Development and Scale Up of a Robust End-to-End Manufacturing Process for High Quality AAV Production

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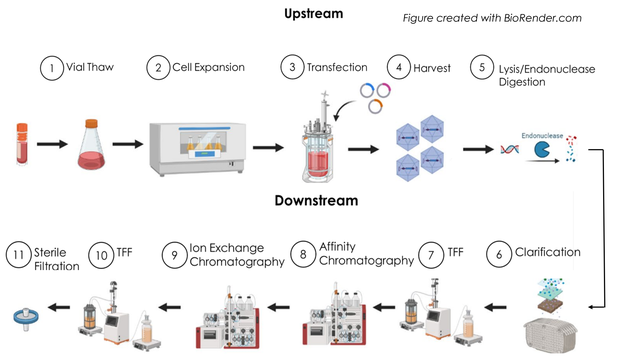
Introduction

Adeno-associated virus (AAV)-based gene therapies have demonstrated substantial therapeutic benefit for the treatment of a variety of disorders, with some having received regulatory approval. With promising clinical data and continued success in pre-clinical and clinical studies, AAV-based gene therapies have emerged as a new class of molecular medicines. Despite the growing demand, there is limited AAV GMP manufacturing capacity and robust processes readily available to support the development of novel AAV therapeutics.

Here, we describe an end-to-end AAV manufacturing process that was optimized for AAV5 vectors which achieved high genomic titers and improved overall downstream processing yield. Multiple DoE studies were performed to optimize critical process parameters. As a result of the studies conducted, these data showed significant improvement in productivity as well as 60% increase in full capsid from upstream crude harvest. The downstream process was also fully developed based on a two-column chromatography strategy. Several DoE screens were conducted to improve the affinity capture and the ion exchange chromatography (IEX) purification steps. The recovery of AAV5 affinity capture increased from 45% to >80%. Importantly, the step recovery of IEX was greatly improved with full capsid enrichment ≥70%, which was confirmed by analytical ultracentrifugation (AUC). In addition, a significant reduction in the impurity levels was achieved using this purification scheme, including >7 logs reduction in host cell protein (HCP) levels bringing those levels below the detectable limit in final AAV product. Moreover, data also demonstrated multiple logs of DNA impurities reduction in downstream process. The end-to-end process was successfully scaled up from 2 L to 50L to generate representative material for non-human primate (NHP) studies.

Schematic Map of GOI Plasmid

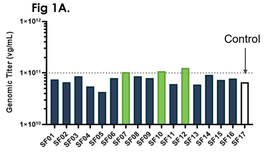
Process Overview



Results

Using DoE to Improve Genomic Titer by 2-Fold

Fig 1A.



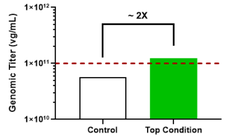


Fig 1B.

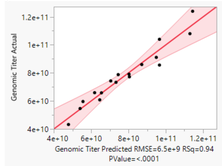


Fig 1. Transfection Process Optimization. (1A) Multi-factor DoE performed in shake flasks to optimize transfection conditions. Dotted lines indicate the targeted titer for this study. (1B) Model from transfection optimization DoE.

Plasmid Ratio Optimization Results in Improved Genome Titer and Percent of Full AAV Capsids

Fig 2A.

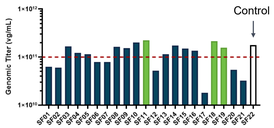


Fig 2B.

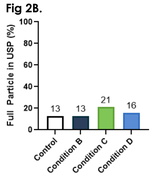


Fig 2C.

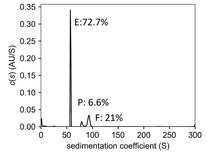


Fig 2. Plasmid Ratio Optimization. (2A) Shake flask DoE performed to optimize the plasmid ratio. (2B) Conditions with highest titer from Fig 2A were scaled up and affinity purified to determine the percent of full capsids using Dynamic Light Scattering (DLS). (2C) AUC data from affinity purified 2L STR material of Condition C.

Optimization of Affinity Chromatography Led to >80% Step Recovery

Fig 3A.

DoE Parameters
Salt Concentration
Supplement 1
Supplement 2

Fig 3B.

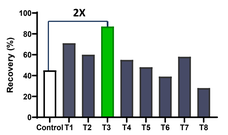


Fig 3. Elution Buffer Optimization. (3A) 3 factor DoE performed in affinity purification to optimize step recovery (3B) Affinity purification step recovery.

IEX Optimization Results in ~70% AAV Full Capsid Content

Fig 4A.

DoE Parameters
Salt type
Supplement 1
pH

Fig 4B.

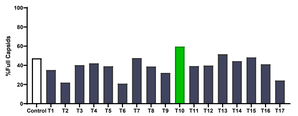


Fig 4. Elution Buffer Optimization. (4A) 3 factor DoE performed in IEX purification to optimize the percentage of full capsids. (4B) Percent of full capsids of IEX eluate.

Fig 5A.

DoE Parameters
Conductivity
pH

Fig 5B.

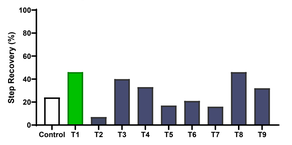


Fig 5. Load Adjustment Optimization. (5A) 2 factor DoE performed in IEX purification to optimize column binding condition. (5B) IEX step recovery.

Fig 6A.

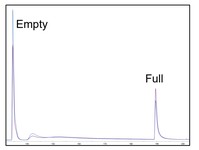


Fig 6B.

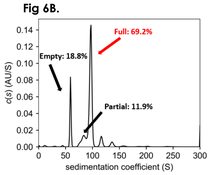


Fig 6. Empty and Full Capsids Separation via IEX Chromatography. (6A) IEX chromatogram of empty/full separation. (6B) AUC data of IEX full eluate.

Assessment of Process Scalability from 2L to 50L STR

Fig 7A.

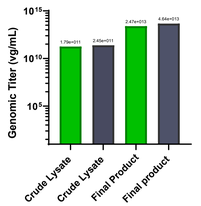


Fig 7B.

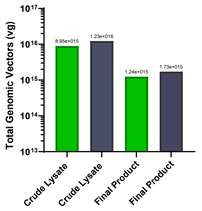


Fig 7. AAV Titer at 2L and 50L Scales . (7A) Genome titer concentration. (7B) Total productivity per batch.

Fig 8A.

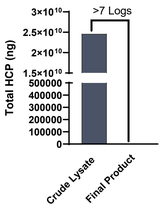


Fig 8B.

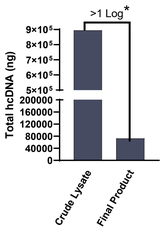


Fig 8C.

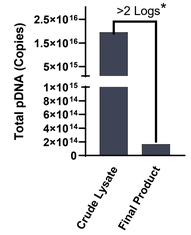


Fig 8. Impurities Reduction throughout Downstream Purification at 50L Scale. (8A) Total HCP. (8B) Total host cell DNA (hcDNA). (8C) Total plasmid DNA (pDNA). Asterisks show additional DNA reduction not accounted for by nuclease digestion.

Conclusion

- ElevateBio developed a robust, flexible, and scalable process that generates high titer and quality AAV material for clinical applications.
- An experimental approach that systematically optimizes critical upstream and downstream process parameters using DoE principles has been established. This tailored approach ensures efficient adaptation for each new AAV construct and serotype to the established process.
- Most of upstream and downstream unit operations can be seamlessly applied to any novel construct and serotype. Optimization work primarily focuses on three critical steps including transient transfection, affinity chromatography, and IEX chromatography for new serotypes.
- Process materials, equipment, and analytics (except for product specific assays) can be leveraged from construct to construct. This inherent flexibility simplifies the transition when working with diverse AAV vectors and various therapeutic indications.
- Based on our refined AAV process, the overall time required for construct-specific parameter optimization can be reduced from over a year to just a couple of months, thereby accelerating critical CMC timelines to get in the clinic as well as reducing cost.

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