Optimizing Scale-up of AAV Gene Therapy in Upstream Processing

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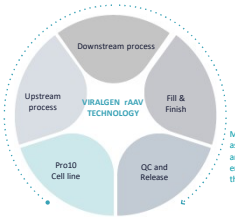
Abstract

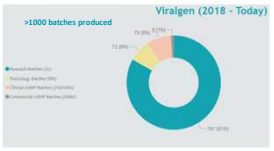
Viralgen specializes in the **production of Adeno-Associate Virus (AAV) gene therapy vectors using our proprietary suspension, triple transfection platform**. This includes a Human Embryonic Kidney (HEK)293-derived suspension cell line, a scalable upstream and robust purification process, coupled with full support for Drug Product Fill and Finish, Quality Control testing, and regulatory support from preclinical to commercial requirements. We have recently completed studies to achieve the **2000L scale-up which have been continuously optimized through both process characterization and experimental approaches to better understand key process steps** such as mixing dynamics and transfection cocktail maturation kinetics. All **knowledge is reintroduced into the process to improve yield and recovery while also maintaining product quality**.

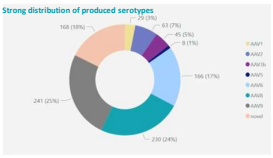
Capability of continuously processing 50L to 2000L batches with depth filtration, affinity and Ion exchange chromatography & tangential flow filtration

Plasmid based triple transfection by using 50 to 2000L single-use bioreactors (SUB) for clinical and commercial production



Manufacturing includes inhouse aseptic DP fill & finish and analytical QC testing services to ensure high quality and efficacy of the product





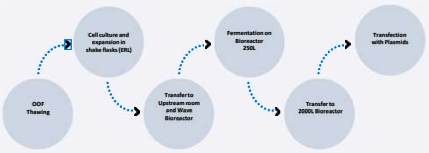
Methods

AAV Platform Production Process

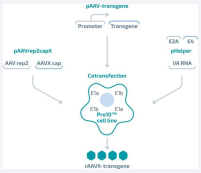
The AAV production process consists three main stages:

- Upstream process where the cells are growing in multi stages seed train, from cell expansion in the flask up to at scale bioreactor
- Triple-plasmid transfection for AAV production containing Gene of Interest (GOI)
- Multistage DSP process for material purification

Upstream Process (USP)



Triple-plasmid transfection



Downstream Process (DSP)

Scaling up of the Production Process

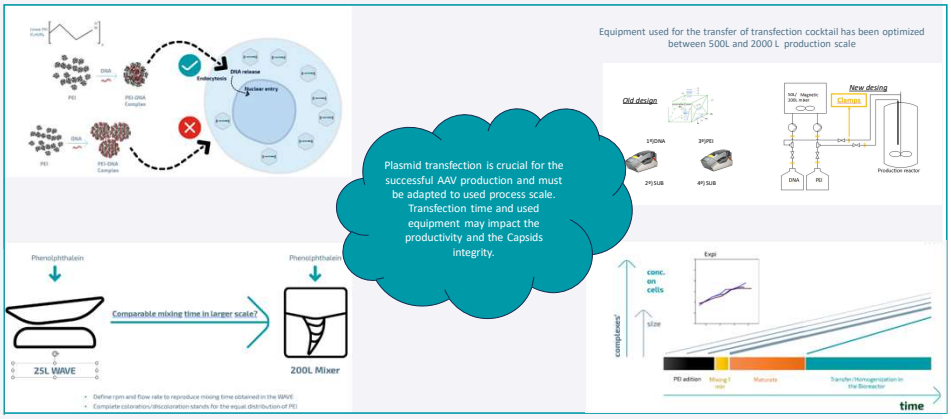
Scaling of the production process from 50L Batches starts collecting information to define larger scale set up. Going from 50L up to 2000L requires usage of additional 250L bioreactor as part of the seed train and adapting necessary process parameters.

Key Engineering Process Characteristics

- Oxygen input; kLa
- Carbon dioxide stripping and pH
- Power Input: P/V, Tip Speed
- Shear stress
- Bubble rise and break up
- Gas entrance velocity
- Homogeneity; Mixing studies

Challenges

- Not all parameters can be kept constant during scaling or vessel change
- Characterization and modelling delivers knowledge how much the conditions deviate



Discussion

How to Find the Best Scale up Conditions?

Small Scale Models (SSM)

- Small scale models (250mL and 2L) implemented in MSAT department are used to characterize the production process, define critical process parameters and to set up optimal scaling up conditions.
- Additionally, the models are used to process improvement deviation handling clarification activities

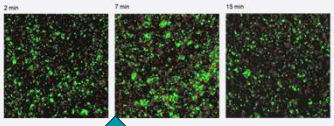
Scale up Conditions

- Cell expansions parameters (cell concentration, gassing) are kept equally between various scales
- The equipment used during the seed train (like Erlenmeyer, 10L waves, 50L waves, 250L SUB) depends on the production scale
- Bioreactor settings (mixing Power to volume or volume of gas per volume of liquid per minute (VVM)) have to be adapted to the scale to assure consistent process performance, The parameters are calculated using various mathematic models

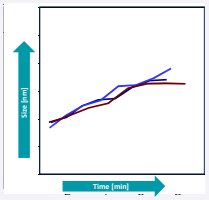
Does Time of Transfection Matter?

Objectives

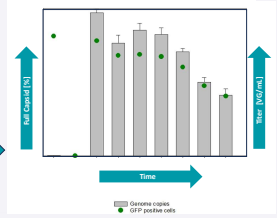
- Increase process knowledge for the transfection cocktail molecular mechanism and their relationship with process parameters to increase productivity and full/empty ratio.
- Small-scale model system with triple transfection



Impact of the time on the PEI-DNA complex building showed using Transmission electron microscopy



Correlation between transfection time and size of the PEI-DNA complexes using DLS



Correlation between transfection time-VG Titer and Full Capsid%

Conclusion

Conclusions

- The scale up study was presented using late-stage development project.
- To successfully perform the scale up several small-scale studies were performed in MSAT lab in order to characterize the process and to find critical process parameters
- Critical process parameters were divided into two groups
  - Parameters which have to stay equal between all scales (like VCD (Viable Cell Density))
  - Parameters which need to be adapted to the used scale (P/V, VVM)
- It has been shown that the transfection time correlates with the size of PEI-DNA complexes having an impact on the AAV production.

The 50L Production Process was Successfully Scaled up from 50L to 2000L

- The Titer values measured in Transfection Pool samples were comparable in all three scales (within method variability range)
- The Recovery% calculation over all DSP steps was comparable for all three scales
- The Capsid integrity (Full/Empty ratio) measured by the Transmission electron cryomicroscopy (cryoTEM) showed comparable results. Approx 90% of the produced AAV capsid for all three scales were full.
- The calculated Total Virus Genomes (VG) values suggested linear scalability between tested scales (50L, 500L and 2000L)
- Scaling up of the process showed no negative impact on the product quality and final process productivity