibitor

In some embodiments, the inhibited parvovirus viral capsid proteins are inhibited from assembling by multiple assembly inhibitors, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly inhibitors. In some
embodiments, the assembly inhibitor comprises multiple assembly inhibitors, such as 1, 2, 3, 4, 5, 6, 7, 8,
25 9 or 10 assembly inhibitors.

In some embodiments, the assembly inhibitor is selected from the group consisting of a chemically assembly inhibitor, a steric assembly inhibitor and/or a kinetically assembly inhibitor.

- 30 In some embodiments, the assembly inhibitor is selected from the group consisting of inhibitory pH, inhibitory salt concentration, denaturing, precipitation conditions and/or an antibody.

In some embodiments, the assembly inhibitor is selected from the group consisting of a retainment of the viral capsid proteins in a compartment and/or physical blockade, such as a cleavable amino acid

- 35 sequence attached to the viral capsid proteins.

In some embodiments, wherein the assembly inhibitor is selected from the group consisting of inhibitory temperature and/or inhibitory viral capsid protein concentration.

- 5 In some embodiments, the assembly inhibitor is selected from the group consisting of an inhibitory modification to the viral capsid proteins, such as a cleavable amino acid sequence, an inhibitory exogenous molecules, inhibitory pH, inhibitory ionic strength, inhibitory conductivity, time, inhibitory temperature, hydrophobicity, inhibiting viral capsid protein concentration, inhibitory viscosity, denaturation, pressure and radiation, such as light, such as ultraviolet light.
- 10 In some embodiments, the assembly inhibitor is an inhibitory modification to the viral capsid proteins. In some embodiments, wherein the assembly inhibitor is a cleavable amino acid sequence attached to the viral capsid proteins. In some embodiments, the cleavable amino acid sequence is a SUMO tag and/or a thrombin cleavage site.
- 15 In some embodiments, the assembly inhibitor is denaturation of the viral capsid proteins.

- 20 In some embodiments the denaturation is thermally-induced denaturation and/or chemically-induced denaturation. In some embodiments, denaturation include a set of conditions which allow the denaturation of parvovirus capsids. In some embodiments, denaturation conditions may include conditions selected from the list including pH, sonication, ionic strength and/or conductivity, or temperature. In some embodiments, denaturation conditions include the factor of pH. In some embodiments, denaturation conditions include sonication. In some embodiments, denaturation conditions include the factor of ionic strength and/or conductivity. In some embodiments, denaturation conditions include the factor of temperature. Denaturation can be performed by any method known in the art.

- 25 In some embodiments, the assembly inhibitor is urea, such as 1 to 15 M urea, 2 to 14 M urea, 3 to 13 M urea, 4 to 12 M urea, 5 to 11 M urea, 6 to 10 M urea or 7 to 9 M urea. In some embodiments, the assembly inhibitor is NaCl, such as 1 to 15 M NaCl, 2 to 14 M NaCl, 3 to 13 M NaCl, 4 to 12 M NaCl, 5 to 11 M NaCl, 6 to 10 M NaCl or 7 to 9 M NaCl. In some embodiments, the assembly inhibitor is KCl, such as 1 to 15 M KCl, 2 to 14 M KCl, 3 to 13 M KCl, 4 to 12 M KCl, 5 to 11 M KCl, 6 to 10 M KCl or 7 to 9 M KCl.
- 30

- 35 In some embodiments, the inhibitory pH is pH below or equal to 5, such as pH below or equal to 4.5, such as pH below or equal to 4, such as pH below or equal to 3.5, such as pH below or equal to 3, such as pH below or equal to 2.5, such as pH below or equal to 2, such as pH below or equal to 1.5. In some embodiments, the inhibitory pH is pH above or equal to 8.5, such as pH above or equal to 9, such as pH

above or equal to 9.5, such as pH above or equal to 10, such as pH above or equal to 10.5, such as pH above or equal to 11, such as pH above or equal to 11.5, such as pH above or equal to 12, such as pH above or equal to 12.5, such as pH above or equal to 13.

- 5 In some embodiments, the inhibitory temperature is a temperature between -196 °C to 0 °C, such as -190 °C to 0 °C, such as -180 °C to 0 °C, such as -170 °C to 0 °C, such as -160 °C to 0 °C, such as -150 °C to 0 °C, such as -140 °C to 0 °C, such as -130 °C to 0 °C, such as -120 °C to 0 °C, such as -110 °C to 0 °C, such as -100 °C to 0 °C, such as -90 °C to 0 °C, such as -80 °C to 0 °C, such as -70 °C to 0 °C, such as -60 °C to 0 °C, such as -50 °C to 0 °C, such as -40 °C to 0 °C, such as -30 °C to 0 °C, such as -20 °C to 0 °C, such as -10 °C to 0 °C. In some embodiments, the inhibitory temperature is a temperature between -196 °C to -1 °C, such as -196 °C to -10 °C, such as -196 °C to -20 °C, such as -196 °C to -40 °C, such as -196 °C to -50 °C, such as -196 °C to -60 °C, such as -196 °C to -70 °C, such as -196 °C to -80 °C, such as -196 °C to -90 °C, such as -196 °C to -100 °C, such as -196 °C to -110 °C, such as -196 °C to -120 °C, such as -196 °C to -130 °C, such as -196 °C to -140 °C, such as -196 °C to -150 °C, such as -196 °C to -160 °C, such as -196 °C to -170 °C, such as -196 °C to -180 °C, such as -196 °C to -190 °C.

- 20 In some embodiments, the inhibitory temperature is a temperature between 40 °C to 280 °C, such as between 40 °C to 250 °C, such as between 40 °C to 220 °C, such as between 40 °C to 210 °C, such as between 40 °C to 200 °C, such as between 40 °C to 190 °C, such as between 40 °C to 180 °C, such as between 40 °C to 170 °C, such as between 40 °C to 160 °C, such as between 50 °C to 160 °C, such as between 60 °C to 160 °C, such as between 70 °C to 160 °C, such as between 80 °C to 160 °C, such as between 80 °C to 150 °C, such as between 80 °C to 140 °C, such as between 90 °C to 140 °C, such as between 90 °C to 140 °C, such as between 100 °C to 140 °C, such as between 100 °C to 130 °C, such as between 110 °C to 130 °C.

- 25 In some embodiments, the inhibitory exogenous molecule is selected from the group consisting of an antibody, a nucleic acid molecule, a peptide, a protein, a nanobody, a lipid, a sugar, a carbohydrate, a polymer, a small molecule, and/or an aptamer.

- 30 In some embodiments, the inhibitory ionic strength and/ inhibitory conductivity is a solution comprising NaCl, KCl and/or urea, such as a solution comprising 1 to 15 M NaCl, KCl and/or urea, 2 to 14 M NaCl, KCl and/or urea, 3 to 13 M NaCl, KCl and/or urea, 4 to 12 M NaCl, KCl and/or urea, 5 to 11 M NaCl, KCl and/or urea, 6 to 10 M NaCl, KCl and/or urea or 7 to 9 M NaCl, KCl and/or urea.

35 ***Assembly activator***

In some embodiments, the step of contacting the assembly activator and the inhibited parvovirus viral

capsid proteins of step (b) comprise contacting multiple assembly activators, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly activators. In some embodiments, the assembly activator comprises multiple assembly activators, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly activators.

- 5 In some embodiments, the assembly activator is selected from the group consisting of a chemically assembly activator, a sterically assembly activator and/or a kinetically assembly activator.

In some embodiments, the chemically assembly activator is selected from the group consisting of activating pH, activating salt concentration, renaturing, solubilization conditions and/or removal of the
10 antibody.

In some embodiments, the sterically assembly activator is selected from the group consisting of a release of the viral capsid proteins from the compartment and/or removal of the physical blockade, such as the cleavable amino acid sequence attached to the viral capsid proteins.

15 In some embodiments, the kinetically assembly activator is selected from the group consisting of activating temperature and/or activating viral capsid protein concentration.

In some embodiments, the assembly activator is selected from the group consisting of a removal of the
20 inhibitory modification to the viral capsid proteins, such as a cleavage of the cleavable amino acid sequence, removal of the inhibitory exogenous molecules, activating pH, activating ionic strength, activating conductivity, activating time, activating temperature, hydrophobicity, activating viral capsid protein concentration, renaturation, activating viscosity, pressure and radiation, such as light, such as ultraviolet light. In some embodiments, the assembly activator is renaturation.

25 In some embodiments, the assembly activator is a removal of the inhibitory modification to the viral capsid proteins. In some embodiments, the assembly activator is a cleavage of the cleavable amino acid sequence attached to the viral capsid proteins. In some embodiments, the assembly activator is a protease. In some embodiments, the assembly activator is thrombin and/or a SUMO protease, such as
30 SUMO protease 2. In some embodiments, the cleavable amino acid sequence is a SUMO tag and/or a thrombin cleavage site, and wherein the assembly activator is thrombin and/or a SUMO protease, such as SUMO protease 2.

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, such as 1 to 500 mM
35 MES pH 6.0, such as 1 to 450 mM MES pH 6.0, such as 1 to 400 mM MES pH 6.0, such as 1 to 350 mM MES pH 6.0, such as 1 to 300 mM MES pH 6.0, such as 1 to 250 mM MES pH 6.0, such as 1 to 200 mM

MES pH 6.0, such as 1 to 150 mM MES pH 6.0, such as 1 to 100 mM MES pH 6.0, such as 1 to 90 mM
MES pH 6.0, such as 1 to 80 mM MES pH 6.0, such as 1 to 70 mM MES pH 6.0, such as 10 to 70 mM
MES pH 6.0, such as 20 to 70 mM MES pH 6.0, such as 30 to 70 mM MES pH 6.0, such as 40 to 60 mM
MES pH 6.0, such as 45 to 55 mM MES pH 6.0.

5

In some embodiments, the assembly activator is a buffer comprising NaCl, such as 100 to 1000 mM
NaCl, such as 100 to 900 mM NaCl, such as 100 to 800 mM NaCl, such as 100 to 700 mM NaCl, such as
100 to 600 mM NaCl, such as 200 to 600 mM NaCl, such as 300 to 600 mM NaCl, such as 400 to 600
mM NaCl, such as 450 to 550 mM NaCl.

10

In some embodiments, the assembly activator is a buffer comprising NaCl, such as 100 to 400 mM NaCl,
such as 150 to 400 mM NaCl, such as 200 to 350 mM NaCl, such as 200 to 300 mM NaCl, such as 200
to 290 mM NaCl, such as 210 to 280 mM NaCl, such as 220 to 270 mM NaCl, such as 230 to 240 mM
NaCl.

15

In some embodiments, the assembly activator is a buffer comprising NaCl, such as 1 to 20 mM NaCl,
such as 1 to 15 mM NaCl, such as 5 to 15 mM NaCl, such as 6 to 14 mM NaCl, such as 7 to 13 mM
NaCl, such as 6 to 12 mM NaCl, such as 8 to 12 mM NaCl, such as 9 to 11 mM NaCl.

20

In some embodiments, the assembly activator is a buffer comprising KCl, such as 1 to 1000 mM KCl,
such as 1 to 900 mM KCl, such as 1 to 800 mM KCl, such as 1 to 700 mM KCl, such as 1 to 600 mM KCl,
such as 1 to 600 mM KCl, such as 1 to 600 mM KCl, such as 1 to 500 mM KCl, such as 1 to 400 mM KCl,
such as 1 to 300 mM KCl, such as 1 to 200 mM KCl, such as 10 to 200 mM KCl, such as 20 to 200 mM
KCl, such as 30 to 200 mM KCl, such as 40 to 200 mM KCl, such as 50 to 200 mM KCl, such as 50 to

25

150 mM KCl.

In some embodiments, the assembly activator is a buffer comprising KCl, such as 1 to 20 mM KCl, such
as 1 to 15 mM KCl, such as 5 to 15 mM KCl, such as 6 to 14 mM KCl, such as 7 to 13 mM KCl, such as 6
to 12 mM KCl, such as 8 to 12 mM KCl, such as 9 to 11 mM KCl

30

In some embodiments, the assembly activator is a buffer comprising KCl, such as 0.1 to 10 mM KCl, 0.1
to 5 mM KCl, 0.1 to 2 mM KCl, 0.1 to 1 mM KCl, 0.1 to 0.5 mM KCl.

In some embodiments, the assembly activator is a buffer comprising MgCl_2 , such as 0.1 to 20 mM MgCl_2 ,
such as 0.5 to 20 mM MgCl_2 , such as 1 to 20 mM MgCl_2 , such as 1 to 10 mM MgCl_2 , such as 1 to 9 mM
 MgCl_2 , such as 1 to 8 mM MgCl_2 , such as 1 to 7 mM MgCl_2 , such as 1 to 6 mM MgCl_2 , such as 1 to 5 mM

35

MgCl₂, such as 1 to 4 mM MgCl₂, such as 1 to 3 mM MgCl₂.

In some embodiments, the assembly activator is a buffer comprising CaCl₂, such as 0.1 to 20 mM CaCl₂, such as 0.5 to 20 mM CaCl₂, such as 1 to 20 mM CaCl₂, such as 1 to 10 mM CaCl₂, such as 1 to 9 mM CaCl₂, such as 1 to 8 mM CaCl₂, such as 1 to 7 mM CaCl₂, such as 1 to 6 mM CaCl₂, such as 1 to 5 mM CaCl₂, such as 1 to 4 mM CaCl₂, such as 1 to 3 mM CaCl₂.

In some embodiments, the assembly activator is a buffer comprising Tris-Cl, such as 1 to 100 mM Tris-Cl, such as 1 to 90 mM Tris-Cl, such as 1 to 80 mM Tris-Cl, such as 1 to 70 mM Tris-Cl, such as 10 to 70 mM Tris-Cl, such as 20 to 70 mM Tris-Cl, such as 30 to 70 mM Tris-Cl, such as 40 to 60 mM Tris-Cl.

In some embodiments, the assembly activator is a buffer comprising Tris-Cl, such as 1 to 50 mM Tris-Cl, such as 1 to 40 mM Tris-Cl, such as 1 to 30 mM Tris-Cl, such as 5 to 30 mM Tris-Cl, such as 10 to 30 mM Tris-Cl, such as 15 to 25 mM Tris-Cl.

In some embodiments, the assembly activator is a buffer comprising Guanidine HCl, such as 0.1 to 5 mM Guanidine HCl, such as 0.1 to 4 mM Guanidine HCl, such as 0.1 to 3 mM Guanidine HCl, such as 0.1 to 2 mM Guanidine HCl, such as 0.5 to 1.5 mM Guanidine HCl.

In some embodiments, the assembly activator is a buffer comprising non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, such as 0.01 to 5 % non-ionic surfactant, such as 0.01 to 4 % non-ionic surfactant, such as 0.01 to 3 % non-ionic surfactant, such as 0.01 to 2 % non-ionic surfactant, such as 0.1 to 2 % non-ionic surfactant, such as 0.5 to 2 % non-ionic surfactant, such as 0.5 to 1.5 % non-ionic surfactant, such as 0.5 to 1 % non-ionic surfactant.

In some embodiments, the assembly activator is a buffer comprising DTT, such as 0.01 to 1 mM DTT, such as 0.01 to 0.5 mM DTT, such as 0.01 to 0.4 mM DTT, such as 0.01 to 0.3 mM DTT, such as 0.01 to 0.2 mM DTT, such as 0.5 to 0.2 mM DTT.

In some embodiments, the assembly activator is a buffer comprising arginine, such as 0.01 to 10 M arginine, such as 0.01 to 5 M arginine, such as 0.01 to 4 M arginine, such as 0.01 to 3 M arginine, such as 0.01 to 2 M arginine, such as 0.1 to 2 M arginine, such as 0.1 to 1 M arginine, such as 0.2 to 0.9 M arginine, such as 0.3 to 0.8 M arginine, such as 0.4 to 0.7 M arginine, such as 0.4 to 0.6 M arginine.

In some embodiments, the assembly activator is a buffer comprising polyethylene glycol 3,550, such as 0.001 to 10 % polyethylene glycol 3,550, such as 0.001 to 5 % polyethylene glycol 3,550, such as 0.001

to 1 % polyethylene glycol 3,550, such as 0.001 to 0.5 % polyethylene glycol 3,550, such as 0.01 to 0.5 % polyethylene glycol 3,550, such as 0.01 to 0.1 % polyethylene glycol 3,550.

5 In some embodiments, the assembly activator is a buffer comprising GSH, such as 0.01 to 10 mM GSH, such as 0.01 to 5 mM GSH, such as 0.01 to 1 mM GSH, such as 0.01 to 0.5 mM GSH, such as 0.05 to 0.5 mM GSH, such as 0.05 to 0.4 mM GSH, such as 0.05 to 0.3 mM GSH, such as 0.05 to 0.2 mM GSH.

10 In some embodiments, the assembly activator is a buffer comprising GSSH, such as 0.001 to 1 mM GSSH, such as 0.001 to 0.5 mM GSSH, such as 0.001 to 0.4 mM GSSH, such as 0.001 to 0.3 mM GSSH, such as 0.001 to 0.2 mM GSSH, such as 0.001 to 0.1 mM GSSH, such as 0.005 to 0.1 mM GSSH, such as 0.005 to 0.05 mM GSSH.

15 In some embodiments, the assembly activator is a buffer comprising EDTA, such as 0.1 to 10 mM EDTA, such as 0.1 to 5 mM EDTA, such as 0.1 to 4 mM EDTA, such as 0.5 to 4 mM EDTA, such as 0.5 to 3 mM EDTA, such as 0.5 to 2 mM EDTA.

20 In some embodiments, the assembly activator is a buffer comprising sucrose, such as 0.01 to 10 M sucrose, such as 0.01 to 5 M sucrose, such as 0.01 to 2 M sucrose, such as 0.01 to 1 M sucrose, such as 0.1 to 1 M sucrose, such as 0.01 to 0.9 M sucrose, such as 0.01 to 0.8 M sucrose, such as 0.01 to 0.8 M sucrose, such as 0.1 to 0.7 M sucrose, such as 0.1 to 0.6 M sucrose, such as 0.2 to 0.6 M sucrose, such as 0.3 to 0.6 M sucrose, such as 0.3 to 0.5 M sucrose.

25 In some embodiments, the assembly activator is a buffer comprising urea, such as 1 to 15 M urea, 2 to 14 M urea, 3 to 13 M urea, 4 to 12 M urea, 5 to 11 M urea, 6 to 10 M urea or 7 to 9 M urea.

30 In some embodiments, the assembly activator is a buffer comprising Na_2HPO_4 , such as 1 to 100 mM Na_2HPO_4 , such as 1 to 90 mM Na_2HPO_4 , such as 1 to 80 mM Na_2HPO_4 , such as 1 to 70 mM Na_2HPO_4 , such as 1 to 60 mM Na_2HPO_4 , such as 1 to 50 mM Na_2HPO_4 , such as 1 to 40 mM Na_2HPO_4 , such as 1 to 30 mM Na_2HPO_4 , such as 1 to 20 mM Na_2HPO_4 , such as 5 to 20 mM Na_2HPO_4 , such as 5 to 15 mM Na_2HPO_4 .

35 In some embodiments, the assembly activator is a buffer comprising KH_2PO_4 , such as 1 to 100 mM KH_2PO_4 , such as 1 to 90 mM KH_2PO_4 , such as 1 to 80 mM KH_2PO_4 , such as 1 to 70 mM KH_2PO_4 , such as 1 to 60 mM KH_2PO_4 , such as 1 to 50 mM KH_2PO_4 , such as 1 to 40 mM KH_2PO_4 , such as 1 to 30 mM KH_2PO_4 , such as 1 to 20 mM KH_2PO_4 , such as 5 to 20 mM KH_2PO_4 , such as 5 to 15 mM KH_2PO_4 .

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl₂ and/or CaCl₂, such as 1 to 100 mM MES pH 6.0, 1 to 20 mM NaCl, 0.01 to 5 mM KCl, 0.1 to 10 mM MgCl₂ and/or 0.1 to 10 mM CaCl₂, such as 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.1 to 1 mM KCl, 0.5 to 4 mM MgCl₂ and/or 0.5 to 4 mM CaCl₂.

5

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl and/or KCl, such as 1 to 100 mM MES pH 6.0, 200 to 300 mM NaCl and/or 1 to 50 mM KCl, such as 30 to 70 mM MES pH 6.0, 220 to 260 mM NaCl and/or 1 to 20 mM KCl,

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl₂,
10 CaCl₂, Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or DTT.

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl₂, CaCl₂, arginine, polyethylene glycol 3,550, GSH and/or GSSH.

15

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, EDTA, KCl, sucrose, M Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550 and/or DTT.

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl₂, CaCl₂, arginine, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, GSH and/or mM GSSH.

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, EDTA, sucrose, Guanidine HCl and/or DTT.

25

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, EDTA, arginine, sucrose, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550, GSH and/or GSSH.

30

In some embodiments, the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl₂, CaCl₂, Guanidine HCl, polyethylene glycol 3,550 and/or DTT.

In some embodiments, the assembly activator is a buffer comprising mM Tris-Cl pH 8.5, NaCl, KCl, MgCl₂, CaCl₂, sucrose, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550, GSH and GSSH.

35

In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, EDTA, arginine, Guanidine HCl, polyethylene glycol 3,550 and/or DTT.

- 5 In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, MgCl₂, CaCl₂, arginine, sucrose, Guanidine HCl, mM GSH and/or GSSH.

In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, mM NaCl, KCl, EDTA, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or DTT.

10

In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, EDTA, polyethylene glycol 3,550, GSH and/or GSSH.

- 15 In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, 1 mM EDTA, arginine, Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or DTT.

- 20 In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, MgCl₂, CaCl₂, arginine, sucrose, Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550, GSH and/or GSSH.

In some embodiments, the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, MgCl₂, CaCl₂, sucrose and/or DTT.

- 25 In some embodiments, the assembly activator is a buffer comprising Tris-Cl and/or urea, having pH 8.0.

In some embodiments, the assembly activator is a buffer comprising NaCl, KCl, Na₂HPO₄ and/or 10 mM KH₂PO₄, having pH 8.0.

- 30 In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.1 to 1 mM KCl, 1 to 5 mM MgCl₂, 1 to 5 mM CaCl₂, 0.5 to 1 M Guanidine HCl, 0.1 to 2 % non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or 0.01 to 0.5 mM DTT.

- 35 In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.1 to 1 mM KCl, 1 to 5 mM MgCl₂, 1 to 5 mM CaCl₂, 0.05 to 1 M arginine, 0.05 to 0.5 % polyethylene glycol 3,550, 0.01 to 1 mM GSH and/or 0.001 to 0.05 mM GSSH.

In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.5 to 5 mM EDTA, 0.1 to 1 mM KCl, 0.1 to 1 M sucrose, 0.5 to 1 M Guanidine HCl, 0.05 to 1 % non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.005 to 0.1% polyethylene glycol 3,550 and/or 0.01 to 1 mM DTT.

In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 200 to 300 mM NaCl, 1 to 20 mM KCl, 1 to 5 mM MgCl₂, 1 to 2 mM CaCl₂, 0.05 to 1 M arginine, 0.05 to 1% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.01 to 1 mM GSH and/or 0.001 to 0.1 mM GSSH.

In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 200 to 300 mM NaCl, 1 to 20 mM KCl, 0.5 to 5 mM EDTA, 0.1 to 1 M sucrose, 0.5 to 1 M Guanidine HCl and/or 0.01 to 1 mM DTT.

In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.4 M sucrose, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.

In some embodiments, the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.75 M Guanidine HCl, 0.05% polyethylene glycol 3,550 and/or 0.1 mM DTT.

In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.4 M sucrose, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.

In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.75 M Guanidine HCl, 0.05% polyethylene glycol 3,550 and/or 0.1 mM DTT.

In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.1 mM GSH and/or 0.01 mM GSSH.

In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 1 mM EDTA, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.1 mM DTT

- 5 In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.

- In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.75 M Guanidine HCl, 0.5% non-ionic surfactant, such
10 as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or 0.1 mM DTT.

- In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.05% polyethylene glycol
15 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.

In some embodiments, the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.4 M sucrose and/or 0.1 mM DTT.

- 20 In some embodiments, the assembly activator is a buffer comprising 20 mM Tris-Cl and/or 8 M urea, having pH 8.0.

- In some embodiments, the assembly activator is a buffer comprising 500 mM NaCl, 100 mM KCl, 10 mM Na₂HPO₄ and/or 10 mM KH₂PO₄, having pH 8.0.
25

In some embodiments, the activating time is between 30 minutes and 24 hours, such between 1 hour and 24 hours, such between 1 hour and 20 hours, such between 1 hour and 16 hours.

- In some embodiments, the activating time is between 15 minutes and 5 hours, such as between 30 minutes and 5 hours, such as between 30 minutes and 4 hours, such as between 30 minutes and 3
30 hours, such as between 30 minutes and 2 hours, such as between 30 minutes and 1 hour and 30 minutes.

- In some embodiments, the activating temperature is between 1 °C and 45 °C, such as between 10 °C and 45 °C, such as between 10 °C and 40 °C, such as between 10 °C and 35 °C, such as between 10 °C and
35

30 °C, such as between 10 °C and 25 °C

In some embodiments, the activating temperature is between 1 °C and 30 °C, such as between 10 °C and 30 °C, such as between 10 °C and 25 °C, such as between 15 °C and 25 °C, such as between 20 °C and 25 °C.

In some embodiments, the activating temperature is between 15 °C and 25 °C, such as between 16 °C and 25 °C, such as between 17 °C and 25 °C, such as between 17 °C and 24 °C, such as between 18 °C and 24 °C, such as between 19 °C and 24 °C, such as between 20 °C and 24 °C, such as between 21 °C and 24 °C, such as between 21 °C and 23 °C,

In some embodiments, the activating temperature is between 1 °C and 10 °C, such as between 1 °C and 9 °C, such as between 1 °C and 8 °C, such as between 1 °C and 7 °C, such as between 1 °C and 6 °C, such as between 2 °C and 6 °C, such as between 3 °C and 5 °C.

Assembly enhancer

In some embodiments the method comprises an assembly enhancer which enhances *in vitro* assembly.

In some embodiments the assembly enhancer is selected from the group consisting of membrane-associated accessory protein, assembly activation protein, an E2A transcriptional regulator, an ICP8 single-stranded DNA-binding protein, presence of any cleavable protein or amino acid sequence, an enzyme that mediates glycosylation, and/or an enzyme that mediates a post translational modification.

In some embodiments the assembly enhancer is selected from the group consisting of membrane-associated accessory protein and/or assembly activation protein.

***In vitro* capsid filling**

The present inventors have developed an *in vitro* parvovirus capsid filling method using scalable and GMP-compatible techniques which enable for clinically relevant and drug development use. Furthermore, the present inventors have identified several key factors for the *in vitro* capsid filling method which can be used to further improve the filling method. The *in vitro* filling method also results in reduced DNA truncation, a significant advantage over current manufacturing technologies as truncation has been identified as a major concern by the FDA. The *in vitro* filling method works for many different AAV serotypes and achieves clinically relevant titers. In addition, the filled capsids are more potent.

In some embodiments, the present disclosure relates to a method of *in vitro* filling parvovirus capsids, comprising the steps of:

- a. providing:
 - i. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
 - ii. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*; and
 - iii. a Rep protein *in vitro*;
- b. combining the one or more populations of assembled parvovirus capsids, the nucleic acid molecule, and the Rep protein *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids and providing filled parvovirus capsids.

In some embodiments, the present disclosure relates to a method of *in vitro* filling parvovirus capsids according, comprising the steps of:

- a. providing:
 - i. a population of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
 - ii. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*;
 - iii. a Rep proteins *in vitro*; and
 - iv. a reaction mixture *in vitro*;
- b. combining the population of assembled parvovirus capsids, the nucleic acid molecule, the Rep protein and the reaction mixture *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids and providing filled parvovirus capsids.

Nucleic acid molecule

In some embodiments, the nucleic acid molecule is a single-stranded nucleic acid molecule or a double-stranded nucleic acid molecule.

In some embodiments, the nucleic acid molecule is a viral nucleic acid molecule and/or a non-viral nucleic acid molecule. In some embodiments, the nucleic acid molecule is a viral nucleic acid molecule. In some embodiments, the nucleic acid molecule is a non-viral nucleic acid molecule.

In some embodiments, the nucleic acid molecule comprises deoxyribonucleic acid (DNA) and/or

ribonucleic acid (RNA). In some embodiments, the nucleic acid molecule comprises DNA. In some embodiments, the nucleic acid molecule comprises RNA. In some embodiments, the nucleic acid molecule comprises AAV DNA.

- 5 In some embodiments, the reaction mixture comprises adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), deoxythymidine triphosphate (dTTP), a pH buffer, such as HEPES or MOPS buffer, dithiothreitol, creatine phosphate, creatine phosphokinase (CPK), and/or magnesium chloride. In some embodiments, the
- 10 reaction mixture comprises adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), deoxythymidine triphosphate (dTTP), a pH buffer, such as HEPES or MOPS buffer, dithiothreitol, and/or magnesium chloride.

15 ***Filling conditions***

- In some embodiments, the filling conditions are selected from the group consisting of ATP concentration, filling temperature, filling pH, CPK concentration, parvovirus capsid concentration and/or filling time. In some embodiments, the filling conditions are selected from the group consisting of ATP concentration, filling temperature, filling pH, CPK concentration, an ATP regeneration system, and/or parvovirus capsid
- 20 concentration.

- In some embodiments, the filling conditions comprise ATP concentration. In some embodiments, the ATP concentration is between 0.1 and 181.44 mM, such as between 0.1 and 180 mM, such as between 0.1 and 170 mM, such as between 0.1 and 160 mM, such as between 0.1 and 150 mM, such as between 0.1 and 140 mM, such as between 0.1 and 130 mM, such as between 0.1 and 120 mM, such as between 0.1 and 110 mM, such as between 0.1 and 100 mM. In some embodiments, the ATP concentration is
- 25 between 0.1 and 100 mM, such as between 1 and 90 mM, such as between 1 and 80 mM, such as between 1 and 70 mM, such as between 1 and 60 mM, such as between 1 and 50 mM, such as between 1 and 40 mM, such as between 1 and 30 mM, such as between 1 and 20 mM, such as between 1 and 15 mM, such as between 1 and 10 mM. In some embodiments, the ATP concentration is between 0.01 and 10 mM, such as between 0.05 and 10 mM, such as between 0.05 and 9 mM, such as between 0.05 and 8 mM, such as between 0.05 and 7 mM, such as between 0.05 and 6 mM, such as between 0.05 and 5 mM, such as between 0.05 and 4 mM, such as between 0.05 and 3 mM, such as between 0.05 and 2.5 mM, such as between 0.1 and 2 mM. In some embodiments, the ATP concentration is between 0.05 and
- 30 15 mM.
- 35

- In some embodiments, the filling conditions comprise filling temperature. In some embodiments, the filling temperature is between 0 °C and 89.7 °C, such as between 5 °C and 80 °C, such as between 5 °C and 70 °C, such as between 5 °C and 60 °C, such as between 5 °C and 50 °C, such as between 10 °C and 50 °C, such as between 15 °C and 50 °C, such as between 20 °C and 50 °C, such as between 25 °C and 50 °C, such as between 30 °C and 50 °C. In some embodiments, the filling temperature is between 30 °C and 50 °C, such as between 30 °C and 45 °C, such as between 30 °C and 45 °C, such as between 31 °C and 45 °C, such as between 32 °C and 45 °C, such as between 33 °C and 45 °C, such as between 34 °C and 45 °C, such as between 30 °C and 44 °C, such as between 30 °C and 43 °C, such as between 30 °C and 42 °C, such as between 30 °C and 41 °C. In some embodiments, the filling temperature is between 33 °C and 42 °C, such as between 34 °C and 41 °C. In some embodiments, the filling temperature is between 35 °C and 40 °C. In some embodiments, the filling temperature is between 1 °C and 40 °C, such as between 5 °C and 40 °C, such as between 10 °C and 40 °C, such as between 15 °C and 40 °C, such as between 20 °C and 40 °C, such as between 25 °C and 40 °C, such as between 26 °C and 40 °C, such as between 27 °C and 40 °C, such as between 28 °C and 40 °C, such as between 29 °C and 40 °C, such as between 29 °C and 39 °C. In some embodiments, the filling temperature is between 20 °C and 50 °C, such as between 20 °C and 45 °C, such as between 20 °C and 40 °C, such as between 25 °C and 40 °C, such as between 26 °C and 40 °C, such as between 27 °C and 40 °C, such as between 28 °C and 40 °C, such as between 29 °C and 40 °C, such as between 29 °C and 39 °C.
- 20 In some embodiments, the filling conditions comprise filling pH. In some embodiments, the filling pH is between 3 and 10, such as between 4 and 9, such as between 4.5 and 9. In some embodiments, the filling pH is between 5 and 8.8, such as between 5.5 and 8.8, such as between 6 and 8.8, such as between 6 and 8.5, such as between 6 and 8. In some embodiments, the filling pH is between 6 and 7.5.
- 25 In some embodiments, the filling conditions comprise the CPK concentration. In some embodiments, the CPK concentration is between 1 and 1000 ug/ml, such as between 1 and 500 ug/ml, such as between 1 and 400 ug/ml, such as between 1 and 300 ug/ml, such as between 1 and 200 ug/ml, such as between 1 and 100 ug/ml. In some embodiments, the CPK concentration is between 1 and 100 ug/ml, such as between 1 and 90 ug/ml, such as between 1 and 80 ug/ml, such as between 1 and 70 ug/ml, such as between 1 and 60 ug/ml, such as between 1 and 50 ug/ml, such as between 1 and 40 ug/ml, such as between 1 and 30 ug/ml, such as between 1 and 20 ug/ml, such as between 1 and 10 ug/ml. In some embodiments, the CPK concentration is between 5 and 110 ug/ml, such as between 5 and 100 ug/ml, between 5 and 90 ug/ml. In some embodiments, the CPK concentration is between 10 and 100 ug/ml.
- 30 In some embodiments, the filling conditions comprise an ATP regenerating system. In some embodiments, the ATP regenerating system is acetate kinase using acetyl phosphate as phosphate
- 35

donor, creatine kinase using creatine phosphate as phosphate donor, pyruvate kinase using phosphoenolpyruvate as phosphate donor, and polyphosphate kinase using polyphosphate as phosphate donor.

5 In some embodiments, the filling conditions comprise the parvovirus capsid concentration. In some
embodiments, the parvovirus capsid concentration is between $1e4$ and $5e16/mL$, such as between $1e4$
and $5e15/mL$, such as between $1e4$ and $1e15/mL$, such as between $1e4$ and $1e15/mL$, such as
between $1e4$ and $1e14/mL$, such as between $1e4$ and $1e13/mL$, such as between $1e4$ and $1e12/mL$,
such as between $1e4$ and $1e11/mL$, such as between $1e4$ and $1e10/mL$, such as between $1e4$ and $1e9/mL$,
10 such as between $1e4$ and $1e8/mL$, such as between $1e4$ and $1e7/mL$, such as between $1e4$ and
 $1e6/mL$, such as between $1e4$ and $1e5/mL$. In some embodiments, the parvovirus capsid concentration
is between 0.01 and $100e10/mL$, such as between 0.1 and $100e10/mL$, such as between 0.1 and $90e10/mL$.
such as between 0.1 and $80e10/mL$. such as between 0.1 and $70e10/mL$. such as between 0.1
and $60e10/mL$. such as between 0.1 and $50e10/mL$. such as between 0.1 and $40e10/mL$. such as
15 between 0.1 and $30e10/mL$. such as between 0.1 and $20e10/mL$. such as between 0.1 and $10e10/mL$.
such as between 0.1 and $5e10/mL$. In some embodiments, the parvovirus capsid concentration is
between 0.01 and $10e10/mL$, such as between 0.1 and $10e10/mL$, such as between 0.5 and $10e10/mL$,
such as between 0.5 and $9e10/mL$, such as between 0.5 and $8e10/mL$, such as between 0.5 and $7e10/mL$,
such as between 0.5 and $6e10/mL$, such as between 0.5 and $5e10/mL$. In some embodiments,
20 the parvovirus capsid concentration is between 0.5 and $7e10/mL$.

In some embodiments, the reaction mixture comprises HEPES, such as $30mM$ HEPES, MES buffer,
phosphate buffer, Tris buffer or MOPS buffer. In some embodiments, the filling conditions comprises
HEPES, such as $30mM$ HEPES, MES buffer, phosphate buffer, Tris buffer or MOPS buffer.

25

In some embodiments, the filling conditions comprises the following:

- a. the ATP concentration is between 0.05 and $15mM$;
- b. the filling temperature is between $35^{\circ}C$ and $40^{\circ}C$;
- c. the CPK concentration is between 10 and $100ug/ml$; and/or
- 30 d. the parvovirus capsid concentration is between 0.5 and $7e10/mL$.

In some embodiments, the filling conditions comprises filling time. In some embodiments, the filling time is
between 0.5 and 24 hours, such as between 0.5 and 20 hours, such as between 0.5 and 16 hours, such
as between 0.5 and 12 hours, such as between 0.5 and 10 hours, such as between 1 and 10 hours, such
35 as between 1 and 9 hours, such as between 2 and 8 hours, such as between 2 and 7 hours, such as
between 3 and 6 hours, such as between 3 and 5 hours. In some embodiments, the filling time is between

1 and 7 hours, such as between 2 and 7 hours, such as between 2.5 and 6.5 hours, such as between 3 and 6 hours, such as between 3 and 5 hours, such as between 3.5 and 4.5 hours. In some embodiments, the filling time is between 3 and 5 hours.

- 5 In some embodiments, the filling conditions comprises the following:
- a. the ATP concentration is between 0.05 and 15 mM;
 - b. the filling temperature is between 35 °C and 40 °C;
 - c. the CPK concentration is between 10 and 100 ug/ml;
 - d. the parvovirus capsid concentration is between 0.5 and 7 e10/mL; and/or
 - 10 e. the filling time is between 3 and 5 hours.

In some embodiments, the nucleic acid molecule comprises a first inverted terminal repeat (ITR1) and/or a second inverted terminal repeat (ITR2). In some embodiments, the ITR1 and/or the ITR2 is from AAV. In some embodiments, the ITR1 and/or the ITR2 comprises the Rep binding domain. In some
 15 embodiments, the nucleic acid molecule, the ITR1 and/or the ITR2 are operably linked to each other in a 5'-to-3' direction. In some embodiments, the nucleic acid molecule, the ITR1 and/or the ITR2 are operably linked to each other in a 3'-to-5' direction

In some embodiments, the method comprises a filling enhancer which enhances *in vitro* filling. filling
 20 enhancer which enhances *in vitro* assembly. In some embodiments, the filling enhancer is selected from the group consisting of membrane-associated accessory protein, assembly activation protein, an E2A transcriptional regulator, an ICP8 single-stranded DNA-binding protein, presence of any cleavable protein or amino acid sequence, an enzyme that mediates glycosylation, and/or an enzyme that mediates a post translational modification. In some embodiments, the filling enhancer is selected from the group
 25 consisting of membrane-associated accessory protein and/or assembly activation protein. In some embodiments, the E2A transcriptional regulator is from adenovirus. In some embodiments, the ICP8 single-stranded DNA-binding protein is from herpes virus. In some embodiments, the enzyme that mediates a post translational modification is synthetic.

30 In some embodiments, the filling conditions are conditions which denature the assembled capsids. In some embodiments, the filling conditions which denature are condition factors selected from this list comprising: pH, sonication, ionic strength/conductivity, or temperature.

In some embodiments, the filled parvovirus capsids comprises an increased amount of intact nucleic acid
 35 molecule compared to parvovirus capsids that have been filled using conventional methods. In some embodiments, the filled parvovirus capsids comprise an 80% intact nucleic acid molecule, such as an

85% intact nucleic acid molecule, such as a 90% intact nucleic acid molecule, such as a 95% intact nucleic acid molecule, such as a 96% intact nucleic acid molecule, such as a 97% intact nucleic acid molecule, such as a 98% intact nucleic acid molecule, such as a 99% intact nucleic acid molecule, such as a 100% intact nucleic acid molecule. In some embodiments, the filled parvovirus capsids comprise a
5 100% intact nucleic acid molecule.

In some embodiments, the nucleic acid molecule comprised in the filled parvovirus capsids has reduced truncation compared to a nucleic acid molecule comprised in parvovirus capsid that have been filled using conventional methods. In some embodiments, the nucleic acid molecule comprised in the filled parvovirus
10 capsids has 20% truncation, such as 15% truncation, such as 10% truncation, such as 5% truncation, such as 4% truncation, such as 3% truncation, such as 2% truncation, such as 1% truncation, such as 0% truncation. In some embodiments, the nucleic acid comprises the ITR1, the transgene of interest and the ITR2.

15 In some embodiments, at least 80% of the assembled parvovirus capsids are filled by said method, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at
20 least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or wherein at least 99% of the assembled parvovirus capsids are filled by said method. In some embodiments, 100% of the assembled parvovirus capsids are filled by said method.

In some embodiments, at least 80% of the assembled parvovirus capsids are filled with said transgene of interest by said method, such as at least 81%, such as at least 82%, such as at least 83%, such as at
25 least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or wherein at least 99% of the assembled parvovirus capsids are filled by said method. In some embodiments, 100% of the assembled parvovirus capsids are filled with said transgene of interest by said
30 method.

In some embodiments, the method yields at least 80% filled parvovirus capsids, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at
35 least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% filled parvovirus

capsids. In some embodiments, the method yields 100% filled parvovirus capsids.

***In vitro* manufacturing of filled capsids**

The present inventors have developed an *in vitro* method of manufacturing complete assembled and filled parvovirus capsids. Furthermore, the present inventors have identified several key factors for the *in vitro* capsid assembly method and the *in vitro* capsid filling method which can be used to further improve the *in vitro* manufacturing method.

In some embodiments, the present disclosure relates to a method of *in vitro* manufacturing filled parvovirus capsids, comprising the steps of:

- a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;
- b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
- c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids, thereby providing one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
- d. providing:
 - i. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*; and
 - ii. a Rep protein *in vitro*;
- e. combining the population of assembled parvovirus capsids obtained in c., the nucleic acid molecule and the Rep protein *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids, thereby manufacturing filled parvovirus capsids.

In some embodiments, the present disclosure relates to a method of *in vitro* manufacturing filled parvovirus capsids, comprising the steps of:

- a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;
- b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and

- c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids,
- 5 thereby providing one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*
- d. providing:
- i. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*;
 - 10 ii. a Rep protein *in vitro*; and
 - iii. a reaction mixture *in vitro*
- e. combining the population of assembled parvovirus capsids obtained in c., the nucleic acid molecule, the Rep protein and the reaction mixture *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein,
- 15 thereby filling parvovirus capsids,
- thereby manufacturing filled parvovirus capsids.

In some embodiments, the assembly step and/or the filling step is as defined elsewhere in the present disclosure.

20

Viral capsid proteins

In some embodiments, the viral capsid proteins are from a Densovirinae, Hamaparvovirinae, or Parvovirinae.

- 25 In some embodiments, the viral capsid proteins are from a Parvovirinae selected from the group consisting of an Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus, preferably Dependoparvovirus.

- 30 In some embodiments, the viral capsid proteins are from an adeno-associated virus (AAV).

In some embodiments, the viral capsid proteins are synthetic viral capsid proteins and/or recombinant viral capsid proteins.

- 35 In some embodiments, the viral capsid protein is a synthetic protein (e.g., an engineered protein and/or a protein composed of synthetic nucleic or amino acids). For example, in some embodiments, a synthetic

capsid may include the synthetic capsids described in WO 2011/054976, WO 2019/158619, and WO 2020/068990, incorporated herein in their entirety by reference. A synthetic viral capsid protein may be any suitable synthetic viral capsid protein now known in the art or later discovered.

5 In some embodiments, the viral capsid proteins are selected from the group consisting of viral protein 1 (VP1), viral protein 2 (VP2), and/or viral protein (VP3). In some embodiments, the viral capsid proteins are VP1. In some embodiments, the viral capsid proteins are VP2. In some embodiments, the viral capsid proteins are VP3. In some embodiments, the viral capsid proteins are VP1 and VP2. In some
10 embodiments, the viral capsid proteins are VP1 and VP3. In some embodiments, the viral capsid proteins are VP2 and VP3. In some embodiments, the viral capsid proteins are VP1, VP2, and VP3. In some embodiments, the VP1, VP2 and/or VP3 are AAV VP1, AAV VP2 and/or AAV VP3.

In some embodiments, the AAV is an AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13,
15 avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, rh10 AAV, rh.39 AAV, or any other AAV now known and later discovered.

In some embodiments, the VP1, VP2 and/or VP3 are synthetic VP1, VP2 and/or VP3 and/or recombinant VP1, VP2 and/or VP3.

20 In some embodiments, the VP is a VP from an AAV type 1. In some embodiments, the VP is a VP from an AAV type 2. In some embodiments, the VP is a VP from an AAV type 3. In some embodiments, the VP is a VP from an AAV type 4. In some embodiments, the VP is a VP from an AAV type 5. In some
25 embodiments, the VP is a VP from an AAV type 6. In some embodiments, the VP is a VP from an AAV type 7. In some embodiments, the VP is a VP from an AAV type 8. In some embodiments, the VP is a VP from an AAV type 9. In some embodiments, the VP is a VP from an AAV type 10. In some embodiments, the VP is a VP from an AAV type 11. In some embodiments, the VP is a VP from an AAV type 12. In
30 some embodiments, the VP is a VP from an AAV type 13. In some embodiments, the VP is a VP from an avian AAV. In some embodiments, the VP is a VP from a bovine AAV. In some embodiments, the VP is a VP from a canine AAV. In some embodiments, the VP is a VP from a goat AAV. In some embodiments, the VP is a VP from a snake AAV. In some embodiments, the VP is a VP from an equine AAV. In some
35 embodiments, the VP is a VP from an ovine AAV. In some embodiments, the VP is a VP from a goat AAV. In some embodiments, the VP is a VP from a shrimp AAV. In some embodiments, the VP is a VP from a rh10 AAV. In some embodiments, the VP is a VP from a rh74 AAV.

In some embodiments, the VP1 is a VP1 from AAV serotype 1, serotype 2, serotype 3A, serotype 3B,

serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74. In some embodiments, the VP2 is a VP2 from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74. In some embodiments, the VP3 is a VP3 from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.

In some embodiments, the VP1, VP2, and/or VP3 are from different AAV serotypes. In some embodiments, the VP1, VP2, and/or VP3 are from the same AAV serotype.

In some embodiments, the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced in an eukaryotic microbial system, such as yeast, such as a methylotrophic yeast. In some embodiments, the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced in an eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*. In some embodiments, the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced in an prokaryotic microbial system, such as *E. Coli*. In some embodiments, the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced a mammalian cell system, such as HEK, CHO, HeLa or Vero cells, an insect cell system, such as Sf9, Sf21 or High Five cells, a plant system, such as tobacco BY, an algae system, a metazoan cell system, a zebrafish and/or cell-free expression systems.

In some embodiments, the viral capsid proteins further comprise a purification tag, such as a His-tag, a C-tag (EPEA), a streptavidin tag and/or biotin tag.

In some embodiments, the viral capsid protein is a viral capsid protein exemplified by the Parvoviridae viral proteins in **Table 1**, below:

Table 1: Exemplary wild-type amino acid sequences of Parvoviridae viral proteins

Parvoviridae Subfamily	Genera	Species	Exemplary wild-type amino acid sequences
Densovirinae	Aquambidensovirus	Asteroid aquambidensovirus 1	VP1 UniProt ID Nos.: A0A2H4YD43 and A0A2H4YD47
Densovirinae	Aquambidensovirus	Decapod aquambidensovirus	VP1 UniProt ID No.: A0A0E3XUQ8

Parvoviridae Subfamily	Genera	Species	Exemplary wild-type amino acid sequences
Densovirinae	Blattambidensovirus	Blattodean blattambidensovirus 1	VP1 UniProt ID No.: Q80PY6, VP2 UniProt ID No.: Q80PY5, and VP3 UniProt ID No.: A0A1Z4CGB4
Densovirinae	Iteradensovirus	Lepidopteran iteradensovirus 1	VP1 UniProt ID No.: Q8V0M6
Densovirinae	Iteradensovirus	Lepidopteran iteradensovirus 2	VP1 UniProt ID No.: Q8V5A7
Densovirinae	Iteradensovirus	Lepidopteran iteradensovirus 3	VP1 UniProt ID No.: Q66NY3
Densovirinae	Iteradensovirus	Lepidopteran iteradensovirus 4	VP1 UniProt ID No.: J7GME5
Densovirinae	Iteradensovirus	Lepidopteran iteradensovirus 5	VP1 UniProt ID No.: I3W890, VP2 UniProt ID No.: I3W891, VP3 UniProt ID No.: I3W892, and VP4 UniProt ID No.: I3W893
Densovirinae	Miniambidensovirus	Orthopteran miniambidensovirus 1	VP1 UniProt ID No.: V5KE12
Densovirinae	Muscodensovirus	Dipteran muscodensovirus 1	VP1 RefSeq ID No.: QCH41363.1
Densovirinae	Protoambidensovirus	Dipteran protoambidensovirus 1	VP1 UniProt ID Nos.: A0A6B9LJ84, A0A6B9LJQ3, A0A6B9LJY5, A0A6B9LSU3, A0A6B9LLF6, A0A6B9LJT8, A0A6B9LST1, A0A6B9LYI6, A0A6B9LKE5, A0A6B9LJC3, A0A6B9LSA2, A0A6B9LK30, A0A6B9LUX4, A0A6B9LK17, A0A6B9LSR8, A0A6B9LYQ0, A0A6B9LYH3, A0A6B9LK00, A0A6B9LLD6, A0A6B9LYP1, A0A6B9LJA8, A0A6B9LS73,

Parvoviridae Subfamily	Genera	Species	Exemplary wild-type amino acid sequences
			A0A6B9LSU8, A0A6B9LS85, A0A6B9LSB6, A0A6B9LYL9, A0A6B9LJS2, A0A6B9LLG7, A0A6B9LYK0, A0A6B9LKF9, A0A6B9LJ96, A0A6B9LLE6, A0A6B9LUV0, A0A6B9LKH3, A0A6B9LYN2, and A0A6B9LJV8
Densovirinae	Protoambidensovirus	Lepidopteran protoambidensovirus 1	VP1 UniProt ID No.: Q90125
Densovirinae	Scindoambidensovirus	Orthopteran scindoambidensovirus 1	VP1 UniProt ID Nos.: F2Y985 R9YVE7, R9YVF8, R9YVE1, R9YVF5, R9YVD6, R9YVC9, R9YVF1, A0A218L3Q4, and A0A7G7S060
Densovirinae	Scindoambidensovirus	Hymenopteran scindoambidensovirus 1	VP1 UniProt ID No.: U5TU71 and VP2 UniProt ID No.: U5TU55
Densovirinae	Scindoambidensovirus	Hemipteran scindoambidensovirus 1	VP4 UniProt ID Nos.: A0A218L3Q5 and PF02336
Hamaparvovirinae	Brevihamaparvovirus	Dipteran brevihamaparvovirus 1	VP1 UniProt ID Nos.: P27453 and Q90187
Hamaparvovirinae	Brevihamaparvovirus	Dipteran brevihamaparvovirus 2	VP1 UniProt ID No.: CAA52901.1 and VP1 RefSeq ID NO.: NP_694829.3
Hamaparvovirinae	Chaphamaparvovirus	Carnivore chaphamaparvovirus 1	VP1 RefSeq ID Nos.: QOR29546.1, QOR29545.1, and QOR29542.1
Hamaparvovirinae	Chaphamaparvovirus	Rodent chaphamaparvovirus 1	VP1 RefSeq ID No.: QLM06163.1, YP_009553677.1,

Parvoviridae Subfamily	Genera	Species	Exemplary wild-type amino acid sequences
			AXX39022.2, and AXX39019.1
Parvovirinae	Amdoparvovirus	Aleutian mink disease parvovirus (strain G)	VP1 UniProt ID No.: P24029
Parvovirinae	Amdoparvovirus	Galliform aveparvovirus 1	VP1 UniProt ID No.: A0A159ZQG2
Parvovirinae	Bocaparvovirus	Carnivore bocaparvovirus 6	VP1 UniProt ID No.: A0A5J6KHC3
Parvovirinae	Bocaparvovirus	Chiropteran bocaparvovirus 1	VP1 UniProt ID No.: R4L110 and VP2 UniProt ID No.: R4L9D4
Parvovirinae	Bocaparvovirus	Lagomorph bocaparvovirus 1	VP1 UniProt ID No.: A0A0U3BGQ5
Parvovirinae	Bocaparvovirus	Pinniped bocaparvovirus 1	VP1 UniProt ID No.: G1JYW8
Parvovirinae	Bocaparvovirus	Pinniped bocaparvovirus 2	VP1 UniProt ID No.: G1JYY5
Parvovirinae	Bocaparvovirus	Human bocavirus type 2	VP1 UniProt ID No.: B9UYK7
Parvovirinae	Bocaparvovirus	Human bocavirus 3	VP1 UniProt ID No.: C1IWT2
Parvovirinae	Bocaparvovirus	Human bocavirus type 4	VP1 UniProt ID No.: C5IY46
Parvovirinae	Bocaparvovirus	Primate bocaparvovirus 1	VP1 UniProt ID No.: Q3YPH4
Parvovirinae	Bocaparvovirus	Primate bocaparvovirus 2	VP1 UniProt ID No.: B9UYK7
Parvovirinae	Bocaparvovirus	Primate bocaparvovirus 3	VP1 UniProt ID No.: C1IWT2
Parvovirinae	Bocaparvovirus	Rodent bocaparvovirus 1	VP1 UniProt ID No.: A0A0Y0B7Q6
Parvovirinae	Bocaparvovirus	Ungulate bocaparvovirus 1	VP1 UniProt ID No.: P07297

Parvoviridae Subfamily	Genera	Species	Exemplary wild-type amino acid sequences
Parvovirinae	Copiparvovirus	Ungulate copiparvovirus 1	VP1 UniProt ID No.: A0A345ALY7
Parvovirinae	Dependoparvovirus	Goose parvovirus	VP1 UniProt ID No.: Q67666
Parvovirinae	Dependoparvovirus	Muscovy duck parvovirus	VP1 UniProt ID No.: A0A140HEY6
Parvovirinae	Dependoparvovirus	Muscovy duck parvovirus	VP1 UniProt ID No.: A0A089X5B1
Parvovirinae	Dependoparvovirus	Avian dependoparvovirus 1	VP1 UniProt ID No.: C7FFS0
Parvovirinae	Dependoparvovirus	Pinniped dependoparvovirus 1	VP1 UniProt ID No.: 1JYZ5
Parvovirinae	Dependoparvovirus	Squamate dependoparvovirus 1	VP1 UniProt ID No.: Q6V7U2
Parvovirinae	Protoparvovirus	Carnivore chapparvovirus 1	VP1 UniProt ID No.: A0A6M4C6Q4
Parvovirinae	Protoparvovirus	Carnivore protoparvovirus 1 feline parvovirus	VP1 UniProt ID Nos.: P04864 and P24840
Parvovirinae	Protoparvovirus	Carnivore protoparvovirus 1 canine parvovirus	VP1 UniProt ID No.: A0A5B8P5H0
Parvovirinae	Protoparvovirus	Carnivore protoparvovirus 2	VP1 UniProt ID Nos.: Q11213, P12930, P04863, and P17455; VP2 UniProt ID No.: P61826
Parvovirinae	Protoparvovirus	Eulipotyphla protoparvovirus 1	VP1 UniProt ID No.: A0A0E4B1Y8
Parvovirinae	Protoparvovirus	Primate protoparvovirus 1	VP1 UniProt ID No.: I6XXD8

Parvoviridae Subfamily	Genera	Species	Exemplary wild-type amino acid sequences
Parvovirinae	Protoparvovirus	Primate protoparvovirus 3	VP1 UniProt ID Nos.: A0A1B0VGA7, A0A1B0VBZ8, A0A1B0VBQ6, A0A1B0VBZ3, A0A1B0VEL1, A0A1B0VBP3, and A0A1B0VJ47
Parvovirinae	Protoparvovirus	Rodent protoparvovirus 1	VP1 UniProt ID No.: Q83430
Parvovirinae	Protoparvovirus	Ungulate protoparvovirus 1	VP1 UniProt ID No.: P52501
Parvovirinae	Tetraparvovirus	Ungulate protoparvovirus 1	VP1 UniProt ID No.: P52501
Parvovirinae	Tetraparvovirus	Chimp parvo	VP1 UniProt ID No.: A0A3G2LVC1
Parvovirinae	Tetraparvovirus	Mink enteritis virus (strain Abashiri) (MEV)	VP1 UniProt ID No.: P27437
Parvovirinae	Tetraparvovirus	Murine minute virus (strain MVM prototype)	VP1 UniProt ID Nos.: P03137 and P07302
Parvovirinae	Tetraparvovirus	Porcine parvovirus (strain 90HS)	VP1 UniProt ID No.: P33484
Parvovirinae	Tetraparvovirus	Porcine parvovirus (strain Kresse)	VP1 UniProt ID No.: P52501
		Porcine parvovirus (strain NADL-2)	VP1 UniProt ID No.: P18546
		Primate bocaparvovirus 1	VP1 UniProt ID No.: Q3YPH4
		Chimpanzee parv4	VP1 UniProt ID No.: E1AYG9 and VP2 UniProt ID No.: E1AYH0

In some embodiments, the VP1 is a VP1 from AAV serotype 1. In some embodiments, the VP1 is a VP1 from AAV serotype 2. In some embodiments, the VP1 is a VP1 from AAV serotype 3. In some embodiments, the VP1 is a VP1 from AAV serotype 4. In some embodiments, the VP1 is a VP1 from AAV

serotype 5. In some embodiments, the VP1 is a VP1 from AAV serotype 6. In some embodiments, the VP1 is a VP1 from AAV serotype 7. In some embodiments, the VP1 is a VP1 from AAV serotype 8. In some embodiments, the VP1 is a VP1 from AAV serotype 9. In some embodiments, the VP1 is a VP1 from AAV serotype 10. In some embodiments, the VP1 is a VP1 from AAV serotype 11. In some
5 embodiments, the VP1 is a VP1 from AAV serotype 12. In some embodiments, the VP1 is a VP1 from AAV serotype 13. In some embodiments, the VP1 is a VP1 from AAV serotype rh10. In some
embodiments, the VP1 is a VP1 from AAV serotype rh74.

In some embodiments, the VP1 is a VP1 exemplified by the AAV VP1 in **Table 2** and **Table 3**, below:
10

Table 2: Exemplary wild-type amino acid sequences of AAV VP1

AAV Serotype	Exemplary wild-type VP1 amino acid sequence
1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGP PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTS FGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQ QPAKKRLNFGQTGDSESVDPDPQLGEPPATPAAVGPTTMASGGGAPMADNNEGA DGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHY FGYSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFPRKRLNFKLFNIQVKEVTTND GVTTIANNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGS QAVGRSSFYCLEYFPSQMLRTGNNTFTSYTFEEVFPFHSSYAHSSQLDRLMNPLIDQ YLYLNRNQSGSAQNKDLLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTD NNNSNFTWTGASKYNLNGRESIINPGTAMASHKDEDEDKFFPMMSGVMIFGKESAGAS NTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSTD PATGDVHAMGALPGMVW QDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKNPPPQILIKNTVPANPPAEFSA TKFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGL YTEPRPIGTRYLTRPL (SEQ ID NO: 7)
2	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGP FNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADADEFQERLKEDTSF GGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQ PARKRLNFGQTGDADSVDPDPQLGQPPAAPSGLGTNTMATGSGAPMADNNEGAD GVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFG YSTPWGYFDFNRFHCHFSRPDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNDGT TTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQA VGRSSFYCLEYFPSQMLRTGNNTFTSYTFEDVPFHSSYAHSSQLDRLMNPLIDQYLY

AAV Serotype	Exemplary wild-type VP1 amino acid sequence
	YLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNS EYSWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDI EKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDR DVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFA SFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEP RPIGTRYLTRNL (SEQ ID NO: 1)
3	MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYL PGNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLQEDTS FGGNLGRAVFQAKKRILEPLGLVEEAAKTAPGKKRPVDQSPQEPDSSSGVGKSGKQ PARKRLNFGQTGDSESVDPDQPLGEPPAAPTSLGSNTMASGGGAPMADNNEGAD GVGNSSGNWHCDSQWLGDRTTSTRTWALPTYNNHLYKQISSQSGASNDNHYFG YSTPWGYFDNRFHCHFSRPDWQRLINNNWGFPRPKLSFKLFNIQVKEVTQNDGTT TIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTNNGSQA VGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVPFHSSYAHSQSLDRMLNPLIDQYL YYLNRTQGTTSGTTNQSRLFSQAGPQSMQLARNWLPGPCYRQQRVSKTANDNN NSNFPWTAASKYHLNGRDSLVPNPGPAMASHKDDEEKFFPMHGNLIFGKEGTTASN AELDNVMITDEEEIRTTNPVATEQYGTVANLQSSNTAPTTRTVNDQGALPGMVWQ DRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPA KFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTVDTNGVYS EPRPIGTRYLTRNL (SEQ ID NO: 8)
5	MSFVDHPPDWLEEVGEGLREFLGLAEGPPKPKPNQQHQDQARGLVLPGYNYLGPG NGLDRGEPVNRADDEVAREHDISYNEQLEAGDNPYLKYNHADADEFQEKLADDTSF NLGKAVFQAKKRVLEPFGLVEEAKTAPTGRIDDHFKRKKARTEEDSKPSTSSDA EAGPSGSQQLQIPAPASSLGADTMSAGGGGGLGDNNQGADGVGNASGDWHCD STWMGDRVTKSTRTWLPSYNNHLYREIKSGSVDGSNANAYFGYSTPWGYFDN RFHSHWSPRDWQRLINNYWGFPRSLRVKIFNIQVKEVTQDSTTTIANNLTSTVQV FTDDDYQLPYVVGNGTEGCLPAFPQVFTLPQYGYATLNRDNTENPTERSFFCLE YFPSKMLRTGNNFETYNFEEVPFHSSFAPSQNLFLANPLVDQYLYRFVSTNNTG GVQFNKNLAGRYANTYKNWFGPMGRTQGWNLGSGVNRASVSFATTNRMELEG ASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESET QPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEI VPGSVWMERDVYLQGPWAK IPETGAHFHPSAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVT

AAV Serotype	Exemplary wild-type VP1 amino acid sequence
	VEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL (SEQ ID NO: 2)
6	MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYL PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAERLQEDTS FGGNLGRAVFQAKKRVLEPFLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQ QPAKKRLNFGQTGDSESVDPQPPLGEPPATPAAVGPTTMASGGGAPMADNNEGA DVGNGASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNH FGYSTPWGYFDFNRFHCHFSRWDWQRLINNNWGFPRKRLNFKLFNIQVKEVTTND GVTTIANNLSTVQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGS QAVGRSSFYCLEYFPSQMLRTGNNTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQ YLYYLNRTQNGSGSAQNKDLLFSRGSAPGMSVQPKNWLPGPCYRQQRVSKTKTD NNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKDKFFPMMSGVMIFGKESAGAS NTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVW QDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSA TKFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNG YTEPRPIGTRYLTRPL (SEQ ID NO: 3)
7	MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYL PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAERLQEDTS FGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPAKKRPVEPSPQRSPDSSTGIGKKGQ QPARKRLNFGQTGDSESVDPQPPLGEPPAAPSSVSGSTVAAGGGAPMADNNEGA DVGNGASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSETAGSTNDNTYF GYSTPWGYFDFNRFHCHFSRWDWQRLINNNWGFPRKKLRFKLFNIQVKEVTTNDG VTTIANNLSTIQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQS VGRSSFYCLEYFPSQMLRTGNNEFFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYL YYLARTQSNPGGTAGNRELQFYQGGPSTMAEQAKNWLPGPCFRQQRVSKTLDQN NNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDERFFPSSGVLIFGKTGATNKT TLENVMTNEEEIRPTNPVATEEYGVSSNLQAANTAAQTQVNNQ GALPGMVWQN RDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPANPPEVFTPAKF ASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNFEKQTGVDFAVDSQGVYSE PRPIGTRYLTRNL (SEQ ID NO: 9)

AAV Serotype	Exemplary wild-type VP1 amino acid sequence
8	MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVDPDQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDFNRFHCHFSRDPWQRLINNNWGFPRKRLSFKLFIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTGGTANTQTLGFSQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAARDNADYSDVMLTSEEEIKTTNPVATEEYGIVADNLQQQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 4)
9	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPFGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRILLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTEVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFYGYSTPWGYFDFNRFHCHFSRDPWQRLINNNWGFPRKRLNFKLFIQVKEVTDNNGVKTIANNLTSTVQVFTDSYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYQQVATNHQSAQAQAQTGWVQNGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 10)

Table 3: Exemplary wild-type amino acid sequences of AAV VP1

AAV Serotype	Exemplary wild-type VP1 amino acid sequence(s)
1	UniProt ID No.: Q9WBP8
2	UniProt ID No.: P03135

AAV Serotype	Exemplary wild-type VP1 amino acid sequence(s)
3	UniProt ID Nos.: Q65310 and O56139
4	UniProt ID No.: O41855
5	UniProt ID No.: Q9YIJ1
6	UniProt ID No.: O56137
7	UniProt ID Nos.: Q8JQG1, Q8JQG0
8	UniProt ID No.: Q8JQF8
9	UniProt ID Nos.: Q6JC40 and AY530579.1
10	UniProt ID No.: Q5Y9B4
11	UniProt ID No.: Q5Y9B2
12	UniProt ID No.: A9RAI0
13	UniProt ID No.: B5SUY7
Avian	UniProt ID Nos.: A0A5C0PVX0 and C7FFS0
Chicken	UniProt ID No.: A0A1S6EMA7
Bovine	UniProt ID No.: P07297
Fox	UniProt ID No.: N0A7G5
Canine	UniProt ID No.: R9QBU0
Snake	UniProt ID No.: Q6V7U2
Bat	UniProt ID No.: A0A2Z4K548
Rat	UniProt ID No.: A0A3Q8HA51
AAVrh10	UniProt ID No.: AAO88201.1
AAVrh.39	UniProt ID No.: ACB55313.1

In some embodiments, the VP1 is a VP1 from AAVrh74, for example, as described in WO 2015/013313, WO 2006/110689, WO 2013/123503, or WO 2013/158879, incorporated herein in their entirety by reference.

5

In some embodiments, the VP2 is a VP2 from AAV serotype 1. In some embodiments, the VP2 is a VP2 from AAV serotype 2. In some embodiments, the VP2 is a VP2 from AAV serotype 3. In some embodiments, the VP2 is a VP2 from AAV serotype 4. In some embodiments, the VP2 is a VP2 from AAV serotype 5. In some embodiments, the VP2 is a VP2 from AAV serotype 6. In some embodiments, the VP2 is a VP2 from AAV serotype 7. In some embodiments, the VP2 is a VP2 from AAV serotype 8. In some embodiments, the VP2 is a VP2 from AAV serotype 9. In some embodiments, the VP2 is a VP2 from AAV serotype 10. In some embodiments, the VP2 is a VP2 from AAV serotype 11. In some

10

embodiments, the VP2 is a VP2 from AAV serotype 12. In some embodiments, the VP2 is a VP2 from AAV serotype 13. In some embodiments, the VP2 is a VP2 from AAV serotype rh10. In some embodiments, the VP2 is a VP2 from AAV serotype rh74.

- 5 In some embodiments, the VP2 is a VP2 exemplified by the AAV VP2 in **Table 4**, below:

Table 4: Exemplary wild-type amino acid sequences of AAV VP2

AAV Serotype	Exemplary wild-type VP2 amino acid sequence
1	TAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPPLGEPPAT PAAVGPTTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRIITSTRTWALP TYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGF RPKRLNFKLFNIQVKEVTNDGVTTIANNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFP ADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEEVPFHSSY AHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGSPAGMSVQPKNWLPGP CYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKFFPMSG VMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSDPATGDVHA MGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPPPQILIKNTPVPA NPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFT VDNNGLYTEPRPIGTRYLTRPL (SEQ ID NO: 11)
2	TAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPPLGQPPAA PSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITSTRTWALP TYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFR PKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPA DVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYA HSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCY RQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNGPAMASHKDDEEKFFPQSGVL IFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQG VLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANP STTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSNNVDFTVDT NGVYSEPRPIGTRYLTRNL (SEQ ID NO: 5)

AAV Serotype	Exemplary wild-type VP2 amino acid sequence
3b	TAPGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGDSESVDPDQPLGEPPAA PTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRTTSTRTWALP TYNNHLYKQISSQSGASNDNHYFGYSTPWGYDFNRFHCHFSPRDWQRLINNNWGFR PKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPA DVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVPFHSSY AHSQSLDRLMNPLIDQYLYLNRTQGTTSCTTNQSRLLFSQAGPQSMQLQARNWLPGP CYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVPNGPAMASHKDDEEKFFPMH GNLIFGKEGTTASNAELDNVMITDEEEIRTTNPVATEQYGTVANLQSSNTAPTTRTVND QGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMIKNTPVPA NPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYSKSVNVDFTV DTNGVYSEPRPIGTRYLTRNL (SEQ ID NO: 12)
5	TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQQLQIPAPASSLGADTMS AGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVTKSTRTWWLPSYNNHQYR EIKSGSVDGSNANAYFGYSTPWGYDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVK IFNIQVKEVTQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPQVFTLPQ YGYATLNRDNTENPTERSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLF KLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFGPMGRTQGWNLGSG VNRASVSATFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPG TTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIIVPGSV WMERDVYLQGPWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDV PVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRT TRPIGTRYLTRPL (SEQ ID NO: 13)
6	TAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPDQPLGEPPAT PAAVGPTTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTTSTRTWALP TYNNHLYKQISSASTGASNDNHYFGYSTPWGYDFNRFHCHFSPRDWQRLINNNWGF RPKRLNFKLFNIQVKEVTNDGVTTIANNLTSTVQVFSSEYQLPYVLGSAHQGCLPPFP ADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSY AHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGSPPAGMSVQPKNWLPGP CYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKDKFFPMSG VMIFGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSDPATGDVHV MGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPA NPPAEFSATKFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFT VDNNGLYTEPRPIGTRYLTRPL (SEQ ID NO: 14)

AAV Serotype	Exemplary wild-type VP2 amino acid sequence
7	TAPAKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVDPDPQLGEPPA APSSVSGSTVAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVTTSTRTWAL PTYNNHLYKQISSETAGSTNDNTYFGYSTPWGYFDNRFHCHFSRDLWQRLINNNWG FRPKKLRFLFNIQVKEVTTNDGVTTIANNLSTIQVFSSEYQLPYVLGSAHQGCLPPF PADVFMIPQYGYLTLNNGSQSVGRSSFYCLEYFPSQMLRTGNNFEFSYSFEDVPFHSS YAHSQSLDRLMNPLIDQYLYLARTQSNPGGTAGNRELQFYQGGPSTMAEQAKNWLP GPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDEDRFFP SSGVLIFGKTGATNKTTLENVMTNEEEIRTPNPVATEEYGVSSNLQAANTAAQTQVVN NQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPPQILIKNTPVP ANPPEVFTPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNFEKQTGVDF VDSQGVYSEPRPIGTRYLTRNL (SEQ ID NO: 15)
8	TAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVDPDPQLGEPPA APSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRTVTTSTRTWAL PTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDNRFHCHFSRDLWQRLINNNW GFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPP FPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSS SYAHSQSLDRLMNPLIDQYLYLSRTQTTGGTANTQTLGFSQGGPNTMANQAKNWLP GPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPS NGILIFGKQNAARDNADYSDVMTSEEEIKTTNPVATEEYGVADNLQQQNTAPQIGTVN SQGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPPQILIKNTPVP ADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSTSVDF VNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 16)
9	TAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVDPDPQPIGEPPAAP SGVGSGLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRTVTTSTRTWALPT YNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDNRFHCHFSRDLWQRLINNNWGF RPAKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSYQLPYVLGSAHEGCLPPF PADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVFPFHSS YAHSQSLDRLMNPLIDQYLYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPS YRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSG SLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQ NQGILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPMLGGFGMKHPPPQILIKNTPVP ADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFA VNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 17)

In some embodiments, the VP3 is a VP3 from AAV serotype 1. In some embodiments, the VP3 is a VP3 from AAV serotype 2. In some embodiments, the VP3 is a VP3 from AAV serotype 3. In some
embodiments, the VP3 is a VP3 from AAV serotype 4. In some embodiments, the VP3 is a VP3 from AAV
5 serotype 5. In some embodiments, the VP3 is a VP3 from AAV serotype 6. In some embodiments, the
VP3 is a VP3 from AAV serotype 7. In some embodiments, the VP3 is a VP3 from AAV serotype 8. In
some embodiments, the VP3 is a VP3 from AAV serotype 9. In some embodiments, the VP3 is a VP3
from AAV serotype 10. In some embodiments, the VP3 is a VP3 from AAV serotype 11. In some
embodiments, the VP3 is a VP3 from AAV serotype 12. In some embodiments, the VP3 is a VP3 from
10 AAV serotype 13. In some embodiments, the VP3 is a VP3 from AAV serotype rh10. In some
embodiments, the VP3 is a VP3 from AAV serotype rh74.

In some embodiments, the VP3 is a VP3 exemplified by the AAV VP3 in **Table 5**, below:

Table 5: Exemplary wild-type amino acid sequences of AAV VP3

AAV Serotype	Exemplary wild-type VP3 amino acid sequence
1	MASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYK QISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFK LFNIQVKEVTTNDGVTTIANLSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQ YGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEEVPPFHSSYAHSSQLDR LMNPLIDQYLYLNRTQNGSGSAQNKDLLFSRGSPPAGMSVQPKNWLPGPCYRQQRVS KTKTDNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDEDKFFPMSGVMIFGKESA GASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSTDPATGDVHAMGALPGMV WQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSAT KFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTE PRPIGTRYLTRPL (SEQ ID NO: 18)
2	MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYK QISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKL FNIQVKEVTQNDGTTTIANLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQ YGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPPFHSSYAHSSQLDR LMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSK TSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGS EKTNDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMV WQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSA

AAV Serotype	Exemplary wild-type VP3 amino acid sequence
	KFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN YNKS VNVDFTVDTNGVYSEP RPIGTRYLTRNL (SEQ ID NO: 6)
3b	MASGGGAPMADNNEGADGVGNSSGNWHCD SQWL GDRVIT TSTR TWALPT YNNHLYK QISSQSGASNDNHYFGYSTPWGYFDNRFHCHFS PRDWQRLINNNW GFRPKKLSFKL FNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQ YGYLT LNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVPFHSSYAHSQSLDR LMNPLIDQYLYYLNRTQGT TSGTTNQSRLFSQAGPQSMSLQARNWLPGPCYRQQRL SKTANDNNNSNFPWTAASKYHLNGRDSL VNPGPAMASHKDDEEKFFPMHGNLIFGKE GTTASNAELDNVMITDEEEIRTTNPVATEQYGTVAN NLQSSNTAPTTRTVNDQGALPGM VWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSP AKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSN YNKS VNVDFTVDTNGVYSE PRPIGTRYLTRNL (SEQ ID NO: 19)
5	MSAGGGGPLGDNNQGADGVGNASGDWHCDSTW MGDRVTKSTR TWLPSYNNHQ YREIKSGSVDGSNANAYFGYSTPWGYFDNRFHSHWSPRDWQRLINNYWGFRPRSLR VKIFNIQVKEVTVDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPQVFTL PQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVFPFHSSFAPSQ NLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFP GPMGRTQGWNL GSGVNRASVSATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPA NPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVP GSVWMERDVYLQGPIWAKIPETGAHFHPS PAMGGFGLKHPPPMMLIKNTPVPGNITSF SDVPVSSFITQYSTGQVT VEMEWELKKENS KRWNPEIQYTN NYNDPQFVDFAPDSTGE YRTRPIGTRYLTRPL (SEQ ID NO: 20)
6	MASGGGAPMADNNEGADGVGNASGNWHCDSTW LGDRVIT TSTR TWALPT YNNHLYK QISSASTGASNDNHYFGYSTPWGYFDNRFHCHFS PRDWQRLINNNW GFRPKRLNFK LFNIQVKEVTTNDGVT TIANNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQ YGYLT LNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDR LMNPLIDQYLYYLNRTQNQSGSAQNKDLLFSRGS PAGMSVQPKNWLPGPCYRQQRVS KTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESA GASNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSDPATGDVHVMGALPGMV WQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSAT

AAV Serotype	Exemplary wild-type VP3 amino acid sequence
	KFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTE RPIGTRYLTRPL (SEQ ID NO: 21)
7	VAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITSTRTWALPTYNNHLYK QISSETAGSTNDNTYFGYSTPWGYDFNRFHCHFSPRDWQRLINNNWGFRPKLRFKL FNIQVKEVTNDGVTIANNLSTIQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQY GYLTLNNGSQSVGRSSFYCLEYFPSQMLRTGNNFEFSYSFEDVPFHSSYAHSQSLDRL MNPIDQYLYLARTQSNPGGTAGNRELQFYQGGPSTMAEQAKNWLPGPCFRQQRVS KTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDEDRFFPSSGVLIFGKTGA TNKTTLENVMTNEEEIRPTNPVATEEYGIVSSNLQAANTAAQTQVVNNQGALPGMWV QNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPPQILIKNTPVPANPPEVFTPAK FASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNFEKQTGVDFAVDSQGVYSEP RPIGTRYLTRNL (SEQ ID NO: 22)
8	MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITSTRTWALPTYNNHLYK QISNGTSGGATNDNTYFGYSTPWGYDFNRFHCHFSPRDWQRLINNNWGFRPKRLSF KLFNIQVKEVTQNEGTIANNLSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIP QYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLD RLMNPIDQYLYLSRTQTTGGTANTQTLGFSQGGPNTMANQAKNWLPGPCYRQQRV STTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQN AARDNADYSDVMLTSEEEIKTNPVATEEYGIVADNLQQQNTAPQIGTVNSQGALPGMV WQNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPPQILIKNTPVPADPPTTFNQS KLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEP RPIGTRYLTRNL (SEQ ID NO: 23)

AAV Serotype	Exemplary wild-type VP3 amino acid sequence
9	MASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRTTSTRTWALPTYNNHLYK QISNSTSGGSSNDNAYFGYSTPWGYDFNRFHCHFSPRDWQRLINNNWGFRPKRLNF KLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSYQLPYVLGSAHEGCCLPPFPADVFMIP QYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSL DRLMNPLIDQYLYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRV TTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQG TGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQATGWVQNQGILPG MVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPQILIKNTPVPADPPTAF NKDKLNSFITQYSTGQVSVEIEWELQKENSkrwnPEIQYTSNYYKSNNVEFAVNTEGVY SEPRPIGTRYLTRNL (SEQ ID NO: 24)

In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 85% (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some

5 In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 86% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 87% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 88% identical to any one of the amino acid sequences of SEQ ID

10 NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 89% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 91% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 92% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 93% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some

15 In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 94% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 96% identical to any one of the amino acid sequences of SEQ ID

20

NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 97% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is at least 98% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1
5 comprises or consists of an amino acid sequence that is at least 99% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10. In some embodiments, the VP1 comprises or consists of an amino acid sequence that is identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.

10 In some embodiments, the VP1 has an amino acid sequence that is at least 85% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.

In some embodiments, the VP1 is inhibited from assembling by an assembly inhibitor.

15 In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 85% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some
20 embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 86% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 87% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 88% identical to any one of the amino acid sequences of SEQ ID
25 NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 89% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2
30 comprises or consists of an amino acid sequence that is at least 91% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 92% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 93% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some
35 embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 94% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an

- amino acid sequence that is at least 96% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 97% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 98% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is at least 99% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17. In some embodiments, the VP2 comprises or consists of an amino acid sequence that is identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
- 10 In some embodiments, the VP2 has an amino acid sequence that is at least 85% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.

In some embodiments, the VP2 is inhibited from assembling by an assembly inhibitor.

- 15 In some embodiments, the VP2 is a VP2 from AAV serotype 2, such as, for example, a VP2 with UniProt ID No.: P03135-2 (SEQ ID NO: 5).

- 20 In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 85% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 86% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 87% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 88% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 89% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 91% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 92% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 93% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 94% identical to

any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 96% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 97% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 98% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is at least 99% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24. In some embodiments, the VP3 comprises or consists of an amino acid sequence that is identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.

In some embodiments, the VP3 has an amino acid sequence that is at least 85% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%) identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.

In some embodiments, the VP3 is inhibited from assembling by an assembly inhibitor.

Ratio of viral capsid proteins

In some embodiments, the one or more populations of inhibited parvovirus viral capsid proteins and/or the one or more populations of assembled parvovirus capsids comprise a specific ratio between VP1, VP2 and/or VP3. In some embodiments, the one or more populations of inhibited parvovirus viral capsid proteins and/or the one or more populations of assembled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$.

In some embodiments, the assembled parvovirus capsids and/or filled parvovirus capsids comprise a specific ratio between VP1, VP2 and/or VP3. In some embodiments, the assembled parvovirus capsids and/or filled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$.

Further method steps

Purification

In some embodiments, the methods disclosed here further comprises a step of purifying, a step of formulating and/or a step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids.

The assembled parvovirus capsids and/or the filled parvovirus capsids disclosed herein may be purified

by any suitable purification method known in the art, such as, for example, chromatography, ultra-centrifugation, centrifugation, flocculation, filtration, and/or ultrafiltration/diafiltration.

In some embodiments, the method further comprises a step of purifying the assembled parvovirus capsids and/or the filled parvovirus capsids. In some embodiments, the step of purifying comprises chromatography, ultra-centrifugation, centrifugation, flocculation, filtration, and/or ultrafiltration/diafiltration. In some embodiments, the capsids are purified by chromatography. In some embodiments, the capsids are purified by ultra-centrifugation. In some embodiments, the capsids are purified by centrifugation. In some embodiments, the capsids are purified by flocculation. In some embodiments, the capsids are purified by filtration. In some embodiments, the capsids are purified by ultrafiltration/diafiltration

Formulation

Mixtures of the capsids described herein may be prepared in water suitably mixed with one or more excipients, carriers, and diluents. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

In some embodiments, the method further comprises a step of formulating the assembled parvovirus capsids and/or the filled parvovirus capsids into a formulation, optionally wherein the assembled parvovirus capsids and/or the filled parvovirus capsids are purified assembled parvovirus capsids and/or purified filled parvovirus capsids. In some embodiments, the step of formulating comprises formulating the assembled parvovirus capsids and/or the filled parvovirus capsids into a formulation with trehalose, sugar, sucrose, polyols, and/or dimethylsulfoxide. In some embodiments, the step of formulating comprises formulating the assembled parvovirus capsids and/or the filled parvovirus capsids into a formulation with a pharmaceutically acceptable carrier, diluent and/or excipient. In some embodiments, the pharmaceutically acceptable carrier, diluent and/or excipient is selected from the group consisting of tween 20, tween 80, trehalose, Pluronic F68, sorbitol and/or glycerol, such as 0.0001 to 0.01% Pluronic F68, 1 to 10 % sorbitol and/or 1 to 10% glycerol.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions and dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions and dispersions. In any case the formulation may be sterile and may be fluid to the extent that easy syringability exists. Formulations may be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent and dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils.

Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars and sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Furthermore, the capsids described herein can be formulated into pharmaceutical compositions for administration to a patient in a biologically compatible form suitable for administration *in vivo*. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations may meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biologics standards.

A pharmaceutical composition containing, for example, a capsid described herein would typically include a pharmaceutically acceptable diluent and carrier. A pharmaceutical composition may include (e.g., consist of), e.g., a sterile saline solution and a nucleic acid. The sterile saline is typically a pharmaceutical grade saline. A pharmaceutical composition may include (e.g., consist of), e.g., sterile water and a nucleic acid. The sterile water is typically a pharmaceutical grade water. A pharmaceutical composition may include (e.g., consist of), e.g., phosphate-buffered saline (PBS) and a nucleic acid. The sterile PBS is typically a pharmaceutical grade PBS.

In certain embodiments, pharmaceutical compositions include one or more capsids and one or more excipients. In certain embodiments, excipients are selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone.

In certain embodiments, capsids may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions and formulations. Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, extent of disease to be treated, and dose to be administered.

In certain embodiments, pharmaceutical compositions including a capsid encompass any pharmaceutically acceptable salts of the inhibitor, esters of the inhibitor, and salts of such esters. In certain embodiments, pharmaceutical compositions including a capsid, upon administration to a subject

(e.g., a human), are capable of providing (directly and indirectly) the polynucleotide molecule (e.g., payload). Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of inhibitors, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. In certain embodiments, prodrugs include one or more conjugate group attached to a capsid, wherein the conjugate group is cleaved by endogenous nucleases within the body.

In certain embodiments, pharmaceutical compositions include a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical compositions including those including hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

In certain embodiments, pharmaceutical compositions include one or more tissue-specific delivery molecules designed to deliver the one or more pharmaceutical agents of the present invention to specific tissues and cell types. For example, in certain embodiments, pharmaceutical compositions include liposomes coated with a tissue-specific antibody.

In certain embodiments, pharmaceutical compositions include a co-solvent system. Certain of such co-solvent systems include, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. In certain embodiments, such co-solvent systems are used for hydrophobic compounds. A non-limiting example of such a co-solvent system is the VPD co-solvent system, which is a solution of absolute ethanol including 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™ and 65% w/v polyethylene glycol 300. The proportions of such co-solvent systems may be varied considerably without significantly altering their solubility and toxicity characteristics. Furthermore, the identity of co-solvent components may be varied: for example, other surfactants may be used instead of Polysorbate 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars and polysaccharides may substitute for dextrose.

In certain embodiments, pharmaceutical compositions are prepared for oral administration. In certain embodiments, pharmaceutical compositions are prepared for buccal administration. In certain embodiments, a pharmaceutical composition is prepared for administration by injection (e.g., intraocular (e.g., intravitreal), intravenous, subcutaneous, intramuscular, intrathecal, intracerebroventricular, etc.). In certain of such embodiments, a pharmaceutical composition includes a carrier and is formulated in aqueous solution, such as water and physiologically compatible buffers such as Hanks's solution, Ringer's solution, and physiological saline buffer. In certain embodiments, other ingredients are included

(e.g., ingredients that aid in solubility and serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules and in multi-dose containers. Certain pharmaceutical compositions for injection are suspensions, solutions, and emulsions in oily and aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate and triglycerides, and liposomes.

10 **Storage**

The assembled parvovirus capsids and/or the filled parvovirus capsids disclosed herein may be stored under conditions that maintain capsid integrity. For example, storage conditions may include temperature, state of the capsid (e.g., solid, aerosolized, lyophilized), storage reagents, pH, and pressure.

- 15 In some embodiments, the method further comprises a step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids, optionally wherein the storing is under conditions that maintain capsid integrity. In some embodiments, the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids is at room temperature. In some embodiments, the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids is at 1 °C to 25 °C, such as 1 °C to 20 °C, such as 5 °C to 25 °C, such as 10 °C to 25 °C, such as 15 °C to 25 °C. In some embodiments, the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids comprises lyophilizing and aerosolizing the assembled parvovirus capsids and/or the filled parvovirus capsids. In some embodiments, the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids at a low pH or a high pH. In some embodiments, the method comprises a step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids under pressure.

Rep Proteins

Filling of the capsids described herein may include filling with one or more Rep proteins, which may be a synthetic Rep protein or a naturally occurring Rep protein. In some embodiments, the synthetic Rep protein is an engineered protein and/or a protein composed of synthetic amino acids.

In some embodiments, Rep protein is a recombinant Rep protein and/or a recombinantly produced Rep protein.

- 35 In some embodiments, the Rep protein is a purified Rep protein. In some embodiments, the Rep protein is purified from *Escherichia Coli*.

In some embodiments, the Rep protein comprises multiple Rep proteins, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Rep proteins. In some embodiments, the step of providing a Rep protein is a step of providing multiple Rep proteins, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Rep proteins

5

In some embodiments, the Rep protein is a synthetic Rep protein. In some embodiments, the Rep protein is produced in an eukaryotic microbial system, such as yeast, such as a methylotrophic yeast. In some embodiments, the Rep protein is produced in an eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*. In some embodiments, the Rep protein is produced in an prokaryotic microbial system, such as *E. Coli*. In some embodiments, the Rep protein is produced in a mammalian cell system, such as HEK, CHO, HeLa or Vero cells, an insect cell system, such as Sf9, Sf21 or High Five cells, a plant system, such as tobacco BY, an algae system, a metazoan cell system, a zebrafish and/or cell-free expression systems

10

In some embodiments, the one or more Rep proteins is a Rep protein of Parvoviridae (e.g., Densovirinae, Hamaparvovirinae, or Parvovirinae). In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Densovirinae, Hamaparvovirinae, or Parvovirinae. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Densovirinae. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Hamaparvovirinae.

15

In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Parvovirinae

20

(e.g., a Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus). In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Amdoparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Artiparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Aveparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Bocaparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Copiparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Dependoparvovirus (e.g., an AAV). In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Erythroparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Loriparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Protoparvovirus. In some embodiments, the one or more Rep proteins is a Rep protein is a Rep protein of Tetraparvovirus.

25

30

35

In some embodiments, the one or more Rep proteins may include Rep40, Rep52, Rep68, and Rep78. In some embodiments, the one or more Rep proteins may include AAV Rep40, AAV Rep52, AAV Rep68, and AAV Rep78.

- 5 In some embodiments, one or more Rep proteins is a Rep protein from an AAV. In some embodiments, the AAV is an AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, rh10 AAV, rh.39 AAV, or any other AAV now known and later discovered. For example, in some embodiments, the one or
- 10 more Rep proteins is a Rep protein from an AAV type 1. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 2. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 3. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 4. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 5. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 6. In
- 15 some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 7. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 8. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 9. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 10. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 11. In some embodiments, the one or more Rep proteins is a
- 20 Rep protein from an AAV type 12. In some embodiments, the one or more Rep proteins is a Rep protein from an AAV type 13. In some embodiments, the one or more Rep proteins is a Rep protein from an avian AAV. In some embodiments, the one or more Rep proteins is a Rep protein from a bovine AAV. In some embodiments, the one or more Rep proteins is a Rep protein from a canine AAV. In some embodiments, the one or more Rep proteins is a Rep protein from a goat AAV. In some embodiments,
- 25 the one or more Rep proteins is a Rep protein from a snake AAV. In some embodiments, the one or more Rep proteins is a Rep protein from an equine AAV. In some embodiments, the one or more Rep proteins is a Rep protein from an ovine AAV. In some embodiments, the one or more Rep proteins is a Rep protein from a goat AAV. In some embodiments, the one or more Rep proteins is a Rep protein from a shrimp AAV. In some embodiments, the one or more Rep proteins is a Rep protein from a rh10 AAV.
- 30 In some embodiments, the one or more Rep proteins is a Rep protein from a rh74 AAV. In some embodiments, the one or more Rep proteins is a Rep protein from an any other AAV now known and later discovered.

- In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74. For
- 35

example, in some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 1. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 2. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 3. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 4. In some
5 embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 5. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 6. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 7. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 8. In some
10 embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 9. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 10. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 11. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 12. In some
15 embodiments, the one or more Rep proteins is a Rep protein from AAV serotype 13. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype rh10. In some embodiments, the one or more Rep proteins is a Rep protein from AAV serotype rh74.

Any suitable combination of one or more Rep proteins may be used in the methods described herein. For example, in some embodiments, the one or more Rep proteins is Rep40. In some embodiments, the one or more Rep proteins is Rep52. In some embodiments, the one or more Rep proteins is Rep40 and
20 Rep52. In some embodiments, the one or more Rep proteins is Rep68. In some embodiments, the one or more Rep proteins is Rep78. In some embodiments, the one or more Rep proteins is Rep68 and Rep78. In some embodiments, the one or more Rep proteins is Rep40, Rep52, Rep68, and Rep78. In some embodiments, the one or more Rep proteins is Rep40, Rep52, and Rep68. In some embodiments, the one or more Rep proteins is Rep40, Rep52, and Rep78.

25 In some embodiments, the one or more Rep proteins is AAV Rep40. In some embodiments, the one or more Rep proteins is AAV Rep52. In some embodiments, the one or more Rep proteins is AAV Rep40 and AAV Rep52. In some embodiments, the one or more Rep proteins is AAV Rep68. In some
30 embodiments, the one or more Rep proteins is AAV Rep78. In some embodiments, the one or more Rep proteins is AAV Rep68 and AAV Rep78. In some embodiments, the one or more Rep proteins is AAV Rep40, AAV Rep52, AAV Rep68, and AAV Rep78. In some embodiments, the one or more Rep proteins is AAV Rep40, AAV Rep52, and AAV Rep68. In some embodiments, the one or more Rep proteins is AAV Rep40, AAV Rep52, and AAV Rep78.

35 In some embodiments, the Rep40, Rep52, Rep68, and/or Rep78 are from different AAV serotypes. In some embodiments, the Rep40, Rep52, Rep68, and/or Rep78 are from the same AAV serotypes.

[illegible]

In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 35. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 36. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 37. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of

SEQ ID NO: 38. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 39. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 40. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 41. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 42. In some embodiments, the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 43.

For example, in some embodiments, the Rep protein is a Rep protein exemplified by the AAV Rep proteins in Table 6, below:

10

Table 6: Exemplary AAV Rep Proteins

Rep Protein	Exemplary UniProt ID Number
Rep40	Q89269
Rep52	Q89270
Rep68	P03132
Rep78	Q89268

In some embodiments, the Rep protein concentration is between 0.01 and 100 μM , such as between 0.01 and 10 μM , between 0.01 and 5 μM , between 0.01 and 2.5 μM , between 0.01 and 1 μM , between 0.01 and 0.5 μM , between 0.01 and 0.25 μM , between 0.01 and 0.1 μM , between 0.1 and 100 μM , between 0.1 and 10 μM , between 0.1 and 5 μM , between 0.1 and 2.5 μM , between 0.1 and 1 μM , between 0.1 and 0.5 μM , between 0.1 and 0.25 μM , between 0.25 and 100 μM , between 0.25 and 10 μM , between 0.25 and 5 μM , between 0.25 and 2.5 μM , between 0.25 and 1 μM , between 0.25 and 0.5 μM , between 0.5 and 100 μM , between 0.5 and 10 μM , between 0.5 and 5 μM , between 0.5 and 2.5 μM , between 0.5 and 1 μM , between 1 and 100 μM , between 1 and 10 μM , between 1 and 5 μM , between 1 and 2.5 μM , between 2.5 and 100 μM , between 2.5 and 10 μM , between 2.5 and 5 μM , between 5 and 100 μM , between 5 and 10 μM , or such as wherein the Rep protein concentration is between 10 and 100 μM . In some embodiments, the Rep protein concentration is 0.1 to 10 μM , such as 0.1 to 9 μM , such as 0.1 to 8 μM , such as 0.1 to 7 μM , such as 0.1 to 6 μM , such as 0.1 to 5 μM , such as 0.5 to 5 μM , such as 0.5 to 4 μM , such as 0.5 to 3 μM , such as 0.5 to 2 μM , such as 0.5 to 1.5 μM . In some embodiments, the Rep protein concentration is 1 μM .

ITR

In some embodiments, the ITR1 and/or the ITR2 are from a Parvoviridae. In some embodiments, the ITR1 and/or the ITR2 are from a Densovirinae, Hamaparvovirinae, or Parvovirinae. In some

30

embodiments, the ITR1 and/or the ITR2 are from a Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus. In some embodiments, the ITR1 and/or the ITR2 are from a Dependoparvovirus. In some embodiments, the ITR1 and/or the ITR2 are from an AAV. In some embodiments, the ITR1 and/or the ITR2 are from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74. In some embodiments, the ITR1 and/or the ITR2 are from AAV serotype 2. In some embodiments, the VP1, VP2 and/or VP3, the Rep protein, and/or ITR1 and/or ITR2 are from different AAV serotypes. In some embodiments, the VP1, VP2 and/or VP3, the Rep protein, and/or ITR1 and/or ITR2 are from the same AAV serotype.

AAV Rep fusion proteins

In some embodiments, the present disclosure relates to an AAV Rep fusion protein comprising:

- a. An AAV Rep domain or a AAV Rep protein, and
- b. A cleavable amino acid sequence, such as a SUMO tag.

In some embodiments, the AAV Rep protein is selected from the group consisting of Rep40, Rep52, Rep68 and/or Rep78. In some embodiments, the AAV Rep fusion protein further comprises a purification tag, such as a His-tag, a C-tag (EPEA), a streptavidin tag and/or biotin tag.

In some embodiments, the AAV Rep protein is as defined elsewhere in the present disclosure.

In some embodiments, the present disclosure relates to a fusion protein, such as a MBP-Rep78 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 35, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 35.

In some embodiments, the present disclosure relates to a fusion protein, such as a MBP-Rep78 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep40 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 36, such as 81%, such as 82%, such as 83%, such as 84%, such

as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 36.

- 5 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep40 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 36.

- 10 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep52 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 37, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 37.

- 15 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep52 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 37.

- 20 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep68 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 38, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 38.

- 25 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep68 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 38.

- 30 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep78 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 39, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 39.

- 35 In some embodiments, the present disclosure relates to a fusion protein, such as a SUMO-Rep78 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep40 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 40, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 40.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep40 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 40.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep52 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 41, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 41.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep52 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 41.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep68 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 42, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 42.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep68 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 42.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep78 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 43, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as

99% sequence identity to the amino acid sequence of SEQ ID NO: 43.

In some embodiments, the present disclosure relates to a fusion protein, such as a Smt3-Rep78 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 43.

5

Vectors encoding AAV Rep fusion proteins and method of producing AAV Rep fusion proteins

In some embodiments, the present disclosure relates to a vector encoding the AAV Rep fusion protein according to the present disclosure.

10 In some embodiments, the present disclosure relates a method of producing the AAV Rep fusion protein according to the present disclosure s, comprising the steps of:

- a. Transforming a prokaryotic microbial system, such as *E. coli*, with the vector encoding the AAV Rep fusion protein according to the present disclosure; and
- b. Incubating the prokaryotic microbial system, such as *E. coli*,
15 thereby producing the AAV Rep fusion protein.

In some embodiments, the present disclosure relates a method of producing the AAV Rep fusion protein according to the present disclosure, comprising the steps of:

- a. Transforming an eukaryotic microbial system, such as yeast, such as *P. pastoris* or *S. cerevisiae*, with the vector encoding the AAV Rep fusion protein according to the present disclosure,
20
- b. Incubating the eukaryotic microbial system, such as *P. pastoris*, or *S. cerevisiae*, thereby producing the AAV Rep fusion protein.

25 In some embodiments, the method further comprises a step of inoculating the prokaryotic microbial system or the eukaryotic microbial system. In some embodiments, the method further comprises a step of purifying the fusion protein. In some embodiments, the eukaryotic microbial system is a methylotrophic yeast.

30 **AAV VP fusion protein**

In some embodiments, the present disclosure relates an AAV VP fusion protein comprising:

- a. An AAV VP domain or an AAV VP protein, and
- b. A cleavable amino acid sequence, such as a SUMO tag.

35 In some embodiments, the AAV VP protein is selected from the group consisting of AAV VP1, AAV VP2,

and/or AAV VP. In some embodiments, the AAV VP fusion protein further comprises a purification tag, such as a His-tag, a C-tag (EPEA), a streptavidin tag and/or biotin tag.

In some embodiments, the AAV VP protein comprises:

- 5 a. A purification tag, such as a His-tag,
- b. A cleavable amino acid sequence, such as a SUMO tag,
- c. An AAV VP protein, such as AAV VP1, AAV VP2 and/or AAV VP3,
- d. A thrombin cleavage site,
- e. A cleavable amino acid sequence, such as a SUMO tag, and
- 10 f. A purification tag, such as C-tag (EPEA).

In some embodiments, the AAV VP protein is as defined elsewhere in the present disclosure.

Vectors encoding AAV VP fusion proteins and method of producing AAV VP fusion proteins

- 15 In some embodiments, the present disclosure relates to a vector encoding the AAV VP fusion protein according to the present disclosure.

In some embodiments, the present disclosure relates to a method of producing a method of producing the AAV VP fusion protein according to the present disclosure, comprising the steps of:

- 20 a. Transforming a prokaryotic microbial system, such as *E. coli*, with the vector encoding the AAV VP fusion protein according to the present disclosure; and
- b. Incubating the prokaryotic microbial system, such as *E. coli*, thereby producing the AAV VP fusion protein.

- 25 In some embodiments, the present disclosure relates to a method of producing the AAV fusion protein according to the present disclosure, comprising the steps of:

- a. Transforming a eukaryotic microbial system, such as yeast, such as *P. pastoris* or *S. cerevisiae*, with the vector encoding the AAV VP fusion protein according to the present disclosure
- 30 b. Incubating the eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*, thereby producing the AAV fusion protein.

- In some embodiments, the method further comprises a step of inoculating the prokaryotic microbial system or the eukaryotic microbial system. In some embodiments, the method further comprises a step of
- 35 purifying the fusion protein. In some embodiments, the eukaryotic microbial system is a methylotrophic

yeast.

Kit of parts

In some embodiments, the present disclosure relates to a kit of parts comprising:

- 5 a. one or more populations of inhibited parvovirus viral capsid proteins of the present disclosure;
- b. an assembly inhibitor of the present disclosure;
- c. an assembly activator of the present disclosure; and
- 10 d. optionally instructions for use.

In some embodiments, the present disclosure relates to a kit of parts comprising:

- a. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins of the present disclosure;
- b. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain of
- 15 the present disclosure;
- c. a Rep protein of the present disclosure; and
- d. optionally instructions for use.

In some embodiments, the present disclosure relates to a kit of parts comprising:

- a. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins of the present disclosure;
- 20 b. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain of the present disclosure;
- c. a Rep protein of the present disclosure;
- d. a reaction mixture of the present disclosure; and
- 25 e. optionally instructions for use.

In some embodiments, the present disclosure relates to a kit of parts comprising:

- a. one or more populations of inhibited parvovirus viral capsid proteins of the present disclosure;
- b. an assembly inhibitor of the present disclosure;
- 30 c. an assembly activator of the present disclosure;
- d. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain of the present disclosure;
- e. a Rep protein of the present disclosure; and
- f. optionally instructions for use.

In some embodiments, the present disclosure relates to a kit of parts comprising:

- a. one or more populations of inhibited parvovirus viral capsid proteins of the present disclosure;
- b. an assembly inhibitor of the present disclosure;
- 5 c. an assembly activator of the present disclosure;
- d. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain of the present disclosure;
- e. a Rep protein of the present disclosure;
- f. a reaction mixture of the present disclosure; and
- 10 g. optionally instructions for use.

Population of assembled or filled capsids

In some embodiments, the present disclosure relates to a population of assembled parvovirus capsids obtainable by the method according to the present disclosure. In some embodiments, the population of assembled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$. In some embodiments, the population of assembled parvovirus capsids comprises VP1, VP2 and VP3 in a ratio of 1:1:10 (VP1:VP2:VP3).

In some embodiments, the present disclosure relates to a population of filled parvovirus capsids obtainable by the method according to the present disclosure. In some embodiments, the population of filled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$. In some embodiments, the population of filled parvovirus capsids comprises VP1, VP2 and VP3 in a ratio of 1:1:10 (VP1:VP2:VP3).

Alternative methods for *in vitro* capsid filling

In some embodiments, the present disclosure relates to a method of *in vitro* filling parvovirus capsids, comprising:

- a) providing:
 - i) a population of assembled parvovirus capsids comprising one or more parvovirus viral proteins; and
 - 30 ii) a nucleic acid molecule; and
- b) combining the population of assembled parvovirus capsids and the nucleic acid molecule comprising the nucleic acid molecule under conditions which allow the encapsidation of the nucleic acid molecule into the assembled capsids, wherein at least 1% of the population of assembled capsids comprise capsids encapsidating one or more nucleic acid molecules,
- 35

thereby manufacturing capsids.

In some embodiments, the present disclosure relates to a method of *in vitro* filling a method of manufacturing capsids, comprising:

- 5 (a) providing:
 - (i) a population of assembled parvovirus capsids comprising parvovirus viral capsid proteins;
 - (ii) a set of conditions which allow the denaturation of the assembled parvovirus capsids;
 - and
 - 10 (iii) a nucleic acid molecule (e.g., containing a transgene of interest); and
 - (b) combining the population of assembled parvovirus capsids and the nucleic acid molecule comprising the nucleic acid molecule under conditions which denature the assembled capsids; and
 - (c) contacting an activator and the denatured capsids comprising parvovirus capsid proteins,
 - 15 wherein the activator allows the re-assembly of capsids comprising parvovirus capsid proteins into assembled capsids and allows the encapsidation of the nucleic acid molecule into the assembled capsids,
 - thereby manufacturing capsids.
- 20 In some embodiments, step
- (a) further comprises providing
 - (iv) one or more Rep proteins, and wherein step
 - (c) further comprises contacting an activator and the denatured capsids, wherein the activator
 - allows the re-assembly of capsids comprising parvovirus capsid proteins into assembled capsids
 - 25 and allows filling of the assembled capsids with the nucleic acid molecule by the one or more Rep proteins.

In some embodiments the denaturation is thermally-induced denaturation and/or chemically-induced denaturation. In some embodiments, denaturation include a set of conditions which allow the denaturation

30 of parvovirus capsids. In some embodiments, denaturation conditions may include conditions selected from the list including pH, sonication, ionic strength and/or conductivity, or temperature. In some embodiments, denaturation conditions include the factor of pH. In some embodiments, denaturation conditions include sonication. In some embodiments, denaturation conditions include the factor of ionic strength and/or conductivity. In some embodiments, denaturation conditions include the factor of

35 temperature. Denaturation can be performed by any method known in the art.

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used and evaluated and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

Example 1: Scalable, lysate based capsid filling

Introduction

Adeno-Associated Virus (AAV) is the most widely used viral vector for gene therapy and the leading viral vector for direct in-vivo clinical applications (Wang, 2019). AAV consists of two major components: A protein capsid, and a single-stranded DNA genome. In a clinical setting, the viral genome can be replaced by an Inverted Terminal Repeat (ITR) flanked Gene of Interest (GoI). AAV first assembles an icosahedral procapsid, genome packaging proteins, Rep protein, bind to the capsid creating a portal at the 5-fold vertex. Rep proteins demonstrate helicase and nuclease activity, translocating single-stranded DNA into the capsid, in an ATP- dependent fashion. A major challenge in AAV drug development is achieving a high proportion of vectors that are packaged with DNA – also referred to as filled capsids. In industrial processes, most viral capsids will often not contain genomic material, and filled capsid yields are highly variable (Heldt, et al., 2023). It has been postulated that this could be due to the method these vectors are produced: In cell-based expression systems. Such systems are (1) inherently complex; (2) highly variable in the sense that minor process variations can have an amplified effect on drug product quality; (3) not solely geared to the production of a therapeutic agent, rendering such platforms highly inefficient for production of viral vectors.

Based on the above, the present inventors have hypothesized that producing AAV in an *in vitro* (i.e., cell-free) system can produce a high proportion of filled capsids in a consistent and predictable manner. Zhou *et al* (Zhou, 1998) conducted a study to understand the mechanisms of AAV propagation by AAV capsid filling using a cell lysate-based system.. The work described by Zhou et al is not an ideal capsid filling process and is not implementable for drug development or clinical applications due to the following limitations:

- Requirement of adherent cells, rendering the method poorly scalable.
- Requirement of medium containing serum, rendering the method incompatible with GMP-grade production
- Requirement of medium containing antibiotics, rendering the method incompatible with GMP-grade production

- Requirement of pre-infection with Adenovirus to enable AAV protein expression, rendering the method less compatible with GMP-grade production
 - Requirement of ultra-gradient centrifugation, rendering the method poorly scalable and incompatible with GMP-grade production.
- 5 • Lack of analytical resolution for process monitoring or making quantitative conclusions about product quality.

The present invention addresses the above limitations and provides an improved *in vitro* (i.e., cell free) method of filling capsids. The present invention relates to a scalable, clinically relevant cell-free (*in vitro*) AAV capsid filling method as shown in Figure 1. The *in vitro* filling method of the present invention has the following advantages:

- Increased process scalability
 - GMP compatibility
 - Increased capsid filling yield.
 - Use of quantitative assays (ELISA and qPCR) for process monitoring and assessing filling efficiency.
- 10
- 15

Equipment and materials

Equipment

Operation	Unit (model and manufacturer)
Incubator	Humidified, CO ₂ incubator. ThermoFisher
Shake platform	Multi-platform shaker (15594060). Fisher Scientific.
qPCR analysis	Quant studio 5. ThermoFisher Scientific
Thermomixer	Thermomixer. ThermoFisher Scientific
Plate reader	Genios Pro. Tecan
Cell counter	Vi-CELL XR. Beckman Coulter

Materials

Material type	Cat. no and/or source
VPC 2.0 cells	Suspension-based HEK293 cells, adapted for virus production. ThermoFisher Scientific
AAV2 Rep and capsid plasmid	VPK-402. Cell Biolabs
AAV Helper plasmid	VPK-422. Cell Biolabs
AAV GFP plasmid	AAV-400. Cell Biolabs

NEB-DH5a cells	Synvolux B.V.
NEB-stable cells	Synvolux B.V.
Viral Production Medium	A4817902, Gibco
GlutaMAX	35050061, Gibco
Maxi Prep kit	K210006, Invitrogen
AAV Max transfection kit	A50515, Gibco
Baffled shake flasks 500mL	4110-0500, ThermoFisher Scientific
Baffled shake flasks 1L	4110-1000, ThermoFisher Scientific
Vented cell culture shake flasks 125mL - 2000mL	4115-0125, 4115-0250, 4115-0500, 4115-1000, 4115-2000
qPCR kit	6233, Takara
dNTP set	10297018, Invitrogen
NTP set	11863933, Invitrogen
Creatine Phosphokinase (CPK) from rabbit muscle, Type 1	Merck -C3755-3.5KU; Source SLCK 9545; Cas No.: 9001-15-4
Creatine phosphate disodium salt	Thermo Scientific Alfa Aesar: A15362.06
AAV2 ELISA kit	PRAAV2XP, Progen

Methods

Preparative procedures

Component generation

5

Plasmid generation

Three plasmids were purchased from Cell Biolabs:

- pAAV-RC2 (cat no.: VPK-402): Containing the AAV2 Rep gene and the AAV2 Cap gene. The annotated sequence is listed in "Sequence Overview" (SEQ ID NO: 25).
- pHelper Vector (cat no.: VPK-422): Containing the Adenovirus E2A gene; the Adenovirus E4 gene; the Adenovirus VA gene. The annotated sequence is listed in "Sequence Overview" (SEQ ID NO: 26).
- pAAV-GFP Control Vector (cat no.: AAV-400): Containing GFP reporter gene; a CMV promotor region; a Poly A sequence; and two flanking ITR regions. The annotated sequence is listed in "Sequence Overview" (SEQ ID NO: 27).

15

E. coli transformations for all vectors were performed by Synvolux BV (Leiden, The Netherlands). pAAV-RC2 and pHelper were transformed into NEB-DH5 α cells; pAAV-GFP was transformed into NEB-stable cells (data not shown).

Plasmids were amplified through fermentation as follows:

- 5 • +/- 10 μ L of glycerol stock was used to inoculate LB medium with 0.1mg/mL ampicillin in baffled Nalgene shake flasks. For pAAV-RC2, this was done using a 1L flask with 400mL medium; for pAAV-GFP and pHelper this was done with 2 x 500mL with 200mL medium.
- Fermentation was performed at 30°C, 180 rpm for 20 hours.
- For plasmid purification, a Maxi Prep procedure (ThermoFisher Scientific, 2011) was followed,
10 with the following observations and deviations:
 - Pellets post-harvest centrifugation for pAAV-GFP and pHelper were significantly larger than for the other plasmids. This is likely due to the culture being overgrown, thus having more biomass.
 - Due to a lack of resuspension buffer (R3), 10mL of dPBS was added to 30mL of R3 to
15 achieve the minimum total resuspension volume. An excess of RNAase (100 μ L) was added to this 10mL dPBS fraction.
 - Lysis was carried out for 7 minutes, instead of 5 minutes.
 - Plasmid were resuspended in 500 μ L of TE buffer.
- Plasmid yields were as follows:
20
 - pAAV-RC2: 1086.1 ng/ μ L
 - pAAV-RC5: 2826.3 ng/ μ L
 - pAAV-GFP: 446.2 ng/ μ L
 - pHelper: 747.9 ng/ μ L
- Plasmids were diluted in TE buffer to a target concentration of 0.25mg/mL (250ng/ μ L).

25

Cell lysate generation

- 30 VPC 2.0 cells were expanded for five passages in Viral Production Medium with GlutaMax. Incubation was at 8% CO₂, 37°C, 85% RH, and 120rpm (10mm orbital diameter). On day 2 of the fifth passage, the Viable Cell Density (VCD) was 4.75 x 10⁶ VC/mL; with a viability of 98.7%. This was diluted in pre-warmed medium to a concentration of 3 x 10⁶ VC/mL. For transfection the AAV-MAX Helper-Free AAV Production System guide was used (Gibco, 2023), with the following notes:

- Culture volume: 240mL (in 2L unbaffled vented shake flask)
- VCD: 3×10^6 VC/mL
- AAV Max enhancer: 2400 μ L
- Viral plex complexation buffer volume: 24mL
- 5 • Transfection booster: 720 μ L
- Transfection reagent: 1440 μ L
- pHelper: 556 μ L
- pAAV-RC2: 884 μ L
- Complexation incubation time: 25 minutes

10

After 70 hours, VCD was 4.45×10^6 VC/mL and the culture viability was 86.5%. The culture was then split into two 125mL cultures in 1L shake flasks. The first flask was treated with 12.5mL AAV Max lysis buffer; the second flasks was treated with 12.5mL AAV Max lysis buffer, 250 μ L 1M MgCl₂, and 500 μ L of Pierce nuclease solution (@25kU/mL).

- 15 After a two-hour incubation at 37°C and 120rpm, lysates were centrifuged in 50mL Greiner tubes at 4500xg, 30 minutes, 4°C.

Glycerol was added to each aliquot to achieve 20% v/v concentration. Material was stored at -20 $\pm$ 5°C. Capsid concentration was determined as described under "Capsid quantification". Note, Rep concentration was not determined.

- 20 Upon use for this work, one 55mL aliquot (lacking nuclease) was thawed and distributed in 2mL aliquots. One of these aliquots was required for this experiment. The remaining aliquots were stored at -20°C.

Capsid AAV Packaging Matrix preparation

Stock solution preparation

- 25 The following (stock) solutions were prepared:

- HEPES 30mM, pH adjusted with NaOH and HCl to 7.5, 0.2 μ m sterile filtered, stored at RT.
- DTT 0.87M in milliQ water. 0.2 μ m sterile filtered, stored at -20 $\pm$ -5°C.
- Creatine Phosphate (CP) disodium salt tetrahydrate 0.2M; in aforementioned HEPES buffer. 0.2 μ m sterile filtered, stored at -20 $\pm$ -5°C.

- 30 • Creatine Phosphokinase (CPK) from rabbit muscle, Type 1 (1mg/mL; 310u/mL); Merck -C3755-3.5KU; Source SLCK 9545; Cas No.: 9001-15-4; in aforementioned HEPES buffer. 0.2 μ m sterile filtered, stored at -20 $\pm$ -5°C.

Inactive CAPM preparation

20mL inactive CAPM (lacking enzymes) medium was prepared as described in Table 7.

Table 7: Inactive CAPM components

Component	Volume	Final concentration
HEPES 30mM, pH 7.5	14818µL	7mM
MgCl ₂ 1M	140µL	0.5mM
DTT 0.87 M	11.5µL	0.1mM
dATP	20µL	0.1mM
dGTP	20µL	0.1mM
dCTP	20µL	0.1mM
dTTP	20µL	0.1mM
ATP	80µL	0.4mM
GTP	40µL	0.2mM
CTP	40µL	0.2mM
UTP	40µL	0.2mM
CP solution, 0.2M	4000µL	40mM

5

Inactive CAPM was *not* filtered and stored in 1.925 mL aliquots at -20+/-5°C.

In vitro capsid filling

To activate CAPM medium, 75µL CPK solution was added to 1.925 mL inactive CAPM.

10

Six experimental conditions were performed (Table 8):

Table 8: Cell free capsid filling conditions

Condition	Active CAPM volume	HEPES volume	CCL volume	Plasmid volume	Plasmid addition concentration	Plasmid final concentration
XX: Negative control with buffer only	230µL	270µL	0	0	0	0
X0: Negative	230µL	40µL	230µL	0	0	0

control with CCL						
0.1X Plasmid	230µL	0	230µL	40µL	0.25µg/mL	0.02µg/mL
1X Plasmid	230µL	0	230µL	40µL	2.5µg/mL	0.2µg/mL
10X Plasmid	230µL	0	230µL	40µL	25µg/mL	2µg/mL
100X Plasmid	230µL	0	230µL	40µL	250µg/mL	20µg/mL

Analytics

Capsid quantification

- 5 Progen's AAV2 Xpress ELISA kit protocol was used to determine capsid concentration (Progen, 2023), with the following notes:
- Measurement wavelength: 450nm
 - Reference wavelength: 620nm
 - Measurement performed on a Tecan Genios Pro plate reader.
- 10
- Duplicate samples
 - Undiluted samples and 100-fold diluted samples were measured.

Results are shown in Figure 2.

15 *Genome copy quantification*

Takara's AAVpro® Titration Kit (for Real Time PCR) Ver.2 kit protocol was used to determine genome copy concentration (Takara, 2023), with the following notes:

- During DNA extraction (protocol step VI-2) extravirion DNA impurities are digested using DNase, before capsid lysis.
- 20
- To test for extravirion DNA digestion efficiency using DNase two samples of pure pGFP were added (250µg/mL) as negative controls: one to be measured directly without DNase digestion, and one digested as per the protocol.
 - Measurement was performed on a ThermoFisher Scientific QuantStudio5 Real-Time PCR System.

- For each sample a 10-, 100-, 1000- and 10000-fold diluted samples were measured.

Results are shown in Figures 3-5.

Conclusions

- 5 In summary, the present inventors have developed a method using scalable and GMP-compatible techniques to enable *in vitro* AAV capsid filling for clinically relevant and drug development use.

Furthermore, several observations can be made from the generated data:

- 10 Figure 2 shows a standard curve used to determine total capsid concentration, which was found to be 1.86e10 capsids/mL for the material used in capsid filling experiments. Adjusting for experimental dilution, this was fixed at fixed at 8.6e9 capsid/mL. This value is almost 1000X higher than the estimate by Zhou et al (1e7 capsid/mL). This demonstrates that the developed method is much more suitable for generating material for drug development and clinical purposes.

- 15 Figure 3 and Figure 5 show that increasing plasmid additions results in higher genome copy levels when capsid concentration is kept constant, demonstrating that plasmid concentration can be used as a parameter to control in vitro capsid filling.

Figure 3 also indicates that the achieved filling titers are significantly higher (>1000X) than suggested by Zhou et al – 1.37e8 gc/mL versus an approximated amount of 1e5 infectious particles/mL. This was further confirmed in Example 3 by providing a direct comparison with a cell-based assay.

- 20 The trend in Figure 4 shows that filled capsid are being detected with qPCR as opposed to an artifact generated during qPCR preparations by undigested unencapsulated plasmid. This was further confirmed in Example 3 by addition of a purification (and free-DNA removal) step.

- The plasmid packing efficiency trend in Figure 5 indicates that an increase in added plasmid results in a decrease in plasmid packaging efficiency. This implies that plasmid concentration is not the only driver of
25 in vitro capsid filling. As such, Example 2 investigated additional parameters aiming to further improve the described method.

Example 2: Capsid filling improvement

30 Introduction

Example 1 demonstrated a method using scalable and GMP-compatible techniques to enable *in vitro* AAV capsid filling for clinically relevant and drug development use.

- The *in vitro* method of Example 1 outperformed methods described by Zhou et al, and the highest observed filling ratio was 1.6% of all capsids. Industry standards typically range from 5-80% and the present inventors
35 decided to further improve the method of Example 1. Furthermore, Example 1 indicated that plasmid

packaging was somehow being limited by yet unidentified bottlenecks. The present inventors sought to improve *in vitro* capsid filling reactions by performing a half-fractional 2-level factorial screening experiment for the following factors and ranges:

- 5
- pH: 6; 6.7; 7.5
 - Temperature (°C): 35; 37; 39
 - [ATP] (mM): 0.1; 1.05; 2
 - [CPK] (µg/mL): 10; 55; 100
 - Empty capsid concentration at start of reaction (e10/mL): 1, 3, 5
- 10

Equipment and materials

Equipment

Operation	Unit (model and manufacturer)
qPCR analysis	Quant studio 5. ThermoFisher Scientific
Thermomixer	Thermomixer. ThermoFisher Scientific

Materials

Material type	Cat. no and/or source
qPCR kit	6233, Takara
dNTP set	10297018, Invitrogen
NTP set	11863933, Invitrogen
Creatine Phosphokinase (CPK) from rabbit muscle, Type 1	Merck -C3755-3.5KU; Source SLCK 9545; Cas No.: 9001-15-4
Creatine phosphate disodium salt	Thermo Scientific Alfa Aesar: A15362.06

Methods

Experimental design

The following experimental design was generated use JMP 17 software (Table 9):

Table 9 Screening design

Run number	Pattern	[ATP] (mM)	T (°C)	pH	[CPK]	[Capsids] (e10/mL)
1	--++	0.1	39	6	100	5
2	---+	0.1	35	7.5	100	5

3	00000	1.05	37	6.75	55	3
4	+-----	2	35	6	10	1
5	+---++	2	35	6	100	5
6	-+++-	0.1	39	7.5	100	1
7	00000	1.05	37	6.75	55	3
8	++---+	2	39	6	10	5
9	+++++	2	39	7.5	100	5
10	-+++-	0.1	39	7.5	10	5
11	-+----	0.1	39	6	10	1
12	+---+-	2	35	7.5	100	1
13	----+-	0.1	35	6	100	1
14	--+--	0.1	35	7.5	10	1
15	++-+-	2	39	6	100	1
16	-----+	0.1	35	6	10	5
17	+--+--	2	35	7.5	10	5
18	+++--	2	39	7.5	10	1
19	00000	1.05	37	6.75	55	3

Preparative procedures

Buffer preparation

Three 10mL solutions were prepared:

- 5
 - HEPES 30mM, pH 7.5
 - HEPES 30mM, pH 6.75
 - HEPES 30mM, pH 6.0

To each, 327 mg Creatine Phosphate disodium tetrahydrate (CP) was added to achieve a final concentration of 0.1M. This was finally supplemented with the following (Table 10):

10

Table 10 Base buffer components

Component	Volume	Final concentration
MgCl ₂ 1M	70μL	7mM
DTT 0.87 M	5.75μL	0.5mM
dATP	10μL	0.1mM
dGTP	10μL	0.1mM

dCTP	10µL	0.1mM
dTTP	10µL	0.1mM
GTP	20µL	0.2mM
CTP	20µL	0.2mM
UTP	20µL	0.2mM

The following working solutions were used:

- CPK solution (1000µg/mL): prepared as described in Example 1.
- Clarified lysate - containing rep and empty capsids: same material as used in Example 1.
- GFP plasmid (0.41µg/µL): 25µL purified plasmid at 4.1µg/mL with 225µL HEPES 30mM, pH 6.75
- 5 • Empty capsid spiking solution (6.8e11 capsids/mL): 100µL 6.8e12 empty AAV2 capsids/mL (Progen) with 900µL HEPES 30mM, pH 6.75
- ATP solution (10mM): 50µL 100mM solution (Thermo) with 450µL HEPES 30mM, pH 6.75

In vitro capsid filling

- 10 Three *round* deep-well plates were used: one for each temperature condition. These were placed on ice prior to loading reagents. Wells were filled with components as described in Table 11 and in the following sequence:
1. ATP solution was added, as per design
 2. Capsid spiking solution was added, as per design
 - 15 3. 10µL plasmid working stock was added to each well
 4. 245µL Cell lysate from Example 1 was added to each well
 5. To initiate the reaction, CPK was added last, as per design

20 **Table 11 Design space, where appropriate, converted to volumetric additions (µL) of working solutions.**

Run number	HEPES 30mM + CP, pH 6	HEPES 30mM + CP, pH 6.75	HEPES 30mM + CP, pH 7.5	ATP 10mM	CPK 1000µg/mL	Capsid stock 6.8e11 capsids/mL	Incubation temperature (°C)
1	188	0	0	2.5	24.5	30.1	39
2	0	0	188	2.5	24.5	30.1	35
3	0	190.4	0	25.7	13.5	15.4	37

4	192.9	0	0	49	2.5	0.7	35
5	141.4	0	0	49	24.5	30.1	35
6	0	0	217.4	2.5	24.5	0.7	39
7	0	190.4	0	25.7	13.5	15.4	37
8	163.5	0	0	49	2.5	30.1	39
9	0	0	141.4	49	24.5	30.1	39
10	0	0	210	2.5	2.5	30.1	39
11	239.4	0	0	2.5	2.5	0.7	39
12	0	0	170.8	49	24.5	0.7	35
13	217.4	0	0	2.5	24.5	0.7	35
14	0	0	239.4	2.5	2.5	0.7	35
15	170.8	0	0	49	24.5	0.7	39
16	210	0	0	2.5	2.5	30.1	35
17	0	0	163.5	49	2.5	30.1	35
18	0	0	192.9	49	2.5	0.7	39
19	0	190.4	0	25.7	13.5	15.4	37

Deep well plates were sealed, placed in Thermomixers with temperature setpoints as specified in Table 11 and incubated at 750rpm for four hours.

5 ***Analytcs***

qPCR analysis was performed as described in Example 1. Any results below the limit of quantification were considered 0. Results are shown in Figure 6 and corresponding conditions are summarized in Table 12 below.

Table 12 qPCR results from capsid filling condition screening

Run	[ATP] (mM)	T (°C)	pH	[CPK] (µg/mL)	[Capsids] (e10/mL)	Genome copies (gc/mL)
4	2	35	6	10	1	2.39e10
7	1.05	37	6.75	55	3	8.41e9
8	2	39	6	10	5	1.89e10
9	2	39	7.5	100	5	6.93e9
13	0.1	35	6	100	1	1.52e10
15	2	39	6	100	1	1.88e10

18	2	39	7.5	10	1	1.61e10
----	---	----	-----	----	---	---------

Model generation, Analysis of Variance, and local optimum prediction

qPCR results were used as the response in this analysis and were imported into the above design and analyzed using JMP 17.

A main effects and interactions model was generated. This was done by selecting all possible first-power terms of the investigated factors for stepwise regression and generating all possible models. The model taken forward for analysis was selected based on having the lowest RMSE. Model terms and corresponding significance are listed in Table 13. The relative contribution of an individual factor was defined as the total of the sum of squares of each factor-associated term as a percentage of the total of the sum of squares of all terms in the screening model (Table 14).

Table 13 Model terms and significance: Statistically significant ($p < 0.05$) model terms are highlighted with “*”.

Model term	P value
<i>[CPK] x [Capsids]</i>	<i>0.00961*</i>
<i>[ATP] x pH</i>	<i>0.01743*</i>
<i>pH</i>	<i>0.03115*</i>
<i>[ATP] x T</i>	<i>0.04034*</i>
<i>pH x [CPK]</i>	<i>0.04244*</i>
T x [CPK]	0.05939
T x [Capsids]	0.07668
[ATP] x [Capsids]	0.23408
T	0.23553

Table 14 Relative factor contribution to model variance

Factor	Range	Relative contribution
<i>[ATP] (mM)</i>	<i>0.1; 2</i>	<i>40.2%</i>
<i>pH</i>	<i>6; 7.5</i>	<i>20.1%</i>
<i>T (°C)</i>	<i>35; 39</i>	<i>12.5%</i>
<i>[CPK] (μg/mL)</i>	<i>10; 100</i>	<i>11.8%</i>
<i>[Capsids] (e10/mL)</i>	<i>1; 5</i>	<i>15.5%</i>

Conclusion

Through DoE-based screening, within the investigated design space, the present inventors have demonstrated that [ATP], pH, T, [CPK] and [Capsids] significantly drive *in vitro* capsid filling (Table 13, Table 14). In addition, the present inventors have demonstrated that *in vitro* capsid filling is heavily dependent on multiple factor interactions (Table 13). Finally, the present inventors have identified several conditions that significantly improve titers of *in vitro* capsid filling, with a maximum observed titer of 2.39e10 gc/mL (Figure 6, Table 12). This represents a 174.4X increase in genome copy titer compared to results from Example 1.

Example 3: Verification and purification

Introduction

Example 1 demonstrated that the present inventors had developed a scalable and GMP-compatible techniques to enable *in vitro* AAV capsid filling for clinically relevant and drug development use.

Example 2 demonstrated that the *in vitro* capsid filling is driven by [ATP], pH, T and [CPK].

Based on findings from Example 2, the present inventors selected the following conditions to maximize material generation:

- [ATP]=10mM
- T=39°C
- pH=6
- [CPK]=10µg/mL
- [Capsid]=1e10/mL

The experiment was scaled up, this served several purposes:

1. Demonstrated the *in vitro* filling unit operation is scalable.
2. Benchmark *in vitro* filled AAV quality against AAV produced using a scalable manufacturing process. The present inventors expanded the suite QC tests to include potency analysis through a cell based GFP reporter assay.
3. Using *in vitro* filling, produce sufficient material to facilitate a post-filling purification. This maximizes host cell DNA removal, providing the highest degree of confidence in qPCR titers reflecting filled capsid.

This experimental overview is shown in Figure 7.

Equipment and materials

Equipment

Operation	Unit (model and manufacturer)
qPCR analysis	Quant studio 5. ThermoFisher Scientific
Thermomixer	Thermomixer. ThermoFisher Scientific
Flow Cytometer	Attune NxT Flow Cytometer. ThermoFisher Scientific
Purification	ÄKTA Pure 25. Cytiva

Materials

- 5 Additional materials described here cover infectivity analysis, all other materials have been previously described in Example 1 and Example 2

Material type	Cat. no and/or source
Vented cell culture shake flasks 125mL - 2000mL	4115-0125, 4115-0250, 4115-0500, 4115-1000, 4115-2000
HEK293 cells	American Type Culture Collection, CRL-1573 TM
Dulbecco's modified Eagle medium (DMEM)	Sigma Life Sciences Cat. No. D0819-500ML
Penicillin/Streptomycin	Thermo Fischer Scientific, Cat. No. 15140-122
Fetal Bovine Serum (FBS)	Sigma Life Sciences, Cat. No. F7524-100ML

Methods

Preparative procedures

- 10 Buffer preparation
20mL inactive CAPM (lacking enzymes) medium was prepared as described in Table 15.

Table15: Inactive CAPM components

Component	Final concentration
HEPES 30mM, pH 7.5	
MgCl ₂ 1M	7mM
DTT 0.87 M	0.5mM
dATP	0.1mM
dGTP	0.1mM
dCTP	0.1mM

dTTP	0.1mM
GTP	0.2mM
CTP	0.2mM
UTP	0.2mM
CP solution, 0.2M	40mM

The following working solutions were used:

- CPK solution (1000µg/mL): prepared as described in Example 1
- Clarified lysate - containing rep and empty capsids: same material as used in Example 1
- 5 • GFP plasmid (0.41µg/µL): 100µL purified plasmid at 4.1µg/mL with 225µL HEPES 30mM, pH 6.75
- ATP solution (10mM): 50µL 100mM solution (Thermo) with 450µL HEPES 30mM, pH 6.75

In vitro filling of capsids

- 10 Filling was performed by mixing empty AAV2 capsids, cell lysate containing AAV2 Rep proteins, a ITR CMV-GFP ITR containing plasmid, and a Capsid AAV Packaging Matrix (CAMP) together with creatine phosphokinase (CPK) (see Table 15 for CAMP composition and Table 16 for final *in vitro* packaging setup). As controls, *in vitro* filling experiments without either AAV2 empty capsids, or plasmid, or both components were used (Table 16).

15

Table 16. *In vitro* filling setup

Condition	AAV2 capsids	Rep cell lysate	Plasmid (0.41 ug/µL)	CAPM	CPK (1000 µg/mL)
Fuse AAV2-GFP	2*10 ¹¹	2.45 mL	100 µL	2.45 ml	50 µL
Fuse - Buffer				5 ml	50 µL
Fuse – No capsids		2.45 mL	100 µL	2.45 ml	50 µL
Fuse – No DNA	2*10 ¹¹	2.45 mL		2.45 ml	50 µL

The filling was performed by incubation at 39°C for 4 hours and material was subsequently purified as described in "AAV purification".

AAV purification

Chromatography was performed using an ÄKTA Pure, with online measurements of pH, conductivity, and UV absorbance (260 and 280 nm). The 1 ml AAVX adsorbent column was equilibrated and AAV material loaded and eluted as below (Table 17). Upon elution the AAV material was diluted in 1 M Tris, pH 9.0 until a neutral pH (pH 7.4) was achieved.

Table 17. Chromatography phases for AAVX chromatographic purification of AAV.

Step	Phase	Buffer	Vol (CV)	Flow rate (CV/min)	Flow direction
1	Equilibration	50 mM Tris + 150 mM NaCl pH 8.0	10	1	Down flow
2	Load	Capsid material	to air	½	Down flow
3	Wash 1	50 mM Tris pH 9.0 + 1M NaCl	5	1	Down flow
4	Wash 2	50 mM Tris pH 9.0	5	1	Down flow
5	Elution	100 mM glycine pH 2.5 + 50 mM NaCl	5	½	Up flow
6	Regeneration	100 mM phosphoric acid	5	½	Up flow
7	Cleaning	6 M Guanidine HCl	5	½	Up flow
8	Equilibration	50 mM Tris + 150 mM NaCl pH 8.0	10	1	Down flow

Samples were stored at -80°C until analyzed.

Control AAV2-GFP vector

AAV2-GFP control vectors were produced in a standard transient triple transfection system. Briefly, VPC 2.0 Hek293 cells (ThermoFisher Scientific) were transfected with pRC2, pHelper, and pAAV-GFP using AAVX-Max transfection reagent (ThermoFisher Scientific). After three days expression, the cells were harvested and lysed. The obtained lysate was clarified by centrifugation and 0.22 µm filtration, and subsequently purified on a AAVX (ThermoFisher Scientific) chromatography column. The vectors were stored at -80°C until analyzed.

Analytics***Capsid quantification***

Capsid quantification analysis was performed by ELISA as described in Example 1 (results are shown in
5 Figure 10).

Genome copy quantification

qPCR analysis was performed as described in Example 1. Any results below the limit of quantification
10 were considered 0 (results shown in Figure 9).

Cell based infectivity analysis

HEK293 cells were cultured in an incubator at 37°C in a 5% (v/v) CO₂ enriched atmosphere at 95%
humidity, supplemented with 10% (v/v) FBS, 1% (v/v) penicillin/streptomycin. To test infectivity of the *in*
vitro filled AAV vectors, human HEK293 cells were seeded into a 96-well cell culture plate at 15000
15 cells/well. The day after, the cells were transduced with the *in vitro* filled AAV vectors and control AAV
vectors by adding the vectors directly to medium at the following titers: 5 VG/cell, 50 VG/cell, and 500
VG/cell.

48 hours after the transduction, the cells were analyzed by fluorescence microscope and flow cytometry
(Attune Flow Cytometer) for GFP expression. For flow analysis, the gating strategy is shown in Figure 8
20 (results shown in Figure 12).

Conclusions

Through a direct comparison of *in vitro* filled AAV material, and AAV material generated using a
conventional triple transfection method (control AAV2) the present inventors show that:

1. A comparable qPCR titer was observed for the *in vitro* filled AAV2 when comparing to control
25 AAV2 (Figure 9).
2. The percentage of filled capsids is higher for *in vitro* filled AAV2 (11% filled capsids) compared to
control AAV2 (6% filled capsids) (Figure 10B).
3. *In vitro* filled AAV2 are able to infect and transduce HEK293 cells producing an observable signal
when viewed with a microscope (Figure 11).
- 30 4. Using the reporter assay, the present inventors can compare the potency of control AAV2 and *in*
vitro filled AAV2 on two levels:
 - a. On a capsid basis - *in vitro* filled AAVs are 2-3x more potent (Figure 13), this is due to a
higher proportion of filled AAV particles

b. On a per genome basis - *in vitro* filled AAVs are also 40-80% more potent (Figure 12). As viral genome quantity was matched between samples, and capsid quality is identical between samples, this effect can only be explained by differences in genome truncation. Genome truncation has been identified as a major concern by the FDA (Nguyen GN, 2021). The data implies more potent AAV demonstrate reduced DNA truncation, a significant advantage over current manufacturing technologies.

Example 4: Scalable, lysate based capsid filling – serotype expansion

Introduction

Example 1 demonstrated a method for using scalable and GMP-compatible techniques to enable *in vitro* AAV2 capsid filling for clinically relevant and drug development use.

Example 2 identified several conditions that significantly improve titers of *in vitro* capsid filling for AAV2. Although AAV2 is a popular serotype in pre-clinical and clinical settings, there are additional relevant serotypes, such as AAV5. AAV5 is considered the most divergent all the AAV serotypes (Issa et al., 2023).

AAV5 also demonstrates the lowest seroprevalence of all the serotypes (Klamroth et al., 2022). Low pre-existing immunity, and reduced risk of antibody cross reactivity with heterologous AAV serotypes, has made AAV5 a popular AAV serotype for therapeutic applications. However, the therapeutic potential of AAV5 has been limited by the absence of efficient and high quality methods for capsid filling.

The present inventors have found that presently disclosed *in vitro* capsid filling methods also allows for *in vitro* capsid filling of AAV5, hereby confirming that the presently disclosed *in vitro* capsid filling methods can be expanded to other AAV serotypes.

Equipment and materials

Equipment

Operation	Unit (model and manufacturer)
qPCR analysis	Quant studio 5. ThermoFisher Scientific
Thermomixer	Thermomixer. ThermoFisher Scientific

Materials

Material type	Cat. no and/or source
qPCR kit	6233, Takara
dNTP set	10297018, Invitrogen
NTP set	11863933, Invitrogen
Creatine Phosphokinase	Merck -C3755-3.5KU; Source SLCK 9545; Cas No.: 9001-15-4

(CPK) from rabbit muscle, Type 1	
Creatine phosphate disodium salt	Thermo Scientific Alfa Aesar: A15362.06

Methods

Preparative procedures

Component generation

5 *Plasmid generation*

pHelper plasmid and pAAV-GFP plasmid were sourced as described in Example 1. In addition to this, a plasmid preparation encoding AAV5 capsid and Rep proteins was generated as described below. pAAV-RC5 (cat no.: VPK-425) was purchased from Cell Biolabs. This contained the AAV2 Rep gene and the AAV5 Cap gene. The annotated sequence is listed in "Sequence Overview" (SEQ ID NO: 28).

10 pAAV-RC5 plasmid was transformed into NEB-DH5 α cells by Synvolux BV (Leiden, The Netherlands), and amplified as described in Example 1.

Cell lysate generation

VPC 2.0 cells were expanded in Viral Production Medium with GlutaMax. Incubation was at 8% CO₂, 15 37°C, and 85% RH. The culture was diluted in pre-warmed medium to a concentration of 3 x 10⁶ VC/mL. For transfection the AAV-MAX Helper-Free AAV Production System guide was used (Gibco, 2023), with the following notes:

- Culture volume: 30mL (in 125mL unbaffled vented shake flask)
- VCD: 3 x 10⁶ VC/mL
- 20 • AAV Max enhancer: 300 μ L
- Viral plex complexation buffer volume: 3mL
- Transfection booster: 90 μ L
- Transfection reagent: 180 μ L
- pHelper (1.5 μ g/ μ L): 18.4 μ L
- 25 • pAAV-RC5 (0.252 μ g/ μ L): 68.9 μ L
- Complexation incubation time: 25 minutes

After 72 hours ,the culture was subjected to a 2-hour lysis with 3mL AAV Max lysis buffer supplemented with MgCl₂, at 37°C. Lysates were centrifuged at 13000xg, 15 minutes and supernatant was stored at -

20+/-5°C.

Capsid AAV Packaging Matrix preparation

Inactive CAPM (lacking enzymes) medium was prepared by supplementing HEPES 30mM, pH

5 6.0 solution with the following (Table 18):

Table 18 Base buffer components

Component	Volume	Final concentration
MgCl ₂ 1M	70µL	7mM
DTT 0.87 M	5.75µL	0.5mM
dATP	10µL	0.1mM
dGTP	10µL	0.1mM
dCTP	10µL	0.1mM
dTTP	10µL	0.1mM
GTP	20µL	0.2mM
CTP	20µL	0.2mM
UTP	20µL	0.2mM

The following working solutions were used:

- 10
- CPK solution (1000µg/mL): prepared as described in Example 1
 - Clarified lysate as described in Example 1.
 - GFP plasmid (0.9µg/µL)
 - ATP solution (200mM)

15 ***In vitro AAV5 capsid filling***

To activate CAPM medium, CPK solution was added to inactive CAPM medium.

The following conditions were prepared on ice (Tables 19-20):

Table 19 *In vitro* reaction mixture for filling AAV5 capsids

Component	Stock Concentration	Volume	Final Concentration
-----------	------------------------	--------	------------------------

AAV5 cell lysate	Na	0.5 ml	na
ATP	50 µg/µl (200mM)	20 µl	4 mM
CPK	1000 ug/ml	10 µl	10 µg/mL
pAAV-GFP	0.9 ug/µl	9µl	0.0082 ug/ul
Matrix buffer	na	0.5 ml	na

Table 20 *In vitro* reaction mixture control: no plasmid addition

Component	Stock Concentration	Volume	Final Concentration
AAV5 cell lysate	Na	0.5 ml	N.A.
ATP	50 µg/µl (200mM)	20 µl	4 mM
CPK	1000 µg/ml	10 µl	10 µg/mL
Matrix buffer	N.A.	0.5 ml	N.A.

- 5 Reactions were incubated at 300 rpm, 39°C for 4 hours on a heat block and analyzed with qPCR as described in "Analytical procedures".

Analytical procedures

- qPCR analysis was performed as described in Example 1. Any results below the limit of quantification were considered 0. Results are shown in Figure 14. Figure 14 shows that the present *in vitro* capsid filling method achieve clinically significant titers (1.1×10^{11} gc/mL) for AAV5.
- 10

Conclusion

- The present inventors have successfully demonstrated that the presently disclosed *in vitro* capsid filling methods can be extended to other AAV serotypes, including the clinically relevant serotype (AAV5). The present *in vitro* capsid filling method achieves clinically significant titers (1.1×10^{11} gc/mL).
- 15

Example 5: Chemically defined capsid filling

Introduction

Example 1 demonstrated a method for using scalable and GMP-compatible techniques to enable *in vitro* AAV2 capsid filling for clinically relevant and drug development use.

- 5 Example 2 identified several conditions that significantly improve titers of *in vitro* capsid filling for AAV2. Example 4 demonstrated that the method could be used for the most divergent AAV serotype (AAV5) thus illustrating the broad applicability of the method across all AAV serotypes.

Previous examples have used Rep proteins in a clarified cell lysate. *In vitro* filling using a purified stock of Rep proteins has several advantages, including:

- 10 • Significant impurity removal during protein purification, reducing purification burden post *in vitro* filling, minimizing drug substance loss.
- 15 • *In vitro* filling of AAV in crude lysate (Examples 1-4) may contain host cell-impurities. Impurity removal improves *in vitro* filling reaction control and optimization by using Rep concentrations as process parameters.
- 20 • Impurities present in Examples 1-4 may impact *in vitro* filling reaction kinetics. Using purified protein starting material will improve *in vitro* filling process control and optimization, by negating any possible interference of host cell components not required for *in vitro* filling.
- Increased process robustness and reproducibility due to chemically defined processing.

Equipment and materials

Equipment

Operation	Unit (model and manufacturer)
2 L baffled flasks	Thermo Scientific 4110-2000
Benchtop shaker	Eppendorf ThermoMixer C (with SmartBlock 1.5 mL)
Shaking incubator	New Brunswick Scientific Innova® 43
Shaking incubator	New Brunswick Innova®44
Sonicator	Vibra-Cell VCX130, ¼ inch probe
Centrifuge	Sorvall RC6 Plus
Rotor head	F12S-6x500 LEX
Centrifuge	VWR MegaStar 1.6R
Rotor head	High Conic II 75003620
Benchtop centrifuge	Hettich Mikro 220R 0005766-13
Äkta purifier	Cytiva Äkta Pure 25
SEC Superdex 200 GL 10/300	Cytiva 28990944

iBlot 2 apparatus	Invitrogen IB21001
SnakeSkin™ Dialysis tubing	Thermo Fisher 88243
CaptureSelect C-tagXL MiniChrom	Thermo Fisher 5943072001
HisTrap excel	Cytiva 29048586

Materials

Material type	Cat. no and/or source
E. coli BL21 (DE3) competent cells	New England Biolabs C2527H
2xYT broth	Cold Spring Harbour Protocols
LB broth	Cold Spring Harbour Protocols
Kanamycin	Thermo Scientific J17924.06
Isopropyl β -D-1-thiogalactopyranoside (IPTG)	VWR 437144N
TRIS	Invitrogen 15506-017
cOmplete protease inhibitor	Roche 11697498001
anti-AAV2 Replicase mouse mAb	Progen 61069
goat anti-mouse HRP conjugate	Thermo Fisher A16066
Tergitol 15-S-9	Sigma-Aldrich 15S9-500ML
DTT	Thermo Fisher R0862
Denarase	c-Lecta 20804-100k
SUMO protease	Merck SAE0067
Blotting membranes	Invitrogen 15286066
NuPage™ 4-12% Bis-Tris mini gels	Thermo Fisher NP0321BOX

Methods

5 Preparative procedures

Component generation

Empty capsid generation

- AAV5 plasmid and empty capsid generation was performed as described in Example 4. Purification techniques as adopted in Example 3.

Rep sequence design

Four Rep proteins are encoded in a single *Rep* gene in the wtAAV genome. The different proteins are expressed from a combination of alternate splicing and leaky start codons. In order to separately express

Rep40, Rep52, Rep 68, Rep78 as four separate proteins, the amino acid sequences had to be extrapolated from *Rep*. Once determined, each *Rep* protein sequence was combined with an N-terminal cleavable SUMO fusion domain containing a purification tag, improving solubility, and a C-terminal 'c-tag' (aa sequence: EPEA) for purification (sequences in "Sequence Overview" SEQ ID NOs: 29-32). Thus four
5 plasmid designs were created, one for each *Rep* protein. For each *Rep* protein a new cell bank was created. Methods described in sections "Transformation and cell bank", "Production", "Cell lysis and purification" and "Dialysis and proteolytic activation" were run four times in parallel, one for each of the four *rep* proteins.

10 *Transformation and cell bank*

The codon-optimized synthetic gene sequence of the target gene coding for His₆-SUMO-Rep-EPEA was transformed into 25 µL competent BL21 (DE3) strain by adding 0.5 µL 50 ng/µL of target plasmid DNA. The mixture is flicked to thoroughly mix and incubated on ice for 30 min. Cells are then heat shocked for exactly 10 seconds at 42°C and placed on ice for 5 min. 475 µL room temperature SOC medium was
15 added, and the cells was placed at 37°C for 60 min with 250 rpm shaking in a benchtop shaker. 200 µL was then spread onto a selective plate consisting of LB medium containing 50 mg/L kanamycin as selection marker and incubated overnight at 37°C. A scrape of transformed *E. coli*-cultures from selective plates was used to inoculate 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. The culture was incubated overnight in an incubator with shaking at 250 rpm at 37°C. The following morning, 25 mL
20 autoclaved LB medium supplemented with 50 mg/L kanamycin was inoculated with 250 µL of the overnight culture and placed at 37°C with 250 rpm shaking in an incubator. When OD₆₀₀ reached 1-2, sterilized glycerol was added to a final concentration of 15% and shaken until homogenous. The medium was aliquoted in 10x1 mL labelled vials and stored at -70°C as MCB. The presence of the full-length *Rep* fusion protein was confirmed by SDS-PAGE.

25

Production

To scale up expression, a scrape from 1 MCB vial of each of the four constructs was used to inoculate a 15 mL falcon tube with 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. Cells were cultured overnight in a shaking incubator at 37.0°C with 250 rpm shaking. The following day, 4 x 2L sterile
30 baffled shake flasks with 500 mL 2xYT medium supplemented with 50 mg/L kanamycin was inoculated with 5 mL of the overnight culture. Flasks were placed in a shaking incubator at 250 rpm at 37.0°C for approximately 3 hours. When the OD₆₀₀ reached ~0.8-1.2, cells were induced by adding IPTG to a final concentration of 1 mM. The shake flasks were incubated overnight at 18°C in the same incubator and same shaking. The following morning, cells were pelleted by centrifugation at 15,000g for 15 minutes at
35 4°C using a fixed-angle rotor. Supernatants were discarded and pellets were stored at -20°C until further use.

Cell lysis and purification

Cells were resuspended in 15 mL lysis buffer (20 mM Tris·HCl pH 8.0, cOmplete protease inhibitor, 5 mM DTT, 7 U/ml Denarase) in a 50 mL falcon tube and lysed by sonication for 10 minutes with pulses of 2
 5 seconds on and 4 seconds off at an amplitude of 70%, keeping the sample in an ice bath to prevent heating. Insoluble material was removed by centrifugation at 15,000g for 15 minutes using a fixed angle rotor at 4°C. The supernatant was sterile filtered through a 0.2 µm filter and loaded onto a CaptureSelect C-tagXL MiniChrom column equilibrated in equilibration buffer (100 mM Tris·HCl pH 8.0). The column
 10 was washed with a wash buffer (50 mM Tris·HCl pH 8.0, 150 mM NaCl) and the protein of interest was eluted using an elution buffer (50 mM Tris·HCl pH 8.0, 2 M MgCl₂). SDS-PAGE (Figure 15 and Figure 16) and Western blotting (data not shown) using an anti-AAV2 Replicase mouse monoclonal antibody was performed to positively identify the presence, purity, and identity of the proteins of interest.

Dialysis and proteolytic activation

15 Purified samples were dialyzed against PBS pH 7.4 containing 10% glycerol using a 10 kDa cutoff. Dialysis was performed at 5°C while slowly stirring. After at least 3 hours, the dialysis buffer was exchanged with fresh dialysis buffer and continued overnight. The following morning, the samples were centrifuged at 15,000g for 10 min at 5°C in a benchtop centrifuge. SUMO protease cleavage was carried out under these conditions: all target protein was adjusted to 10 µM in a 500 µL setup and incubated for
 20 16 hours at 5°C and at room temperature with 0.1 U/µL SUMO protease and 1 mM DTT. Cleavage was verified by SDS-PAGE (Figure 17 and Figure 18) and Western blot (data not shown) using an anti-AAV2 Replicase mouse monoclonal antibody.

Capsid AAV Packaging Matrix preparation

25 Inactive CAPM (lacking enzymes) medium was prepared by supplementing HEPES 30mM, pH 6.0 solution with the following (Table 21):

Table 21 Base buffer components

Component	Volume	Final concentration
MgCl ₂ 1M	70µL	7mM
DTT 0.87 M	5.75µL	0.5mM
dATP	10µL	0.1mM
dGTP	10µL	0.1mM
dCTP	10µL	0.1mM
dTTP	10µL	0.1mM

GTP	20µL	0.2mM
CTP	20µL	0.2mM
UTP	20µL	0.2mM

The following working solutions were used:

- 5
- CPK solution (1000µg/mL): prepared as described in Example 1
 - Clarified lysate as described in Example 1.
 - GFP plasmid (0.9µg/µL)
 - ATP solution (200mM)

In vitro AAV5 capsid filling

The following conditions were prepared on ice (Tables 22-26):

10

Table 22. AAV5 in vitro filled particles

Component	Stock Conc	Vol	Final Conc
AAV5 empty capsid	6,9 E+12 cap/ml	70 µl	2.42E+12 cap/mL
Rep40	10µM*	20 µl	1µM
Rep52	10µM*	20µl	1µM
Rep68	10µM*	20 µl	1µM
Rep72	10µM*	62 µl	1µM
ATP	50 µg/µl (200mM)	5 ul	4 mM
CPK	1000 µg/ml	2 µl	10 µg/mL
pAAV-GFP	0.9 µg/µl	2.4 µl	0.0109 µg/ul
Matrix buffer	na	43.6 µl	na

*10µM of SUMO protease treated Rep protein.

Table 23. Control without Rep

Component	Stock Conc	Vol	Final Conc
-----------	------------	-----	------------

AAV5 empty capsid	6.9E+12 cap/ml	70 µl	2.42E+12 cap/mL
ATP	50 µg/µl (200mM)	5 µl	4 mM
CPK	1000 ug/ml	2 µl	10 µg/mL
pAAV-GFP	0.9 ug/µl	2.4 µl	0.0109 µg/ul
Matrix buffer	na	117.9 µl	na
H2O		5.7 µl	

Table 24. Control without pDNA

Component	Stock Conc	Vol	Final Conc
AAV5 empty capsid	6.9E+12 cap/ml	70 µl	2.42E+12 cap/mL
Rep40	10µM	20 µl	1µM
Rep52	10µM	20 µl	1µM
Rep68	10µM	20 µl	1µM
Rep72	10µM	20 µl	1µM
ATP	50 µg/µl (200mM)	5 µl	4 mM
CPK	1000 ug/ml	2,µl	10 µg/mL
Matrix buffer	na	40.7 µl	na
H2O		5.3 µl	

Table 25. Control buffer only

Component	Stock Conc	Vol	Final Conc
ATP	50 µg/µl (200mM)	6.7 µl	4 mM
CPK	1000 ug/ml	2.6 µl	10 µg/mL

Matrix buffer	na	114.2 µl	na
H2O		76.5 µl	

Table 26. control without capsids

Component	Stock Conc	Vol	Final Conc
Rep40	10µM	20 µl	1µM
Rep52	10µM	20µl	1µM
Rep68	10µM	20 µl	1µM
Rep72	10µM	20 µl	1µM
ATP	50 µg/µl (200mM)	5 ul	4 mM
CPK	1000 µg/ml	2 µl	10 µg/mL
pAAV-GFP	0.9 µg/µl	2.4µl	0.0109 µg/ul
Matrix buffer	na	110.6 µl	na

Reactions were incubated at 300 rpm, 39°C for 4 hours on a heat block and analyzed with qPCR as described in “Analytical procedures – qPCR”. Results shown in Figure 19.

Analytical procedures

qPCR

qPCR analysis was performed as described in Example 1. Any results below the limit of quantification were considered 0. Data shown in Figure 19.

SDS page

Expression, purification and SUMO enzymatic cleavage of Rep was assessed using SDS-Page. Data shown in Figure 15, Figure 16, Figure 17 and Figure 18.

Conclusion

The present Example describes:

1. The novel method of *in vitro* filling of AAV5 using purified Rep proteins, the first demonstration of chemically defined capsid filling (Figure 18)
2. The successful development of novel fusion proteins that contain:
 - a. An AAV Rep domain ("Sequence Overview" SEQ ID NOs: 29-32)
 - 5 b. A cleavable SUMO domain that enhances expression and purification ("Sequence Overview" SEQ ID NOs: 29-32)
3. The expression of individual AAV rep proteins in the prokaryotic microbial system, *Escherichia coli* (Figure 15 and Figure 16).
- 10 4. Methods for SUMO protease digestion liberating Rep proteins of SUMO fusion element, where time and temperature are critical parameters (Figure 17 and Figure 18).

Due to supporting data in Examples 1-4, the method described here for AAV5 can be applied to all AAV serotypes.

Example 6: Capsid assembly and stoichiometry control through precipitation

15 Introduction

Adeno-associated virus (AAV) consists of a single-stranded DNA genome of 4.7 kilobases packed in a non-enveloped capsid of 60 proteins. AAV serotype 2 (AAV2) is the best-studied member of the genus which currently comprises several serotypes possessing diverging tropism (Issa *et al.*, 2023). The AAV genome consists of three open reading frames (ORFs) that encode the four Rep proteins required for genome assembly, the assembly-activating protein that promotes assembly of the capsid, and the three capsid proteins VP1, VP2 and VP3. The major capsid protein VP3 assembles the icosahedral capsid, while the minor capsid protein VP1 and VP2 are required for intracellular trafficking, escape, nuclear localization and genome release. All VPs share the VP3 domain and are assembled with the relative abundance of VP1/2/3 as 1:1:10 (Wörner *et al.*, 2021). Recombinant AAV (rAAV) can be produced in 20 HEK293 cells using a triple-plasmid method or in Sf9 cells using baculovirus. These production methods suffer from considerable problems with scalability and product heterogeneity (Samulski *et al.*, 2014).

Le *et al.*, 2019 has shown recombinant production of AAV2 VP3 protein as inclusion bodies in *Escherichia coli* and subsequent refolding (Le *et al.*, 2019). While this proof-of-principle study makes 30 some advances, the primary application remains rather limited, i.e., it only involves the production virus-like particles (VLPs) used in vaccination and therefore only makes use of the major capsid protein. The present inventors have discovered novel *in vitro* capsid assembly methods. These improved methods makes it possible to incorporate VP1 and VP2 in a titratable manner and expand applicability by including

- different serotypes. The present inventors produce all three capsid proteins from AAV5 as inclusion bodies in *E. coli* and, after solubilization, purify them to a degree compatible with refolding. The ability to refold AAV5 VP3 is first screened in a host of different buffers. The present inventors show that AAV5 VP3-only capsid can indeed be formed under certain conditions. Incubating for shorter periods improves titer. The present inventors then proceed to purify AAV5 VP1, AAV5 VP2 and AAV5 VP3 further. Mixing these preparations at different ratios results in the formation of capsid particles, which can be purified using the assembled capsid-specific AAVX purification resin. The results described here offer control of the stoichiometry of the different coat proteins within the viral capsid.

10 **Equipment and materials**

Equipment

Operation	Unit (model and manufacturer)
2 L baffled flasks	Thermo Scientific 4110-2000
Shaking incubator	New Brunswick Innova 43
Sonicator	Vibra-Cell VCX130, ¼ inch probe
Purifier	Cytiva Äkta Pure 25
Gel filtration column	SEC Superdex 200 GL 10/300 Cytiva 28990944
Ion exchange column	HiTrap Q FF Cytiva 17505301
iBlot 2 apparatus	Invitrogen IB21001
Dialysis membrane	Thermo Fisher 88243

Materials

Material type	Cat. no and/or source
<i>E. coli</i> BL21 (DE3)	New England Biolabs C2527H
pET29-GOI	Twist Biosciences
2-YT broth	Cold Spring Harbour Protocols
LB broth	Cold Spring Harbour Protocols
Kanamycin	Thermo Scientific J17924.06
IPTG	VWR 437144N
Urea	Sigma-Aldrich U5128-1KG
Tergitol 15-S-9	Sigma-Aldrich 15S9-500ML
TRIS	Invitrogen 15506-017
EDTA	Fluka 03659
DTT	Thermo Fisher R0862
NaCl	Sigma-Aldrich S9888

MgCl ₂	Thermo Scientific 223210010
KCl	Sigma-Aldrich P9541
Na ₂ HPO ₄	Sigma-Aldrich S0876
KH ₂ PO ₄	Supelco 1.04873.1000
cOmplete protease inhibitor	Roche 11697498001
anti-AAV VP1/VP2/VP3 mAb	Progen 690058
Blotting membranes	Invitrogen 15286066
POROS CaptureSelect AAVX	Thermo Fisher A36739
QuickFold Refolding Kit	AthenaES 0600
AAV5 Xpress ELISA kit	Progen PRAAV5XP
10 kDa spin concentrator	Pierce 88528
NuPage™ 4-12% Bis-Tris mini gels	Thermo Fisher NP0321BOX

Methods

Preparative procedures

Transformation and protein production

- 5 The following expression constructs were transformed into *E. coli* BL21 (DE3) and plated out on kanamycin selective growth plates (LB agar):

- pET29-AAV5-VP1
- pET29-AAV5-VP2
- pET29-AAV5-VP3

10

The protein sequences of the proteins of interest produced from these constructs can be found in "Sequence Overview" (SEQ ID NOs: 2, 20, 33). One colony was taken from these plates after growth overnight at 37°C and was used to inoculate 5 ml LB media containing 50 µg mL⁻¹ kanamycin. These were incubated overnight (16 hours) at 37°C shaking at 250 rpm. The next morning, six sterile 2L baffled
 15 flasks were prepared with 500 mL 2YT media and inoculated with 5 ml overnight culture. Cultures were grown at 37°C shaking at 250 rpm to an OD of 1.0 whereupon 0.5 mL 1 M IPTG was added. Cultures were grown overnight at 37°C shaking at 250 rpm. Cells were harvested the next day at 10000g for 30 minutes in 500 mL sterile centrifuge tubes and pellets were stored at -20°C until further use.

20 Inclusion body preparation and solubilization

Cells were resuspended in a 50 mL tube on ice in 40 mL lysis buffer (100 mM Tris·Cl, pH 8.0, 5 mM EDTA pH 8.0, cOmplete protease inhibitor, 5 mM DTT) after which the volume was adjusted to 50 mL. Cells were lysed by sonication for 10 min in a 100 mL glass beaker placed in an ice bath, with pulses of 2

sec on and 4 sec off, at an amplitude of 70%, keeping the sample in an ice bath to prevent heating. The lysate was centrifuged at 8000g for 15 minutes at 10°C and the supernatant was discarded. The pellet was washed three times with wash buffer 1 (100 mM Tris·Cl pH 8.0, 5 mM EDTA pH 8.0, 2 M urea, 2% (w/v) Tergitol™ 15-S-9, 5 mM DTT) and twice with wash buffer 2 (100 mM Tris·Cl pH 8.0, 5 mM EDTA pH 8.0, 2 M urea, 5 mM DTT). Inclusion bodies were solubilized while rotating overnight in solubilization buffer (20 mM Tris·Cl pH 8.0, 8 M urea, 5 mM DTT). The next morning, any insoluble residue was removed by centrifugation at 15000g for 30 minutes at 4°C. The supernatant was kept at 4°C until further processing.

10 *Protein purification*

AAV5 VP1, AAV5 VP2, and AAV5 VP3 were purified using ion exchange and size exclusion chromatography (SEC). In short, solubilized inclusion bodies were loaded onto a cation exchange column equilibrated in 20 mM Tris·Cl pH 8.0, 8 M urea and the protein of interest was eluted with a gradient of 20 mM Tris·Cl pH 8.0, 8 M urea, 1 M NaCl over 20 column volumes. The AAV5 VP3 protein was then used for refolding experiments. The final concentration of AAV5 VP3 was 0.56 g l⁻¹. To increase purity, samples were concentrated to 2 mL using a 10 kDa spin concentrator and 0.5 mL was loaded onto a Superdex 200 SEC column equilibrated in 20 mM Tris·Cl pH 8.0, 8 M urea, 150 mM NaCl. The mobile phase consisted of 20 mM Tris·Cl pH 8.0, 8 M urea, 150 mM NaCl. The peaks containing the proteins of interest were collected and used in subsequent experiments.

20

Capsid purification

AAVX resin was equilibrated 3 times with 1 volume of 50 mM Tris·Cl pH 8.0, 150 mM NaCl and resuspended to a 62.5% slurry. Samples containing refolded capsid particles were incubated with 400 µL equilibrated slurry resin for 1 hour at 4°C while mixing. After pelleting the resin and discarding the supernatant, the resin was resuspended in 500 µL wash buffer 1 (50 mM Tris·Cl pH 9.0, 1 M NaCl) and incubated for 5 minutes at 4°C. This process was repeated with wash buffer 2 (50 mM Tris·Cl pH 9.0) and mQ water. Finally, elution of capsid particles was performed by incubating the pelleted resin with 200 µL elution buffer (100 mM glycine pH 2.5, 50 mM NaCl) for 5 minutes at 4°C. The resin was pelleted at 1000g for 5 minutes at 4°C in between equilibration, wash, and elution steps.

30

Refolding of AAV5 VP3 into capsid particles

Refolding properties of AAV5 VP3 were screened by rapid dilution (100x) into 17 different buffers (listed below) and incubation for 16 hours at 4°C and 22°C. All buffers were shown to support the formation of capsid particles, i.e., allowed for capsid assembly. Buffer 11 was shown to support formation of capsid particles particularly well. Subsequent refolding experiments in this buffer were performed for 1 hour and 16 hours at room temperature (22°C).

35

Buffers:

- Buffer 1: 50 mM MES pH 6.0, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.75 M Guanidine HCl, 0.5% Triton X-100, 0.1 mM DTT
- 5 Buffer 2: 50 mM MES pH 6.0, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.05% polyethylene glycol 3,550, 0.1 mM GSH, 0.01 mM GSSH
- Buffer 3: 50 mM MES pH 6.0, 9.6 mM NaCl, 1 mM EDTA, 0.4 mM KCl, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.5% Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM DTT
- Buffer 4: 50 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.5% Triton X-100, 0.1 mM GSH, 0.01 mM GSSH
- 10 Buffer 5: 50 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.1 mM DTT
- Buffer 6: 50 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.4 M sucrose, 0.5% Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH, 0.01 mM GSSH
- 15 Buffer 7: 50 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.75 M Guanidine HCl, 0.05% polyethylene glycol 3,550, 0.1 mM DTT
- Buffer 8: 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.4 M sucrose, 0.5% Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH, 0.01 mM GSSH
- Buffer 9: 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.75 M Guanidine HCl, 0.05% polyethylene glycol 3,550, 0.1 mM DTT
- 20 Buffer 10: 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.1 mM GSH, 0.01 mM GSSH
- Buffer 11: 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 1 mM EDTA, 0.5% Triton X-100, 0.1 mM DTT
- 25 Buffer 12: 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.05% polyethylene glycol 3,550, 0.1 mM GSH, 0.01 mM GSSH
- Buffer 13: 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.75 M Guanidine HCl, 0.5% Triton X-100, 0.1 mM DTT
- Buffer 14: 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.5% Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH, 0.01 mM GSSH
- 30 Buffer 15: 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.4 M sucrose, 0.1 mM DTT
- Buffer 16: 20 mM Tris-Cl, 8 M urea, pH 8.0
- 35 Buffer 17: 500 mM NaCl, 100 mM KCl, 10 mM Na₂HPO₄, 10 mM KH₂PO₄, pH 8.0

Stoichiometric control

Partially purified AAV5 VP1, AAV5 VP2 and AAV5 VP3 proteins were mixed in ratios of 1:1:20, 1:1:1, and 10:10:1 based on densitometric image analysis of an SDS PAGE purity gel. The volume was adjusted to 5 mL 20 mM Tris·Cl, 8 M urea, pH 8.0, 1 mM DTT and the sample was dialyzed against 500 mM NaCl, 100 mM KCl, 10 mM Na₂HPO₄, 10 mM KH₂PO₄, pH 8.0 using a 10 kDa cutoff membrane. Dialysis was performed for 3 hours at 4°C while stirring, whereupon the buffer was replaced with fresh dialysis buffer. Dialysis was continued overnight (16 hours) at 4°C while stirring. The next morning, samples were centrifuged at 15000g for 20 minutes at 4°C. Capsid particles were purified as described above before further analysis.

Analytical procedures*SDS page and Western blotting*

Identity of AAV5 VP1, AAV5 VP2, and AAV5 VP3 proteins and stoichiometric control of capsid particles was confirmed by SDS page and western blotting using an anti-AAV antibody (Figure 20).

Capsid ELISA

AAV5 capsid formation was confirmed using the AAV5 Xpress ELISA kit .

Conclusion

The present Examples shows:

- AAV5 VP1, AAV5 VP2, and AAV5 VP3 can be produced as inclusion bodies in *E. coli* (Figure 20).
 - AAV5 VP1, AAV5 VP2, and AAV5 VP3 can be purified to a level compatible with refolding (Figure 21, Figure 24.)
 - Buffer screening revealed specific conditions that enable VPs to refold into AAV5 capsids (Figure 22). All of the tested buffers enabled VPs to refold into AAV5 capsids.
 - The titer of AAV5 VP3 only capsids obtained in AAV5 particle ELISA is $1.7\text{-}5.3 \cdot 10^8$ capsids mL⁻¹ and is temperature dependent and time dependent (Figure 23).
 - Simultaneous, mixed refolding of VP1; VP2; and VP3, followed by affinity-based capsid purification revealed capsid populations with different VP1:VP2:VP3 stoichiometries (Figure 25).
- This demonstrates that the method can be used to control capsid VP stoichiometry.

Example7: Capsid assembly through SUMO proteolysis**Introduction**

The present inventors have found a novel way of assembling AAV capsids. One possible route to achieve

this is using cleavable fusion proteins that enable capsid protein stoichiometry control further downstream in a cell-free environment. The fusion proteins contain several elements of interest: a His-tag for purification purposes, a SUMO fusion protein, VP proteins, a thrombin cleavage site, SUMO fusion protein, and a C-tag (EPEA) for purification purposes (SEQ ID NO: 34). SUMO proteins enable (1) increased solubility of protein of interest (VP) and (2) steric hindrance between capsid proteins to prevent or minimize spontaneous intracellular assembly. Each SUMO protein has a cleavable amino acid sequence towards its C-terminus. Two SUMO moieties are fused N- and C-terminally to VP3 protein of AAV2.

This example describes (1) *P. pastoris*-based expression of the gene coding for His-SUMO-AAV2-VP3-SUMO-EPEA and associated purification methods; and (2) induced assembly of VP3 proteins into capsids upon proteolytic cleavage.

Equipment and materials

Equipment

Operation	Unit (model and manufacturer)
2 L baffled flasks	Thermo Scientific 4110-2000
Shaking incubator	New Brunswick Innova 43
Sonicator	Vibra-Cell VCX130, ¼ inch probe
Äkta purifier	Cytiva Äkta Pure 25
SEC Superdex 200 GL 10/300	Cytiva 28990944
iBlot 2 apparatus	Invitrogen IB21001
CaptureSelect C-tagXL MiniChrom	Thermo Fisher 5943072001

Materials

Material type	Cat. no and/or source
E. coli BL21 (DE3)	New England Biolabs C2527H
PET29b+ GOI	Twist Biosciences
2-YT broth	Cold Spring Harbour Protocols
LB broth	Cold Spring Harbour Protocols
Kanamycin	Thermo Scientific J17924.06
IPTG	VWR 437144N
TRIS	Invitrogen 15506-017
cOmplete protease inhibitor	Roche 11697498001
AAV5 Xpress ELISA kit	Progen PRAAV5XP
AAV2 Xpress ELISA kit	Progen PRAAV2XP

anti-AAV VP1/VP2/VP3 mAb	Progen 690058
Tergitol 15-S-9	Sigma-Aldrich 15S9-500ML
DTT	Thermo Fisher R0862
Denerase	c-Lecta 20804-100k
Methanol	VWR 20846.292
SUMO protease	Merck SAE0067
SUMO protease 2	LifeSensors 4020
Thrombin	Cytiva 27084601
POROS CaptureSelect AAVX	Thermo Fisher A36739
Blotting membranes	Invitrogen 15286066

Methods

Preparative procedures

Construct selection

- 5 The codon-optimized synthetic gene sequence of the target gene coding for His-SUMO-AAV2-VP3-SUMO-EPEA was cloned via EcoRI/NotI restriction into an appropriately digested expression plasmid pPZ carrying a wild type PAOX1 promoter. After transformation into *E. coli* TOP10F' cells, recultivation of transformants and plasmid preparation, correct insertion of the target gene was checked by restriction pattern analysis, and authenticity of the gene sequence was confirmed by sequencing (LGC Genomics,
- 10 Berlin, Germany). The plasmid was linearized using SacI endonuclease and desalted for transformation into *P. pastoris*. DNA concentration was determined by spectrophotometric measurement and adjusted to be roughly 1 µg µL⁻¹.
- Electroporation was carried out using competent cells of basic production strain CBS7435 MutS (genotype Δ *aox1*; phenotype MutS) applying a standard procedure and standard equipment for
- 15 electroporation. After regeneration at 28°C, preparations were plated on agar plates containing the antibiotics Zeocin and Geneticin. Single colonies (8 per transformation) were picked from transformation plates into single wells of a 96-deep well plate filled with optimized cultivation media. Remaining wells were inoculated with a mock strain. After an initial growth phase to generate biomass, expression was induced by addition of an optimized liquid mixture containing a defined concentration of methanol. At
- 20 defined points of time, further induction with methanol was performed. After a total of 50 hours from the initial methanol induction, cells were collected by centrifugation. The presence of the full-length AAV2 VP3 fusion protein was confirmed by Western blotting. A master cell bank (MCB) in glycerol was prepared of the clones expressing the fusion protein.

Production

To scale up expression, four vials of the MCB were defrosted and added in 4 x 2L flasks containing 250 mL BMGY medium supplemented with biotin. Flasks were placed in the shaker at 250 rpm at 30°C for approximately 18 hours. When the OD reached ~30, cells were pelleted by centrifugation at 3200g for 20 minutes at 4°C. After the supernatant with spent media was discarded, cells were resuspended in 250 mL BMGY medium supplemented with biotin. When the OD reached ~100, 1.5% (v/v) MeOH was added. After 48 hours of incubation shaking at 250 rpm at 30°C, cells were harvested by centrifugation at 15000g for 20 minutes at 4°C. Pellets were stored at -20°C until further use.

10 *Cell lysis and purification*

Cells were resuspended in 50 mL lysis buffer (20 mM Tris·Cl pH 8.0, 2 mM MgCl₂, cOmplete protease inhibitor, 5 mM DTT, 7 U/ml Denerase) and lysed by sonication for 3 minutes with pulses of 20 seconds on and 40 seconds off at an amplitude of 90%, keeping the sample in an ice bath to prevent heating. Directly after, 0.1% (v/v) Tergitol 15-S-9 was added, and the sample was incubated on ice for 20 minutes. Insoluble material was removed by centrifugation at 15000g for 15 minutes at 4°C. The supernatant was loaded onto a CaptureSelect C-tagXL MiniChrom column equilibrated in binding buffer (100 mM Tris·Cl pH 8.0). The column was washed with wash buffer 1 (100 mM Tris·Cl pH 8.0, 150 mM NaCl) and the protein of interest was eluted using elution buffer (50 mM Tris·Cl pH 8.0, 2 M MgCl₂). SDS PAGE and Western blotting using an anti-AAV antibody was performed to positively identify the presence and identity of the proteins of interest.

Induced assembly by proteolytic activation

After dialysis overnight into PBS using a 10 kDa cutoff membrane, SUMO- and thrombin cleavage was carried out simultaneously under these conditions: all target protein was incubated for 16 hours overnight at room temperature (22°C) with 12 µL 0.5 U/µL Thrombin and 2 µL 10 U/µL SUMO protease 2.

Analytical procedures

Western blotting

Cleavage of the proteins of interest was confirmed by SDS PAGE and western blotting (Figure 26) using an anti-AAV antibody.

Capsid ELISA

After concentrating a hundredfold using a 100 kDa spin concentrator, formation of capsid particles was assayed with an AAV2-specific capsid ELISA at 10x and 100x dilutions. Capsid titers were calculated using a best fit four parameter logistic equation (Figure 27).

Conclusion

The present Example describes:

1. The successful development of a novel fusion protein that contains:
 - a. An AAV VP domain
 - 5 b. Two different, cleavable, sterically inhibiting domains preventing spontaneous self-assembly and enabling increased capsid assembly process control
 - c. Two different purification tags, enabling the use of pure full-length fusion proteins for *in vitro* assembly reactions
2. The expression of AAV capsid proteins in the eukaryotic microbial system, *Pichia pastoris*. This
10 unlocks a wide array of expression features and process controls limited strictly to methylotrophic yeasts, such as control of protein glycosylation and methanol-based induction.
3. Methods for SUMO digestion and resultant capsid assembly, where time and temperature are critical parameters.
4. Method development for sterically controlled *in vitro* AAV capsid assembly for high capsid titers.
- 15 The methods described here can also be applied to enable capsid stoichiometric VP control as described in Example 6.

Example 8: Chemically Defined High Fill Rate (AAV5)

- 20 Example 5 demonstrated a novel method of *in vitro* filling of AAV5 using purified Rep proteins, the first demonstration of chemically defined capsid filling. Methods were provided for the successful development of novel fusion proteins that contain an AAV Rep domain and a cleavable SUMO domain that enhances expression and purification. These fusion proteins can be individually expressed in the prokaryotic microbial expression system, *Escherichia coli*, and the Rep moiety can be liberated by SUMO protease
25 digestion, where time and temperature might be critical parameters.
- This research laid an essential groundwork for transitioning *in vitro* filling technology to an industrially feasible process. The present inventors further improved the *in vitro* filling technology. Firstly, it was determined how different combinations of Rep proteins can influence capsid filling rates. Secondly, previously used Rep moieties were prepared with cleavable SUMO-histidine tags to simplify purification.
- 30 This technique necessitates the use of imidazole and cleavage enzymes, rendering it less translatable for clinical applications and Good Manufacturing Practice (GMP) production. Therefore, a GMP-compatible Rep construct had to be developed.

The following example demonstrates achieving an industrially viable in vitro filling process by (1) maximizing AAV fill rates with (2) GMP-compatible methods. This was accomplished by:

- Examining optimal combinations of Rep proteins
- Expanding the range of expression-enhancing purification tags
- 5 • Further engineering a Rep protein to eliminate the need for his-tags (GMP compatibility) and cleavable elements
- Achieving 100% observed fill rates of AAV

Equipment and materials

10 **Equipment**

Operation	Unit (model and manufacturer)
2 L baffled flasks	Thermo Scientific 4110-2000
Benchtop shaker	Eppendorf ThermoMixer C (with SmartBlock 1.5 mL)
Shaking incubator	New Brunswick Scientific Innova® 43
Shaking incubator	New Brunswick Innova®44
Sonicator	Vibra-Cell VCX130, ¼ inch probe
Centrifuge	Sorvall RC6 Plus
Rotor head	F12S-6x500 LEX
Centrifuge	VWR MegaStar 1.6R
Rotor head	High Conic II 75003620
Benchtop centrifuge	Hettich Mikro 220R 0005766-13
Äkta purifier	Cytiva Äkta Pure 25
iBlot 2 apparatus	Invitrogen IB21001

Materials

Material type	Cat. no and/or source
E. coli BL21 (DE3) competent cells	New England Biolabs C2527H
2xYT broth	Cold Spring Harbour Protocols
LB broth	Cold Spring Harbour Protocols
Autoinduction medium	Overnight Express Instant TB – Novagen 71491
Kanamycin	Thermo Scientific J17924.06
Isopropyl β-D-1-thiogalactopyranoside (IPTG)	VWR 437144N
TRIS	Invitrogen 15506-017

HEPES	Sigma-Aldrich SRE0065
Sodium creatine phosphate dibasic tetrahydrate	Roche 10621714001
EDTA	Thermo Scientific 17892
MgCl ₂	Thermo Scientific J61014
Maltose	Thermo Scientific A16266.22
Glycerol	Thermo Scientific T17904-k2
PBS	Thermo Scientific AM9625
cOmplete protease inhibitor	Roche 11697498001
anti-AAV2 Replicase mouse mAb	Progen 61069
goat anti-mouse HRP conjugate	Thermo Fisher A16066
DTT	Thermo Fisher R0862
Denarase	c-Lecta 20804-100k
SUMO protease	Merck SAE0067
Blotting membranes	Invitrogen 15286066
NuPage™ 4-12% Bis-Tris mini gels	Thermo Fisher NP0321BOX
MBPTrap HP	Cytiva 28918780
HiTrap Q HP	Cytiva 17115301
Superose 6	HiLoad 16/600 Superose 6 pg 29323952
BCA Protein assay kit	Thermo Fisher, 23225
Spin concentrator	Merck UFC905008
SnakeSkin™ Dialysis tubing	Thermo Fisher 88243
CaptureSelect C-tagXL MiniChrom	Thermo Fisher 5943072001
HisTrap excel	Cytiva 29048586

Methods

Preparative procedures

Component generation

5 Empty capsid generation

AAV5 plasmid and empty capsid generation was performed as described in Example 4. Purification techniques as adopted in Example 3.

SUMO-Rep production

SUMO-Rep sequence design

- 10 In the wtAAV genome, the rep gene, through the use of two promoters and alternative splicing, encodes

four regulatory proteins that are dubbed Rep78, Rep68, Rep52 and Rep40. In order to separately express Rep40, Rep52, Rep 68, Rep78 as four separate proteins, the amino acid sequences had to be extrapolated from Rep. Once determined, each Rep protein sequence was combined with an N-terminal cleavable SUMO fusion domain containing a purification tag, improving solubility, and a C-terminal 'c-tag' (aa sequence: EPEA) for purification (sequences of SEQ ID NOs: 36-39 listed in "Sequence Overview").

Thus, four plasmid designs were created, one for each Rep protein. For each Rep protein a new cell bank was created. Methods described in sections below were run four times in parallel, one for each of the four rep proteins.

10 Transformation and cell bank

The codon-optimized synthetic gene sequence of the target gene coding for His₆-SUMO-Rep-EPEA was transformed into 25 µL competent BL21 (DE3) strain by adding 0.5 µL 50 ng/µL of target plasmid DNA. The mixture was flicked to thoroughly mix and incubated on ice for 30 min. Cells were then heat shocked for exactly 10 seconds at 42°C and placed on ice for 5 min. 475 µL room temperature SOC medium was added, and the cells was placed at 37°C for 60 min with 250 rpm shaking in a benchtop shaker. 200 µL was then spread onto a selective plate consisting of LB medium containing 50 mg/L kanamycin as selection marker and incubated overnight at 37°C. A scrape of transformed E. coli-cultures from selective plates was used to inoculate 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. The culture was incubated overnight in an incubator with shaking at 250 rpm at 37°C. The following morning, 25 mL autoclaved LB medium supplemented with 50 mg/L kanamycin was inoculated with 250 µL of the overnight culture and placed at 37°C with 250 rpm shaking in an incubator.

When OD₆₀₀ reached 1-2, sterilized glycerol was added to a final concentration of 15% and shaken until homogenous. The medium was aliquoted in 10x1 mL labelled vials and stored at -70°C as MCB. The presence of the full-length Rep fusion protein was confirmed by SDS-PAGE.

25

Production

To scale up expression, a scrape from 1 MCB vial of each of the four constructs was used to inoculate a 15 mL falcon tube with 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. Cells were cultured overnight in a shaking incubator at 37.0°C with 250 rpm shaking. The following day, 4 x 2L sterile baffled shake flasks with 500 mL 2xYT medium supplemented with 50 mg/L kanamycin was inoculated with 5 mL of the overnight culture. Flasks were placed in a shaking incubator at 250 rpm at 37.0°C for approximately 3 hours. When the OD₆₀₀ reached ~0.8-1.2, cells were induced by adding IPTG to a final concentration of 1 mM. The shake flasks were incubated overnight at 18°C in the same incubator and same shaking. The following morning, cells were pelleted by centrifugation at 15,000g for 15 minutes at 4°C using a fixed-angle rotor. Supernatants were discarded and pellets were stored at -20°C until further use.

35

Cell lysis and purification

Cells were resuspended in 15 mL lysis buffer (20 mM Tris·HCl pH 8.0, cOmplete protease inhibitor, 5 mM DTT, 7 U/ml Denarase) in a 50 mL falcon tube and lysed by sonication for 10 minutes with pulses of 2
5 seconds on and 4 seconds off at an amplitude of 70%, keeping the sample in an ice bath to prevent heating. Insoluble material was removed by centrifugation at 15,000g for 15 minutes using a fixed angle rotor at 4°C. The supernatant was sterile filtered through a 0.2 µm filter and loaded onto a CaptureSelect C-tagXL MiniChrom column equilibrated in equilibration buffer (100 mM Tris·HCl pH 8.0). The column
10 was washed with a wash buffer (50 mM Tris·HCl pH 8.0, 150 mM NaCl) and the protein of interest was eluted using an elution buffer (50 mM Tris·HCl pH 8.0, 2 M MgCl₂). SDS-PAGE and Western blotting using an anti-AAV2 Replicase mouse monoclonal antibody was performed to positively identify the presence, purity, and identity of the proteins of interest (data not shown).

Dialysis and proteolytic activation

15 Purified samples were dialyzed against PBS pH 7.4 containing 10% glycerol using a 10 kDa cutoff. Dialysis was performed at 5°C while slowly stirring. After at least 3 hours, the dialysis buffer was exchanged with fresh dialysis buffer and continued overnight. The following morning, the samples were centrifuged at 15,000g for 10 min at 5°C in a benchtop centrifuge. SUMO protease cleavage was carried out under these conditions: all target protein was adjusted to 10 µM in a 500 µL setup and incubated for
20 16 hours at 5°C and at room temperature with 0.1 U/µL SUMO protease and 1 mM DTT. Cleavage was verified by SDS-PAGE and Western blot (data not shown) using an anti-AAV2 Replicase mouse monoclonal antibody. Cleavage was verified by SDS-PAGE (data not shown) and Western blot (data not shown) using an anti-AAV2 Replicase mouse monoclonal antibody.

MBP-Rep78 production

25 MBP-Rep78 sequence design

In the wtAAV genome, the rep gene, through the use of two promoters and alternative splicing, encodes four regulatory proteins that are dubbed Rep78, Rep68, Rep52 and Rep40. In order to separately express Rep78 as a separate protein, the amino acid sequences had to be extrapolated from Rep. Once determined, the Rep protein sequence was combined with an N-terminal cleavable MBP fusion domain,
30 which improves solubility and acts as an affinity purification tag (sequence of SEQ ID NO: 35 listed in "Sequence Overview"). An expression plasmid was created, harboring the coding sequence of this fusion protein. The plasmid was transformed into an Escherichia coli expression strain and a new cell bank was created. Methods described below were used to purify the fusion protein to homogeneity.

Transformation and cell bank

The codon-optimized synthetic gene sequence of the target gene coding for MBP-Rep78 was transformed into 25 µL competent BL21 (DE3) strain by adding 0.5 µL 50 ng/µL of target plasmid DNA. The mixture is flicked to thoroughly mix and incubated on ice for 30 min. Cells are then heat shocked for exactly 10 seconds at 42°C and placed on ice for 5 min. 475 µL room temperature SOC medium was added, and the cells was placed at 37°C for 60 min with 250 rpm shaking in a benchtop shaker. 200 µL was then spread onto a selective plate consisting of LB medium containing 50 mg/L kanamycin as selection marker and incubated overnight at 37°C. A scrape of transformed E. coli-cultures from selective plates was used to inoculate 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. The culture was incubated overnight in an incubator with shaking at 250 rpm at 37°C. The following morning, 25 mL autoclaved LB medium supplemented with 50 mg/L kanamycin was inoculated with 250 µL of the overnight culture and placed at 37°C with 250 rpm shaking in an incubator. When OD₆₀₀ reached 1-2, sterilized glycerol was added to a final concentration of 15% and shaken until homogenous. The medium was aliquoted in 10x1 mL labelled vials and stored at -70°C as MCB.

Production

To scale up expression, a scrape from 1 MCB vial was used to inoculate a 15 mL falcon tube with 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. Cells were cultured overnight in a shaking incubator at 37°C with 250 rpm shaking. The following day, a 2L sterile baffled shake flasks with 500 mL autoinduction medium with 50 mg/L kanamycin was inoculated with 5 mL of the overnight culture. Flasks were placed in a shaking incubator at 250 rpm at 37.0°C for approximately 3 hours. When the OD₆₀₀ reached ~1.0-1.2, the temperature was lowered to 18°C and the shake flasks were incubated overnight in the same incubator and at the same shaking speed. The following morning, cells were pelleted by centrifugation at 15,000g for 15 minutes at 4°C using a fixed-angle rotor. Supernatants were discarded and pellets were stored at -20°C until further use.

Cell lysis and purification

Cells were resuspended in 200 mL lysis buffer (100 mM Tris·HCl pH 8.0, cOmplete protease inhibitor, 5 mM DTT, 2 mM MgCl₂, 10.5 U/ml Denarase, 1 mM EDTA) and lysed by sonication for 10 minutes with pulses of 2 seconds on and 4 seconds off at an amplitude of 70%, keeping the sample in an ice bath to prevent heating. Insoluble material was removed by centrifugation at 15,000g for 15 minutes using a fixed angle rotor at 4°C. The supernatant was sterile filtered through a 0.2 µm filter and loaded onto a MBPTrap HP column equilibrated in equilibration buffer (20 mM Tris·HCl pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM DTT). The column was washed with 5 column volumes of equilibration buffer and the protein of interest was eluted using elution buffer (20 mM Tris·HCl pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 10 mM maltose). The eluate containing the protein of interest was loaded onto a HiTrap Q HP column

equilibrated in equilibration buffer. The column was washed with 2 column volumes of equilibration buffer and elution was performed by a linear gradient of 10 column volumes to 25% elution buffer and a step elution with 5 column volumes of elution buffer (20 mM Tris·HCL pH 7.4, 2M NaCl, 1 mM DTT). The flow through fractions containing the protein of interest were pooled and concentrated to 10 mL using spin concentrators (4000g, 4°C). The protein of interest was finally polished size exclusion chromatography using a Superose 6 column. Two runs were performed with a 5 mL load on a column equilibrated in PBS, 10% glycerol (w/v). The protein of interest eluted at a retention time of ~84 mL and corresponding fractions were pooled and concentrated to 5 mL by spin concentrators. The presence, purity, and identity of MBP-Rep78 was determined by SDS-PAGE (Figure 29) and Western blotting using an anti-AAV2 Replicase mouse monoclonal antibody (Figure 30). The protein was flash frozen in N2 (l) and stored at -70°C.

In vitro filling buffer preparation

100 mM creatine phosphate was prepared in 30 mM HEPES, pH 6.0 and supplemented with the following components.

Table 27 Base buffer components

Component	Volume	Final concentration
MgCl ₂ 1M	17.5 µL	7 mM
DTT 0.87 M	1.44 µL	0.5 mM
dATP	2.5 µL	0.1 mM
dGTP	2.5 µL	0.1 mM
dCTP	2.5 µL	0.1 mM
dTTP	2.5 µL	0.1 mM
GTP	5 µL	0.2 mM
CTP	5 µL	0.2 mM
UTP	5 µL	0.2 mM

The following working solutions and components were used:

- CPK solution (1000 µg/mL); prepared as described in Example 1
- GFP plasmid (0.9 µg/µL); prepared as described in Example 1
- ATP solution (100 mM)

In vitro AAV5 capsid filling

To generate the data presented in Figure 28, the following components were thawed on ice.

Table 28. Component list AAV5 in vitro filled particles

Component	Stock Conc	Final Conc
AAV5 empty capsid	1.3E+13 cap/ml	2.4E+12 cap/mL
Rep40	55.4 μ M	1 μ M
Rep52	20.5 μ M	1 μ M
SUMO-Rep78	11.1 μ M	1 μ M
SUMO-Rep68	15.1 μ M	1 μ M
ATP	50 μ g/ μ l (200mM)	2.5 mM
CPK	1000 μ g/ml	10 μ g/mL
pAAV-GFP	0.9 μ g/ μ l	0.0109 μ g/ μ l
Matrix buffer	na	na

To generate the data presented in Figure 28, the following reactions were prepared.

Table 29. AAV5 particle in vitro filling reaction volume (μ l)

Component	all Reps	Rep40/ SUMO- Rep68	Rep40/ SUMO- Rep78	Rep52/ SUMO- Rep68	Rep52/ SUMO- Rep78	no capsids	no DNA	No Reps
AAV5 empty capsid	18.5	18.5	18.5	18.5	18.5		18.5	18.5
Rep40	1.8	1.8	1.8			1.8	1.8	
Rep52	4.9			4.9	4.9	4.9	4.9	
SUMO-Rep78	9.0		9.0		9.0	9.0	9.0	
SUMO-Rep68	6.6	6.6		6.6		6.6	6.6	
ATP	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
CPK	1	1	1	1	1	1	1	1
pAAV-GFP	1.2	1.2	1.2	1.2	1.2	1.2		1.2

Matrix buffer	50	50	50	50	50	50	50	50
---------------	----	----	----	----	----	----	----	----

Figure 28A shows AAV titers determined by qPCR of chemically defined *in vitro* filling reactions, using different combinations of recombinant Rep proteins.

- 5 Figure 28B shows AAV fill rates determined by capsid ELISA and qPCR of chemically defined *in vitro* filling reactions, using different combinations of recombinant Rep proteins.

To generate the data presented in Figure 31, the following reaction was prepared on ice.

- 10 Table 30. AAV5 *in vitro* filled particles

Component	Stock Conc	Vol	Final Conc
AAV5 empty capsid	4.92E+13 cap/ml	244 μ l	2.4E+12 cap/mL
Rep40	55.4 μ M	90.5 μ l	1 μ M
MBP-Rep78	41.7 μ M	120 μ l	1 μ M
ATP	50 μ g/ μ l (200mM)	125 μ l	2.5 mM
CPK	1000 μ g/ml	50 μ l	10 μ g/mL
pAAV-GFP	0.9 μ g/ μ l	61 μ l	0.0109 μ g/ μ l
Matrix buffer	na	2500 μ l	na

Reactions were incubated at 300 rpm, 39°C for 4 hours on a heat block. Place on ice after incubation.

AAVX purification

- 15 The *in vitro* filling reaction was diluted with one volume of PBS and subject to AAVX purification as described in Example 3. The sample was analyzed with qPCR as described in the "Analytical procedures" section below. Results are shown in Figure 31. Figure 31 shows AAV titers determined by qPCR; and fill rates as determined by ELISA and qPCR of chemically defined *in vitro* filling reactions using optimal combinations of recombinant Rep proteins.

20

Analytical procedures

qPCR

qPCR analysis was performed essentially as described in Example 1 with the major difference that the

primer amplicon was located in the transgene region of the AAV genome. Data shown in Figure 31.

SDS-PAGE

Expression and purification MBP-Rep78 was assessed using SDS-PAGE and Western blotting. Data shown in Figure 29 and Figure 30, respectively.

5

Conclusions

This works describes:

1. The first demonstration of chemically defined *in vitro* capsid filling demonstrating maximum fill rates (~100%) of transgene in AAV5
- 10 2. The novel method of *in vitro* filling of AAV using a non-liberated Rep fusion protein, MBP-Rep78
3. Versatility of fusion proteins (SUMO and MBP) that enhance solubility of Rep proteins while retaining *in vitro* activity.
4. How different combinations of rep proteins lead to different capsid fill rates. In this example, the combination of exclusively using Rep40 and Rep78 showed maximum capsid filling rates.

15

Example 9 Truncation Analysis of *in vitro* filled capsids: proof of genome preservation

Introduction

Adeno-associated viruses (AAVs) exhibit significant variability in composition, payload, and infectivity. Truncation notably diminishes payload expression, thereby reducing treatment efficacy and overall therapeutic potency, which can compromise patient safety. To date, studies specifically targeting fully filled particles are limited, although extensive research has been conducted on quantifying the ratio of empty, partially filled, and fully filled AAVs (Ebberink *et al.* 2022, Wagner *et al.* 2024, Wu *et al.* 2022, and McColl-Carboni *et al.* 2025). Herein, the inventors delineate a method to quantify the ratio of full, partially filled, and empty particles, and present data illustrating this ratio.

20 This data commences with purified chemically defined *in vitro* filled AAVs, the production of which is described in Example 8.

For this data, truncation was determined using mass photometry. This technique analyzes biomolecules at the single-particle level in their native state, obviating the need for labels. Mass photometry (MP) experiments provide insights into the molecular weight of all components present in a sample. In MP, a single molecule that comes in contact with a measurement surface (e.g. a coverslip) is exposed to a beam of light and produces a small but measurable light scattering signal, which is directly proportional to the molecule's mass. The greater the mass of the molecule, the more intense the signal. Due to the differences in mass between empty, partially filled, and fully filled particles, it is feasible to distinguish between these particle types. By quantifying the counts of each particle type, the ratio between them can be accurately determined.

35

Here the inventors show, using in vitro filling and analysing by mass photometry, significantly higher AAV particles containing the complete nucleic acid payload when compared to the current state of the art AAV production methods.

5 **Materials**

Equipment

Operation	Unit (model and manufacturer)
Mass Photometer	Refeyn OneMP
Sonicator	Branson 2800
2 L baffled flasks	Thermo Scientific 4110-2000
Benchtop shaker	Eppendorf ThermoMixer C (with SmartBlock 1.5 mL)
Shaking incubator	New Brunswick Scientific Innova® 43
Shaking incubator	New Brunswick Innova®44
Sonicator	Vibra-Cell VCX130, ¼ inch probe
Centrifuge	Sorvall RC6 Plus
Rotor head	F12S-6x500 LEX
Centrifuge	VWR MegaStar 1.6R
Rotor head	High Conic II 75003620
Benchtop centrifuge	Hettich Mikro 220R 0005766-13
Åkta purifier	Cytiva Åkta Pure 25
SEC Superdex 200 GL 10/300	Cytiva 28990944
iBlot 2 apparatus	Invitrogen IB21001
SnakeSkin™ Dialysis tubing	Thermo Fisher 88243
CaptureSelect C-tagXL MiniChrom	Thermo Fisher 5943072001
HisTrap excel	Cytiva 29048586
2 L baffled flasks	Thermo Scientific 4110-2000
Benchtop shaker	Eppendorf ThermoMixer C (with SmartBlock 1.5 mL)

Materials

Material type	Cat. no and/or source
Chemically defined in vitro filled AAVs	Produced according to Example 8
HEK-filled AAVs	Produced according to Gibco's AAV-MAX Helper-free AAV Production System user guide (Publication number

	MAN0019619)
PBS	Sigma Aldrich P4417
Mass standards	Refeyn MassFERENCE P2 (MP-CON-41015)
MilliQ	N/A
Silicon gasket	Refeyn (MP-CON-31004)
Glass cover slips #1	VWR (631-1339)
E. coli BL21 (DE3) competent cells	New England Biolabs C2527H
2xYT broth	Cold Spring Harbour Protocols
LB broth	Cold Spring Harbour Protocols
Kanamycin	Thermo Scientific J17924.06
Isopropyl β -D-1-thiogalactopyranoside (IPTG)	VWR 437144N
TRIS	Invitrogen 15506-017
cOmplete protease inhibitor	Roche 11697498001
anti-AAV2 Replicase mouse mAb	Progen 61069
goat anti-mouse HRP conjugate	Thermo Fisher A16066
Tergitol 15-S-9	Sigma-Aldrich 15S9-500ML
DTT	Thermo Fisher R0862
Denarase	c-Lecta 20804-100k
SUMO protease	Merck SAE0067
Blotting membranes	Invitrogen 15286066
NuPage™ 4-12% Bis-Tris mini gels	Thermo Fisher NP0321BOX

Methods

Preparative procedures

Components generation

- 5 As per Example 8 and outlined below.

Empty capsid generation

AAV5 plasmid and empty capsid generation was performed as described in Example 4. Purification techniques as adopted in Example 3.

HEK-AAV reference material generation

- 10 The HEK-filled AAV material was generated using the previously stated AAV-MAX Helper-free AAV Production System user guide (Publication number MAN0019619) from Gibco. Purification techniques as adopted in Example 3.

Rep Protein generation

Rep sequence design

Four Rep proteins are encoded in a single Rep gene in the wtAAV genome. The different proteins are expressed from a combination of alternate splicing and leaky start codons. In order to separately express

5 Rep40, Rep52, Rep 68, Rep78 as four separate proteins, the amino acid sequences had to be extrapolated from Rep. Once determined, each Rep protein sequence was combined with an N-terminal cleavable SUMO fusion domain containing a purification tag, improving solubility, and a C-terminal 'c-tag' (aa sequence: EPEA) for purification (sequences of SEQ ID NOs: 40-43 listed in "Sequence Overview").

Thus four plasmid designs were created, one for each Rep protein. For each Rep protein a new cell bank

10 was created. Methods described in sections below were run four times in parallel, one for each of the four rep proteins.

Transformation and cell bank

The codon-optimized synthetic gene sequence of the target gene coding for His6-SUMO-Rep-EPEA was

15 transformed into 25 µL competent BL21 (DE3) strain by adding 0.5 µL 50 ng/µL of target plasmid DNA. The mixture was flicked to thoroughly mix and incubated on ice for 30 min. Cells were then heat shocked for exactly 10 seconds at 42°C and placed on ice for 5 min. 475 µL room temperature SOC medium was added, and the cells were placed at 37°C for 60 min with 250 rpm shaking in a benchtop shaker. 200 µL was then spread onto a selective plate consisting of LB medium containing 50 mg/L kanamycin as

20 selection marker and incubated overnight at 37°C. A scrape of transformed E. coli-cultures from selective plates was used to inoculate 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. The culture was incubated overnight in an incubator with shaking at 250 rpm at 37°C. The following morning, 25 mL autoclaved LB medium supplemented with 50 mg/L kanamycin was inoculated with 250 µL of the overnight culture and placed at 37°C with 250 rpm shaking in an incubator. When OD₆₀₀ reached 1-2,

25 sterilized glycerol was added to a final concentration of 15% and shaken until homogenous. The medium was aliquoted in 10x1 mL labelled vials and stored at -70°C as MCB. The presence of the full-length Rep fusion protein was confirmed by SDS-PAGE.

Production

30 To scale up expression, a scrape from 1 MCB vial of each of the four constructs was used to inoculate a 15 mL falcon tube with 5 mL sterile filtered LB medium containing 50 mg/L kanamycin. Cells were cultured overnight in a shaking incubator at 37.0°C with 250 rpm shaking. The following day, 4 x 2L sterile baffled shake flasks with 500 mL 2xYT medium supplemented with 50 mg/L kanamycin was inoculated with 5 mL of the overnight culture. Flasks were placed in a shaking incubator at 250 rpm at 37.0°C for

35 approximately 3 hours. When the OD₆₀₀ reached ~0.8-1.2, cells were induced by adding IPTG to a final concentration of 1 mM. The shake flasks were incubated overnight at 18°C in the same incubator and

same shaking. The following morning, cells were pelleted by centrifugation at 15,000g for 15 minutes at 4°C using a fixed-angle rotor. Supernatants were discarded and pellets were stored at -20°C until further use.

5 Cell lysis and purification

Cells were resuspended in 15 mL lysis buffer (20 mM Tris·HCl pH 8.0, complete protease inhibitor, 5 mM DTT, 7 U/ml Denarase) in a 50 mL falcon tube and lysed by sonication for 10 minutes with pulses of 2 seconds on and 4 seconds off at an amplitude of 70%, keeping the sample in an ice bath to prevent heating. Insoluble material was removed by centrifugation at 15,000g for 15 minutes using a fixed angle rotor at 4°C. The supernatant was sterile filtered through a 0.2 µm filter and loaded onto a CaptureSelect C-tagXL MiniChrom column equilibrated in equilibration buffer (100 mM Tris·HCl pH 8.0). The column was washed with a wash buffer (50 mM Tris·HCl pH 8.0, 150 mM NaCl) and the protein of interest was eluted using an elution buffer (50 mM Tris·HCl pH 8.0, 2 M MgCl₂). SDS-PAGE and Western blotting (data not shown) using an anti-AAV2 Replicase mouse monoclonal antibody was performed to positively identify the presence, purity, and identity of the proteins of interest.

Dialysis and proteolytic activation

Purified samples were dialyzed against PBS pH 7.4 containing 10% glycerol using a 10 kDa cutoff. Dialysis was performed at 5°C while slowly stirring. After at least 3 hours, the dialysis buffer was exchanged with fresh dialysis buffer and continued overnight. The following morning, the samples were centrifuged at 15,000g for 10 min at 5°C in a benchtop centrifuge. SUMO protease cleavage was carried out under these conditions: all target protein was adjusted to 10 µM in a 500 µL setup and incubated for 16 hours at 5°C and at room temperature with 0.1 U/µL SUMO protease and 1 mM DTT. Cleavage was verified by SDS-PAGE and Western blot (data not shown) using an anti-AAV2 Replicase mouse monoclonal antibody.

In vitro filling buffer preparation

HEPES 30mM, pH 6.0 solution was supplemented with the following:

Table 31 Base buffer components

Component	Volume	Final concentration
MgCl ₂ 1M	70µL	7mM
DTT 0.87 M	5.75µL	0.5mM
dATP	10µL	0.1mM
dGTP	10µL	0.1mM
dCTP	10µL	0.1mM

dTTP	10µL	0.1mM
GTP	20µL	0.2mM
CTP	20µL	0.2mM
UTP	20µL	0.2mM

The following working solutions were used:

- 5
- CPK solution (1000µg/mL): prepared as described in Example 1
 - Clarified lysate as described above.
 - GFP plasmid (0.9µg/µL)
 - ATP solution (200mM)

In vitro AAV5 capsid filling

The following conditions were prepared on ice:

10

Table 32. AAV5 in vitro filled particles

Component	Stock Conc	Vol	Final Conc
AAV5 empty capsid	6,9 E+12 cap/ml	70 µl	2.42E+12 cap/mL
Rep40	10µM	20 µl	1µM
Rep52	10µM	20µl	1µM
Rep68	10µM	20 µl	1µM
Rep72	10µM	62 µl	1µM
ATP	50 µg/µl (200mM)	5 ul	4 mM
CPK	1000 µg/ml	2 µl	10 µg/mL
pAAV-GFP	0.9 µg/µl	2.4 µl	0.0109 µg/ul
Matrix buffer	na	43.6 µl	na

Table 33. Control without Rep

Component	Stock Conc	Vol	Final Conc
-----------	------------	-----	------------

AAV5 empty capsid	6.9E+12 cap/ml	70 µl	2.42E+12 cap/mL
ATP	50 µg/µl (200mM)	5 µl	4 mM
CPK	1000 ug/ml	2 µl	10 µg/mL
pAAV-GFP	0.9 ug/µl	2.4 µl	0.0109 µg/ul
Matrix buffer	na	117.9 µl	na
H2O		5.7 µl	

Table 34. Control without pDNA

Component	Stock Conc	Vol	Final Conc
AAV5 empty capsid	6.9E+12 cap/ml	70 µl	2.42E+12 cap/mL
Rep40	10µM	20 µl	1µM
Rep52	10µM	20 µl	1µM
Rep68	10µM	20 µl	1µM
Rep72	10µM	20 µl	1µM
ATP	50 µg/µl (200mM)	5 µl	4 mM
CPK	1000 ug/ml	2,µl	10 µg/mL
Matrix buffer	na	40.7 µl	na
H2O		5.3 µl	

Table 35. Control buffer only

Component	Stock Conc	Vol	Final Conc
ATP	50 µg/µl (200mM)	6.7 µl	4 mM
CPK	1000 ug/ml	2.6 µl	10 µg/mL
Matrix buffer	na	114.2 µl	na

H2O		76.5 µl	
-----	--	---------	--

Table 36. control without capsids

Component	Stock Conc	Vol	Final Conc
Rep40	10µM	20 µl	1µM
Rep52	10µM	20µl	1µM
Rep68	10µM	20 µl	1µM
Rep72	10µM	20 µl	1µM
ATP	50 µg/µl (200mM)	5 ul	4 mM
CPK	1000 µg/ml	2 µl	10 µg/mL
pAAV-GFP	0.9 µg/µl	2.4µl	0.0109 µg/ul
Matrix buffer	na	110.6 µl	na

Reactions were incubated at 300 rpm, 39°C for 4 hours on a heat block and analyzed with qPCR.

5

Cleaning of the glass cover slips

Glass cover slips were placed in a custom Teflon holder and immersed in a 1:1 ethanol:acetone solution, followed by sonication for 20 minutes. The cover slips were then subjected to three wash cycles with MilliQ water. Subsequently, the cover slips were immersed in MilliQ water and sonicated for an additional 20 minutes, followed by three more wash cycles with MilliQ water. Finally, the cover slips were immersed in pure ethanol and sonicated for 20 minutes. The cover slips were dried using a nitrogen stream.

10

Analytical procedures

Mass Photometry

15

All mass photometry experiments were carried out at room temperature using a OneMP instrument from Refeyn. The steps below summarize the MP measurement and analysis process.

20

1. Adhere a 6-well silicone gasket to a clean cover glass.
2. Add 18 µL of PBS to a new sample well for each measurement.
3. Focus the interface between the buffer and the cover glass surface.
4. Introduce 2 µL of sample into the same well.

5. Binding to the cover glass surface was imaged and recorded for 2 minutes at a frame rate of 1 kHz.

Each sample well was used only once. Reproducibility of the mass photometry measurements was validated through independent experiments using the same sample.

5

Data processing

All mass photometry images were processed and analyzed using DiscoverMP (Refeyn). The interference intensities of the vectors were converted to masses through calibration with known mass standards.

Gaussian functions were fitted to the individual peaks, with weights assigned to represent the empty, partially filled, and fully filled adeno-associated viruses (AAVs).

10

Results and observations

Upon analyzing the chemically defined *in vitro* filled AAVs (Figure 32, longdashed line) and the HEK-filled AAVs (Figure 32, dotted line), it becomes evident that the distribution spread is significantly broader for the former. Both AAVs exhibited an empty capsid peak around 2.5 MDa; however, only the chemically defined *in vitro* filled AAVs reached a molecular weight of up to 3.5 MDa (Figure 32). In contrast, the HEK-filled material did not exceed a molecular weight of 3.1 MDa (Figure 32). Given that the full payload of the plasmid used for packaging was 2,778 base pairs (corresponding to a molecular weight of 0.9 MDa), it appeared that only the chemically defined *in vitro* filled AAVs were capable of packaging the full payload.

15

20

The peak with the highest molecular weight for HEK-filled AAVs (Figure 32, 3.1 MDa) did not surpass the 0.9 MDa threshold required for a full genome, suggesting the presence of a separate subpopulation of partially filled AAVs. It is known and previously published that the distributions for partially filled AAVs can be relatively broad (McColl-Carboni *et al* 2024, Wu *et al.*, 2010).

25

These observations supported the hypothesis that the HEK-filled material contained an increased proportion of partially filled AAVs and was unable to package the full payload, whereas the chemically defined *in vitro* filled material included a population with the full genome encapsulated within the particles.

Conclusions

30

- The chemically defined *in vitro* filled AAVs contained a significant proportion of the population with full-length genomes (2.8 kbp, corresponding to 0.9 MDa difference from empty capsids).
- The HEK-filled material exhibited a higher proportion of partially filled AAVs and lacked fully filled particles. Alternatively, the population of fully filled particles may be so small that it fell below the detection threshold for accurate quantification using mass photometry.

- In vitro filling for producing AAVs yielded a significantly higher population of AAV particles that contained 100% of the gene of interest (GoI) payload. In contrast, the standard methods currently in use produce AAV populations where no detectable amount of AAVs contained the full genomic payload. This advancement represents a substantial improvement over the existing state of the art, as it enhances the efficiency and precision of gene delivery by ensuring that a much greater proportion of AAV vectors carry the complete intended therapeutic payload.

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication and patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, and adaptations of the invention following, in general, the principles of the invention and including such departures from the invention that come within known and customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

References

- Bantel-Schaal U, Delius H, Schmidt R, zur Hausen H. Human adeno-associated virus type 5 is only distantly related to other known primate helper-dependent parvoviruses. *J Virol.* 1999 Feb;73(2):939-47. doi: 10.1128/JVI.73.2.939-947.1999
- 5 Chiorini JA, Kim F, Yang L, Kotin RM. Cloning and characterization of adeno-associated virus type 5. *J Virol.* 1999 Feb;73(2):1309-19. doi: 10.1128/JVI.73.2.1309-1319.1999.
- Chiorini JA, Yang L, Liu Y, Safer B, Kotin RM. Cloning of adeno-associated virus type 4 (AAV4) and generation of recombinant AAV4 particles. *J Virol.* 1997 Sep;71(9):6823-33. doi: 10.1128/JVI.71.9.6823-6833.1997.
- 10 Ebberink EHTM, Ruisinger A, Nuebel M, Thomann M, Heck AJR. Assessing production variability in empty and filled adeno-associated viruses by single molecule mass analyses. *Mol Ther Methods Clin Dev.* 2022;27. doi:10.1016/j.omtm.2022.11.003
- 15 Fields et al. *Virology*, 4th ed. Lippincott-Raven Publishers, Philadelphia, 1996.
- Gao G, Vandenberghe LH, Alvira MR, Lu Y, Calcedo R, Zhou X, Wilson JM. Clades of Adeno-associated viruses are widely disseminated in human tissues. *J Virol.* 2004 Jun;78(12):6381-8. doi: 10.1128/JVI.78.12.6381-6388.2004.
- 20 Gao GP, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. *Proc Natl Acad Sci U S A.* 2002 Sep 3;99(18):11854-9. doi: 10.1073/pnas.182412299. Epub 2002 Aug 21.
- 25 Heldt CL, Areo O, Joshi PU, Mi X, Ivanova Y, Berrill A. Empty and Full AAV Capsid Charge and Hydrophobicity Differences Measured with Single-Particle AFM. *Langmuir.* 2023 Apr 25;39(16):5641-5648. doi: 10.1021/acs.langmuir.2c02643. Epub 2023 Apr 11.
- 30 Issa SS, Shaimardanova AA, Solovyeva VV, Rizvanov AA. Various AAV Serotypes and Their Applications in Gene Therapy: An Overview. *Cells.* 2023 Mar 1;12(5):785. doi: 10.3390/cells12050785. PMID: 36899921; PMCID: PMC10000783.
- 35 KLAMROTH, R., HAYES, G., ANDREEVA, T., GREGG, K., SUZUKI, T., MITHA, I. H., HARDESTY, B., SHIMA, M., POLLOCK, T., SLEV, P., OLDENBURG, J., OZELO, M. C., STIELTJES, N., CASTET, S. M., MAHLANGU, J., PEYVANDI, F., KAZMI, R., SCHVED, J. F., LEAVITT, A. D., CALLAGHAN, M., PAN-

- PETESCH, B., QUON, D. V., ANDREWS, J., TRINH, A., LI, M. & WONG, W. Y. 2022. Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A. *Hum Gene Ther*, 33, 432-441.
- 5 Le DT, Radukic MT, Müller KM. Adeno-associated virus capsid protein expression in *Escherichia coli* and chemically defined capsid assembly. *Sci Rep*. 2019 Dec 9;9(1):18631. doi: 10.1038/s41598-019-54928-y. PMID: 31819093; PMCID: PMC6901487.
- McColl-Carboni A, Dollive S, Laughlin S, et al. Analytical characterization of full, intermediate, and empty AAV capsids. *Gene Ther*. 2024;31(5-6):285-294. doi:10.1038/s41434-024-00444-2
- 10 Mori S, Wang L, Takeuchi T, Kanda T. Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein. *Virology*. 2004 Dec 20;330(2):375-83. doi: 10.1016/j.virol.2004.10.012.
- 15 Muramatsu S, Mizukami H, Young NS, Brown KE. Nucleotide sequencing and generation of an infectious clone of adeno-associated virus 3. *Virology*. 1996 Jul 1;221(1):208-17. doi: 10.1006/viro.1996.0367.
- Nguyen GN, Everett JK, Kafle S, Roche AM, Raymond HE, Leiby J, Wood C, Assenmacher CA, Merricks EP, Long CT, Kazazian HH, Nichols TC, Bushman FD, Sabatino DE. A long-term study of AAV gene therapy in dogs with hemophilia A identifies clonal expansions of transduced liver cells. *Nat Biotechnol*. 2021 Jan;39(1):47-55. doi: 10.1038/s41587-020-0741-7. Epub 2020 Nov 16.
- 20 Ruffing M, Heid H, Kleinschmidt JA. Mutations in the carboxy terminus of adeno-associated virus 2 capsid proteins affect viral infectivity: lack of an RGD integrin-binding motif. *J Gen Virol*. 1994 Dec;75 (Pt 12):3385-92. doi: 10.1099/0022-1317-75-12-3385.
- Rutledge EA, Halbert CL, Russell DW. Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. *J Virol*. 1998 Jan;72(1):309-19. doi: 10.1128/JVI.72.1.309-319.1998.
- 30 Samulski RJ, Muzyczka N. AAV-Mediated Gene Therapy for Research and Therapeutic Purposes. *Annu Rev Virol*. 2014 Nov;1(1):427-51. doi: 10.1146/annurev-virology-031413-085355. PMID: 26958729.
- 35

- Schmidt M, Govindasamy L, Afione S, Kaludov N, Agbandje-McKenna M, Chiorini JA. Molecular characterization of the heparin-dependent transduction domain on the capsid of a novel adeno-associated virus isolate, AAV(VR-942). *J Virol.* 2008 Sep;82(17):8911-6. doi: 10.1128/JVI.00672-08.
- 5 Shade RO, Blundell MC, Cotmore SF, Tattersall P, Astell CR. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. *J Virol.* 1986 Jun;58(3):921-36. doi: 10.1128/JVI.58.3.921-936.1986.
- Srivastava A, Lusby EW, Berns KI. Nucleotide sequence and organization of the adeno-associated virus 2 genome. *J Virol.* 1983 Feb;45(2):555-64. doi: 10.1128/JVI.45.2.555-564.1983.
- 10 Wagner C, Fuchsberger FF, Innthaler B, et al. Automated Mass Photometry of Adeno-Associated Virus Vectors from Crude Cell Extracts. *Int J Mol Sci.* 2024;25(2). doi:10.3390/ijms25020838
- Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov.* 2019 May;18(5):358-378. doi: 10.1038/s41573-019-0012-9.
- 15 Wu D, Hwang P, Li T, Piszczek G. Rapid characterization of adeno-associated virus (AAV) gene therapy vectors by mass photometry. *Gene Ther.* 2022;29(12). doi:10.1038/s41434-021-00311-4
- 20 Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. *Mol Ther.* 2010;18(1):80-86. doi:10.1038/mt.2009.255
- Wörner TP, Bennett A, Habka S, Snijder J, Friese O, Powers T, Agbandje-McKenna M, Heck AJR. Adeno-associated virus capsid assembly is divergent and stochastic. *Nat Commun.* 2021 Mar 12;12(1):1642. doi: 10.1038/s41467-021-21935-5. PMID: 33712599; PMCID: PMC7955066.
- 25 Xiao W, Chirmule N, Berta SC, McCullough B, Gao G, Wilson JM. Gene therapy vectors based on adeno-associated virus type 1. *J Virol.* 1999 May;73(5):3994-4003. doi: 10.1128/JVI.73.5.3994-4003.1999.
- 30 Zhou X, Muzyczka N. In vitro packaging of adeno-associated virus DNA. *J Virol.* 1998 Apr;72(4):3241-7. doi: 10.1128/JVI.72.4.3241-3247.1998.
- US 6,156,303; Adeno-associated virus (AAV) isolates and AAV vectors derived therefrom

WO 00/28061, ADENO-ASSOCIATED VIRUS SEROTYPE 1 NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME.

WO 98/11244; AAV4 VECTOR AND USES THEREOF

5

WO 99/61601, AAV5 VECTOR AND USES THEREOF

Sequence Overview

SEQ ID NO:1 (VP1 AAV Serotype 2)

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEA
 5 DAAALEHDKAYDRQLDSDGNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVK
 TAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPPAAPSGGLGTNTMA
 TGS GAPMADNNEGADGVGNSSGNWHCDSTWMGDRVTTSTRTWALPTYNNHLYKQISSQSGASNDNH
 YFGYSTPWGYFDFNRHFCHFSRPDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTV
 QVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNN
 10 FTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWL
 PGPCYRQQRVSKTSADNNSEYSWTGATKYHLNGRDSLVPNGPAMASHKDDEEKFFPQSGVLIFGKQG
 SEKTNDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQG
 PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQ
 KENSKRWNPFIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

15

SEQ ID NO:2 (VP1 AAV Serotype 5)

MSFVDHPPDWLEEVGEGLREFLGLGAGPPKPKPNQQHQDQARGLVLPGYNYLGPNGLDRGEPVNRA
 DEVAREHDISYNEQLEAGDNPYLYNHADADEFQEKLADDTSGGNLKGAVFQAKKRVLEPGLVEEGAK
 TAPTGKRIDDHFKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAPASSLGADTMSAGGGGGLGDN
 20 NQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWLPSYNNHQQYREIKSGSVDGSNANAYFGYSTPW
 GYFDFNRHFHSHWSPRDWQRLINNYWGFRPSRLVKIFNIQVKEVTQDSTTTTIANNLTSTVQVFTDDDYQ
 LPYVVGNGTEGCLPAFPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFE
 EVPFHSSFAPSQNLFLKANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNL
 GSGVNRASVSFAATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEG
 25 NMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPWAKIPET
 GAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRW
 NPEIQYTNNYNNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL

SEQ ID NO:3 (VP1 AAV Serotype 6)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNA
 30 ADAAALEHDKAYDQQLKAGDNPYLYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRVLEPGLVEEG
 AKTAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPDQPLGEPPATPAAVGPTTM
 ASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTTSTRTWALPTYNNHLYKQISSASTGASND
 NHYFGYSTPWGYFDFNRHFCHFSRPDWQRLINNNWGFRPKRLNFKLFNIQVKEVTNDGVTTTIANNLTS
 35 TVQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGN
 NFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNNQSGSAQNKDLLFSRGPAGMSVQPKN

WLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGK
ESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYL
QGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEW
ELQKENSkrwnPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

5

SEQ ID NO:4 (VP1 AAV Serotype 8)

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNA
ADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEG
AKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVDPQPLGEPPAAPSGVGPNP
10 MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGAT
NDNTYFGYSTPWGYFDNRFHCHFSRPDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNL
TSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTQTTGGTANTQTLGFSQGGPNPMTANQAK
NWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGK
15 QNAARDNADYSDVMLTSEEEIKTTNPVATEEYGIVADNLQQQNTAPQIGTVNSQGALPGMVWQNRDVYL
QGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEW
ELQKENSkrwnPEIQYTSNYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO:5 (VP2 AAV Serotype 2)

20 TAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLTNTMA
TGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNH
YFGYSTPWGYFDNRFHCHFSRPDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTV
QVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNN
FTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRQFSQAGASDIRDQSRNWL
25 PGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNGPAMASHKDDEEKFFPQSGVLIFGKQG
SEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQG
PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAKFASFITQYSTGQVSVEIEWELQ
KENSkrwnPEIQYTSNYNKSVDFTVDNNGVYSEPRPIGTRYLTRNL

30 SEQ ID NO:6 (VP3 AAV Serotype 2)

MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASND
NHYFGYSTPWGYFDNRFHCHFSRPDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTST
TVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRQFSQAGASDIRDQSRN
35 WLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNGPAMASHKDDEEKFFPQSGVLIFGK
QGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYL

QGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEW
ELQKENSkrwnPEIQYTSNYNKSvNVDFTVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO:7 (VP1 AAV Serotype 1)

5 MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNGLDKGEPVNA
ADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEG
AKTAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPQPPLGEPPATPAAVGPTTM
ASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRIITTSTRTWALPTYNNHLYKQISSASTGASND
NHYFGYSTPWGYFDFNRHFCHFSRPDWQRLINNNWGFPRKRLNFKLFNIQVKEVTTNDGVTTIANNLTS
10 TVQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGN
NFTFSYTFEEVPPFHSSYAHSQSLDRLMNPLIDQYLYLNRQTQNSGSAQNKDLLFSRGSPAGMSVQPKN
WLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDEDEKFFPMSGVMIFGK
ESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSTDPATGDVHAMGALPGMVWQDRDRVYL
QGPIWAKIPHTDGHFHPSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEW
15 ELQKENSkrwnPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

SEQ ID NO:8 (VP1 AAV Serotype 3)

MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPFGNGLDKGEPVNE
ADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEAA
20 KTAPGKKRPVDQSPQEPDSSSGVGKSGKQPAKKRLNFGQTGDSESVDPQPPLGEPPAAPTSLGSNTM
ASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRIITTSTRTWALPTYNNHLYKQISSQSGASNDN
HYFGYSTPWGYFDFNRHFCHFSRPDWQRLINNNWGFPRKLSFKLFNIQVKEVTQNDGTTTIANNLTST
VQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGN
NFQFSYTFEDVPPFHSSYAHSQSLDRLMNPLIDQYLYLNRQTGTTSGTTNQSRLFSQAGPQSMQLQAR
25 NWLPGPCYRQQRSLKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASHKDEEEKFFPMHGNLIF
GKEGTTASNAELDNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDRV
YLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEI
EWELQKENSkrwnPEIQYTSNYNKSvNVDFTVDTNGVYSEPRPIGTRYLTRNL

30 SEQ ID NO:9 (VP1 AAV Serotype 7)

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFGNGLDKGEPVNA
ADAAALEHDKAYDQQLKAGDNPYLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEG
AKTAPAKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLNFGQTGDSESVDPQPPLGEPPAAPSSVSGST
VAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRIITTSTRTWALPTYNNHLYKQISSSETAGSTN
35 DNTYFGYSTPWGYFDFNRHFCHFSRPDWQRLINNNWGFPRKLRFKLFNIQVKEVTTNDGVTTIANNLT
STIQVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQSVGRSSFYCLEYFPSQMLRTG

NNFEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLARTQSNPGGTAGNRELQFYQGGPSTMAEQAK
KNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDERFFPSSGVLIF
GKTGATNKTTLENVLMTNEEEIRPTNPVATEEYGIVSSNLQAANTAAQTQVVNNQGALPGMVWQNRDQV
LQGPWAKIPHTDGNFHPSPMLMGFGGLKHPPQILIKNTPVPANPPEVFTPAKFASFITQYSTGQVSVEIE
5 WELQKENS KRWNPEIQYTSNFEKQTGVDFAVDSQGVYSEPRPIGTRYLTRNL

SEQ ID NO:10 (VP1 AAV Serotype 9)

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLPGNGLDKGEPVNA
ADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKRILLEPLGLVEEAA
10 KTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTEVPDPQPIGEPPAAPSGVSLTMAS
GGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRTVTTSTRTWALPTYNNHLYKQISNSTSGGSSNDN
AYFGYSTPWGYDFDNRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLTST
VQVFTDSYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNN
FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP
15 GPSYRQQRVSTTVTQNNNSEFAWPAGSSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQG
TGRDNVDADKVMITNEEEIKTTNPVATESYGGVATNHQSAQAQAQTGWVWQNGILPGMVWQDRDQVYLQ
GPIWAKIPHTDGNFHPSPMLMGFGGMKHPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWE
LQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

20 SEQ ID NO:11 (VP2 AAV Serotype 1)

TAPGKKRPVEQSPQEPDSSSGIGKTGQPAKKRLNFGQTGDSESVDPDPQLGEPPATPAAVGPTTMA
GGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVTTSTRTWALPTYNNHLYKQISSASTGASNDNH
YFGYSTPWGYDFDNRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTV
QVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNF
25 TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNGSGSAQNKDLLFSRGPAGMSVQPKNWL
PGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKFFPMGSGVMIFGKESA
GASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSTDPATGDVHAMGALPGMVWQDRDQVYLQ
PIWAKIPHTDGHFHPSPMLMGFGGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ
KENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

30

SEQ ID NO:12 (VP2 AAV Serotype 3b)

TAPGKKRPVDQSPQEPDSSSGVGKSGKQPAKKRLNFGQTGDSESVDPDPQLGEPPAAPTSLSNTMA
SGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRTVTTSTRTWALPTYNNHLYKQISSQSGASNDNH
YFGYSTPWGYDFDNRFHCHFSPRDWQRLINNNWGFPRKLSFKLFNIQVKEVTQNDGTTTIANNLTSTV
35 QVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNN
FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQGTTS GTTNQSRLLFSQAGPQSMQLQARN

WLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVPNPGPAMASHKDDEEKFFPMHGNLIFGK
EGTTASNAELDNVMITDEEEIRTTNPVATEQYGTVANLQSSNTAPTTRTVNDQGALPGMVWQDRDVYL
QGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEW
ELQKENSkrwnPEIQYTSNYKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

5

SEQ ID NO:13 (VP2 AAV Serotype 5)

TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAPASSLGADTMSAGGGGGLGDN
NQGADGVGNASGDWHCDSTWMGDRVTKSTRTWLPSYNNHQRREIKSGSVDGSNANAYFGYSTPW
GYFDNRFHSHWSPRDWQRLINNYWGRFRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQ
10 LPYVVGNGTEGCLPAFPQVFTLPQYGYATLNDRNTENPTERSFFCLEYFPSKMLRTGNNEFTYNFE
EVPFHSSFAPSQNLFLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFGPMGRTQGWNL
GSGVNRASVSFAATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEG
NMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIIVPGSVWMERDVYLQGPWAKIPET
GAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRW
15 NPEIQYTNNYNDPQFVDFAPDSTGEYRTRPIGTRYLTRPL

SEQ ID NO:14 (VP2 AAV Serotype 6)

TAPGKKRPVEQSPQEPDSSSGIGKTGQPAKKRLNFGQTGDSESVDPQPLGEPPATPAAVGPTTMAS
GGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVTTSTRTWALPTYNNHLYKQISSASTGASNDNH
20 YFGYSTPWGYFDNRFHCHFSRPDWQRLINNNWGRFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTV
QVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNF
TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNGSGSAQNKDLLFSRGSPPAGMSVQPKNWL
PGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESA
GASNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQG
25 PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ
KENSKRWNPVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

SEQ ID NO:15 (VP2 AAV Serotype 7)

TAPAKKRPVEPSPQRSPDSSTGIGKKGQPAKRLNFGQTGDSESVDPQPLGEPPAAPSSVSGSTVA
30 AGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVTTSTRTWALPTYNNHLYKQISSETAGSTNDN
TYFGYSTPWGYFDNRFHCHFSRPDWQRLINNNWGRFRPKLRFLNFKLFNIQVKEVTTNDGVTTIANNLTSTI
QVFSDEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQSVGRSSFYCLEYFPSQMLRTGNF
EFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLARTQSNPGGTAGNRELQFYQGGPSTMAEQAKN
WLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDedrffpssgvlifgk
35 TGATNKTTLENVLMTNEEEIRPTNPVATEEYGIVSSNLQAANTAAQTQVNNQALPGMVWQNRDVYLQ

GPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPANPPEVFTPAKFASFITQYSTGQVSVEIEWEL
QKENS KRWNPEIQYTSNFEKQTGVDFAVDSQGVYSEPRPIGTRYLTRNL

SEQ ID NO:16 (VP2 AAV Serotype 8)

5 TAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVDPQPPLGEPPAAPSGVGPNTMA
AGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDREVTTSTRTWALPTYNNHLYKQISNGTSGGATND
NTYFGYSTPWGYFDFNRHFCHFSRDPWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTS
TIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGN
NMQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTQTTGGTANTQTLGFSQGGPNTMANQAKN
10 WLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQ
NAARDNADYSDVMLTSEEEIKTTNPVATEEYGIVADNLQQQNTAPQIGTVNSQGALPGMVWQNRDVYLQ
GPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWE
LQKENS KRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL

15 SEQ ID NO:17 (VP2 AAV Serotype 9)

TAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTEVPDPQPIGEPPAAPSGVGSLTMAS
GGGAPVADNNEGADGVGSSSGNWHCDSTWLGDREVTTSTRTWALPTYNNHLYKQISNSTSGGSSNDN
AYFGYSTPWGYFDFNRHFCHFSRDPWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTST
VQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGN
20 FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP
GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQG
TGRDNVDADKVMITNEEEIKTTNPVATESYGGQVATNHQSAQAQAQTGWQNNQILPGMVWQDRDVYLQ
GPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWE
LQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

25

SEQ ID NO:18 (VP3 AAV Serotype 1)

MASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDREVTTSTRTWALPTYNNHLYKQISSASTGASN
DNHYFGYSTPWGYFDFNRHFCHFSRDPWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLT
STVQVFSQSEYQLPYVLGSAHQGCLPPFPADVFMPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
30 NNFTFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNNQSGSAQNKDLLFSRGSPAGMSVQPK
NWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDDEKFFPMMSGVMIFG
KESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNFQSSSTD PATGDVHAMGALPGMVWQDRDVY
LQGPIWAKIPHTDGHFHPSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIE
WELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

35

SEQ ID NO:19 (VP3 AAV Serotype 3b)

MASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRTVTTSTRTWALPTYNNHLYKQISSQSGASND
NHYFGYSTPWGYFDFNRHFCHFSRDLWQRLINNNWGFPRPKLSFKLFNIQVKEVTQNDGTTTIANNLTS
TVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQGTTS GTTNQSRLLFSQAGPQSMQLQA
5 RNWLPGPCYRQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVPNGPAMASHKDDEEKFFPMHGNLIF
GKEGTTASNAELDNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDV
YLQGPWAKIPHTDGHFHPSPMLGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEI
EWELQKENS KRWNPEIQYTSNYSNKS NVNDFTVDTNGVYSEPRPIGTRYLTRNL

10 SEQ ID NO:20 (VP3 AAV Serotype 5)

MSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVTKSTRTWLPSYNNHQQYREIKSGSVDG
SNANAYFGYSTPWGYFDFNRHSHWSPRDWQRLINNYWGFPRSLRVKIFNIQVKEVTQDSTTTIANN
LTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPQPQVFTLPQYGYATLNRDNTENPTERSSSFFCLEYFPSKM
LRTGNNFEFTYNFEEVFPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNW
15 FPGPMGRTQGWNLGSGVNRASVSFAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNS
QPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEI VPGSVWMMER
DVYLQGPWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVTE
MEWELKKENS KRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL

20 SEQ ID NO:21 (VP3 AAV Serotype 6)

MASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVTTSTRTWALPTYNNHLYKQISSASTGASN
DNHYFGYSTPWGYFDFNRHFCHFSRDLWQRLINNNWGFPRKRLNFKLFNIQVKEVTNDGVTTIANNLT
STVQVFS DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNNQSGSAQNKDLLFSRGSPAGMSVQPK
25 NWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFG
KESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTD PATGDVHVMGALPGMVWQDRDVY
LQGPWAKIPHTDGHFHPSPMLGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIE
WELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

30 SEQ ID NO:22 (VP3 AAV Serotype 7)

VAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRTVTTSTRTWALPTYNNHLYKQISSSETAGSTN
DNTYFGYSTPWGYFDFNRHFCHFSRDLWQRLINNNWGFPRPKLRFKLFNIQVKEVTNDGVTTIANNLT
STIQVFS DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQSVGRSSFYCLEYFPSQMLRTG
NNFEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLARTQSNPGGTAGNRELQFYQGGPSTMAEQ A
35 KNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDERFFPSSGVLIF
GKTGATNKTTLENVLMTNEEEIRPTNPVATEEYGVSSNLQAANTAAQTQVNNQ GALPGMVWQNRDVY

LQGPWAKIPHTDGNFHPSPMLMGGFGLKHPPPQILIKNTPVPANPPEVFTPAKFASFITQYSTGQVSVEIE
WELQKENS KRWNPEIQYTSNFEKQTGVDFAVDSQGVYSEPRPIGTRYLTRNL

SEQ ID NO:23 (VP3 AAV Serotype 8)

5 MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGD RVITTSTRTWALPTYNNHLYKQISNGTSGGAT
NDNTYFGYSTPWGYDFNRFHCHFS PRDWQRLINNNWGF RPKRLSFKLFNIQVKEVTQNEGKTIANNL
TSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTQTTGGTANTQTLGFSQGGPNTMANQAK
NWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDEERFFPSNGILIFGK
10 QNAARDNADYS DVMILTSEEEIKTTNPVATEEY GIVADNLQQQNTAPQIGTVNSQ GALPGMVWQNRDVYL
QGPIWAKIPHTDGNFHPSPMLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEW
ELQKENS KRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO:24 (VP3 AAV Serotype 9)

15 MASGGGAPVADNNEGADGVGSSSGNWHCD SQWLGD RVITTSTRTWALPTYNNHLYKQISNSTSGGSS
NDNAYFGYSTPWGYDFNRFHCHFS PRDWQRLINNNWGF RPKRLNFKLFNIQVKEVTDNNGVKTIANNL
TSTVQVFTDSYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRT
GNNFQFSYEFENVFPFHSSYAHSQSLDRLMNPLIDQYLYLSKTINGSGQNQQTLKFSVAGPSNMAVQGR
NYIPGPSYRQQRVSTTVTQNNNSEFAWP GASSWALN GRNSLMNPGPAMASHKEGEDRFFPLSGSLIFG
20 KQGTGRDNVDADKVMITNEEEIKTTNPVATESY GQVATNHQSAQAQAQTGWQNGILPGMVWQDRD
VYLQGPIWAKIPHTDGNFHPSPMLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVE
IEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO:25 (pAAV-RC2 Vector sequence) [131-1996: AAV-2 Rep gene, 2013-4220: AAV-2 Cap gene

25 5292-6152: Ampicillin Resistance (bla) gene]
GCGCGCCGATATCGTTAACGCCCCGCGCCGCGCTCTAGAACTAGTGGATCCCCCGGAAGATCA
GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAAGTATAGGAACTTCTGATCTGCGCAGCCGCCATG
CCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCGGCATTTCTG
ACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTGA
30 ATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGCGACTTTCTGACGGAATGGC
GCCGTGTGAGTAAGGCCCCCGAGGCCCTTTCTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTCC
ACATGCACGTGCTCGTGAAACCACCGGGGTGAAATCCATGGTTTTGGGACGTTTCCTGAGTCAGAT
TCGCGAAAAACTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAACTGGTTCGCGGTC
ACAAAGACCAGAAATGGCGCCGAGGCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTAC
35 TTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAATATGGAACAGTATTTAAGCGCCT
GTTTGAATCTCACGAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGACAGCGCAGG

AGCAGAACAAAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAG
GTACATGGAGCTGGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGG
AGGACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCAAGGCTGCCTT
GGACAAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGTGGGCCAGCAGCC
5 CGTGGAGGACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAACGGGTACGATCCCCAATATG
CGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTGCGCAAGAGGAACACCATCTGGCTGTTTG
GGCCTGCAACTACCGGAAGACCAACATCGCGGAGGCCATAGCCCACACTGTGCCCTTCTACGGGT
GCGTAAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTGACAAGATGGTGATCTGGTGGGA
GGAGGGGAAGATGACCGCCAAGGTCGTGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGC
10 GCGTGGACCAGAAATGCAAGTCCTCGGCCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACA
CCAACATGTGCGCCGTGATTGACGGGAAGTCAACGACCTTGAACACCAGCAGCCGTTGCAAGACC
GGATGTTCAAATTTGAACTACCCGCGCTGCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGT
CAAAGACTTTTTCCGGTGGGCAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAAG
GGTGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGCGCGA
15 GTCAGTTGCGCAGCCATCGACGTGACGCGGAAGCTTCGATCAACTACGCAGACAGGTACCAAAA
CAAATGTTCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAAT
CAGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGCTTCCCGTGTCAGAATC
TCAACCCGTTTCTGTCGTCAAAAAGGCGTATCAGAACTGTGCTACATTATCATATCATGGGAAAGG
TGCCAGACGCTTGCACTGCCTGCGATCTGGTCAATGTGGATTTGGATGACTGCATCTTTGAACAATA
20 AATGATTTAAATCAGGTATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGAA
GGAATAAGACAGTGGTGGAAAGCTCAAACCTGGCCCACCACCACCAAAGCCCCGAGAGCGGCATAAG
GACGACAGCAGGGGTCTTGTGCTTCCCTGGGTACAAGTACCTCGGACCCTTCAACGGACTCGACAAG
GGAGAGCCGCTCAACGAGGCAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCAGC
TCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCGGAGTTTCAGGAGCGCCTTA
25 AAGAAGATACGTCTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAGAGGGTTCTTG
AACCTCTGGGCCTGGTTGAGGAACCTGTAAAGACGGCTCCGGGAAAAAAGAGGCCCGGTAGAGCACT
CTCCTGTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCCAGCAGCCTGCAAGAAAAAGA
TTGAATTTTGGTCAGACTGGAGACGCAGACTCAGTACCTGACCCCCAGCCTCTCGGACAGCCACCA
GCAGCCCCCTCTGGTCTGGGAAGTAATACGATGGCTACAGGCAGTGGCGCACCAATGGCAGACAAT
30 AACGAGGGCGCCGACGGAGTGGGTAATTCCCTCGGGAAATTGGCATTGCGATTCCACATGGATGGGC
GACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCACCTACAACAACCACCTCTACAAA
CAAATTTCCAGCCAATCAGGAGCCTCGAACGACAATCACTACTTTGGCTACAGCACCCCTTGGGGGT
ATTTTGACTTCAACAGATTCCACTGCCACTTTTACCACGTGACTGGCAAAGACTCATCAACAACAAC
TGGGGATTCCGACCCAAGAGACTCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGA
35 ATGACGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTTACTGACTCGGAGTA
CCAGCTCCCGTACGTCTCGGCTCGGCGCATCAAGGATGCCTCCCGCCGTTCCAGCAGACGTCTT

CATGGTGCCACAGTATGGATACCTCACCCCTGAACAACGGGAGTCAGGCAGTAGGACGCTCTTCATTT
TACTGCCTGGAGTACTTTCTTCTCAGATGCTGCGTACCGGAAACAACTTTACCTTCAGCTACACTTT
TGAGGACGTTCCTTTCCACAGCAGCTACGCTCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTC
ATCGACCAGTACCTGTATTACTTGAGCAGAACAACACTCCAAGTGAACCACCACGCAGTCAAGGC
5 TTCAGTTTTCTCAGGCCGGAGCGAGTGACATTCGGGACCAGTCTAGGAAGTGGCTTCCTGGACCCT
GTTACCGCCAGCAGCGAGTATCAAAGACATCTGCGGATAACAACAACAGTGAATACTCGTGGAAGTGG
AGCTACCAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGGGCCCCGGCCATGGCAAGCCA
CAAGGACGATGAAGAAAAGTTTTTCTCAGAGCGGGGTTCTCATCTTTGGGAAGCAAGGCTCAGAG
AAAACAAATGTGGACATTGAAAAGGTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCCCG
10 TGGCTACGGAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGAGGCAACAGACAAGCAGCTACCG
CAGATGTCAACACACAAGGCGTTCTTCCAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAGG
GGCCCATCTGGGCAAAGATTCCACACACGGACGACATTTTCACCCCTCTCCCCTCATGGGTGGATT
CGGACTTAAACACCCCTCCTCCACAGATTCTCATCAAGAACACCCCGGTACCTGCGAATCCTTCGACC
ACCTTCAGTGCGGCAAAGTTTGCTTCCTTCATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGA
15 TCGAGTGGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCCGAAATTCAGTACACTTCCAATA
CAACAAGTCTGTTAATGTGGACTTTACTGTGGACACTAATGGCGTGTATTCAGAGCCTCGCCCCATT
GGCACCAGATACCTGACTCGTAATCTGTAATTGCTTGTTAATCAATAAACCGTTTAATTCGTTTCAGTT
GAACTTTGGTCTCTGCGTATTTCTTTCTTATCTAGTTTCCATGGCTACGTAGATAAGTAGCATGGCGG
GTTAATCATTAATACTACAGCCCGGGCGTTTAAACAGCGGGCGGAGGGGTGGAGTCGTGACGTGAATT
20 ACGTCATAGGGTTAGGGAGGTCCTGTATTAGAGGTCACGTGAGTGTGTTTTCGACATTTTTCGACACC
ATGTGGTCTCGCTGGGGGGGGGGGGCCCCGAGTGAGCACGCAGGGTCTCCATTTTGAAGCGGGAGGT
TTGAACGAGCGCTGGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG
TTACCCAACTTAATCGCCTTGACAGCACATCCCCCTTTCCGAGCTGGCGTAATAGCGAAGAGGCCCCG
CACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAAATTGTAAGCGTTAATATTT
25 TGTTAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTTAACCAATAGGCCGAAATCGGCAAA
ATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCC
ACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTA
CGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCCTA
AAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAGCCGCGAACGTGGCGAGAAAGGAAGGGAAG
30 AAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCAACAC
ACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAATGTGCGCG
GAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCCTGAT
AAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATCCCT
TTTTTTCGCGCATTTTGCCTTCCTGTTTTTGTCTACCCAGAAACGCTGGTGAAGTAAAGATGCTGAA
35 GATCAGTTGGGTGCACGAGTGGGTACATCGAAGTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT
TTCGCCCCGAAGAACGTTTTCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCC

CGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGT
ACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCAT
AACCATGAGTGATAAACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAAC
CGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA
5 GCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAAC TA
TTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAG
TTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCG
GTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG
TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTG
10 CCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAC
TTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAAC
GTGAGTTTTTCTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT
TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTGCCGG
ATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTT
15 CTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTC
TGCTAATCCTGTTACCACTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAAGTCAAG
ACGATAGTTACCGGATAAGGCGCAGCGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCT
TGGAGCGAACGACCTACCCGAAGTGAAGTACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTC
CCGAAGGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG
20 GGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGGTTTCGCCACCTCTGACTTGAG
CGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCCTTT
TTACGGTTCCTGGCCTTTTGTGCGCTTTTGTCTACATGTTCTTCTGCGTTATCCCTGATTCTGT
GGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAG
CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGC
25 CGATTTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAAGCGGGCAGTGAGCGCAACGCAA
TTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTG
TGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAA

SEQ ID NO:26 (pHelper Vector sequence) [1-5336: Adenovirus E2A gene, 5337-8537: Adenovirus E4
30 gene, 8538-9280: Adenovirus VA gene, 10182-11042: Ampicillin Resistance (bla) gene (complement)]
GGTACCCAACCTCATGCTTAACAGTCCCCAGGTACAGCCCACCCTGCGTCGCAACCAGGAACAGCT
CTACAGCTTCCTGGAGCGCCACTCGCCCTACTTCCGCAGCCACAGTGCGCAGATTAGGAGCGCCAC
TTCTTTTTGTCACTTGAAAAACATGTAAAAATAATGTACTAGGAGACACTTTCAATAAAGGCAAATGTT
TTTATTTGTACACTCTCGGGTGATTATTTACCCCCACCCTTGCCGTCTGCGCCGTTAAAAATCAAA
35 GGGGTTCTGCCGCGCATCGCTATGCGCCACTGGCAGGGACACGTTGCGTACTGGTGTTTGTAGTGCT
CCACTTAACTCAGGCACAACCATCCGCGGCAGCTCGGTGAAGTTTTCACTCCACAGGCTGCGCAC

CATCACCAACGCGTTTAGCAGGTCGGGCGCCGATATCTTGAAGTCGCAGTTGGGGCCTCCGCCCTG
CGCGCGCGAGTTGCGATACACAGGGTTGCAGCACTGGAACACTATCAGCGCCGGGTGGTGCACGC
TGGCCAGCACGCTCTTGTCTGGAGATCAGATCCGCGTCCAGGTCTCCGCGTTGCTCAGGGCGAAC
GGAGTCAACTTTGGTAGCTGCCTTCCCAAAAAGGGTGCATGCCCAGGCTTTGAGTTGCACTCGCAC
5 CGTAGTGGCATCAGAAAGGTGACCGTGCCCGGTCTGGGCGTTAGGATACAGCGCCTGCATGAAAGC
CTTGATCTGCTTAAAGCCACCTGAGCCTTTGCGCCTTCAGAGAAGAACATGCCGCAAGACTTGCCG
GAAAACTGATTGGCCGGACAGGCCGCGTCATGCACGCAGCACCTTGCGTCGGTGTTGGAGATCTGC
ACCACATTTTCGGCCCCACCGGTTCTTCACGATCTTGCCCTTGCTAGACTGCTCCTTCAGCGCGCGCT
GCCCCGTTTTGCTCGTCATCATCCATTTCAATCACGTGCTCCTTATTTATCATAATGCTCCCGTGTAGA
10 CACTTAAGCTCGCCTTCGATCTCAGCGCAGCGGTGCAGCCACAACGCGCAGCCCGTGGGCTCGTG
GTGCTTGTAGGTTACCTCTGCAAACGACTGCAGGTACGCCTGCAGGAATCGCCCCATCATCGTCACA
AAGGTCTTGTTGCTGGTGAAGGTCAGCTGCAACCCGCGGTGCTCCTCGTTTAGCCAGGTCTTGATA
CGGCCGCCAGAGCTTCCACTTGGTCAGGCAGTAGCTTGAAGTTTGCCTTTAGATCGTTATCCACGTG
GTACTTGTCCATCAACGCGCGCGCAGCCTCCATGCCCTTCTCCACGCAGACACGATCGGCAGGCT
15 CAGCGGGTTTATCACCGTGCTTTCACTTTCCGCTTCACTGGACTCTTCCTTTTCTCTTGCGTCCGCA
TACCCCGCGCCACTGGGTGCTCTTCAATCAGCCGCCGACCGTGCGCTTACCTCCCTTGCCGTGCT
TGATTAGCACCGGTGGGTGCTGAAACCCACCATTTGTAGCGCCACATCTTCTCTTTCTTCTCGCT
GTCCACGATCACCTCTGGGGATGGCGGGCGCTCGGGCTTGGGAGAGGGGCGCTTCTTTTTCTTTTT
GGACGCAATGGCCAAATCCGCCGTCGAGGTGATGGCCGCGGGCTGGGTGTGCGCGGCACCAGC
20 GCATCTTGTGACGAGTCTTCTTCGTCCTCGGACTCGAGACGCCGCCTCAGCCGCTTTTTTGGGGGC
GCGCGGGGAGGCGGCGGCGACGGCGACGGGGACGACAGTCTCCTCATGGTTGGTGGACGTCGCG
CCGCACCGCGTCCGCGCTCGGGGGTGGTTTCGCGCTGCTCCTCTTCCCGACTGGCCATTTCTTCT
CCTATAGGCAGAAAAAGATCATGGAGTCAGTCGAGAAGGAGGACAGCCTAACCGCCCCCTTTGAGT
TCGCCACCACCGCTCCACCGATGCCGCCAACGCGCCTACCACCTTCCCCGTCGAGGCACCCCCG
25 CTTGAGGAGGAGGAAGTGATTATCGAGCAGGACCCAGGTTTTGTAAGCGAAGACGACGAGGATCGC
TCAGTACCAACAGAGGATAAAAAAGCAAGACCAGGACGACGCAGAGGCAAACGAGGAACAAGTCGGG
CGGGGGGACCAAAGGCATGGCGACTACCTAGATGTGGGAGACGACGTGCTGTTGAAGCATCTGCA
GCGCCAGTGCGCCATTATCTGCGACGCGTTGCAAGAGCGCAGCGATGTGCCCCCTCGCCATAGCGG
ATGTCAGCCTTGCCACGAACGCCACCTGTTCTACCGCGCGTACCCCCCAAACGCCAAGAAAACG
30 GCACATGCGAGCCCAACCCGCGCCTCAACTTCTACCCCGTATTTGCCGTGCCAGAGGTGCTTGCCA
CCTATCACATCTTTTTCCAAAAGTCAAGATACCCCTATCCTGCCGTGCCAACCGCAGCCGAGCGGA
CAAGCAGCTGGCCTTGCGGCAGGGCGCTGTACATACCTGATATCGCCTCGCTCGACGAAGTGCCAAA
AATCTTTGAGGGTCTTGACGCGACGAGAAACGCGCGGCAAACGCTCTGCAACAAGAAAACAGCGA
AAATGAAAGTCACTGTGGAGTGCTGGTGGAATTGAGGGTGACAACGCGCGCCTAGCCGTGCTGAA
35 ACGCAGCATCGAGGTCACCCACTTTGCCTACCCGGCACTTAACCTACCCCCAAGGTTATGAGCACA
GTCATGAGCGAGCTGATCGTGCGCGTGCACGACCCCTGGAGAGGGATGCAAACCTTGCAAGAACA

ACCGAGGAGGGCCTACCCGAGTTGGCGATGAGCAGCTGGCGCGCTGGCTTGAGACGCGCGAGC
CTGCCGACTTGAGGAGCGACGCAAGCTAATGATGGCCGAGTGCTTGTTACCGTGAGCTTGAGT
GCATGCAGCGGTTCTTTGCTGACCCGGAGATGCAGCGCAAGCTAGAGGAAACGTTGCACTACACCT
TTCGCCAGGGCTACGTGCGCCAGGCCTGCAAAATTTCCAACGTGGAGCTCTGCAACCTGGTCTCCT
5 ACCTTGGAATTTTGACGAAAACCGCCTCGGGCAAAACGTGCTTCATTCCACGCTCAAGGGCGAGG
CGCGCCGCGACTACGTCCGCGACTGCGTTTACTTATTTCTGTGCTACACCTGGCAAACGGCCATGG
GCGTGTGGCAGCAATGCCTGGAGGAGCGCAACCTAAAGGAGCTGCAGAAGCTGCTAAAGCAAACT
TGAAGGACCTATGGACGGCCTTCAACGAGCGCTCCGTGGCCGCGCACCTGGCGGACATTATCTTCC
CCGAACGCCTGCTTAAACCCCTGCAACAGGGTCTGCCAGACTTCACCACTCAAAGCATGTTGCAAAA
10 CTTTAGGAATTTATCCTAGAGCGTTCAGGAATTCTGCCGCCACCTGCTGTGCGCTTCTAGCGAC
TTTGTGCCCATTAAGTACCGTGAATGCCCTCCGCCGCTTTGGGGTCACTGCTACCTTCTGCAGCTAG
CCAATACTTGCCTACCACTCCGACATCATGGAAGACGTGAGCGGTGACGGCCTACTGGAGTGTC
ACTGTGCGTGCAACCTATGCACCCCGCACCGCTCCCTGGTCTGCAATTCGCAACTGCTTAGCGAAA
GTCAAATTATCGGTACCTTTGAGCTGCAGGGTCCCTCGCCTGACGAAAAGTCCGCGGCTCCGGGGT
15 TGAAACTCACTCCGGGGCTGTGGACGTGCGCTTACCTTCGCAAATTTGTACCTGAGGACTACCACGC
CCACGAGATTAGTTCTACGAAGACCAATCCCGCCCGCCAAATGCGGAGCTTACCGCCTGCGTCAT
TACCCAGGGCCACATCCTTGGCCAATTGCAAGCCATCAACAAAGCCCGCCAAGAGTTTCTGCTACGA
AAGGGACGGGGGTTTACCTGGACCCCGAGTCCGGCGAGGAGCTCAACCCAATCCCCCGCCGCC
GCAGCCCTATCAGCAGCCGCGGGCCCTTGCTTCCAGGATGGCACCCAAAAAGAGCTGCAGCTG
20 CCGCCGCCGCCACCCACGGACGAGGAGGAATACTGGGACAGTCAGGCAGAGGAGGTTTTGGACGA
GGAGGAGGAGATGATGGAAGACTGGGACAGCCTAGACGAAGCTTCCGAGGCCGAAGAGGTGTCAG
ACGAAACACCGTCAACCTCGGTGCGATTCCCCTCGCCGGCGCCCCAGAAATTGGCAACCGTTCCCA
GCATCGCTACAACCTCCGCTCCTCAGGCGCCGCCGGCACTGCCTGTTGCGCGACCCAACCGTAGAT
GGGACACCACTGGAACCAGGGCCGGTAAGTCTAAGCAGCCGCCGCCGTTAGCCCAAGAGCAACAA
25 CAGCGCCAAGGCTACCGCTCGTGCGCGGGCACAAGAACGCCATAGTTGCTTGCTTGCAAGACTGT
GGGGGCAACATCTCCTTCGCCCCGCCGCTTTCTTCTCTACCATCACGGCGTGCGCTTCCCCCGTAAC
ATCCTGCATTACTACCGTCATCTCTACAGCCCCTACTGCACCGGCGGCAGCGGCAGCGGCAGCAAC
AGCAGCGGTACACAGAAGCAAAGGCGACCGGATAGCAAGACTCTGACAAAGCCCAAGAAATCCAC
AGCGGCGGCAGCAGCAGGAGGAGGAGCGCTGCGTCTGGCGCCCAACGAACCCGTATCGACCCGC
30 GAGCTTAGAAATAGGATTTTTCCCACTCTGTATGCTATATTTCAACAAAGCAGGGGCCAAGAACAAAG
GCTGAAAAATAAAAAACAGGTCTCTGCGCTCCCTCACCCGAGCTGCCTGTATCAGAAAAGCGAAGAT
CAGCTTCGGCGCACGCTGGAAGACGCGGAGGCTCTCTTCAGCAAATACTGCGCGCTGACTCTTAAG
GACTAGTTTCGCGCCCTTTCTCAAATTTAAGCGCGAAAACCTACGTCTCTCCAGCGGCCACACCCGG
CGCCAGCACCTGTCGTCAGCGCCATTATGAGCAAGGAAATCCCACGCCCTACATGTGGAGTTACC
35 AGCCACAAATGGGACTTGCGGCTGGAGCTGCCCAAGACTACTCAACCCGAATAAACTACATGAGCG
CGGGACCCACATGATATCCCGGGTCAACGGAATCCGCGCCCAACGAACCGAATTCTCCTCGAAC

AGGCGGCTATTACCACCACACCTCGTAATAACCTTAATCCCCGTAGTTGGCCCGCTGCCCTGGTGTA
CCAGGAAAGTCCCGCTCCCACCACTGTGGTACTTCCCAGAGACGCCAGGCCGAAGTTCAGATGAC
TAACTCAGGGGCGCAGCTTGCGGGCGGCTTTCGTACAGGGTGCGGTGCGCCGGGCGTTTTAGGG
CGGAGTAACTTGCATGTATTGGGAATTGTAGTTTTTTAAAATGGGAAGTGACGTATCGTGGGAAAAAC
5 GGAAGTGAAGATTTGAGGAAGTTGTGGTTTTTTGGCTTTCGTTTCTGGGCGTAGGTTGCGGTGCGG
TTTTCTGGGTGTTTTTTGTGGACTTTAACCGTTACGTCATTTTTTAGTCCTATATATACTCGCTCTGTA
CTTGGCCCTTTTTTAACTGTGACTGATTGAGCTGGTGCCGTGTCGAGTGGTGTTTTTTAATAGGTTTT
TTTACTGGTAAGGCTGACTGTTATGGCTGCCGCTGTGGAAGCGCTGTATGTTGTTCTGGAGCGGGA
GGGTGCTATTTTGCCTAGGCAGGAGGGTTTTTCAGGTGTTTATGTGTTTTTCTCTCTATTAATTTTGT
10 TATACCTCCTATGGGGGCTGTAATGTTGTCTCTACGCCTGCGGGTATGTATCCCCGGGCTATTTTC
GGTCGCTTTTTAGCACTGACCGATGTTAACCAACCTGATGTGTTTACCGAGTCTTACATTATGACTCC
GGACATGACCGAGGAAGTGTGCGGTGGTGCTTTTTTAATCACGGTGACCAGTTTTTTTACGGTCACGCC
GGCATGGCCGTAGTCCGTCTTATGCTTATAAGGGTTGTTTTTCTGTTGTAAGACAGGCTTCTAATGT
TTAAATGTTTTTTTTTTGTTATTTTATTTTGTGTTAATGCAGGAACCCGCAGACATGTTTGAGAGAAA
15 AATGGTGTCTTTTTCTGTGGTGGTTCCGGAACCTACCTGCCTTTATCTGCATGAGCATGACTACGATG
TGCTTGCTTTTTTGC GCGAGGCTTTCCTGATTTTTTGAAGCAGCACCTTGCAATTTATATCGCCGCCC
ATGCAACAAGCTTACATAGGGGCTACGCTGGTTAGCATAGCTCCGAGTATGCGTGTGTAATCAGTG
TGGGTTCTTTTGTGATGTTTCTGGCGGGGAAGTGCCGCGCTGGTCCGTGAGACCTGCACGATT
ATGTTGAGCTGGCCCTGCGAAGGGACCTACGGGATCGCGGTATTTTTGTTAATGTTCCGCTTTTGAA
20 TCTTATACAGGTCTGTGAGGAACCTGAATTTTTGCAATCATGATTCGCTGCTTGAGGCTGAAGGTGG
AGGGCGCTCTGGAGCAGATTTTACAATGGCCGGACTTAATATTCGGGATTTGCTTAGAGACATATT
GATAAGGTGGCGAGATGAAAATTATTTGGGCATGTTTGAAGGTGCTGGAATGTTTATAGAGGAGATT
CACCTGAAGGGTTTAGCCTTTACGTCCACTTGACGTGAGGGCAGTTTGCTTTTGAAGCCATTG
TGCAACATCTTACAAATGCCATTATCTGTTCTTTGGCTGTAGAGTTTGACCACGCCACCGAGGGGA
25 GCGCGTTCACTTAATAGATCTTCATTTTGAAGTTTTGGATAATCTTTTGAATAAAAAAAAAAAAAACAT
GGTTCTTCCAGCTCTTCCCGCTCCTCCCGTGTGTGACTCGCAGAACGAATGTGTAGGTTGGCTGGG
TGTGGCTTATTCTGCGGTGGTGGATGTTATCAGGGCAGCGGCGCATGAAGGAGTTTACATAGAACC
CGAAGCCAGGGGGCGCCTGGATGCTTTGAGAGAGTGATATACTACAACCTACTACACAGAGCGAGC
TAAGCGACGAGACCGGAGACGCAGATCTGTTTGTACGCCCCGCACCTGGTTTTGCTTCAGGAAATAT
30 GACTACGTCCGGCGTTCCATTTGGCATGACACTACGACCAACACGATCTCGGTTGTCTCGGCGCACT
CCGTACAGTAGGGATCGCCTACCTCCTTTTGAAGACAGAGACCCGCGCTACCATACTGGAGGATCAT
CCGCTGCTGCCCCGAATGTAACACTTTGACAATGCACAACGTGAGTTACGTGCGAGGTCTTCCCTGCA
GTGTGGGATTTACGCTGATTCAGGAATGGGTTGTTCCCTGGGATATGGTTCTGACGCGGGAGGAGC
TTGTAATCCTGAGGAAGTGTATGCACGTGTGCTGTGTTGTGCCAACATTGATATCATGACGAGCAT
35 GATGATCCATGGTTACGAGTCCTGGGCTCTCCACTGTCATTGTTCCAGTCCCGGTTCCCTGCAGTGC
ATAGCCGGCGGGCAGGTTTTGGCCAGCTGGTTTAGGATGGTGGTGGATGGCGCCATGTTTAATCAG

AGGTTTATATGGTACCGGGAGGTGGTGAATTACAACATGCCAAAAGAGGTAATGTTTATGTCCAGCG
TGTTTATGAGGGGTCGCCACTTAATCTACCTGCGCTTGTGGTATGATGGCCACGTGGGTTCTGTGGT
CCCCGCCATGAGCTTTGGATACAGCGCCTTGCACTGTGGGATTTTGAACAATATTGTGGTGCTGTGC
TGCAGTTACTGTGCTGATTTAAGTGAGATCAGGGTGCCTGTGTGCCCGGAGGACAAGGCGTCTC
5 ATGCTGCGGGCGGTGCGAATCATCGCTGAGGAGACCACTGCCATGTTGTATTCTGCAGGACGGAG
CGGCGGGCGGCAGCAGTTTATTCGCGCGCTGCTGCAGCACCAACCGCCCTATCCTGATGCACGATTAT
GACTCTACCCCCATGTAGGCGTGGACTTCCCCTTCGCCGCCCGTTGAGCAACCGCAAGTTGGACAG
CAGCCTGTGGCTCAGCAGCTGGACAGCGACATGAACTTAAGCGAGCTGCCCCGGGAGTTTATTAAT
ATCACTGATGAGCGTTTGGCTCGACAGGAAACCGTGTGGAATATAACACCTAAGAATATGTCTGTTA
10 CCCATGATATGATGCTTTTTAAGGCCAGCCGGGGAGAAAGGACTGTGTACTCTGTGTGTTGGGAGG
GAGGTGGCAGGTTGAATACTAGGGTTCTGTGAGTTTGATTAAGGTACGGTGATCAATATAAGCTATG
TGGTGGTGGGGCTATACTACTGAATGAAAAATGACTTGAAATTTTCTGCAATTGAAAAATAACACGT
TGAAACATAACATGCAACAGGTTACAGATTCTTTATTCCTGGGCAATGTAGGAGAAGGTGTAAGAGTT
GGTAGCAAAAGTTTCAGTGGTGTATTTTCCACTTTCCAGGACCATGTAAAAGACATAGAGTAAGTGC
15 TTACCTCGCTAGTTTCTGTGGATTCACTAGAATCGATGTAGGATGTTGCCCTCCTGACGCGGTAGG
AGAAGGGGAGGGTGCCCTGCATGTCTGCCGCTGCTCTTGCTCTTGCCGCTGCTGAGGAGGGGGGC
GCATCTGCCGCAGCACCGGATGCATCTGGGAAAAGCAAAAAGGGGCTCGTCCCTGTTTCCGGAGG
AATTTGCAAGCGGGGTCTTGATGACGGGGAGGCAAAACCCCGTTGCGCGCAGTCCGGCCGGCCC
GAGACTCGAACCAGGGGCTCCTGCGACTCAACCCCTTGAAAAATAACCCTCCGGCTACAGGGAGCGA
20 GCCACTTAATGCTTTTCGCTTTCCAGCCTAACCGCTTACGCCGCGCGCGGCCAGTGCCAAAAAAGC
TAGCGCAGCAGCCGCCGCGCCTGGAAGGAAGCCAAAAGGAGCGCTCCCCCGTTGTCTGACGTCGC
ACACCTGGGTTTCGACACGCGGGCGGTAACCGCATGGATCACGGCGGACGGCCGGATCCGGGGTTCC
GAACCCCGGTGCTCCGCCATGATACCCTTGCGAATTTATCCACCAGACCACGGAAGAGTGCCCGCT
TACAGGCTCTCCTTTTGCACGGTCTAGAGCGTCAACGACTGCGCACGCCTCACCGGCCAGAGCGTC
25 CCGACCATGGAGCACTTTTTGCCGCTGCGCAACATCTGGAACCGCGTCCGCGACTTTCCGCGCGCC
TCCACCACCGCCCGCGGCATCACCTGGATGTCCAGGTACATCTACGGATTACGTGACGTTTAAACC
ATATGATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA
ACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCC
ATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGA
30 CAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCCT
GCCGCTTACCGGATACCTGTCCGCCCTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACG
CTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGT
TCAGCCCGACCGCTGCGCCTTATCCGGTAACATCGTCTTGAGTCCAACCCGGTAAGACACGACTTA
TCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG
35 TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGA
AGCCAGTTACCTTCGGAAGAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACACCGCTGGTAGCG

GTGGTTTTTTTGTGGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATC
TTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT
CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGA
GTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCT
5 GTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGG
CCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCA
GCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAA
TTGTTGCCGGAAGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCT
ACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTACGCTCCGGTCCCAACGATCAA
10 GGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGT
CAGAAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCA
TGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATG
CGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTA
AAAGTGCTCATCATTGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGAT
15 CCAGTTCGATGTAACCCACTCGTGCACCCACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCT
GGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGA
ATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATAC
ATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC
TAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTTGTAAATCAGCTCATTTTTTAACC
20 AATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTT
CCAGTTTGAACAAGAGTCCACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCT
ATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAA
AGCACTAAATCGGAACCCATAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAGCCGGCGAACGT
GGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTC
25 ACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGATGGATCC

SEQ ID NO:27 (pAAV-GFP Vector sequence) [1-130: Left ITR, 139-798: CMV Promoter, 806-1298:

Human β -globin Intron, 1321-2061: GFP, 2120-2598: PolyA, 2638-2778: Right ITR, 3695-4555:

Ampicillin Resistance]

30 CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCG
CCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGCCAACTCCATCACTAGGGGTTCCCT
GCGGCCGCACGCGTCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGG
AGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCAT
TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT
35 GGAGTATTTACGGTAAACTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT
ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTTC

CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC
AATGGGCGTGGATAGCGGTTTGA CTACG GGGATTTC AAGTCTCCACCCCAT TGACGTCAATGGG
AGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCAT TGACGCA
AATGGGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGAT
5 CGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCG
CGGATT CGAATCCCGGCCGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACGTA
AGTACCGCCTATAGAGTCTATAGGCCCACAAAAAATGCTTTCTTCTTTAATATACTTTTTGTTTATCT
TATTTCTAATACTTTCCCTAATCTCTTTCTTT CAGGGCAATAATGATACAATGTATCATGCCTCTTTGCA
CCATTCTAAAGAATAACAGTGATAATTTCTGGGTAAAGGCAATAGCAATATTTCTGCATATAAATATTT
10 CTGCATATAAATTGTAAGTGTGAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCATT
CTGCTTTTATTTTATGGTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATC
ATGTTCATACCTCTTATCTTCTCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATC
ACTTTGGCAAAGAATTGGGATT CGAACATCGATTGAATTCTGAATGGTGAGCAAGGGCGAGGAGCTG
TTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGT
15 GTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCG
GCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCACCCCTGACCTACGGCGTGCAAGTTCAGC
CGCTACCCCGACCACATGAAGCAGCAGCACTTCTCAAGTCCGCCATGCCCGAAGGCTACGTCCAG
GAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGG
CGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG
20 GGCACAAGCTGGAGTACAAC TACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACG
GCATCAAGGTGAATTC AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAAGTTCGCGGACCACT
ACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC
CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACC
GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTACTCAGATCTCGAGCTCAAGTAGGGA
25 TCCTCTAGAGTCGACCTGCAGAAGCTTGCCTCGAGCAGCGCTGCTCGAGAGATCTACGGGTGGCAT
CCCTGTGACCCCTCCCAAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCAACAGCCTT
GTCCTAATAAAATTAAGTTGCATCATTTTGTCTGACTAGGTGTCCTTCTATAATATTATGGGGTGGAG
GGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGGGGTCTATTGGG
AACCAAGCTGGAGTGCAAGTGGCACAATCTTGGCTCACTGCAATCTCCGCCTCCTGGGTTC AAGCGA
30 TTCTCCTGCCTCAGCCTCCCGAGTTGTTGGGATTCCAGGCATGCATGACCAGGCTCAGCTAATTTTT
GTTTTTTTGGTAGAGACGGGGTTTCAACATATTGGCCAGGCTGGTCTCCAACCTCTAATCTCAGGTG
ATCTACCCACCTTGGCCTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGTCC
TTCTGATTTTGTAGGTAACCACGTGCGGACCGAGCGGCCGAGGAACCCCTAGTGATGGAGTTGGC
CACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGG
35 GCTTTGCCCGGGCGGCCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCG
GTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATACGTCAAAGCAACCATAGTACGCGCC

CTGTAGCGGCGCATTAAAGCGCGGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCA
GCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTCCCTTCTCTCGCCACGTTGCGCCGGCTTTCCCCG
TCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAA
AAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA
5 CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCG
GGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAA
CAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTATGGTGCACTCTCAGTACAATCT
GCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCCGCTGACGCGCCCTGACGG
GCTTGCTGCTCCCAGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAG
10 AGGTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGG
TTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAAC
CCCTATTTGTTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATG
CTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTT
GCGGCATTTTGCCTTCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATC
15 AGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCG
CCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTA
TTGACGCCGGGCAAGAGCAACTCGGTGCGCCGATACACTATTCTCAGAATGACTTGGTTGAGTACTC
ACCAAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACC
ATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTT
20 TTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT
ACCAAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAC
GGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAG
GACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGC
GTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCT
25 ACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCAC
TGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTT
TTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTT
TTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGC
GCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGGCCGATCAAGA
30 GCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAATACTGTCTTCTA
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAA
TCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATA
GTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGC
GAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAG
35 GGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCT
TCCAGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCA

TTTTTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGG
TTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

SEQ ID NO:28 (pAAV-RC5 Vector sequence) [121-1986:AAV-2 Rep gene, 2013-4187:AAV-5 Cap gene,
5 5268-6128:Ampicillin Resistance (bla) gene]

ATCGTTAACGCCCCGCGCCGGCCGCTCTAGAAGTAGTGGATCCCCCGGAAGATCAGAAGTTCCTAT
TCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACCTCTGATCTGCGCAGCCGCCATGCCGGGGTTTT
ACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCGGCATTTCTGACAGCTTTGT
GAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTGAATCTGATTGA
10 GCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGCGACTTTCTGACGGAATGGCGCCGTGTGA
GTAAGGCCCCGGAGGCCCTTTTCTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTCCACATGCACGT
GCTCGTGGAAACCACCGGGGTGAAATCCATGGTTTTGGGACGTTTCCTGAGTCAGATTCGCGAAAAA
CTGATTCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCA
GAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAATTACTTGCTCCCCA
15 AAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCT
CACGGAGCGTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACA
AAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTACATGGA
GCTGGTGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAGGACCAGG
CCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCAAGGCTGCCTTGACAATGC
20 GGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGACTACCTGGTGGGCCAGCAGCCCGTGGAGG
ACATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAACGGGTACGATCCCCAATATGCGGCTTCC
GTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCAAGAGGAACACCATCTGGCTGTTTGGGCCTGCA
ACTACCGGGAAGACCAACATCGCGGAGGCCATAGCCACACTGTGCCCTTCTACGGGTGCGTAAAC
TGGACCAATGAGAACTTTCCCTTCAACGACTGTGTGACAAGATGGTGATCTGGTGGGAGGAGGGG
25 AAGATGACCGCCAAGGTCGTGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGA
CCAGAAATGCAAGTCCTCGGCCAGATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACAT
GTGCGCCGTGATTGACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTT
CAAAATTTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGAAGTCAAAGAC
TTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTGA
30 GCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGCGCGAGTCAGTT
GCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTACGCAGACAGGTACCAAAACAAATGT
TCTCGTCACGTGGGCATGAATCTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATCAGAATT
CAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGCTTTCCCGTGTCAGAATCTCAACCC
GTTTCTGTCTGCAAAAAGGCGTATCAGAACTGTGCTACATTCATCATATCATGGGAAAGGTGCCAG
35 ACGCTTGCACTGCCTGCGATCTGGTCAATGTGGATTGGATGACTGCATCTTTGAACAATAAATGATT
TGTAATAAATTTAGTAGTCATGTCTTTTGTGATCACCTCCAGATTGGTTGGAAGAAGTTGGTGAA

GGTCTTCGCGAGTTTTTGGGCCTTGAAGCGGGCCACCGAAACCAAACCCAATCAGCAGCATCAA
GATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGAAACGGTCTCGATCGA
GGAGAGCCTGTCAACAGGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCT
TGAGGCGGGAGACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCG
5 CCGACGACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG
AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCCTACCGAAAGCGGATAGACGACCACT
TTCCAAAAAGAAAGAAGGCTCGGACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCCGAAG
CTGGACCCAGCGGATCCAGCAGCTGCAAATCCAGCCCAACCAGCCTCAAGTTTGGGAGCTGATA
CAATGTCTGCGGGAGGTGGCGGCCATTGGGCGACAATAACCAAGGTGCCGATGGAGTGGGCAAT
10 GCCTCGGGAGATTGGCATTGCGATTCCACGTGGATGGGGACAGAGTCGTCACCAAGTCCACCCGA
ACCTGGGTGCTGCCCAGCTACAACAACCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGA
AGCAACGCCAACGCCTACTTTGGATACAGCACCCCTGGGGGTACTTTGACTTTAACCGCTTCCACA
GCCACTGGAGCCCCCGAGACTGGCAAAGACTCATCAACAATACTGGGGCTTCAGACCCCGGTCCC
TCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTACGGTGCAGGACTCCACCACCACCATCGC
15 CAACAACCTCACCTCCACCGTCCAAGTGTTACGGACGACGACTACCAGCTGCCCTACGTCGTCGG
CAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTACGCTGCCGCAGTACGGTTA
CGCGACGCTGAACCGCGACAACACAGAAAATCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTA
CTTTCCAGCAAGATGCTGAGAACGGGCAACAATTGAGTTTACCTACAATTTGAGGAGGTGCC
TTCCACTCCAGCTTCGCTCCAGTCAGAACCTGTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACT
20 TGTACCGCTTCGTGAGCACAAATAAACTGGCGGAGTCCAGTTCAACAAGAACCTGGCCGGGAGAT
ACGCCAACACCTACAAAACTGGTTCCCGGGGCCCATGGGCCGAACCCAGGGCTGGAACCTGGGC
TCCGGGGTCAACCGCGCCAGTGTGAGCGCCTTCGCCACGACCAATAGGATGGAGCTCGAGGGCGC
GAGTTACAGGTGCCCCCGCAGCCGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGC
CCTGGAGAACACTATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTCGA
25 GGGCAACATGCTCATCACCAGCGAGAGCGAGACGACGCCGGTGAACCGCGTGGCGTACAACGTCG
GCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACCGGCACGTACAACCTC
CAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTCCAAGGACCCATCTGGGC
CAAGATCCCAGAGACGGGGGCGCACTTTACCCCTCTCCGGCCATGGGCGGATTGCGACTCAAACA
CCCACCGCCCATGATGCTCATCAAGAACACGCCTGTGCCCGGAAATATCACCAGCTTCTCGGACGT
30 GCCCGTCAGCAGCTTCATCACCAGTACAGCACCGGGCAGGTACCCGTGGAGATGGAGTGGGAGC
TCAAGAAGGAAAACTCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAATAACGACCCCCA
GTTTGTGGACTTTGCCCGGACAGCACCGGGGAATACAGAACCACCAGACCTATCGGAACCCGATA
CCTTACCCGACCCCTTTAACCCATTTCATGTCGCATACCCTCAATAAACCGGTTAATTCGTGTCAGTTG
AACTTTGGTCTCATGTCGTTATTATCTTATCTGGTCACCAGATCCCCGTAGATAAGTAGCATGGCGGG
35 TTAATCATTAACCTACAGCCCGGGCGTTTAAACAGCGGGCGGAGGGGTGGAGTCGTGACGTGAATTA
CGTCATAGGGTTAGGGAGGTCCTGTATTAGAGGTCACGTGAGTGTTCGCGACATTTTGCACACCA

TGTGGTCTCGCTGGGGGGGGGGGCCCCGAGTGAGCACGCAGGGTCTCCATTTTGAAGCGGGAGGTT
TGAACGAGCGCTGGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGT
TACCCAACCTTAATCGCCTTGACGACATCCCCCTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCG
CACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAAATTGTAAGCGTTAATATTT
5 TGTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTTAACCAATAGGCCGAAATCGGCAAA
ATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAACAAGAGTCC
ACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTA
CGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTTA
AAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAG
10 AAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCAACAC
ACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCG
GAACCCCTATTTGTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGAT
AAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATCCCT
TTTTTGCGGCATTTTGCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAGTAAAGATGCTGAA
15 GATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT
TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCC
CGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCCGATACACTATTCTCAGAATGACTTGGTTGAGT
ACTACCAAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCAT
AACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAAC
20 CGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA
GCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTA
TTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAG
TTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCG
GTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG
25 TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTG
CCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAC
TTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAAC
GTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT
TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGCGCGG
30 ATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTT
CTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTC
TGCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAG
ACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCTGTGCACACAGCCCAGCT
TGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTC
35 CCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG
GGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAG

CGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTT
TTACGGTTCCTGGCCTTTTGTGCGCCTTTTGTCTACATGTTCTTCTCCTGCGTTATCCCCTGATTCTGT
GGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAG
CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGC
5 CGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAA
TTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTG
TGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCG
CCGAT

10 SEQ ID NO:29 (Smt3-Rep40)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRLFLYDGIRIQADQTPEDLDMEDNDIEAHREQIGGMELVGWLVDKGITSEKQWIQEDQASYISFNAA
SNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKR
NTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKVDKMVIWWEEGKMTAKVVESAKAILGGS
15 KVRVDQKCKSSAQIDPTPVI VTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVK
DFFRWAKDHVVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRLARGHSL
EPEA

SEQ ID NO:30 (Smt3-Rep52)

20 MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRLFLYDGIRIQADQTPEDLDMEDNDIEAHREQIGGMELVGWLVDKGITSEKQWIQEDQASYISFNAA
SNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKR
NTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKVDKMVIWWEEGKMTAKVVESAKAILGGS
KVRVDQKCKSSAQIDPTPVI VTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVK
25 DFFRWAKDHVVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSR
HVGMLNMLFPCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVVKKAYQKLCYIHHIMGKVPDACT
ACDLVNVLDLDDCIFEQEPEA

SEQ ID NO:31 (Smt3-Rep68)

30 MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRLFLYDGIRIQADQTPEDLDMEDNDIEAHREQIGMPGFYEIVIKVPSDLGHLPGISDSFVNWWAE
KEWELPPDSMDLNLIEQAPLTVAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGK
SMVLGRFLSQIREKLIQRIYRGIEPTLPNWFVTKTRNGAGGGNKVDECIYIPNYLLPKTQPELQWAWTN
MEQYLSACLNLTERKRLVAQHLTHVSQTQEKNQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK
35 QWIQEDQASYISFNAA SNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYA
ASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKVDKMVIWWEEGK

MTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELT
RRLDHDFGKVTQKQEVKDFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSD
AEASINYADRLARGHSLEPEA

5 SEQ ID NO:32 (Smt3-Rep78)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMPGFYEIVIKVPSDLGHLPGISDSFVNWWAE
KEWELPPDSDMDLNLIEQAPLTVAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVK
SMVLGRFLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVDECIYIPNYLLPKTQPELQWAWTN
10 MEQYLSACLNLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK
QWQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYA
ASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDQVCKMVIWWEEGK
MTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELT
RRLDHDFGKVTQKQEVKDFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSD
15 AEASINYADRYQNKCSRHVGMNMLFPCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVVKKAY
QKLCYIHHIMGKVPDACTACDLVNVLDLDDCIFEQEPEA

SEQ ID NO:33 (AAV5 VP2)

MTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQQLQIPAPASSLGADTMSAGGGGPLGD
20 NNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWLPSYNNHQYREIKSGSVDGSNANAYFGYSTP
WGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLSTVQVFTDDD
YQLPYVVGNGTEGCLPAFPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFETYN
FEEVPFHSSFAPSQNLFLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFGPMGRTQGWL
NLGSGVNRASVSFAATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLE
25 GNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGGPIWAKIPE
TGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKR
WNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL

SEQ ID NO:34 (Amino acid sequence for His-SUMO-AAV2-VP3-SUMO-EPEA fusion protein)

MHHHHHHHSEKPKKEGVKTENDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSMRQIRFRFDG
30 QPINETDTPAQLEMEDEDTIDVFQQQTGGMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI
TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFPR
KRLNFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYL
TLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHQSGLDRLMNPLIDQYLYLSRT
35 NTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDS
LVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQR

GNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVP
ANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKS VNVDFTVDTNGVYSEPR
PIGTRYLTRNLLVPRGSSEKPKGVKTENDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCERQGLSM
RQIRFRFDGQPINETDTPAQLEMEDEDTIDVFQQQTGGEPEA

5

SEQ ID NO: 35 (MBP-Rep78)

MEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIFWAHDRFGGYA
QSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPKTWEEIPALDKELKAKG
KSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKHKHMNADTDYSIA
10 EAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENY
LLTDEGLEAVNKDKPLGAVALKSYEEELVKDPRIATMENAQKGEIMPNIQMSAFWYAVRTAVINAASG
RQTVDEALKDAQTNSSNNNNNNNNNNLGENLYFQSMPGFYEIVIKVPSDLDGHLPGISDSFVNWWAEK
EWELPPDSMDLNLIEQAPLTVAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVKS
MVLGRFLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTNM
15 EQYLSACLNLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEKQ
WIQEDQASYISFNAA SNRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAA
SVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDCCVDMVIWWEEGKM
TAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTR
RLDHDGFKVTKQEVKDFFRWAKDHHVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDA
20 EASINYADRYQNKCSRHVGMNMLFPCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVVKKAYQ
KLCYIHHIMGKVPDACTACDLVNVLDLDDCIFEQ

SEQ ID NO: 36 (SUMO-Rep40)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQKGKE
25 MDCLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMELVGWLVDKGITSEKQWQEDQASYISFNAA
SNRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAA SVFLGWATKKFGKR
NTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDCCVDMVIWWEEGKMTAKVVESAKAILGGS
KVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGFKVTKQEVK
DFFRWAKDHHVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRLARGHSL
30 EPEA

SEQ ID NO: 37 (SUMO-Rep52)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQKGKE
MDCLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMELVGWLVDKGITSEKQWQEDQASYISFNAA
35 SNRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAA SVFLGWATKKFGKR
NTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDCCVDMVIWWEEGKMTAKVVESAKAILGGS

KVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFFHQQLQDRMFKFELTRRLDHDFGKVTQKEVK
DFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSR
HVGMLNMLFPCRQCERMNQNSNICFTHGQKDCLECFPVSESQPVSVVKKAYQKLCYIHHIMGKVPDACT
ACDLVNVDLDDCIFEQEPEA

5

SEQ ID NO: 38 (SUMO-Rep68)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRFlyDGIRIQADQTPEDLDMEDNDIEAHREQIGGMPGFYEIVIKVPSDLGHLPGISDSFVNWVAE
KEWELPPDSDMDLNLIEQAPLTVAEKLQRDFLTWRRVSKAPEALFFVQFEKGESYFHHVLTETGVK
10 SMVLGRFLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTN
MEQYLSACLNLTERKRLVAQHLTHVSQTQEKNENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK
QWQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYA
ASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDCCVDMVIWWEEGK
MTAKVWESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFFHQQLQDRMFKFELT
15 RRLDHDFGKVTQKEVKDFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSD
AEASINYADRLARGHSLEPEA

SEQ ID NO: 39 (SUMO-Rep78)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
20 MDSLRFlyDGIRIQADQTPEDLDMEDNDIEAHREQIGGMPGFYEIVIKVPSDLGHLPGISDSFVNWVAE
KEWELPPDSDMDLNLIEQAPLTVAEKLQRDFLTWRRVSKAPEALFFVQFEKGESYFHHVLTETGVK
SMVLGRFLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTN
MEQYLSACLNLTERKRLVAQHLTHVSQTQEKNENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK
QWQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYA
25 ASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDCCVDMVIWWEEGK
MTAKVWESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFFHQQLQDRMFKFELT
RRLDHDFGKVTQKEVKDFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSD
AEASINYADRYQNKCSRHVGMLNMLFPCRQCERMNQNSNICFTHGQKDCLECFPVSESQPVSVVKKAY
QKLCYIHHIMGKVPDACTACDLVNVDLDDCIFEQEPEA

30

SEQ ID NO: 40 (Smt3-Rep40)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRFlyDGIRIQADQTPEDLDMEDNDIEAHREQIGGMELVGWLVDKGITSEKQWQEDQASYISFNAA
SNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKR
35 NTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDCCVDMVIWWEEGKMTAKVWESAKAILGGS
KVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFFHQQLQDRMFKFELTRRLDHDFGKVTQKEVK

DFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRLARGHSL
EPEA

SEQ ID NO: 41 (Smt3-Rep52)

5 MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRFlyDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMELVGWLVDKGITSEKQWQEDQASYISFNAA
SNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKR
NTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKMDKMWIWWEEGKMTAKVWESAKAILGGS
KVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVK
10 DFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSR
HVGMLMLFPCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVKKAYQKLCYIHHIMGKVPDACT
ACDLVNVLDLDDCIFEQEPEA

SEQ ID NO: 42 (Smt3-Rep68)

15 MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRFlyDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMPGFYEIVIKVPSDLGHLPGISDSFVNWVAE
KEWELPPDSDMDLNLIEQAPLTVAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVK
SMVLGRFLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVDECIYIPNYLLPKTQPELQWAWTN
MEQYLSACLNLTERKRLVAQHLTHVSQTQEKNQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK
20 QWQEDQASYISFNAAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYA
ASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKMDKMWIWWEEGK
MTAKVWESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELT
RRLDHDGKVTQKEVKDFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSD
AEASINYADRLARGHSLEPEA

25

SEQ ID NO: 43 (Smt3-Rep78)

MHHHHHHGSGMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKE
MDSLRFlyDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMPGFYEIVIKVPSDLGHLPGISDSFVNWVAE
KEWELPPDSDMDLNLIEQAPLTVAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVK
30 SMVLGRFLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVDECIYIPNYLLPKTQPELQWAWTN
MEQYLSACLNLTERKRLVAQHLTHVSQTQEKNQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK
QWQEDQASYISFNAAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYA
ASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKMDKMWIWWEEGK
MTAKVWESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELT
35 RRLDHDGKVTQKEVKDFFRWAKDHWVEVEHEFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSD

AEASINYADRYQNKCSRHVGMNMLFPCRQCERMNQNSNICFTHGQKDCLECFPVSESQPVSVVKKAY
QKLCYIHHIMGKVPDACTACDLVNVDLDDCIFEQEPEA

Claims

1. A method of *in vitro* assembling parvovirus capsids, comprising the steps of:
 - a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;
 - b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
 - c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids, thereby assembling parvovirus capsids and providing assembled parvovirus capsids.
2. A method of *in vitro* filling parvovirus capsids, comprising the steps of:
 - a. providing:
 - i. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
 - ii. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*; and
 - iii. a Rep protein *in vitro*;
 - b. combining the one or more populations of assembled parvovirus capsids, the nucleic acid molecule, and the Rep protein *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids and providing filled parvovirus capsids.
3. A method of *in vitro* filling parvovirus capsids, comprising the steps of:
 - a. providing:
 - i. a population of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*;
 - ii. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*;
 - iii. a Rep proteins *in vitro*; and
 - iv. a reaction mixture *in vitro*;
 - b. combining the population of assembled parvovirus capsids, the nucleic acid molecule, the Rep protein and the reaction mixture *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids and providing filled parvovirus capsids.

4. A method of *in vitro* manufacturing filled parvovirus capsids, comprising the steps of:
- providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;
 - combining the inhibited parvovirus viral capsid proteins *in vitro*; and
 - contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids, thereby providing one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*
 - providing:
 - a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*; and
 - a Rep protein *in vitro*;
 - combining the population of assembled parvovirus capsids obtained in c., the nucleic acid molecule and the Rep protein *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids, thereby manufacturing filled parvovirus capsids.
5. A method of *in vitro* manufacturing filled parvovirus capsids, comprising the steps of:
- providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor;
 - combining the inhibited parvovirus viral capsid proteins *in vitro*; and
 - contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids, thereby providing one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins *in vitro*
 - providing:
 - a nucleic acid molecule comprising a transgene of interest and a Rep binding domain *in vitro*;
 - a Rep protein *in vitro*; and

- iii. a reaction mixture *in vitro*
 - e. combining the population of assembled parvovirus capsids obtained in c., the nucleic acid molecule, the Rep protein and the reaction mixture *in vitro* under filling conditions which allow filling of the assembled capsids with the nucleic acid molecule by the Rep protein, thereby filling parvovirus capsids, thereby manufacturing filled parvovirus capsids.
6. A method of *in vitro* controlling the stoichiometry of parvovirus viral capsid proteins in assembled parvovirus capsids, comprising the step of:
- a. providing one or more populations of inhibited parvovirus viral capsid proteins *in vitro*, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by an assembly inhibitor wherein the one or more populations of inhibited parvovirus viral capsid proteins are provided at a defined stoichiometry;
 - b. combining the inhibited parvovirus viral capsid proteins *in vitro*; and
 - c. contacting an assembly activator and the inhibited parvovirus viral capsid proteins of step (b) *in vitro*, wherein the assembly activator disinhibits the assembly inhibitor, thereby allowing the assembly of parvovirus capsids comprising parvovirus viral capsid proteins into assembled parvovirus capsids, thereby assembling parvovirus capsids and providing assembled parvovirus capsids with a defined stoichiometry of parvovirus viral capsid proteins.
7. The method according to any one of the preceding claims, wherein the viral capsid proteins are from a Densovirinae, Hamaparvovirinae, or Parvovirinae.
8. The method according to any one of the preceding claims, wherein the viral capsid proteins are from a Parvovirinae selected from the group consisting of an Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus, preferably Dependoparvovirus.
9. The method according to any one of the preceding claims, wherein the viral capsid proteins are from an adeno-associated virus (AAV).
10. The method according to any one of the preceding claims, wherein the viral capsid proteins are synthetic viral capsid proteins and/or recombinant viral capsid proteins.

11. The method according to any one of the preceding claims, wherein the viral capsid proteins are selected from the group consisting of viral protein 1 (VP1), viral protein 2 (VP2), and/or viral protein (VP3).
12. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP1.
13. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP2.
14. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP3.
15. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP1 and VP2.
16. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP1 and VP3.
17. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP2 and VP3.
18. The method according to any one of the preceding claims, wherein the viral capsid proteins are VP1, VP2, and VP3.
19. The method according to any one of the preceding claims, wherein the VP1, VP2 and/or VP3 are synthetic VP1, VP2 and/or VP3 and/or recombinant VP1, VP2 and/or VP3.
20. The method according to any one of the preceding claims, wherein the VP1, VP2 and/or VP3 are AAV VP1, AAV VP2 and/or AAV VP3.
21. The method according to any one of the preceding claims, wherein the AAV is an AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, rh10 AAV, rh.39 AAV, or any other AAV now known and later discovered.

22. The method according to any one of the preceding claims, wherein the VP1 is a VP1 from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.
23. The method according to any one of the preceding claims, wherein the VP2 is a VP2 from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.
24. The method according to any one of the preceding claims, wherein the VP3 is a VP3 from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.
25. The method according to any one of the preceding claims, wherein the VP1, VP2, and/or VP3 are from different AAV serotypes.
26. The method according to any one of the preceding claims, wherein the VP1, VP2, and/or VP3 are from the same AAV serotype.
27. The method according to any one of the preceding claims, wherein the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced in a eukaryotic microbial system, such as yeast, such a methylotrophic yeast.
28. The method according to any one of the preceding claims, wherein the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced in an eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*.
29. The method according to any one of the preceding claims, wherein the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced in an prokaryotic microbial system, such as *E. Coli*.
30. The method according to any one of the preceding claims, wherein the viral capsid proteins, such as VP1, VP2 and/or VP3, is produced a mammalian cell system, such as HEK, CHO, HeLa or Vero cells, an insect cell system, such as Sf9, Sf21 or High Five cells, a plant system, such as tobacco BY, an algae system, a metazoan cell system, a zebrafish and/or cell-free expression systems.

31. The method according to any one of the preceding claims, wherein the inhibited parvovirus viral capsid proteins are inhibited from assembling by multiple assembly inhibitors, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly inhibitors.
32. The method according to any one of the preceding claims, wherein the assembly inhibitor comprises multiple assembly inhibitors, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly inhibitors.
33. The method according to any one of the preceding claims, wherein the assembly inhibitor is selected from the group consisting of a chemically assembly inhibitor, a steric assembly inhibitor and/or a kinetically assembly inhibitor.
34. The method according to any one of the preceding claims, wherein the assembly inhibitor is selected from the group consisting of inhibitory pH, inhibitory salt concentration, denaturing, precipitation conditions and/or an antibody.
35. The method according to any one of the preceding claims, wherein the assembly inhibitor is selected from the group consisting of a retainment of the viral capsid proteins in a compartment and/or physical blockade, such as a cleavable amino acid sequence attached to the viral capsid proteins.
36. The method according to any one of the preceding claims, wherein the assembly inhibitor is selected from the group consisting of inhibitory temperature and/or inhibitory viral capsid protein concentration.
37. The method according to any one of the preceding claims, wherein the assembly inhibitor is selected from the group consisting of an inhibitory modification to the viral capsid proteins, such as a cleavable amino acid sequence, an inhibitory exogenous molecule, inhibitory pH, inhibitory ionic strength, inhibitory conductivity, time, inhibitory temperature, hydrophobicity, inhibitory viral capsid protein concentration, inhibitory viscosity, denaturation, pressure and radiation, such as light, such as ultraviolet light.
38. The method according to any one of the preceding claims, wherein the assembly inhibitor is an inhibitory modification to the viral capsid proteins.
39. The method according to any one of the preceding claims, wherein the assembly inhibitor is a cleavable amino acid sequence attached to the viral capsid proteins.

40. The method according to any one of the preceding claims, wherein the cleavable amino acid sequence is a SUMO tag and/or a thrombin cleavage site.
41. The method according to any one of the preceding claims, wherein the assembly inhibitor is denaturation of the viral capsid proteins.
42. The method according to any one of the preceding claims, wherein the denaturation is thermally-induced denaturation and/or chemically-induced denaturation.
43. The method according to any one of the preceding claims, wherein the denaturation is caused by pH, sonication, ionic strength, conductivity and/or temperature.
44. The method according to any one of the preceding claims, wherein the denaturation is caused by pH.
45. The method according to any one of the preceding claims, wherein the denaturation is caused by ionic strength and/or conductivity.
46. The method according to any one of the preceding claims, wherein the denaturation is caused by temperature.
47. The method according to any one of the preceding claims, wherein the assembly inhibitor is guanidine, such as 1 to 15 M urea, 1 to 14 M urea, 1 to 13 M urea, 1 to 12 M urea, 1 to 11 M urea, 1 to 10 M urea or 1 to 9 M urea
48. The method according to any one of the preceding claims, wherein the assembly inhibitor is urea, such as 1 to 15 M urea, 2 to 14 M urea, 3 to 13 M urea, 4 to 12 M urea, 5 to 11 M urea, 6 to 10 M urea or 7 to 9 M urea.
49. The method according to any one of the preceding claims, wherein the assembly inhibitor is NaCl, such as 1 to 15 M NaCl, 2 to 14 M NaCl, 3 to 13 M NaCl, 4 to 12 M NaCl, 5 to 11 M NaCl, 6 to 10 M NaCl or 7 to 9 M NaCl.
50. The method according to any one of the preceding claims, wherein the assembly inhibitor is KCl, such as 1 to 15 M KCl, 2 to 14 M KCl, 3 to 13 M KCl, 4 to 12 M KCl, 5 to 11 M KCl, 6 to 10 M KCl or 7 to 9 M KCl.

51. The method according to any one of the preceding claims, wherein the inhibitory pH is pH below or equal to 5, such as pH below or equal to 4.5, such as pH below or equal to 4, such as pH below or equal to 3.5, such as pH below or equal to 3, such as pH below or equal to 2.5, such as pH below or equal to 2, such as pH below or equal to 1.5.
52. The method according to any one of the preceding claims, wherein the inhibitory pH is pH above or equal to 8.5, such as pH above or equal to 9, such as pH above or equal to 9.5, such as pH above or equal to 10, such as pH above or equal to 10.5, such as pH above or equal to 11, such as pH above or equal to 11.5, such as pH above or equal to 12, such as pH above or equal to 12.5, such as pH above or equal to 13.
53. The method according to any one of the preceding claims, wherein the inhibitory temperature is a temperature between -196 °C to 0 °C, such as -190 °C to 0 °C, such as -180 °C to 0 °C, such as -170 °C to 0 °C, such as -160 °C to 0 °C, such as -150 °C to 0 °C, such as -140 °C to 0 °C, such as -130 °C to 0 °C, such as -120 °C to 0 °C, such as -110 °C to 0 °C, such as -100 °C to 0 °C, such as -90 °C to 0 °C, such as -80 °C to 0 °C, such as -70 °C to 0 °C, such as -60 °C to 0 °C, such as -50 °C to 0 °C, such as -40 °C to 0 °C, such as -30 °C to 0 °C, such as -20 °C to 0 °C, such as -10 °C to 0 °C.
54. The method according to any one of the preceding claims, wherein the inhibitory temperature is a temperature between -196 °C to -1 °C, such as -196 °C to -10 °C, such as -196 °C to -20 °C, such as -196 °C to -40 °C, such as -196 °C to -50 °C, such as -196 °C to -60 °C, such as -196 °C to -70 °C, such as -196 °C to -80 °C, such as -196 °C to -90 °C, such as -196 °C to -100 °C, such as -196 °C to -110 °C, such as -196 °C to -120 °C, such as -196 °C to -130 °C, such as -196 °C to -140 °C, such as -196 °C to -150 °C, such as -196 °C to -160 °C, such as -196 °C to -170 °C, such as -196 °C to -180 °C, such as -196 °C to -190 °C.
55. The method according to any one of the preceding claims, wherein the inhibitory temperature is a temperature between 40 °C to 280 °C, such as between 40 °C to 250 °C, such as between 40 °C to 220 °C, such as between 40 °C to 210 °C, such as between 40 °C to 200 °C, such as between 40 °C to 190 °C, such as between 40 °C to 180 °C, such as between 40 °C to 170 °C, such as between 40 °C to 160 °C, such as between 50 °C to 160 °C, such as between 60 °C to 160 °C, such as between 70 °C to 160 °C, such as between 80 °C to 160 °C, such as between 80 °C to 150 °C, such as between 80 °C to 140 °C, such as between 90 °C to 140 °C, such as between 90 °C to 140 °C, such as between 100 °C to 140 °C, such as between 100 °C to 130 °C, such as between 110 °C to 130 °C.

56. The method according to any one of the preceding claims, wherein the inhibitory exogenous molecule is selected from the group consisting of an antibody, a nucleic acid molecule, a peptide, a protein, a nanobody, a lipid, a sugar, a carbohydrate, a polymer, a small molecule, and/or an aptamer.
57. The method according to any one of the preceding claims, wherein the inhibitory ionic strength and/ inhibitory conductivity is a solution comprising NaCl, KCl and/or urea, such as a solution comprising 1 to 15 M NaCl, KCl and/or urea, 2 to 14 M NaCl, KCl and/or urea, 3 to 13 M NaCl, KCl and/or urea, 4 to 12 M NaCl, KCl and/or urea, 5 to 11 M NaCl, KCl and/or urea, 6 to 10 M NaCl, KCl and/or urea or 7 to 9 M NaCl, KCl and/or urea.
58. The method according to any one of the preceding claims, the step of contacting the assembly activator and the inhibited parvovirus viral capsid proteins of step (b) comprise contacting multiple assembly activators, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly activators.
59. The method according to any one of the preceding claims, wherein the assembly activator comprises multiple assembly activators, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 assembly activators.
60. The method according to any one of the preceding claims, wherein the assembly activator is selected from the group consisting of a chemically assembly activator, a steric assembly activator and/or a kinetically assembly activator
61. The method according to any one of the preceding claims, wherein the assembly activator is selected from the group consisting of activating pH, activating salt concentration, renaturing, solubilization conditions and/or removal of the antibody.
62. The method according to any one of the preceding claims, wherein the assembly activator is selected from the group consisting of a release of the viral capsid proteins from the compartment and/or removal of the physical blockade, such as the cleavable amino acid sequence attached to the viral capsid proteins.
63. The method according to any one of the preceding claims, wherein the assembly activator is selected from the group consisting of activating temperature and/or activating viral capsid protein concentration.
64. The method according to any one of the preceding claims, wherein the assembly activator is selected from the group consisting of a removal of the inhibitory modification to the viral capsid

proteins, such as a cleavage of the cleavable amino acid sequence, removal of the inhibitory exogenous molecules, activating pH, activating ionic strength, activating conductivity, activating time, activating temperature, hydrophobicity, activating viral capsid protein concentration, renaturation, activating viscosity, agitation, mixing, pressure and radiation, such as light, such as ultraviolet light.

65. The method according to any one of the preceding claims, wherein the assembly activator is a removal of the inhibitory modification to the viral capsid proteins.
66. The method according to any one of the preceding claims, wherein the assembly activator is a cleavage of the cleavable amino acid sequence attached to the viral capsid proteins.
67. The method according to any one of the preceding claims, wherein the assembly activator is a protease.
68. The method according to any one of the preceding claims, wherein the assembly activator is thrombin and/or a SUMO protease, such as SUMO protease 2.
69. The method according to any one of the preceding claims, wherein the cleavable amino acid sequence is a SUMO tag and/or a thrombin cleavage site, and wherein the assembly activator is thrombin and/or a SUMO protease, such as SUMO protease 2.
70. The method according to any one of the preceding claims, wherein the assembly activator is renaturation.
71. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, such as 1 to 500 mM MES pH 6.0, such as 1 to 450 mM MES pH 6.0, such as 1 to 400 mM MES pH 6.0, such as 1 to 350 mM MES pH 6.0, such as 1 to 300 mM MES pH 6.0, such as 1 to 250 mM MES pH 6.0, such as 1 to 200 mM MES pH 6.0, such as 1 to 150 mM MES pH 6.0, such as 1 to 100 mM MES pH 6.0, such as 1 to 90 mM MES pH 6.0, such as 1 to 80 mM MES pH 6.0, such as 1 to 70 mM MES pH 6.0, such as 10 to 70 mM MES pH 6.0, such as 20 to 70 mM MES pH 6.0, such as 30 to 70 mM MES pH 6.0, such as 40 to 60 mM MES pH 6.0, such as 45 to 55 mM MES pH 6.0.
72. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising NaCl, such as 100 to 1000 mM NaCl, such as 100 to 900 mM NaCl, such as 100 to 800 mM NaCl, such as 100 to 700 mM NaCl, such as 100 to 600 mM NaCl, such as 200 to

600 mM NaCl, such as 300 to 600 mM NaCl, such as 400 to 600 mM NaCl, such as 450 to 550 mM NaCl.

73. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising NaCl, such as 100 to 400 mM NaCl, such as 150 to 400 mM NaCl, such as 200 to 350 mM NaCl, such as 200 to 300 mM NaCl, such as 200 to 290 mM NaCl, such as 210 to 280 mM NaCl, such as 220 to 270 mM NaCl, such as 230 to 240 mM NaCl.
74. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising NaCl, such as 1 to 20 mM NaCl, such as 1 to 15 mM NaCl, such as 5 to 15 mM NaCl, such as 6 to 14 mM NaCl, such as 7 to 13 mM NaCl, such as 6 to 12 mM NaCl, such as 8 to 12 mM NaCl, such as 9 to 11 mM NaCl.
75. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising KCl, such as 1 to 1000 mM KCl, such as 1 to 900 mM KCl, such as 1 to 800 mM KCl, such as 1 to 700 mM KCl, such as 1 to 600 mM KCl, such as 1 to 600 mM KCl, such as 1 to 600 mM KCl, such as 1 to 500 mM KCl, such as 1 to 400 mM KCl, such as 1 to 300 mM KCl, such as 1 to 200 mM KCl, such as 10 to 200 mM KCl, such as 20 to 200 mM KCl, such as 30 to 200 mM KCl, such as 40 to 200 mM KCl, such as 50 to 200 mM KCl, such as 50 to 150 mM KCl.
76. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising KCl, such as 1 to 20 mM KCl, such as 1 to 15 mM KCl, such as 5 to 15 mM KCl, such as 6 to 14 mM KCl, such as 7 to 13 mM KCl, such as 6 to 12 mM KCl, such as 8 to 12 mM KCl, such as 9 to 11 mM KCl.
77. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising KCl, such as 0.1 to 10 mM KCl, 0.1 to 5 mM KCl, 0.1 to 2 mM KCl, 0.1 to 1 mM KCl, 0.1 to 0.5 mM KCl.
78. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MgCl_2 , such as 0.1 to 20 mM MgCl_2 , such as 0.5 to 20 mM MgCl_2 , such as 1 to 20 mM MgCl_2 , such as 1 to 10 mM MgCl_2 , such as 1 to 9 mM MgCl_2 , such as 1 to 8 mM MgCl_2 , such as 1 to 7 mM MgCl_2 , such as 1 to 6 mM MgCl_2 , such as 1 to 5 mM MgCl_2 , such as 1 to 4 mM MgCl_2 , such as 1 to 3 mM MgCl_2 .
79. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising CaCl_2 , such as 0.1 to 20 mM CaCl_2 , such as 0.5 to 20 mM CaCl_2 , such as 1 to

20 mM CaCl_2 , such as 1 to 10 mM CaCl_2 , such as 1 to 9 mM CaCl_2 , such as 1 to 8 mM CaCl_2 , such as 1 to 7 mM CaCl_2 , such as 1 to 6 mM CaCl_2 , such as 1 to 5 mM CaCl_2 , such as 1 to 4 mM CaCl_2 , such as 1 to 3 mM CaCl_2 .

80. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl, such as 1 to 100 mM Tris-Cl, such as 1 to 90 mM Tris-Cl, such as 1 to 80 mM Tris-Cl, such as 1 to 70 mM Tris-Cl, such as 10 to 70 mM Tris-Cl, such as 20 to 70 mM Tris-Cl, such as 30 to 70 mM Tris-Cl, such as 40 to 60 mM Tris-Cl.
81. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl, such as 1 to 50 mM Tris-Cl, such as 1 to 40 mM Tris-Cl, such as 1 to 30 mM Tris-Cl, such as 5 to 30 mM Tris-Cl, such as 10 to 30 mM Tris-Cl, such as 15 to 25 mM Tris-Cl.
82. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Guanidine HCl, such as 0.1 to 5 mM Guanidine HCl, such as 0.1 to 4 mM Guanidine HCl, such as 0.1 to 3 mM Guanidine HCl, such as 0.1 to 2 mM Guanidine HCl, such as 0.5 to 1.5 mM Guanidine HCl.
83. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, such as 0.01 to 5 % non-ionic surfactant, such as 0.01 to 4 % non-ionic surfactant, such as 0.01 to 3 % non-ionic surfactant, such as 0.01 to 2 % non-ionic surfactant, such as 0.1 to 2 % non-ionic surfactant, such as 0.5 to 2 % non-ionic surfactant, such as 0.5 to 1.5 % non-ionic surfactant, such as 0.5 to 1 % non-ionic surfactant.
84. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising DTT, such as 0.01 to 1 mM DTT, such as 0.01 to 0.5 mM DTT, such as 0.01 to 0.4 mM DTT, such as 0.01 to 0.3 mM DTT, such as 0.01 to 0.2 mM DTT, such as 0.5 to 0.2 mM DTT.
85. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising arginine, such as 0.01 to 10 M arginine, such as 0.01 to 5 M arginine, such as 0.01 to 4 M arginine, such as 0.01 to 3 M arginine, such as 0.01 to 2 M arginine, such as 0.1 to 2 M arginine, such as 0.1 to 1 M arginine, such as 0.2 to 0.9 M arginine, such as 0.3 to 0.8 M arginine, such as 0.4 to 0.7 M arginine, such as 0.4 to 0.6 M arginine.

86. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising polyethylene glycol 3,550, such as 0.001 to 10 % polyethylene glycol 3,550, such as 0.001 to 5 % polyethylene glycol 3,550, such as 0.001 to 1 % polyethylene glycol 3,550, such as 0.001 to 0.5 % polyethylene glycol 3,550, such as 0.01 to 0.5 % polyethylene glycol 3,550, such as 0.01 to 0.1 % polyethylene glycol 3,550.
87. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising GSH, such as 0.01 to 10 mM GSH, such as 0.01 to 5 mM GSH, such as 0.01 to 1 mM GSH, such as 0.01 to 0.5 mM GSH, such as 0.05 to 0.5 mM GSH, such as 0.05 to 0.4 mM GSH, such as 0.05 to 0.3 mM GSH, such as 0.05 to 0.2 mM GSH.
88. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising GSSH, such as 0.001 to 1 mM GSSH, such as 0.001 to 0.5 mM GSSH, such as 0.001 to 0.4 mM GSSH, such as 0.001 to 0.3 mM GSSH, such as 0.001 to 0.2 mM GSSH, such as 0.001 to 0.1 mM GSSH, such as 0.005 to 0.1 mM GSSH, such as 0.005 to 0.05 mM GSSH.
89. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising EDTA, such as 0.1 to 10 mM EDTA, such as 0.1 to 5 mM EDTA, such as 0.1 to 4 mM EDTA, such as 0.5 to 4 mM EDTA, such as 0.5 to 3 mM EDTA, such as 0.5 to 2 mM EDTA.
90. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising sucrose, such as 0.01 to 10 M sucrose, such as 0.01 to 5 M sucrose, such as 0.01 to 2 M sucrose, such as 0.01 to 1 M sucrose, such as 0.1 to 1 M sucrose, such as 0.01 to 0.9 M sucrose, such as 0.01 to 0.8 M sucrose, such as 0.01 to 0.8 M sucrose, such as 0.1 to 0.7 M sucrose, such as 0.1 to 0.6 M sucrose, such as 0.2 to 0.6 M sucrose, such as 0.3 to 0.6 M sucrose, such as 0.3 to 0.5 M sucrose.
91. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising urea, such as 1 to 15 M urea, 2 to 14 M urea, 3 to 13 M urea, 4 to 12 M urea, 5 to 11 M urea, 6 to 10 M urea or 7 to 9 M urea.
92. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Na_2HPO_4 , such as 1 to 100 mM Na_2HPO_4 , such as 1 to 90 mM Na_2HPO_4 , such as 1 to 80 mM Na_2HPO_4 , such as 1 to 70 mM Na_2HPO_4 , such as 1 to 60 mM Na_2HPO_4 , such as 1 to 50 mM Na_2HPO_4 , such as 1 to 40 mM Na_2HPO_4 , such as 1 to 30 mM Na_2HPO_4 , such as 1 to 20 mM Na_2HPO_4 , such as 5 to 20 mM Na_2HPO_4 , such as 5 to 15 mM Na_2HPO_4 .

93. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising KH_2PO_4 , such as 1 to 100 mM KH_2PO_4 , such as 1 to 90 mM KH_2PO_4 , such as 1 to 80 mM KH_2PO_4 , such as 1 to 70 mM KH_2PO_4 , such as 1 to 60 mM KH_2PO_4 , such as 1 to 50 mM KH_2PO_4 , such as 1 to 40 mM KH_2PO_4 , such as 1 to 30 mM KH_2PO_4 , such as 1 to 20 mM KH_2PO_4 , such as 5 to 20 mM KH_2PO_4 , such as 5 to 15 mM KH_2PO_4 .
94. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl_2 and/or CaCl_2 , such as 1 to 100 mM MES pH 6.0, 1 to 20 mM NaCl, 0.01 to 5 mM KCl, 0.1 to 10 mM MgCl_2 and/or 0.1 to 10 mM CaCl_2 , such as 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.1 to 1 mM KCl, 0.5 to 4 mM MgCl_2 and/or 0.5 to 4 mM CaCl_2 .
95. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl and/or KCl, such as 1 to 100 mM MES pH 6.0, 200 to 300 mM NaCl and/or 1 to 50 mM KCl, such as 30 to 70 mM MES pH 6.0, 220 to 260 mM NaCl and/or 1 to 20 mM KCl,
96. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl_2 , CaCl_2 , Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or DTT.
97. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl_2 , CaCl_2 , arginine, polyethylene glycol 3,550, GSH and/or GSSH.
98. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, EDTA, KCl, sucrose, M Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550 and/or DTT.
99. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, MgCl_2 , CaCl_2 , arginine, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, GSH and/or mM GSSH.

100. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, EDTA, sucrose, Guanidine HCl and/or DTT.
101. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, EDTA, arginine, sucrose, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550, GSH and/or GSSH.
102. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising MES pH 6.0, NaCl, KCl, $MgCl_2$, $CaCl_2$, Guanidine HCl, polyethylene glycol 3,550 and/or DTT.
103. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising mM Tris-Cl pH 8.5, NaCl, KCl, $MgCl_2$, $CaCl_2$, sucrose, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550, GSH and GSSH.
104. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, EDTA, arginine, Guanidine HCl, polyethylene glycol 3,550 and/or DTT.
105. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, $MgCl_2$, $CaCl_2$, arginine, sucrose, Guanidine HCl, mM GSH and/or GSSH.
106. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, mM NaCl, KCl, EDTA, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or DTT.
107. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, EDTA, polyethylene glycol 3,550, GSH and/or GSSH.
108. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, 1 mM EDTA, arginine, Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or DTT.

109. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, MgCl₂, CaCl₂, arginine, sucrose, Guanidine HCl, non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, polyethylene glycol 3,550, GSH and/or GSSH.
110. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl pH 8.5, NaCl, KCl, MgCl₂, CaCl₂, sucrose and/or DTT.
111. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising Tris-Cl and/or urea, having pH 8.0.
112. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising NaCl, KCl, Na₂HPO₄ and/or 10 mM KH₂PO₄, having pH 8.0.
113. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.1 to 1 mM KCl, 1 to 5 mM MgCl₂, 1 to 5 mM CaCl₂, 0.5 to 1 M Guanidine HCl, 0.1 to 2 % non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or 0.01 to 0.5 mM DTT.
114. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.1 to 1 mM KCl, 1 to 5 mM MgCl₂, 1 to 5 mM CaCl₂, 0.05 to 1 M arginine, 0.05 to 0.5 % polyethylene glycol 3,550, 0.01 to 1 mM GSH and/or 0.001 to 0.05 mM GSSH.
115. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 5 to 15 mM NaCl, 0.5 to 5 mM EDTA, 0.1 to 1 mM KCl, 0.1 to 1 M sucrose, 0.5 to 1 M Guanidine HCl, 0.05 to 1 % non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.005 to 0.1% polyethylene glycol 3,550 and/or 0.01 to 1 mM DTT.
116. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 200 to 300 mM NaCl, 1 to 20 mM KCl, 1 to 5 mM MgCl₂, 1 to 2 mM CaCl₂, 0.05 to 1 M arginine, 0.05 to 1% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.01 to 1 mM GSH and/or 0.001 to 0.1 mM GSSH.

117. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 200 to 300 mM NaCl, 1 to 20 mM KCl, 0.5 to 5 mM EDTA, 0.1 to 1 M sucrose, 0.5 to 1 M Guanidine HCl and/or 0.01 to 1 mM DTT.
118. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.4 M sucrose, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.
119. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 30 to 70 mM MES pH 6.0, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.75 M Guanidine HCl, 0.05% polyethylene glycol 3,550 and/or 0.1 mM DTT.
120. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.4 M sucrose, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.
121. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 1 mM EDTA, 0.5 M arginine, 0.75 M Guanidine HCl, 0.05% polyethylene glycol 3,550 and/or 0.1 mM DTT.
122. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.1 mM GSH and/or 0.01 mM GSSH.
123. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 9.6 mM NaCl, 0.4 mM KCl, 1 mM EDTA, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.1 mM DTT
124. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 1 mM EDTA, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.
125. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 1 mM EDTA,

0.5 M arginine, 0.75 M Guanidine HCl, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, and/or 0.1 mM DTT.

126. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.5 M arginine, 0.4 M sucrose, 0.75 M Guanidine HCl, 0.5% non-ionic surfactant, such as Tergitol NP-7, Tergitol NP-10 or Triton X-100, 0.05% polyethylene glycol 3,550, 0.1 mM GSH and/or 0.01 mM GSSH.
127. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 50 mM Tris-Cl pH 8.5, 240 mM NaCl, 10 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 0.4 M sucrose and/or 0.1 mM DTT.
128. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 20 mM Tris-Cl and/or 8 M urea, having pH 8.0.
129. The method according to any one of the preceding claims, wherein the assembly activator is a buffer comprising 500 mM NaCl, 100 mM KCl, 10 mM Na₂HPO₄ and/or 10 mM KH₂PO₄, having pH 8.0.
130. The method according to any one of the preceding claims, wherein the activating time is between 30 minutes and 24 hours, such between 1 hour and 24 hours, such between 1 hour and 20 hours, such between 1 hour and 16 hours.
131. The method according to any one of the preceding claims, wherein the activating time is between 15 minutes and 5 hours, such as between 30 minutes and 5 hours, such as between 30 minutes and 4 hours, such as between 30 minutes and 3 hours, such as between 30 minutes and 2 hours, such as between 30 minutes and 1 hour and 30 minutes.
132. The method according to any one of the preceding claims, wherein the activating temperature is between 1 °C and 45 °C, such as between 10 °C and 45 °C, such as between 10 °C and 40 °C, such as between 10 °C and 35 °C, such as between 10 °C and 30 °C, such as between 10 °C and 25 °C.
133. The method according to any one of the preceding claims, wherein the activating temperature is between 1 °C and 30 °C, such as between 10 °C and 30 °C, such as between 10 °C and 25 °C, such as between 15 °C and 25 °C, such as between 20 °C and 25 °C.

134. The method according to any one of the preceding claims, wherein the activating temperature is between 15 °C and 25 °C, such as between 16 °C and 25 °C, such as between 17 °C and 25 °C, such as between 17 °C and 24 °C, such as between 18 °C and 24 °C, such as between 19 °C and 24 °C, such as between 20 °C and 24 °C, such as between 21 °C and 24 °C, such as between 21 °C and 23 °C,
135. The method according to any one of the preceding claims, wherein the activating temperature is between 1 °C and 10 °C, such as between 1 °C and 9 °C, such as between 1 °C and 8 °C, such as between 1 °C and 7 °C, such as between 1 °C and 6 °C, such as between 2 °C and 6 °C, such as between 3 °C and 5 °C.
136. The method according to any one of the preceding claims, wherein the viral capsid proteins further comprise a purification tag, such as a His-tag and/or a C-tag, (EPEA).
137. The method according to any one of the preceding claims, wherein activating viral capsid protein concentration is higher than the inhibitory viral capsid protein concentration.
138. The method according to any one of the preceding claims, wherein the method comprises an assembly enhancer which enhances *in vitro* assembly.
139. The method according to any one of the preceding claims, wherein the assembly enhancer is selected from the group consisting of membrane-associated accessory protein, assembly activation protein, an E2A transcriptional regulator, an ICP8 single-stranded DNA-binding protein, presence of any cleavable protein or amino acid sequence, an enzyme that mediates glycosylation, and/or an enzyme that mediates a post translational modification.
140. The method according to any one of the preceding claims, wherein the assembly enhancer is selected from the group consisting of membrane-associated accessory protein and/or assembly activation protein.
141. The method according to any one of the preceding claims, wherein the one or more populations of inhibited parvovirus viral capsid proteins are provided at a defined stoichiometry.
142. The method according to any one of the preceding claims, wherein the one or more populations of inhibited parvovirus viral capsid proteins comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$.

143. A population of assembled parvovirus capsids obtainable by the method according to any one of preceding claims.
144. The population of assembled parvovirus capsids according to any one of preceding claims, wherein the population of assembled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$.
145. The population of assembled parvovirus capsids according to any one of preceding claims, wherein the population of assembled parvovirus capsids comprises VP1, VP2 and VP3 in a ratio of 1:1:10 (VP1:VP2:VP3).
146. The method according to any one of the preceding claims, wherein the nucleic acid molecule is a single-stranded nucleic acid molecule or a double-stranded nucleic acid molecule.
147. The method according to any one of the preceding claims, wherein the nucleic acid molecule is a viral nucleic acid molecule and/or a non-viral nucleic acid molecule.
148. The method according to any one of the preceding claims, wherein the nucleic acid molecule is a viral nucleic acid molecule.
149. The method according to any one of the preceding claims, wherein the nucleic acid molecule is a non-viral nucleic acid molecule.
150. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprises deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA).
151. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprises DNA.
152. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprises RNA.
153. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprises AAV DNA.

154. The method according to any one of the preceding claims, wherein the reaction mixture comprises adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), deoxythymidine triphosphate (dTTP), a pH buffer, such as HEPES or MOPS buffer, dithiothreitol, creatine phosphate, creatine phosphokinase (CPK), and/or magnesium chloride.
155. The method according to any one of the preceding claims, wherein the reaction mixture comprises adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), deoxythymidine triphosphate (dTTP), a pH buffer, such as HEPES or MOPS buffer, dithiothreitol, and/or magnesium chloride.
156. The method according to any one of the preceding claims, wherein the filling conditions are selected from the group consisting of ATP concentration, filling temperature, filling pH, CPK concentration, an ATP regeneration system, parvovirus capsid concentration and/or filling time.
157. The method according to any one of the preceding claims, wherein the filling conditions are selected from the group consisting of ATP concentration, filling temperature, filling pH, CPK concentration an ATP regeneration system, and/or parvovirus capsid concentration.
158. The method according to any one of the preceding claims, wherein the filling conditions comprise ATP concentration.
159. The method according to any one of the preceding claims, wherein the ATP concentration is between 0.1 and 181.44 mM, such as between 0.1 and 180 mM, such as between 0.1 and 170 mM, such as between 0.1 and 160 mM, such as between 0.1 and 150 mM, such as between 0.1 and 140 mM, such as between 0.1 and 130 mM, such as between 0.1 and 120 mM, such as between 0.1 and 110 mM, such as between 0.1 and 100 mM.
160. The method according to any one of the preceding claims, wherein the ATP concentration is between 0.1 and 100 mM. such as between 1 and 90 mM, such as between 1 and 80 mM, such as between 1 and 70 mM, such as between 1 and 60 mM, such as between 1 and 50 mM, such as between 1 and 40 mM, such as between 1 and 30 mM, such as between 1 and 20 mM, such as between 1 and 15 mM, such as between 1 and 10 mM.

161. The method according to any one of the preceding claims, wherein the ATP concentration is between 0.01 and 10 mM, such as between 0.05 and 10 mM, such as between 0.05 and 9 mM, such as between 0.05 and 8 mM, such as between 0.05 and 7 mM, such as between 0.05 and 6 mM, such as between 0.05 and 5 mM, such as between 0.05 and 4 mM, such as between 0.05 and 3 mM, such as between 0.05 and 2.5 mM, such as between 0.1 and 2 mM.
162. The method according to any one of the preceding claims, wherein the ATP concentration is between 0.05 and 15 mM.
163. The method according to any one of the preceding claims, wherein the filling conditions comprise filling temperature.
164. The method according to any one of the preceding claims, wherein the filling temperature is 0 °C and 89.7 °C, such as between 5 °C and 80 °C, such as between 5 °C and 70 °C, such as between 5 °C and 60 °C, between 5 °C and 50 °C, such as between 5 °C and 50 °C, such as between 10 °C and 50 °C, such as between 15 °C and 50 °C, such as between 20 °C and 50 °C, such as between 25 °C and 50 °C, such as between 30 °C and 50 °C.
165. The method according to any one of the preceding claims, wherein the filling temperature is between 1 °C and 40 °C, such as between 5 °C and 40 °C, such as between 10 °C and 40 °C, such as between 15 °C and 40 °C, such as between 20 °C and 40 °C, such as between 25 °C and 40 °C, such as between 26 °C and 40 °C, such as between 27 °C and 40 °C, such as between 28 °C and 40 °C, such as between 29 °C and 40 °C, such as between 29 °C and 39 °C.
166. The method according to any one of the preceding claims, wherein the filling temperature is between 20 °C and 50 °C, such as between 20 °C and 45 °C, such as between 20 °C and 40 °C, such as between 25 °C and 40 °C, such as between 26 °C and 40 °C, such as between 27 °C and 40 °C, such as between 28 °C and 40 °C, such as between 29 °C and 40 °C, such as between 29 °C and 39 °C.
167. The method according to any one of the preceding claims, wherein the filling temperature is between 30 °C and 50 °C, such as between 30 °C and 45 °C, such as between 30 °C and 45 °C, such as between 31 °C and 45 °C, such as between 32 °C and 45 °C, such as between 33 °C and 45 °C, such as between 34 °C and 45 °C, such as between 30 °C and 44 °C, such as between 30 °C and 43 °C, such as between 30 °C and 42 °C, such as between 30 °C and 41 °C.

168. The method according to any one of the preceding claims, wherein the filling temperature is between 33 °C and 42 °C, such as between 34 °C and 41 °C.
169. The method according to any one of the preceding claims, wherein the filling temperature is between 35 °C and 40 °C.
170. The method according to any one of the preceding claims, wherein the filling conditions comprise filling pH.
171. The method according to any one of the preceding claims, wherein the filling pH is between 3 and 10, such as between 4 and 9, such as between 4.5 and 9.
172. The method according to any one of the preceding claims, wherein the filling pH is between 5 and 8.8, such as between 5.5 and 8.8, such as between 6 and 8.8, such as between 6 and 8.5, such as between 6 and 8.
173. The method according to any one of the preceding claims, wherein the filling pH is between 6 and 7.5.
174. The method according to any one of the preceding claims, wherein the filling conditions comprise ATP regenerating system, such as acetate kinase using acetyl phosphate as phosphate donor, creatine kinase using creatine phosphate as phosphate donor, pyruvate kinase using phosphoenolpyruvate as phosphate donor, and polyphosphate kinase using polyphosphate as phosphate donor.
175. The method according to any one of the preceding claims, wherein the filling conditions comprise the CPK concentration.
176. The method according to any one of the preceding claims, wherein the CPK concentration is between 1 and 1000 ug/ml, such as between 1 and 500 ug/ml, such as between 1 and 400 ug/ml, such as between 1 and 300 ug/ml, such as between 1 and 200 ug/ml, such as between 1 and 100 ug/ml.
177. The method according to any one of the preceding claims, wherein the CPK concentration is between 1 and 100 ug/ml, such as between 1 and 90 ug/ml, such as between 1 and 80 ug/ml, such as between 1 and 70 ug/ml, such as between 1 and 60 ug/ml, such as

between 1 and 50 ug/ml, such as between 1 and 40 ug/ml, such as between 1 and 30 ug/ml, such as between 1 and 20 ug/ml, such as between 1 and 10 ug/ml.

178. The method according to any one of the preceding claims, wherein the CPK concentration is between 5 and 110 ug/ml, such as between 5 and 100 ug/ml, between 5 and 90 ug/ml.
179. The method according to any one of the preceding claims, wherein the CPK concentration is between 10 and 100 ug/ml.
180. The method according to any one of the preceding claims, wherein the filling conditions comprise the parvovirus capsid concentration.
181. The method according to any one of the preceding claims, wherein the parvovirus capsid concentration is between $1e4$ and $5e16$ /mL, such as between $1e4$ and $5e15$ /mL, such as between $1e4$ and $1e15$ /mL, such as between $1e4$ and $1e15$ /mL, such as between $1e4$ and $1e14$ /mL, such as between $1e4$ and $1e13$ /mL, such as between $1e4$ and $1e12$ /mL, such as between $1e4$ and $1e11$ /mL, such as between $1e4$ and $1e10$ /mL, such as between $1e4$ and $1e9$ /mL, such as between $1e4$ and $1e8$ /mL, such as between $1e4$ and $1e7$ /mL, such as between $1e4$ and $1e6$ /mL, such as between $1e4$ and $1e5$ /mL.
182. The method according to any one of the preceding claims, wherein the parvovirus capsid concentration is between 0.01 and $100e10$ /mL, such as between 0.1 and $100e10$ /mL, such as between 0.1 and $90e10$ /mL, such as between 0.1 and $80e10$ /mL, such as between 0.1 and $70e10$ /mL, such as between 0.1 and $60e10$ /mL, such as between 0.1 and $50e10$ /mL, such as between 0.1 and $40e10$ /mL, such as between 0.1 and $30e10$ /mL, such as between 0.1 and $20e10$ /mL, such as between 0.1 and $10e10$ /mL, such as between 0.1 and $5e10$ /mL.
183. The method according to any one of the preceding claims, wherein the parvovirus capsid concentration is between 0.01 and $10e10$ /mL, such as between 0.1 and $10e10$ /mL, such as between 0.5 and $10e10$ /mL, such as between 0.5 and $9e10$ /mL, such as between 0.5 and $8e10$ /mL, such as between 0.5 and $7e10$ /mL, such as between 0.5 and $6e10$ /mL, such as between 0.5 and $5e10$ /mL.
184. The method according to any one of the preceding claims, wherein the parvovirus capsid concentration is between 0.5 and $7e10$ /mL.

185. The method according to any one of the preceding claims, wherein the reaction mixture comprises HEPES, such as 30mM HEPES, MES buffer, phosphate buffer, Tris buffer or MOPS buffer.
186. The method according to any one of the preceding claims, wherein the filling conditions comprises HEPES, such as 30mM HEPES, MES buffer, phosphate buffer, Tris buffer or MOPS buffer.
187. The method according to any one of the preceding claims, wherein the filling conditions comprises the following:
- a. the ATP concentration is between 0.05 and 15 mM;
 - b. the filling temperature is between 35 °C and 40 °C;
 - c. the CPK concentration is between 10 and 100 ug/ml; and/or
 - d. the parvovirus capsid concentration is between 0.5 and 7 e10/mL.
188. The method according to any one of the preceding claims, wherein the filling conditions comprises filling time.
189. The method according to any one of the preceding claims, wherein the filling time is between 0.5 and 24 hours, such as between 0.5 and 20 hours, such as between 0.5 and 16 hours, such as between 0.5 and 12 hours, such as between 0.5 and 10 hours, such as between 1 and 10 hours, such as between 1 and 9 hours, such as between 2 and 8 hours, such as between 2 and 7 hours, such as between 3 and 6 hours, such as between 3 and 5 hours.
190. The method according to any one of the preceding claims, wherein the filling time is between 1 and 7 hours, such as between 2 and 7 hours, such as between 2.5 and 6.5 hours, such as between 3 and 6 hours, such as between 3 and 5 hours, such as between 3.5 and 4.5 hours.
191. The method according to any one of the preceding claims, wherein the filling time is between 3 and 5 hours.
192. The method according to any one of the preceding claims, wherein the filling conditions comprises the following:
- a. the ATP concentration is between 0.05 and 15 mM;
 - b. the filling temperature is between 35 °C and 40 °C;
 - c. the CPK concentration is between 10 and 100 ug/ml;

- d. the parvovirus capsid concentration is between 0.5 and 7 e10/mL; and/or
 - e. the filling time is between 3 and 5 hours.
193. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprises a first inverted terminal repeat (ITR1) and/or a second inverted terminal repeat (ITR2).
194. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 is from AAV.
195. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 comprises the Rep binding domain.
196. The method according to any one of the preceding claims, wherein the nucleic acid molecule, the ITR1 and/or the ITR2 are operably linked to each other in a 5'-to-3' direction.
197. The method according to any one of the preceding claims, wherein the nucleic acid molecule, the ITR1 and/or the ITR2 are operably linked to each other in a 3'-to-5' direction.
198. The method according to any one of the preceding claims, wherein the method comprises a filling enhancer which enhances *in vitro* filling.
199. The method according to any one of the preceding claims, wherein the filling enhancer is selected from the group consisting of membrane-associated accessory protein, assembly activation protein, an E2A transcriptional regulator, an ICP8 single-stranded DNA-binding protein, presence of any cleavable protein or amino acid sequence, an enzyme that mediates glycosylation, and/or an enzyme that mediates a post translational modification.
200. The method according to any one of the preceding claims, wherein the filling enhancer is selected from the group consisting of membrane-associated accessory protein and/or assembly activation protein.
201. The method according to any one of the preceding claims, wherein the E2A transcriptional regulator is from adenovirus.
202. The method according to any one of the preceding claims, wherein the ICP8 single-stranded DNA-binding protein is from herpes virus.

203. The method according to any one of the preceding claims, wherein the enzyme that mediates a post translational modification is synthetic.
204. The method according to any one of the preceding claims, wherein the filling conditions are conditions which denature the assembled capsids.
205. The method according to any one of the preceding claims, wherein the filling conditions which denature are condition factors selected from this list comprising: pH, sonication, ionic strength/conductivity, or temperature.
206. The method according to any one of the preceding claims, wherein the filled parvovirus capsids comprises an increased amount of intact nucleic acid molecule compared to parvovirus capsids that have been filled using conventional methods
207. The method according to any one of the preceding claims, wherein the filled parvovirus capsids comprise an 80% intact nucleic acid molecule, such as an 85% intact nucleic acid molecule, such as a 90% intact nucleic acid molecule, such as a 95% intact nucleic acid molecule, such as a 96% intact nucleic acid molecule, such as a 97% intact nucleic acid molecule, such as a 98% intact nucleic acid molecule, such as a 99% intact nucleic acid molecule, such as a 100% intact nucleic acid molecule.
208. The method according to any one of the preceding claims, wherein the filled parvovirus capsids comprise a 100% intact nucleic acid molecule.
209. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprised in the filled parvovirus capsids has reduced truncation compared to a nucleic acid molecule comprised in parvovirus capsid that have been filled using conventional methods.
210. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprised in the filled parvovirus capsids has 20% truncation, such as 15% truncation, such as 10% truncation, such as 5% truncation, such as 4% truncation, such as 3% truncation, such as 2% truncation, such as 1% truncation, such as 0% truncation.
211. The method according to any one of the preceding claims, wherein the nucleic acid molecule comprised in the filled parvovirus capsids has no truncation.

212. The method according to any one of the preceding claims, wherein the nucleic acid comprises the ITR1, the transgene of interest and the ITR2.
213. A population of filled parvovirus capsids obtainable by the method according to any one of preceding claims.
214. The population of filled parvovirus capsids according to any one of preceding claims, wherein the population of filled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$.
215. The population of filled parvovirus capsids according to any one of preceding claims, wherein the population of filled parvovirus capsids comprises VP1, VP2 and VP3 in a ratio of 1:1:10 (VP1:VP2:VP3).
216. The method according to any one of the preceding claims, wherein the one or more populations of inhibited parvovirus viral capsid proteins and/or the one or more populations of assembled parvovirus capsids comprises VP1, VP2 and VP3 in any ratio of integers where $VP1 + VP2 + VP3 = 60$.
217. The method according to any one of the preceding claims, wherein the one or more populations of inhibited parvovirus viral capsid proteins and/or the one or more populations of assembled parvovirus capsids comprises VP1, VP2 and VP3 in a ratio of 1:1:10 (VP1:VP2:VP3).
218. The method according to any one of the preceding claims, wherein the method further comprises a step of purifying the assembled parvovirus capsids and/or the filled parvovirus capsids.
219. The method according to any one of the preceding claims, wherein the step of purifying comprises chromatography, ultra-centrifugation, centrifugation, flocculation, filtration, and/or ultrafiltration/diafiltration.
220. The method according to any one of the preceding claims, wherein the method further comprises a step of formulating the assembled parvovirus capsids and/or the filled parvovirus capsids into a formulation, optionally wherein the assembled parvovirus capsids and/or the filled parvovirus capsids are purified assembled parvovirus capsids and/or purified filled parvovirus capsids.

221. The method according to any one of the preceding claims, wherein the step of formulating comprises formulating the assembled parvovirus capsids and/or the filled parvovirus capsids into a formulation with trehalose, sugar, sucrose, polyols, and/or dimethylsulfoxide.
222. The method according to any one of the preceding claims, wherein the step of formulating comprises formulating the assembled parvovirus capsids and/or the filled parvovirus capsids into a formulation with a pharmaceutically acceptable carrier, diluent and/or excipient.
223. The method according to any one of the preceding claims, wherein the pharmaceutically acceptable carrier, diluent and/or excipient is selected from the group consisting of tween 20, tween 80, trehalose, Pluronic F68, sorbitol and/or glycerol, such as 0.0001 to 0.01% Pluronic F68, 1 to 10 % sorbitol and/or 1 to 10% glycerol.
224. The method according to any one of the preceding claims, wherein the method further comprises a step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids, optionally wherein the storing is under conditions that maintain capsid integrity.
225. The method according to any one of the preceding claims, wherein the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids is at room temperature.
226. The method according to any one of the preceding claims, wherein the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids is at 1 °C to 25 °C, such as 1 °C to 20 °C, such as 5 °C to 25 °C, such as 10 °C to 25 °C, such as 15 °C to 25 °C.
227. The method according to any one of the preceding claims, wherein the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids comprises lyophilizing and aerosolizing the assembled parvovirus capsids and/or the filled parvovirus capsids.
228. The method according to any one of the preceding claims, wherein the step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids at a low pH or a high pH.
229. The method according to any one of the preceding claims, comprising step of storing the assembled parvovirus capsids and/or the filled parvovirus capsids under pressure.
230. The method according to any one of the preceding claims, wherein the VP1 comprises an amino acid sequence that is at least 85% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.

231. The method according to any one of the preceding claims, wherein the VP1 comprises an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.
232. The method according to any one of the preceding claims, wherein the VP1 comprises an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10, optionally wherein the VP1 comprises an amino acid sequence that is at least 96%, 97%, 98%, and 99% identical to the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.
233. The method according to any one of the preceding claims, wherein the VP1 comprises an amino acid sequence that is identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.
234. The method according to any one of the preceding claims, wherein the VP1 has an amino acid sequence that is at least 85% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.
235. The method according to any one of the preceding claims, wherein the VP1 has an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.
236. The method according to any one of the preceding claims, wherein the VP1 has an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 2 or 4, optionally wherein the VP1 has an amino acid sequence that is at least 96%, 97%, 98%, and 99% identical to any one of the amino acid sequences of SEQ ID NOs: 1-4 or 7-10.
237. The method according to any one of the preceding claims, wherein the VP1 has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-4 or 7-10.
238. The method according to any one of the preceding claims, wherein the VP2 comprises an amino acid sequence that is at least 85% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.

239. The method according to any one of the preceding claims, wherein the VP2 comprises an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
240. The method according to any one of the preceding claims, wherein the VP2 comprises an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17, optionally wherein the VP2 comprises an amino acid sequence that is at least 96%, 97%, 98%, and 99% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
241. The method according to any one of the preceding claims, wherein the VP2 comprises an amino acid sequence that is identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
242. The method according to any one of the preceding claims, wherein the VP2 has an amino acid sequence that is at least 85% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
243. The method according to any one of the preceding claims, wherein the VP2 has an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
244. The method according to any one of the preceding claims, wherein the VP2 has an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 13, 16, or 17, optionally wherein the VP2 has an amino acid sequence that is at least 96%, 97%, 98%, and 99% identical to any one of the amino acid sequences of SEQ ID NOs: 5 or 11-17.
245. The method according to any one of the preceding claims, wherein the VP2 has an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 or 11-17.
246. The method according to any one of the preceding claims, wherein the VP3 comprises an amino acid sequence that is at least 85% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.

247. The method according to any one of the preceding claims, wherein the VP3 comprises an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.
248. The method according to any one of the preceding claims, wherein the VP3 comprises an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24, optionally wherein the VP3 comprises an amino acid sequence that is at least 96%, 97%, 98%, and 99% identical to any one of the amino acid sequences of SEQ ID NOs: 6 and 18-24.
249. The method according to any one of the preceding claims, wherein the VP3 comprises an amino acid sequence that is identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.
250. The method according to any one of the preceding claims, wherein the VP3 has an amino acid sequence that is at least 85% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.
251. The method according to any one of the preceding claims, wherein the VP3 has an amino acid sequence that is at least 90% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.
252. The method according to any one of the preceding claims, wherein the VP3 has an amino acid sequence that is at least 95% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24, optionally wherein the VP3 has an amino acid sequence that is at least 96%, 97%, 98%, and 99% identical to any one of the amino acid sequences of SEQ ID NOs: 6 or 18-24.
253. The method according to any one of the preceding claims, wherein the VP3 has an amino acid sequence of SEQ ID NO: 6 SEQ ID NOs: 6 or 18-24.
254. The method according to any one of the preceding claims, wherein Rep protein is a synthetic Rep protein.
255. The method according to any one of the preceding claims, wherein Rep protein is a recombinant Rep protein and/or a recombinantly produced Rep protein.

256. The method according to any one of the preceding claims, the Rep protein comprises multiple Rep proteins, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Rep proteins.
257. The method according to any one of the preceding claims, the step of providing a Rep protein is a step of providing multiple Rep proteins, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Rep proteins
258. The method according to any one of the preceding claims, wherein the Rep protein is from a Parvoviridae.
259. The method according to any one of the preceding claims, wherein the Rep protein is from a Densovirinae, Hamaparvovirinae, or Parvovirinae.
260. The method according to any one of the preceding claims, wherein the Rep protein is from is a Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvoviru, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus.
261. The method according to any one of the preceding claims, wherein the Rep protein is from a Dependoparvovirus.
262. The method according to any one of the preceding claims, wherein the Rep protein is from an AAV.
263. The method according to any one of the preceding claims, wherein the Rep protein is synthetic Rep protein and/or a recombinant Rep protein.
264. The method according to any one of the preceding claims, wherein the Rep protein is selected from the group consisting of AAV Rep40, AAV Rep52, AAV Rep68, AAV Rep78.
265. The method according to any one of the preceding claims, wherein the Rep40 is from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.
266. The method according to any one of the preceding claims, wherein the Rep52 is from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6,

serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.

267. The method according to any one of the preceding claims, wherein the Rep68 is from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.

268. The method according to any one of the preceding claims, wherein the Rep78 is from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.

269. The method according to any one of the preceding claims, wherein the Rep protein is selected from the list consisting of:

- a. AAV Rep40;
- b. AAV Rep52;
- c. AAV Rep40 and AAV Rep52;
- d. AAV Rep68;
- e. AAV Rep78;
- f. AAV Rep68 and AAV Rep78;
- g. AAV Rep40, AAV Rep52, AAV Rep68, and AAV Rep78;
- h. AAV Rep40, AAV Rep52, and AAV Rep68; and/or
- i. AAV Rep40, AAV Rep52, and AAV Rep78.

270. The method according to any one of the preceding claims, wherein the Rep40, Rep52, Rep68, and/or Rep78 are from different AAV serotypes.

271. The method according to any one of the preceding claims, wherein the Rep40, Rep52, Rep68, and/or Rep78 are from the same AAV serotype.

272. The method according to any one of the preceding claims, wherein the Rep protein is produced in an eukaryotic microbial system, such as yeast, such as a methylotrophic yeast.

273. The method according to any one of the preceding claims, wherein the Rep protein is produced in an eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*.

274. The method according to any one of the preceding claims, wherein the Rep protein is produced in an prokaryotic microbial system, such as *E. Coli*.
275. The method according to any one of the preceding claims, wherein the Rep protein is produced in a mammalian cell system, such as HEK, CHO, HeLa or Vero cells, an insect cell system, such as Sf9, Sf21 or High Five cells, a plant system, such as tobacco BY, an algae system, a metazoan cell system, a zebrafish and/or cell-free expression systems.
276. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from a Parvoviridae.
277. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from a Densovirinae, Hamaparvovirinae, or Parvovirinae.
278. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from a Amdoparvovirus, Artiparvovirus, Aveparvovirus, Bocaparvovirus, Copiparvovirus, Dependoparvovirus, Erythroparvovirus, Loriparvovirus, Protoparvovirus, or Tetraparvovirus.
279. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from a Dependoparvovirus.
280. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from an AAV.
281. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from AAV serotype 1, serotype 2, serotype 3A, serotype 3B, serotype 4, serotype 5, serotype 6, serotype 6.2, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh10, or serotype rh74.
282. The method according to any one of the preceding claims, wherein the ITR1 and/or the ITR2 are from AAV serotype 2.
283. The method according to any one of the preceding claims, wherein the VP1, VP2 and/or VP3, the Rep protein, and/or ITR1 and/or ITR2 are from different AAV serotypes.
284. The method according to any one of the preceding claims, wherein the VP1, VP2 and/or VP3, the Rep protein, and/or ITR1 and/or ITR2 are from the same AAV serotype.

285. An AAV Rep fusion protein comprising:
- An AAV Rep domain or a AAV Rep protein, and
 - A cleavable amino acid sequence, such as a SUMO tag.
286. The AAV Rep fusion protein according to any one of the preceding claims, wherein the AAV Rep protein is selected from the group consisting of Rep40, Rep52, Rep68 and/or Rep78.
287. The AAV Rep fusion protein according to any one of the preceding claims, further comprising a purification tag, such as a His-tag, a C-tag (EPEA), a streptavidin tag and/or biotin tag.
288. A vector encoding the AAV Rep fusion protein according to any one of the preceding claims.
289. A method of producing the AAV Rep fusion protein according to any one of the preceding claims, comprising the steps of:
- Transforming a prokaryotic microbial system, such as *E. coli*, with the vector of claim 288; and
 - Incubating the prokaryotic microbial system, such as *E. coli*, thereby producing the AAV Rep fusion protein.
290. A method of producing the AAV Rep fusion protein according to any one of the preceding claims, comprising the steps of:
- Transforming an eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*, with the vector of claim 288,
 - Incubating the eukaryotic microbial system, such as yeast, such as *P. pastoris*, or *S. cerevisiae*, thereby producing the AAV Rep fusion protein.
291. The method according to claim 289 to 290, further comprising a step of inoculating the prokaryotic microbial system or the eukaryotic microbial system.
292. The method according to any one of claims 289 to 291, further comprising a step of purifying the fusion protein.

293. The method according to any one of claims 289 to 292, wherein the eukaryotic microbial system is a methylotrophic yeast.
294. An AAV VP fusion protein comprising:
- An AAV VP domain or an AAV VP protein, and
 - A cleavable amino acid sequence, such as a SUMO tag.
295. The AAV VP fusion protein according to any one of the preceding claims, wherein the AAV VP protein is selected from the group consisting of AAV VP1, AAV VP2, and/or AAV VP.
296. The AAV VP fusion protein according to any one of the preceding claims, further comprising a purification tag, such as a His-tag, a C-tag (EPEA), a streptavidin tag and/or biotin tag.
297. The AAV VP fusion protein according to any one of the preceding claims, comprising
- A purification tag, such as a His-tag,
 - A cleavable amino acid sequence, such as a SUMO tag,
 - An AAV VP protein, such as AAV VP1, AAV VP2 and/or AAV VP3,
 - A thrombin cleavage site,
 - A cleavable amino acid sequence, such as a SUMO tag, and
 - A purification tag, such as C-tag (EPEA).
298. The AAV VP fusion protein according to any one of the preceding claims, wherein the VP protein is as defined in any one of the preceding claims.
299. A vector encoding the AAV VP fusion protein according to any one of the preceding claims.
300. A method of producing the AAV VP fusion protein according to any one of the preceding claims, comprising the steps of:
- Transforming a prokaryotic microbial system, such as *E. coli*, with the vector of claim 299; and
 - Incubating the prokaryotic microbial system, such as *E. coli*, thereby producing the AAV VP fusion protein.
301. A method of producing the AAV VP fusion protein according to any one of the preceding claims, comprising the steps of:

- a. Transforming a eukaryotic microbial system, such as yeast, such as *P. pastoris* or *S. cerevisiae*, with the vector of claim 299,
 - b. Incubating the eukaryotic microbial system, such as *P. pastoris* or *S. cerevisiae*, thereby producing the AAV fusion protein.
302. The method according to any one of claims 300 to 301, further comprising a step of inoculating the eukaryotic microbial system.
303. The method according to any one of claims 300 to 302, further comprising a step of purifying the fusion protein.
304. The method according to any one of claims 300 to 303, wherein the eukaryotic microbial system is a methylotrophic yeast.
305. A method of producing the AAV Rep fusion protein or the AAV VP fusion protein according to any one of the preceding claims, comprising the steps of:
- a. Transforming an mammalian system, such as HEK cells, with the vector according to any one of the preceding claims,
 - b. Incubating the mammalian system, such as HEK cells, thereby producing the AAV Rep fusion protein or the AAV VP fusion protein.
306. A kit of parts comprising:
- a. one or more populations of inhibited parvovirus viral capsid proteins according to any one of the preceding claims;
 - b. an assembly inhibitor according to any one of the preceding claims;
 - c. an assembly activator according to any one of the preceding claims; and
 - d. optionally instructions for use.
307. A kit of parts comprising:
- a. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins according to any one of the preceding claims;
 - b. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain according to any one of the preceding claims;
 - c. a Rep protein according to any one of the preceding claims; and
 - d. optionally instructions for use.
308. A kit of parts comprising:

- a. one or more populations of assembled parvovirus capsids comprising parvovirus viral capsid proteins according to any one of the preceding claims;
 - b. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain according to any one of the preceding claims;
 - c. a Rep protein according to any one of the preceding claims;
 - d. a reaction mixture according to any one of the preceding claims; and
 - e. optionally instructions for use.
309. A kit of parts comprising:
- a. one or more populations of inhibited parvovirus viral capsid proteins according to any one of the preceding claims;
 - b. an assembly inhibitor according to any one of the preceding claims;
 - c. an assembly activator according to any one of the preceding claims;
 - d. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain according to any one of the preceding claims;
 - e. a Rep protein according to any one of the preceding claims; and
 - f. optionally instructions for use.
310. A kit of parts comprising:
- a. one or more populations of inhibited parvovirus viral capsid proteins according to any one of the preceding claims;
 - b. an assembly inhibitor according to any one of the preceding claims;
 - c. an assembly activator according to any one of the preceding claims;
 - d. a nucleic acid molecule comprising a transgene of interest and a Rep binding domain according to any one of the preceding claims;
 - e. a Rep protein according to any one of the preceding claims;
 - f. a reaction mixture according to any one of the preceding claims; and
 - g. optionally instructions for use.
311. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 35, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 35.

312. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 36, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 36.
313. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 37, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 37.
314. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 38, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 38.
315. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 39, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 39.
316. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 40, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 40.
317. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 41, optionally wherein the Rep protein comprises an amino acid

sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 41.

318. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 42, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 42.
319. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 43, optionally wherein the Rep protein comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 43.
320. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 35.
321. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 36.
322. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 37.
323. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 38.
324. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 39.
325. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 40.

326. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 41.
327. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 42.
328. The method according to any one of the preceding claims, wherein the Rep protein comprises or consists of an amino acid sequence of SEQ ID NO: 43.
329. The method according to any one of the preceding claims, wherein the Rep protein concentration is between 0.01 and 100 μ M, such as between 0.01 and 10 μ M, between 0.01 and 5 μ M, between 0.01 and 2.5 μ M, between 0.01 and 1 μ M, between 0.01 and 0.5 μ M, between 0.01 and 0.25 μ M, between 0.01 and 0.1 μ M, between 0.1 and 100 μ M, between 0.1 and 10 μ M, between 0.1 and 5 μ M, between 0.1 and 2.5 μ M, between 0.1 and 1 μ M, between 0.1 and 0.5 μ M, between 0.1 and 0.25 μ M, between 0.25 and 100 μ M, between 0.25 and 10 μ M, between 0.25 and 5 μ M, between 0.25 and 2.5 μ M, between 0.25 and 1 μ M, between 0.25 and 0.5 μ M, between 0.5 and 100 μ M, between 0.5 and 10 μ M, between 0.5 and 5 μ M, between 0.5 and 2.5 μ M, between 0.5 and 1 μ M, between 1 and 100 μ M, between 1 and 10 μ M, between 1 and 5 μ M, between 1 and 2.5 μ M, between 2.5 and 100 μ M, between 2.5 and 10 μ M, between 2.5 and 5 μ M, between 5 and 100 μ M, between 5 and 10 μ M, or such as wherein the Rep protein concentration is between 10 and 100 μ M.
330. The method according to any one of the preceding claims, wherein the Rep protein concentration is 0.1 to 10 μ M, such as 0.1 to 9 μ M, such as 0.1 to 8 μ M, such as 0.1 to 7 μ M, such as 0.1 to 6 μ M, such as 0.1 to 5 μ M, such as 0.5 to 5 μ M, such as 0.5 to 4 μ M, such as 0.5 to 3 μ M, such as 0.5 to 2 μ M, such as 0.5 to 1.5 μ M.
331. The method according to any one of the preceding claims, wherein the Rep protein concentration is 1 μ M.
332. The method according to any one of the preceding claims, wherein the Rep protein is purified Rep protein.
333. The method according to any one of the preceding claims, wherein the Rep protein is purified from *Escherichia Coli*.

334. The method according to any one of the preceding claims, wherein at least 80% of the assembled parvovirus capsids are filled by said method, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or wherein at least 99% of the assembled parvovirus capsids are filled by said method.
335. The method according to any one of the preceding claims, wherein 100% of the assembled parvovirus capsids are filled by said method.
336. The method according to any one of the preceding claims, wherein at least 80% of the assembled parvovirus capsids are filled with said transgene of interest by said method, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or wherein at least 99% of the assembled parvovirus capsids are filled by said method.
337. The method according to any one of the preceding claims, wherein 100% of the assembled parvovirus capsids are filled with said transgene of interest by said method.
338. The method according to any one of the preceding claims, wherein the method yields at least 80% filled parvovirus capsids, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% filled parvovirus capsids.
339. The method according to any one of the preceding claims, wherein the method yields 100% filled parvovirus capsids.
340. A fusion protein, such as a MBP-Rep78 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 35, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as

93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 35.

341. A fusion protein, such as a MBP-Rep78 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 35.

342. A fusion protein, such as a SUMO-Rep40 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 36, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 36.

343. A fusion protein, such as a SUMO-Rep40 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 36.

344. A fusion protein, such as a SUMO-Rep52 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 37, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 37.

345. A fusion protein, such as a SUMO-Rep52 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 37.

346. A fusion protein, such as a SUMO-Rep68 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 38, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 38.

347. A fusion protein, such as a SUMO-Rep68 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 38.

348. A fusion protein, such as a SUMO-Rep78 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 39, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 39.
349. A fusion protein, such as a SUMO-Rep78 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 39.
350. A fusion protein, such as a Smt3-Rep40 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 40, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 40.
351. A fusion protein, such as a Smt3-Rep40 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 40.
352. A fusion protein, such as a Smt3-Rep52 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 41, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 41.
353. A fusion protein, such as a Smt3-Rep52 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 41.
354. A fusion protein, such as a Smt3-Rep68 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 42, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 42.

355. A fusion protein, such as a Smt3-Rep68 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 42.
356. A fusion protein, such as a Smt3-Rep78 fusion protein, consisting of or comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 43, such as 81%, such as 82%, such as 83%, such as 84%, such as 85%, such as 86%, such as 87%, such as 88%, such as 89%, such as 90%, such as 91%, such as 92%, such as 93%, such as 94%, such as 95%, such as 96%, such as 97%, such as 98%, or such as 99% sequence identity to the amino acid sequence of SEQ ID NO: 43.
357. A fusion protein, such as a Smt3-Rep78 fusion protein, consisting of or comprising the amino acid sequence of SEQ ID NO: 43.