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Speed Up Your AAV Vector Development and Manufacturing With a HEK293 Suspension-Based Platform

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Introduction

Adeno-associated viral vectors (AAVs) are among the most widely used vectors for gene therapy applications. While cultivation systems such as roller bottles, CellSTACKs (Corning), CellFactories (Nunc, ThermoFisher Scientific), fixed bed reactors, or microcarrier-based cell culture are extensively used in large-scale adherent production of viral vectors such as AAV, there are many reasons to adapt your process to suspension:

- Eliminates the need for serum and its associated negative aspects (animal-origin, transmissible spongiform encephalopathy/bovine spongiform encephalopathy risk, costs, ethics, lot-to-lot variation, sourcing, etc.)
- Allows flexibility regarding scale and volumes per process
- Improved scalability offers lower operation costs compared to multiple parallel processes
- Using controlled cultivation systems improves optimization potential
- Enhanced process control enables easy documentation of parameters for regulatory purposes

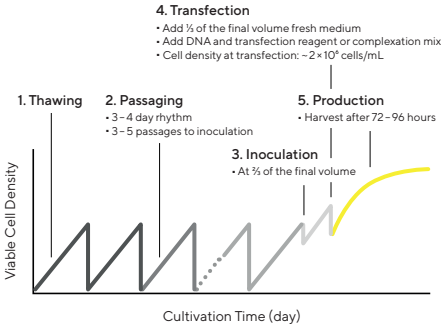
Experimental Approach

Transferring your cells from adherent to suspension culture is the first step in your journey towards a scalable production process for AAV. You should use a culture medium during adaptation that supports transient transfection to eliminate the need for medium exchange later in the process. While you will likely start optimization of transfection and production in suspension in small scale cultivations, e. g., Erlenmeyer shake flasks, your final process will be in a large-scale controlled cultivation system. The move from shake flask to controlled bioreactor is not as challenging as you may think. For our case studies, we show data on production of AAV-2 and AAV-8 with our easy protocol, which was applied in an initial scale-up step from shake flasks to 2 L benchtop bioreactors.

Our Easy Protocol for Seed Train and Production

In this approach (Figure 1), we thawed HEK 293 cells before culturing and expanding them for at least three passages. Small-scale processes are performed in 30 mL volume in 125 mL plain Erlenmeyer shake flasks. The initial scale-up step was performed in 2 L stirred tank benchtop bioreactors as a good scale-down model for larger controlled systems.

Figure 1 *Exemplary Procedure for Seed Train and Production Process.*

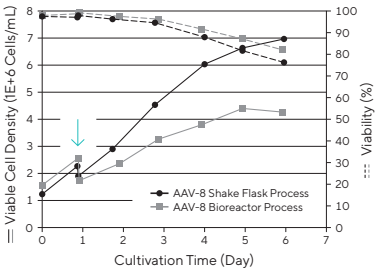


We used HEK ViP NX Medium, which is serum-free, chemically defined and optimized for transfection and virus production, in all steps. We did not add any growth factors, but note that some HEK cell lines require supplementation by – for example, insulin or IGF – to increase titers. We inoculated the production process at a cell density between 1.5 – 2 million cells/mL at half (or more) of the final cultivation volume. After one day (to let the cells equilibrate), we added fresh medium at half (or less) of the final volume, and the pre-complexed transfection mix. Our transfection mix was PEI-MAX (Polysciences) with a 2-plasmid system for AAV-2 and AAV-8 (PlasmidFactory) using 1 µg/mL of DNA at a 4:1 ratio in fresh medium (5 – 10% of total volume or mixed directly in the complete volume of fresh medium added). Addition of medium and transfection mix should set viable cell density to 1.5 – 2.5 million cells/mL. Samples were taken daily from the production processes to analyze viable cell density, viability, transfection efficiency, and AAV titers. Final harvest was done at 96 h (AAV-2) and 120 h (AAV-8) post transfection. See Box 1 for factors to consider and optimize during the procedure.

Case Study 1: Scale-Up Step for AAV-8

Processes for AAV-8 production were inoculated from a common pre-culture at around 1.5 million cells/mL and after one day diluted via fresh medium addition to around 2 million cells/mL for transfection. While the maximum cell density (Figure 2) in the shake flask reached 7 million cells/mL on day 5 (120 h), the bioreactor culture showed a lower maximum cell density of 4.4 million cells/mL.

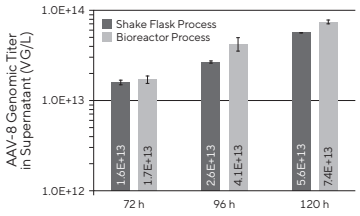
Figure 2: *Cell Density and Viability in AAV-8 Production*



Note. Cell density and viability in shake flask and bioreactor processes from inoculation (day 0) to transfection (day 1) and harvest (day 4). The green arrow indicates the time of transfection.

Genomic AAV-8 titers (Figure 3) were determined via qPCR from culture supernatants (no cell lysis) after DNAse I and Proteinase K digest. Interestingly, though cell density was lower in the bioreactor, genomic titers proved to be higher compared to the shake flask process.

Figure 3: *Genomic Titers in AAV-8 production*

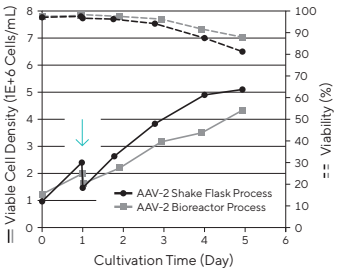


Note. AAV-8 genomic titers in culture supernatants.

Case Study 2: Scale-Up Step for AAV-2

Processes for AAV-2 production were inoculated from a common pre-culture at around 1- 1.5 million cells/mL and after one day diluted via fresh medium addition to around 1.5 million cells/mL for transfection. Similar to AAV-8 processes, the bioreactor process also showed lower cell growth during AAV production, with higher viability at harvest (Figure 4).

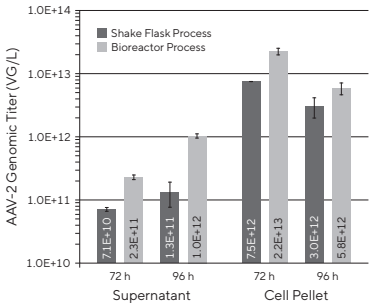
Figure 4: *Cell Density and Viability in AAV-2 Production*



Note. Cell density and viability in shake flask and bioreactor processes from inoculation (day 0) to transfection (day 1) and harvest (day 5). The green arrow indicates the time of transfection.

Genomic AAV-2 titers (Figure 5) were determined via qPCR after DNAse I and Proteinase K digest. In contrast to other serotypes such as AAV-8, AAV-2 is highly retained within the cells[1]. Therefore, titers were measured in supernatants and also in lysed cells from cell pellets. Genomic titer in cell pellets decreased with prolonged cultivation while titer in supernatant increased. Similar to our results with AAV-8, titers for AAV-2 were also higher in the bioreactor process compared to the shake flask process for all samples.

Figure 5: *Genomic Titers in AAV-2 Production*



Note. AAV-2 genomic titers in culture supernatants and cell pellets (lysed cells).

Conclusion

A straight-forward approach allows easy scale-up. For both case studies, processes were successfully transferred in an initial step from uncontrolled shake flask process to a bioreactor process with even higher titers in the controlled system. Future optimization will now focus on pH profiles and inoculation cell density to further increase AAV titers.

Box 1: Factors to Consider and Optimize

- Seed train and optimal passage number at transfection to fit the final process (keep passage number >3 after thawing but below 10 unless tested otherwise)
- Inoculation cell density of final process to allow high cell density throughout the process without running into limitations
- Time of transfection to allow equilibration of culture in controlled environment and exponential growth at transfection
- Ratio of fresh medium introduced before transfection can increase transfection efficiency and titers; feeding lines can be washed after adding transfection mix
- Optimization of pH profiles, temperature shifts, etc. when switching from uncontrolled to controlled systems has a potential to further increase titers

References

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