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, CellSTAXs (Corning), CellFactors (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: 

- Enhanced process control enables easy documentation of parameters for regulatory purposes.
- Improved scalability offers lower operation costs compared to multiple parallel processes.
- Allows flexibility regarding scale and volumes per process.
- 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 optimizing 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 100 mL volume in 20 mL Erlenmeyer shake flasks. The initial scale-up step was performed in 2 L stirred tank bioreactor systems as a good scale-down model for larger controlled systems.

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

Case Study 1: Scale-Up Step for AAV-8
Processes for AAV-8 production were inoculated from a common pre-culture at around 15 million cells/mL, and after one day diluted via fresh medium to 20 million cells/mL for transfection. While the maximal cell density (Figure 2) in the shake flask reached 7 million cells/mL on day 5 (0:20 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

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

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.

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.

References

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