greater than or equal to 45°C, a sample containing helper virus particles, AAV particles, and a buffer. The buffer includes a concentration of 10 mM or greater kosmotropic salts and/or a concentration of 10 mM or greater of divalent or trivalent cations.

[0003a] In one aspect of the present disclosure, there is provided a method of inactivating helper virus in a sample containing helper virus particles, adeno-associated virus particles, and a buffer, the method comprising:

heating the sample to a temperature greater than or equal to 45°C for between 1 minute and 6 hours,

wherein the buffer comprises a concentration of between 25 mM and 500 mM of divalent or trivalent cations, or a concentration of between 0.5 M and 0.6 M of kosmotropic salt.

[0004] The methods include heating the sample to a temperature of greater than or equal to 45°C, greater than or equal to 46°C, greater than or equal to 47°C, greater than or equal to 48°C, greater than or equal to 49°C, greater than or equal to 50°C, or greater than or equal to 51°C. In certain examples the sample is heated to a temperature between 45°C and 65°C, between 45°C and 60°C, between 45°C and 55°C, between 47°C and 53°C, between 48°C and 51°C, or between 48°C and 50°C. For example, in certain examples the sample is heated to between 48°C and 50°C, or to 48°C or 49°C.

[0005] The period of time during which the sample is heated can vary. For example, in certain examples the sample is heated to the target temperature for a time period of between 1 minute and 6 hours, such as between 10 and 180 minutes, between 20 and 180 minutes, between 20 and 60 minutes, or between 20 and 40 minutes. Generally, higher temperatures or other conditions accelerating the inactivation of helper virus particles are used in conjunction with comparatively brief heating times, and vice versa.

[0006] In some methods a buffer containing a concentration of 10 mM or greater of divalent or trivalent metal cations is used. Exemplary cations include those of the following metals: Mg, Ca, Mn, Ni, Zn, Co, Sr, Cu, Cr, Fe, and Sc, which form cations such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Sr^{2+}, Cu^{2+}, Cr^{2+}, and Sc^{3+}. In certain examples the buffer includes $Mg^{2+}$and/or Ca^{2+} at a concentration of 10 mM or greater.

[0007] In a subset of those buffers containing a concentration of 10 mM or greater of divalent or trivalent metal cations, the concentration is greater than 15 mM. For example, the concentration of cations may be greater than 20 mM, greater than 50 mM, greater than 100 mM, or greater than 200 mM. In another subset of buffers containing a concentration of 10 mM or greater of divalent or trivalent metal cations, the concentration is from 10 mM and 500 mM. For example, the concentration of cations may be 20 mM to 400 mM, 30 mM to 300 mM, 50 mM to 250 mM, 70 mM to 200 mM, or at a concentration of 50 mM, 100 mM, 150 mM, or 200 mM.

[0008] In some methods a buffer with a concentration of 10 mM or greater of kosmotropic salts is used. Exemplary kosmotropic salts include ammonium sulfate, ammonium acetate, sodium citrate, sodium acetate, sodium sulfate, potassium phosphate, and cesium chloride, either separately or in any combination. If present, the kosmotropic salts are typically used at a concentration exceeding 10 mM, such as between 0.1 M and 1 M; between 0.2 M and 0.8 M; between 0.3 M and 0.7 M; between 0.4 M and 0.6 M; or 0.5 M.

[0009] In some methods, the buffer used contains a chaotropic salt. In a subset of buffers containing a chaotropic salt, the chaotropic salt is a salt of urea, or a salt of guanidine. In some methods, the buffer used contains a polyol. In a subset of buffers containing a polyol, the polyol is glycerol, propylene glycol, or 1,6-hexanediol.

[0010] In some methods, the buffer maintains pH over a range of temperatures between 4°C and 70°C. A subset of buffers maintain pH between 3.0 and 10.0 at temperatures between 4°C and 70°C. A further subset of these buffers maintains pH between 7.0 and 9.0 at temperatures between 4°C and 70°C.

[0011] In some methods, the buffer is a tris buffer, a phosphate buffer, a triazolamine buffer, or a bis-tris propane buffer. In a subset of the tris and bis-tris propane buffers, the buffer comprises additional ingredients, including HEPES, citrate, NaCl, and Pluronic F68. In a subset of bis-tris propane buffers, the buffer comprises 40 mM bis-tris propane, 20 mM HEPES, 20 mM citrate, 200 mM NaCl, and 0.001% (w/v) Pluronic F68.

[0012] The methods disclosed herein are useful to preserve the genomic integrity of any AAV particle. In some methods AAV particle will comprise a genome of approximately 4.7 kb of DNA, of greater than 4.7 kb of DNA, of greater than 5.0 kb of DNA, or of approximately 5.1 kb of DNA. Alternatively, the AAV particle may comprise a genome of less than 4.0 kb of DNA, or of approximately 3.0 kb of DNA. The methods disclosed herein may be used with the AAV particles with genomes that are single-stranded or are substantially self-complementary.

[0013] Some methods of inactivation of the instant disclosure achieve a log reduction of active helper virus of greater than 5.0. A subset of these methods achieve a log reduction of helper virus of greater than 6.0, or of greater than 6.3. In some methods, the helper virus will

What is claimed is:

1. A method of inactivating helper virus in a sample containing helper virus particles, adeno-associated virus particles, and a buffer, the method comprising:
   heating the sample to a temperature greater than or equal to 45°C for between 1 minute and 6 hours,
   wherein the buffer comprises a concentration of between 25 mM and 500 mM of divalent or trivalent cations, or a concentration of between 0.5 M and 0.6 M of kosmotropic salt.

2. The method of claim 1, wherein the sample is heated to a temperature between 45°C and 65°C; between 45°C and 60°C; between 45°C and 55°C; between 47°C and 53°C; or a temperature of 48°C or 49°C.

3. The method of claim 1, wherein the sample is maintained at the temperature for a time period of between 10 and 180 minutes; between 20 and 180 minutes;between 20 and 60 minutes; orbetween 20 and 40 minutes.

4. The method of claim 1, wherein the method results in a log reduction of helper virus of 6.0 or greater.

5. The method of claim 1, wherein the adeno-associated virus particle comprises a genome

   a) of more than 4.7 kb of DNA;

   b) of approximately 5.1 kb of DNA;

   c) of approximately 4.7 kb of DNA;

   d) of less than 4.0 kb of DNA;

   e) approximately 3.0 kb of DNA; or

   f) that is substantially self-complementary.

6. The method of claim 1, wherein the buffer further comprises

   a) a chaotropic salt; optionally wherein the chaotropic salt is a salt of urea or a salt of guanidine;or

   b) a polyol selected from the group comprising: glycerol, propylene glycol, and 1,6-Hexanediol.

7. The method of claim 1, wherein the buffer maintains pH between 3.0 and 10.0, or between 7.0 and 9.0, at temperatures between 4°C and 70°C.

8. The method of claim 1, wherein the buffer further comprises: 40 mM bis-tris propane, 20 mM HEPES, 20 mM citrate, 200 mM NaCl, and 0.001% (w/v) Pluronic F68.

9. The method of claim 7, wherein the buffer is a Tris buffer; a phosphate buffer; or atriazolapmine buffer.

10. The method of claim 1, wherein the helper virus is an adenovirus; optionally wherein the adenovirus is Ad5.

11. The method of any one of claims 1 to 10, wherein the concentration of divalent or trivalent cations is:

    a) 25 mM to 400 mM

    b) 30 mM to 300 mM;

    c) 50 mM to 250 mM;

    d) 70 mM to 200 mM.

12. The method of any one of claims 1–10, wherein the divalent or trivalent cations are selected from the group consisting of: Mg, Ca, Mn, Ni, Zn, Co, Sr, Cu, Cr, Fe, and Sc;

    optionally wherein:

    a) the divalent cations are selected from the group consisting of: Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Sr^{2+}, $Cu^{2+}$and Cr^{2+}; or

    b) the trivalent cations are Sc_3^{+}.

13. The method of claim 12, wherein the cations are Mg^{2+}.

14. The method of any one of claims 1–10, wherein the kosmotropic salt is selected from the group consisting of ammonium sulfate, ammonium acetate, sodium citrate, sodium acetate, sodium sulfate, potassium phosphate, and cesium chloride.

15. The method of claim 14, wherein the concentration of the kosmotropic salt is 0.5 M