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 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 the following genes are stably integrated into the host cell genome:
the genes encoding the AAV Cap proteins VP1, VP2, and VP3;
the genes encoding the AAV Rep proteins Rep78 and Rep68,
the genes encoding the AAV Rep proteins Rep52 and Rep40;
the genes encoding the adenoviral helper functions E4orf6, E2A, and VA-RNA,
the genes encoding at the Ad5 helper genes E1A and E1B, and
the gene of interest (GOI) flanked by AAV ITRs,
and wherein the expression of the four Rep proteins and the expression of the adenoviral helper functions E2A and E4orf6 are under the control of an inducible system.

2. The method according to claim 1, 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.

3. The method according to claim 1 or claims 2, 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.

4. The method according to any one of claims 1 to 3, wherein the 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.

5. The method according to any one of claims 1 to 4, wherein a 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.

6. The method according to claim 5, wherein the perfusion device used for perfusion culture is a hollow-fiber filter used in ATF mode.

7. The method according to any one of claims 1 to 6, 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.

8. The method according to any one of claims 1 to 7, wherein a cell specific perfusion rate (CSPR) is 0.01 to 0.20 nL/cell/day.

9. The method according to any one of claims 1 to 8, wherein a perfusion rate is 1 to 20 vvd (volume of fresh medium/working volume of reactor/day).

10. The method according to any one of claims 1 to 9, wherein a cell density at the seeding of the perfusion culture is 0.5×10^6 to 5×10^6 cells/mL.

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

12. The method according to any one of claims 1 to 11, wherein said method further comprises the step of harvesting AAV from a perfusion culture retentate.