are unable to provide a therapeutic benefit associated with transgene production, and there is a potential for increasing innate or adaptive immune responses to the vector (e.g., T-cell mediated), which then renders empty capsids a concern in gene therapy contexts. Wright, Molecular Therapy 22: 1–2 (2014).

[0005] Classically, the use of quantitative PCR (qPCR) is the most widely accepted and preferred to method for determining the dose of AAV preparations because it provides a measurement of the vector genomes (e.g., vector genome (vg)/kg subject). *See* Halbert CL et al., J Virol 1997;71:5932–5941; Samulski RJ et al., J Virol 1989;63:3822–3828; Clark KR et al., Hum Gene Ther 1999;10:1031–1039; and Wright JF, Hum Gene Ther 2011;22:519–521. Use of qPCR is limited, however, by the fact that only total DNA is measured, which can lead to significant issues. For example, the readout of qPCR can be impacted by issues such as, the use of inefficient primers, issues with calibration standards, or DNA tertiary structures (e.g., hairpin structures, circular, or supercoiled). Thus, the use of qPCR can lead to inaccurate determination of the dose and ultimately potency of the final AAV preparation due to the inclusion of capsids that contain more than one copy of DNA and/or fractured capsids in the dose calculation. This is because the potency of an AAV preparation consist of two parts: 1) the ability of AAV capsid to transduce cells and 2) the ability of DNA in AAV to produce desired product. Inoperable capsids, which would not be accounted for with qPCR, do not effectively transduce the cells. Moreover, qPCR is also limited due to its inherent variability (i.e., high variability up to 0.5 log steps) which can lead to an inaccurate determination of dose of the final AAV preparation. The inherent variability can also be compounded by the inter-assay variability seen with qPCR due to sample preparation, which involves several steps, including, capsid protein digestion, DNA extraction, and DNA purification. If the dose and/or potency is not accurately determined, patients may receive the wrong dose and/or widely varying doses from treatment to treatment. Thus, an accurate tool to measure the dose and ultimately the potency of AAV preparations, and to monitor gene therapy vector quality and potency is therefore needed.

SUMMARY

[0006] Provided herein are methods of measuring the quantity attributes of gene therapy vector preparations. In certain examples, the gene therapy vector preparations are adeno-associated virus (AAV) preparations. The methods disclosed herein can be used at any point of purification. In certain examples, the methods can be used prior to preparing or packaging

the AAV capsids for use. The methods disclosed herein can be used to improve the determination of the concentration, dose, and/or potency of an AAV preparation. In one aspect, the present disclosure refers to a method of quantifying a preparation of an AAV comprising a gene of interest, comprising quantifying a total number of AAV capsids in the AAV preparation via an AAV-specific ELISA assay and evaluating a percentage or ratio of full versus empty AAV capsids in the AAV preparation or an AAV fraction via cryogenic transmission electron microscopy (CryoTEM). In another aspect, the present disclosure refers to a method of measuring the concentration of full AAV capsids in a preparation of an AAV comprising a gene of interest, comprising quantifying a total number of AAV capsids in the AAV preparation via an AAV-specific ELISA assay and evaluating a percentage of full versus empty AAV capsids in the AAV preparation via cryogenic transmission electron microscopy (CryoTEM). In yet another aspect, the present disclosure refers to a method for preparing a preparation of an AAV comprising a gene of interest at a specific dose, comprising (i) obtaining a purified AAV fraction; (ii) measuring the concentration of AAV capsids in the purified AAV fraction using an AAV-specific ELISA assay; (iii) preparing an AAV preparation with a desired concentration based on the total number of AAV capsids; and (iv) evaluating a percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV faction via cryogenic transmission electron microscopy (CryoTEM). In a further aspect, the present disclosure refers to a method for producing a preparation of an AAV comprising a gene of interest, comprising: (i) transfecting host cells with at least one plasmid comprising the gene of interest; (ii) collecting supernatant or cell suspension of a cell culture comprising AAV capsids to create an AAV fraction; (iii) quantifying a total number of AAV capsids in the AAV fraction using an AAV-specific ELISA assay; (iv) preparing an AAV preparation with a desired concentration based on the total number of AAV capsids; and (v) evaluating a percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV faction via cryogenic transmission electron microscopy (CryoTEM).

[0007] A feature of AAV vector generation in cell culture is the formation of an excess of "empty" capsids, which lack the vector genome. In certain examples, the amount of empty capsids is assessed in order to accurately determine the concentration, dose, and/or potency of an AAV preparation. Empty capsids can also be considered as product related impurities, which should be reduced during purification. In certain examples, it is important to determine the

total amount of empty capsids to reduce an innate and/or adaptive immune response against the capsid antigen.

[0008] Classically, the concentration, dose, and/or potency of an AAV preparation relies on a quantitative determination by quantitative PCR (qPCR). Surprisingly, the inventors found that an AAV-specific enzyme-linked immunosorbent assay (ELISA) alone can be relied upon for the quantitative determination of the AAV preparation and its use can improve the measurement of the concentration and/or dose an AAV preparation and ultimately the potency. The methods of the present disclosure are advantageous over those known in the art because antigen determination with ELISA provides highly reliable results, surprisingly more accurate than that of qPCR. In certain examples, the method does not include a step of measuring concentration, dose, and/or potency via qPCR. In certain examples, the concentration, dose, and/or potency can be determined by the concentration of AAV capsids in an AAV preparation. In certain examples, the methods disclosed herein comprising ELISA result in an AAV preparation with at least about 10% less variability of the concentration, dose, and/or potency as compared to a method using qPCR. In certain examples, the methods disclosed herein comprising ELISA result in an AAV preparation with at least about 20% less variability of the concentration, dose, and/or potency as compared to a method using qPCR. In certain examples, the methods disclosed herein comprising ELISA result in an AAV preparation with about 10% to about 80% less variability of the concentration, dose, and/or potency as compared to a method using qPCR. In certain examples, the methods disclosed herein comprising ELISA result in an AAV preparation with about 15% to about 50% less variability of the concentration, dose, and/or potency as compared to a method using qPCR. In certain examples, the methods disclosed herein comprising ELISA result in an AAV preparation with about 20% to about 33% less variability of the concentration, dose, and/or potency as compared to a method using qPCR.

[0009] In certain examples, methods of the disclosure entail quantifying the dose and/or potency of an AAV preparation. In certain examples, the method comprises testing an AAV preparation via an AAV-specific ELISA assay to quantify the total number of AAV capsids in the AAV preparation. The number of capsids can then be used to determine the dose to administer a subject. The dose can be used to determine the potency (i.e., the dose or concentration required to produce a certain effect (e.g., EC_{50})). In certain examples, the method does not include a step of measuring the dose and/or potency via qPCR.

[0010] In certain examples, methods of the disclosure entail measuring the concentration of AAV capsids in an AAV fraction or preparation. In certain examples, the method comprises testing an AAV fraction or preparation via an AAV-specific ELISA assay to quantify the total number of AAV capsids in the AAV fraction or preparation. In certain examples, the method does not include a step of measuring the concentration via qPCR.

[0011] In certain examples, methods of the disclosure entail measuring the concentration of full AAV capsids in an AAV fraction or preparation. In certain examples, the method comprises testing an AAV fraction or preparation via an AAV-specific ELISA assay to quantify the total number of AAV capsids in the AAV fraction or preparation and evaluating a percentage of full versus empty AAV capsids in the AAV preparation (e.g., using CryoTEM). In certain examples, the method does not include a step of measuring the concentration via qPCR.

[0012] In certain examples, empty capsids are removed from the AAV fraction or preparation resulting in an AAV fraction or preparation with a specific concentration of full capsids and/or a specific ratio of full:empty capsids. In certain examples, the concentration or ratio is constant between batches such that an AAV-specific ELISA assay is sufficient to determine the concentration, dose, and/or potency of the AAV fraction or preparation. In certain examples, the method does not include a step of measuring the concentration, dose, and/or potency via qPCR.

[0013] In certain examples, methods of the disclosure entail quantifying the dose and/or potency of an AAV preparation. In certain examples, the method comprises testing an AAV preparation via an AAV-specific ELISA assay in combination with a method that evaluates or confirms the percentage or ratio of full versus empty (full:empty) AAVcapsids in the AAV

preparation. In certain examples, the method does not include a step of measuring the dose and/or potency via qPCR.

[0014] In certain examples, the method comprises measuring the concentration of AAV capsids in an AAV fraction or preparation via an AAV-specific ELISA assay in combination with a method that evaluates or confirms the percentage or ratio of full:empty AAV capsids in the AAV fraction or preparation. In certain examples, the method does not include a step of measuring the concentration via qPCR.

[0015] In certain examples, the method for quantifying the dose and/or potency of an AAV preparation comprises confirming the biological activity of the AAV preparation. In certain examples, the method comprises confirming the biological activity of the AAV preparation using an *in vitro* method. In certain examples, the method comprises confirming the biological activity of the AAV preparation using an *in vivo* method. In certain examples, the methods of the disclosure do not require confirmation using a biological assay if the AAV-specific ELISA assay is paired with a method to confirm product quality (e.g., measuring the ratio of full:empty). For example, in certain examples, the methods of the invention do not require confirmation using a biological assay if the AAV-specific ELISA assay is combined with CryoTEM. In certain examples, the method does not include a step of measuring dose and/or potency via qPCR.

[0016] In certain examples, methods of the disclosure entail producing an AAV preparation, comprising: (i) transfecting host cells with at least one plasmid comprising the gene of interest; (ii) collecting supernatant or cell suspension of a cell culture comprising AAV capsids to create an AAV fraction; (iii) quantifying the total number of AAV capsids in the AAV fraction using an AAV-specific ELISA assay; and (iv) preparing an AAV preparation with a desired concentration of AAV capsids based on the total number of AAV capsids determined in step (iii). In certain examples, the method comprises concentrating the AAV fraction. In certain examples, the method comprises removing at least a portion of empty capsids from the AAV fraction. In certain examples, at least a portion of the empty capsids are removed to create an AAV fraction or preparation with a specific concentration of full capsids and/or a specific ratio of full:empty capsids. In certain examples, the method comprises evaluating or confirming the percentage or ratio of full versus empty (full:empty) AAV capsids in the AAV fraction or preparation. In certain examples, the method does not include a step of measuring the

concentration, dose, and/or potency via qPCR. In certain examples, the method further comprises lyophilizing the AAV fraction or preparation.

[0017] In certain examples, methods of the disclosure entail administering an AAV preparation to a subject in need thereof, comprising (i) obtaining a purified AAV preparation; (ii) measuring the concentration of the AAV capsids in the purified AAV preparation using an AAV-specific ELISA assay; (iii) administering a therapeutically effective amount of the purified AAV preparation to the subject. In certain examples, the AAV preparation in steps (i) and (ii) is an AAV fraction that can be used to generate a final AAV preparation, in which the AAV fraction is purified or diluted to form the AAV preparation for steps (i), (ii), and/or (iii). In certain examples, the dose and/or potency of the purified AAV fraction or preparation is measured by quantifying the total number of AAV capsids in the AAV fraction or preparation. In certain examples, the method comprises evaluating or confirming the percentage or ratio of full:empty AAV capsids in the AAV fraction or preparation. In certain examples, the method does not include a step of measuring the concentration, dose, and/or potency via qPCR.

[0018] In certain examples, methods of the disclosure entail administering an AAV preparation at a specific dose to a subject in need thereof, comprising (i) obtaining a purified AAV preparation; (ii) measuring the concentration of AAV capsids in the purified AAV preparation using an AAV-specific ELISA assay; (iii) administering a specific dose of the AAV preparation to the subject. In certain examples, the AAV preparation in steps (i) and (ii) is an AAV fraction that can be used to generate a final AAV preparation, in which the AAV fraction is purified or diluted to form the AAV preparation for steps (i), (ii), and/or (iii). In certain examples, the dose of the purified AAV fraction or preparation is measured by quantifying the total number of AAV capsids in the AAV fraction or preparation. In certain examples, the method comprises evaluating or confirming the percentage or ratio of full:empty AAV capsids in the AAV fraction or preparation. In certain examples, the method does not include a step of measuring the concentration, dose, and/or potency via qPCR.

[0019] In certain examples, the ELISA assay can be a sandwich ELISA, direct ELISA, indirect ELISA, or competitive ELISA assay. In certain examples, for the methods disclosed herein, the AAV-specific ELISA assay is a sandwich ELISA assay specific for an AAV antigen. In certain examples, the AAV antigen is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or a chimeric AAV antigen. In certain examples, the AAV

antigen is an AAV8 antigen. In certain examples, the AAV antigen is from a recombinant AAV (rAAV). In certain examples, the AAV antigen is from a genetically engineered AAV, or chemically modified AAV, or both.

[0020] In certain examples, the ELISA comprises an antibody specific for an AAV epitope of the antigen. In certain examples, the AAV epitope is a conformational epitope present on assembled AAV capsids. In certain examples, the AAV epitope is a linear epitope present on assembled AAV capsids. Examples of such epitopes include, but is not limited to, capsid virion proteins VP1, VP2, and/or VP3. In certain examples, the AAVs are genetically engineered to express additional virion proteins on the surface of the capsid, and those engineered proteins can be used/detected in an ELISA assay. In certain examples, the AAV are chemically modified to express variants of virion protein, which can be used/detected in an ELISA assay (e.g., VP1', VP2', VP3', etc…). In certain examples, the antibodies identify serotype specific capsid virion proteins.

[0021] In certain examples, for the methods disclosed herein, the method for evaluating or confirming the percentage or ratio of full:empty AAV capsids in an AAV fraction or preparation can be cryogenic transmission electron microscopy (CryoTEM), negative staining TEM, capillary electrophoresis, analytical ultracentrifugation, or combinations thereof. In certain examples, the method for evaluating or confirming the percentage of full versus empty (full:empty) AAV capsids in an AAV fraction or preparation is CryoTEM. In certain examples, evaluating comprises quantitating the percentage or ratio of full:empty AAV capsids.

[0022] In certain examples, the CryoTEM step comprises: (i) embedding an AAV fraction or preparation in a substrate in an inert support; (ii) flash-freezing the embedded AAV fraction or preparation; (iii) imaging the embedded AAV fraction or preparation using transmission electron microscopy; and (iv) quantitating the percentage of full AAV capsids versus empty AAV capsids in the AAV fraction or preparation. In certain examples, the substrate is amorphous, non-crystalline ice. In certain examples, the AAV fraction or preparation is free from cellular debris. For example, the vector samples do not contain uncertain particles >2%, does not contain broken or oligomerized particles >2%.

[0023] The use of CryoTEM in combination with an AAV-specific ELISA assay is advantageous over those known in the art because CryoTEM provides a direct measurement of

full:empty capsids, unlike that of quantitative PCR (qPCR). In certain examples, the method does not include a step of measuring concentration or dose via qPCR.

[0024] In certain examples, for the methods disclosed herein, the ELISA assay is performed prior to the quantification of full:empty AAV capsids. In certain examples, the ELISA assay is performed prior to CryoTEM. In certain examples, the ELISA assay is performed to dilute the total AAV capsid number to a validated range (e.g., the cp/ml ranges as disclosed herein). In certain examples, the ELISA assay is performed after the quantification of full:empty AAV capsids. In certain examples, the ELISA assay is performed after CryoTEM. In certain examples, the ELISA assay is performed to dilute the total of full AAV capsid number to a validated range. In certain examples, the quantification of full:empty is performed only once. For example, the CryoTEM step can be used once to test that the batch has a consistent empty:full ratio as that of the previous batches.

[0025] In certain examples, for the methods disclosed herein, the use of both ELISA and CryoTEM results in a method that can be applied for all AAV serotypes independent from vector DNA. This is advantageous as it allows the assay to be performed across all platforms.

[0026] In certain examples, for the methods, fractions or preparations disclosed herein, the percentage of full AAV capsids in the AAV fraction or preparation as compared to empty capsids is from about 40% to about 100%. In certain examples, the percentage of full AAV capsids is from about 40% to about 95%, about 40% to about 90%, about 40% to about 85%, about 40% to about 80%, about 45% to about 75%, about 50% to about 70%, or about 55% to about 65%. In certain examples, at least about 60% of the AAV capsids are full AAV capsids. In certain examples, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% of the AAV capsids are full AAV capsids. In certain examples, the methods as disclosed herein are used to generate AAV fractions or preparations with consistent amounts of full capsids between batches of AAV fractions or preparations.

[0027] In certain examples, for the methods and preparations disclosed herein, the concentration of the AAV preparations is between about $1x10^{10}$ cp/ml to about $1x10^{20}$ cp/ml. In certain examples, for the methods and preparations disclosed herein, the concentration of the

AAV preparations is between about $1x10^{11}$ cp/ml to about $1x10^{19}$ cp/ml, about $1x10^{12}$ cp/ml to about $1x10^{18}$ cp/ml, about $1x10^{13}$ cp/ml to about $1x10^{17}$ cp/ml, or about $1x10^{14}$ cp/ml to about $1x10^{16}$ cp/ml. In certain examples, for the methods and preparations disclosed herein, the concentration of the AAV preparations is between about $1x10^{12}$ cp/ml to about $1x10^{15}$ cp/ml, about $1x10^{13}$ cp/ml to about $1x10^{15}$ cp/ml, or about $1x10^{12}$ cp/ml to about $1x10^{13}$ cp/ml. In certain examples, for the methods and preparations disclosed herein, the concentration of the AAV preparations is between about $1x10^{14}$ cp/ml to about $5x10^{14}$ cp/ml, about $2x10^{14}$ cp/ml to about $3x10^{14}$ cp/ml, or about $3.5x10^{14}$ cp/ml to about $5x10^{15}$ cp/ml. In certain examples, cp/ml can be total capsids per ml or full capsids per ml. In certain examples, the cp/ml is total capsids per ml.

[0028] In certain examples, for the methods or preparations disclosed herein, the dose of the AAV preparation is between about 1×10^5 cp/kg to about 1×10^{25} cp/kg. In certain examples, for the methods, fractions, or preparations disclosed herein, the dose of the AAV fraction or preparation is between about 1×10^{10} cp/kg to about 1×10^{20} cp/kg. In certain examples, for the methods, fractions, or preparations disclosed herein, the dose of the AAV fraction or preparation is between about 1×10^{10} cp/kg to about 1×10^{16} cp/kg. In certain examples, cp/kg can be total capsids per kg of the subject or full capsids per kg of the subject. In certain examples, the cp/kg is total capsids per kg of the subject.

[0029] In certain examples, for all methods disclosed herein, the subject is a human or non-human animal (e.g., chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, or geese). In certain examples, the subject is a human.

[0030] In certain examples, for all methods disclosed herein, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or a chimeric AAV vector. In certain examples, the AAV is AAV8. In certain examples, the AAV is AAV9. In certain examples, the AAV is a recombinant AAV (rAAV). In certain examples, the AAV is genetic engineered, chemically modified, or both.

[0031] In certain examples, the disclosure includes an AAV fraction or preparation, wherein the concentration, dose, and/or potency of the fraction or preparation is measured by an AAV-

specific ELISA assay. In certain examples, the disclosure includes an AAV fraction or preparation, wherein the concentration, dose, and/or potency of the fraction or preparation is measured by an AAV-specific ELISA assay in combination with an assay that measures the percentage or ratio of full versus empty AAV capsids in the AAV fraction or preparation. In certain examples, the disclosure includes an AAV fraction or preparation, wherein the concentration, dose, and/or potency of the fraction or preparation is measured by an AAV-specific ELISA assay in combination with CryoTEM. In certain examples, the concentration, dose, and/or potency of the AAV preparation is not measured via qPCR.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] Figure 1 is a graph comparing the percentage of errors for the measurement of AAV vector number and vector genome content using antigen ELISA or qPCR, respectively. AAV ELISA (QC ("Quality Control")): n=60; AAV ELISA (R&D ("Research and Development")): n=83; ITR-qPCR (R&D): n=64; FIX-qPCR (QC): n=40; FIX-qPCR (R&D): n=135.

[0033] Figure 2 is the result from an agarose gel electrophoresis study examining the ability of antigen ELISA and qPCR to estimate the dose of AAV capsids loaded onto the agarose gel ($2.0x10^{10}$ capsids/lane).

[0034] Figure 3 is a graph verifying use of the antigen ELISA method to determine the dose of the AAV preparation administered in an *in vivo* biopotency assay using a mouse model (n=7).

[0035] Figure 4 is a graph comparing batch to batch variation of AAV8 FVIII single stranded DNA using AAV-specific ELISA versus ITR-qPCR. AAV8 : Antigen – Median : 3.03cp/ml ± 0.66cp/ml. ITR-qPCR – Median : 9.11vg/ml ± 3.01vg/ml.

[0036] Figure 5A-5B: Figure 5A is a graph demonstrating the ratio of AAV vector genomes (vg) per AAV8 antigen ELISA capsid particles (cp). Figure 5B is a graph demonstrating the high consistency of "full" capsids in the AAV preparation (e.g., see the process as discussed in the publication of International Application No. PCT/US2017/059967, incorporated herein in its entirety for all intended purposes).

[0037] Figure 6A-6C: Figure 6A is a CryoTEM image of one full AAV8 capsid and one empty AAV8 capsid. Figure 6B is a CryoTEM image of one oligomerized AAV8 capsid (i.e., the AAV capsid is forms a trimeric structure, which could be empty or full). Figure 6C is a CryoTEM image of one broken AAV8 capsid.

WHAT IS CLAIMED IS:

1. A method of quantifying a preparation of an AAV comprising a gene of interest, comprising quantifying a total number of AAV capsids in the AAV preparation via an AAV-specific ELISA assay and evaluating a percentage or ratio of full versus empty AAV capsids in the AAV preparation or an AAV fraction via cryogenic transmission electron microscopy (CryoTEM).

2. A method of measuring the concentration of full AAV capsids in a preparation of an AAV comprising a gene of interest, comprising quantifying a total number of AAV capsids in the AAV preparation via an AAV-specific ELISA assay and evaluating a percentage of full versus empty AAV capsids in the AAV preparation via cryogenic transmission electron microscopy (CryoTEM).

3. A method for preparing a preparation of an AAV comprising a gene of interest at a specific dose, comprising
   (i) obtaining a purified AAV fraction;

   (ii) measuring the concentration of AAV capsids in the purified AAV fraction using an AAV-specific ELISA assay;

   (iii) preparing an AAV preparation with a desired concentration based on the total number of AAV capsids; and

   (iv) evaluating a percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV faction via cryogenic transmission electron microscopy (CryoTEM).

4. A method for producing a preparation of an AAV comprising a gene of interest, comprising:
   (i) transfecting host cells with at least one plasmid comprising the gene of interest;

   (ii) collecting supernatant or cell suspension of a cell culture comprising AAV capsids to create an AAV fraction;

(iii) quantifying a total number of AAV capsids in the AAV fraction using an AAV-specific ELISA assay;

(iv) preparing an AAV preparation with a desired concentration based on the total number of AAV capsids; and

(v) evaluating a percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV faction via cryogenic transmission electron microscopy (CryoTEM).

5. The method of claim 3 or 4, further comprising concentrating the AAV fraction and/or further comprising removing at least a portion of empty capsids from the AAV fraction.

6. The method of any one of claims 3-5, wherein the method further comprises lyophilizing the AAV preparation.

7. The method of claim 1 or 2 wherein evaluating the percentage or ratio of full versus empty AAV capsids in the AAV preparation or in the AAV fraction via CryoTEM is conducted before the ELISA assay.

8. The method of claim 1 or 2, wherein evaluating the percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV fraction via CryoTEM is conducted after the ELISA assay.

9. The method of any one of claims 3-6 wherein evaluating the percentage or ratio of full versus empty AAV capsids in the AAV fraction via CryoTEM is conducted before the ELISA assay.

10. The method of any one of claims 3-6, wherein evaluating the percentage or ratio of full versus empty AAV capsids in the AAV fraction via CryoTEM is conducted after the ELISA assay.

11. The method of any one of claims 1-10, wherein evaluating the percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV fraction via CryoTEM is performed once to test that the AAV preparation or the AAV fraction has a consistent

percentage or ratio of full versus empty AAV capsids as that of previous preparations or fractions of AAV.

12. The method of any one of claims 1-11, further comprising determining the percentage or ratio of full versus empty AAV capsids in the AAV preparation or an AAV fraction by negative staining TEM, capillary electrophoresis, analytical ultracentrifugation, native agarose gel, alkaline agarose gel, southern blot, dot-blot hybridization, UV spectrophotometry, weak anion exchange chromatography, or mass spectrometry.

13. The method of any one of claims 1-12, wherein evaluating the percentage or ratio of full versus empty AAV capsids in the AAV preparation or the AAV fraction via CryoTEM comprises:

(i) embedding the AAV preparation or AAV fraction in a substrate on an inert support;

(ii) flash-freezing the embedded AAV preparation or AAV fraction;

(iii) imaging the embedded AAV preparation or AAV fraction using cryogenic transmission electron microscopy; and

(iv) quantitating the percentage of full AAV capsids versus empty AAV capsids in the AAV preparation or AAV fraction.

14. The method of claim 13, wherein the substrate is amorphous, non-crystalline ice.

15. The method of claim 13 or claim 14, wherein the AAV preparation or AAV fraction is free from cellular debris.

16. The method of any one of claims 1-15, wherein the ELISA assay is a sandwich ELISA, direct ELISA, indirect ELISA, or competitive ELISA assay specific for an AAV antigen.

17. The method of claim 16, wherein the AAV antigen is from an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, chimeric AAV, genetically engineered AAV, or chemically modified AAV.

18. The method of any one of claims 1-17, wherein the method does not include quantitative PCR.

19. The method of any one of claims 1-18, wherein the percentage of full AAV capsids is from 40% to 100%.

20. The method of any one of claims 1-19, wherein at least 60% of the AAV capsids are full AAV capsids.

21. The method of any one of claims 2-20, where in the concentration is between $1x10^{10}$ cp/ml to $1x10^{20}$ cp/ml.

22. The method of any one of claims 1-21, wherein the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or a chimeric AAV vector.

23. The method of claim 22, wherein the AAV is AAV6, AAV8, or AAV9.

24. The method of any one of claims 1-23, wherein the AAV is a genetically engineered AAV, a chemically modified AAV, or both.