Claims

1. A method for the production of Adeno-associated virus (AAV), comprising the steps of:
   (a) providing a stable AAV producer host cell line in which at least some or all genes encoding the components necessary for the production of AAV are stably integrated into the host cell genome;
   (b) culturing said cells in perfusion culture during the AAV production step (*i.e.*, during the N step), wherein said perfusion culture encompasses continuous replacement of spent media with fresh media, and wherein said continuous replacement of spent media with fresh media continues after the induction of AAV production,

   wherein at least the following genes are stably integrated into the host cell genome:
   a gene encoding the AAV Rep protein Rep78 or Rep68,
   a gene encoding the AAV Rep protein Rep52 or Rep40; and
   the genes encoding the adenoviral helper functions E4orf6 and E2A.

2. The method according to claim 1, wherein at least the following genes are stably integrated into the host cell genome:
   the genes encoding the AAV Cap proteins VP1, VP2, and VP3;
   a gene encoding the AAV Rep protein Rep78 or Rep68,
   a gene encoding the AAV Rep protein Rep52 or Rep40;
   the genes encoding the adenoviral helper functions E4orf6 and E2A, and
   the gene of interest (GOI) flanked by AAV ITRs.

3. The method according to claim 1 or 2, wherein the AAV is selected from the group consisting of AAV serotype 1 (AAV1), AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAVDJ, AAVDJ8, AAVrh10, hybrids of two or more different of said serotypes, and said serotypes having mutations that alter the tropism of the AAV serotype.

4. The method according to any one of claims 1 to 3, wherein the genes encoding the components necessary for the production of AAV are selected from the group consisting of genes encoding the AAV Cap proteins VP1, VP2, and VP3; genes encoding the AAV Rep proteins Rep78, Rep68, Rep52, and Rep40; genes encoding the adenoviral helper functions E4orf6, E2A and VA-RNA; genes encoding the Ad5 helper genes E1A and E1B; and the gene of interest (GOI) flanked by AAV inverted terminal repeat sequences (ITRs).

5. The method according to any one of claims 1 to 4, wherein the following additional genes are stably integrated into the host cell genome:
the genes encoding both of the AAV Rep proteins Rep78 and Rep68,
the genes encoding both of the AAV Rep proteins Rep52 and Rep40; and
a gene or genes encoding either one or both of the adenoviral helper functions E1A, E1B.

6. The method according to any one of claims 1 to 5, wherein the stable AAV producer host cell line is a cell line, selected from the group consisting of CAP cells, AGE1.hn, HEK293, PER.C6, NSO1, COS-7, BHK, CHO, CV1, VERO, HeLa, MDCK, BRL3A, W138, and HepG2 cells.

7. The method according to any one of claims 1 to 6, wherein perfusion culture is performed in a stirred-tank bioreactor (STR), orbitally shaken bioreactor (OSB), rocking bed bioreactor, air-lift bioreactor, tubular bioreactor, hollow-fiber bioreactor (HFBR), fixed-bed bioreactor, or fluidized bed bioreactor.

8. The methods according to any one of claims 1 to 7, wherein the perfusion device used for perfusion culture is a hollow-fiber filter used in TFF (tangential flow filtration) mode or ATF (alternating tangential flow) mode; a floating membrane, a spin-filter, a rotating cylindrical filter, a rotating disc filter, a centrifuge, a gravitational settler, a lamella settler, a compact cell settler, an acoustic settler, or a hydrocyclone.

9. The method according to claim 8, wherein the perfusion device used for perfusion culture is a hollow-fiber filter used in ATF mode.

10. The method according to any one of claims 1 to 9, wherein said continuous replacement of spent media with fresh media continues for at least 24 hours, at least 48 hours, or at least 72 hours after the induction of AAV production, and/or until the harvest of AAV.

11. The method according to any one of claims 1 to 10, wherein the cell specific perfusion rate (CSPR) is about 0.01 to about 0.20 nL/cell/day.

12. The method according to any one of claims 1 to 11, wherein the perfusion rate is about 1 to about 20 vvd (volume of fresh medium/working volume of reactor/day).

13. The method according to any one of claims 1 to 12, wherein the cell density at the seeding of the perfusion culture is about 0.5×10^6 to about 5×10^6 cells/mL.

14. The method according to any one of claims 1 to 13, wherein the cell density at the timepoint of the induction of AAV production is about 20×10^6 cells/mL or higher.

15. The method according to any one of claims 1 to 14, wherein said method further comprises the step of harvesting AAV from the perfusion culture retentate.