Upstream Optimization Paved a Straightforward Path for High Percentage of Full Capsids in AAV6

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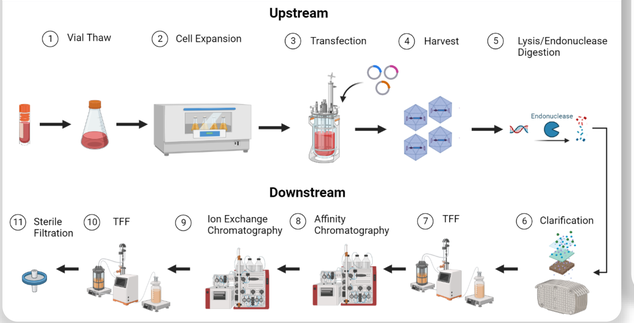
INTRODUCTION

Adeno-Associated viruses (AAV) are a potent tool in the expanding field of cell and gene therapy as they offer low immunogenicity and can facilitate gene transfer or gene editing for *in vivo* or *in vitro* applications via the efficient delivery of genetic material. However, the AAV potency and safety are highly dependent on the genome packaging efficiency, including full particle percentage among the total particles, the distribution of partial particles, and the amount of empty particles. Because clinical applications for AAV are increasing rapidly, there is a high demand for further increasing yields as well as product quality, particularly for a higher percentage of full capsids in the final product.

A platform process for AAV6 production has been well-established, using a GOI with a size of ~4.7kb, and is based on transient transfection of serum-free cells grown in suspension. In this process, we have evaluated multiple parameters in the upstream production process (for example, cell growth, transfection reagents, DNA concentration, etc) to reach titers > 1e11 vg/mL and >15% full particles based on dynamic light scattering (DLS) using Design of Experiment (DoE) studies in shake flasks. Furthermore, our data demonstrated good scalability from shake flask to a 2L stirred tank reactor (STR) in terms of genome titer and full%. The challenge for the baseline process was that final AAV could only reach ~50% full particles even after ION Exchange (IEX) enrichment. To avoid changes in the downstream process and accelerate the development timeline for this project, a follow-up DoE was performed to optimize the ratio among 3 transfection plasmids to increase AAV full percentage. Batch purification was used to determine upstream processes with higher percent full particle via DLS, and the top conditions achieved >1.5e11vg/mL and 17-23% full (DLS). Then, the top 2 processes were scaled up into 2L STR and particles were purified through our platform affinity chromatography using AKTA for further verification. The data showed the affinity purified AAVs from both processes were > 1e13 vg/mL and ~ 20-30% full (via Analytical Ultracentrifugation [AUC]). Since the GMP friendly downstream process usually include IEX chromatography after the affinity column to enrich the full particles, these two upstream processes have been also applied through IEX separation and our data demonstrated the top condition was able enrich particles to >70% full.

Process Overview



RESULTS

3 Factor DoE Improves vg Titer 4X

Fig 1A.

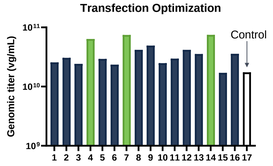


Fig 1B.

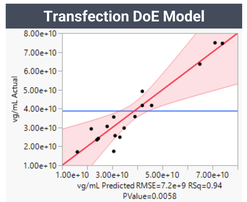


Fig 1. Transfection Optimization Process. (A) 3 factor DoE performed in shake flasks looking at transfection conditions. (B) Model from transfection optimization DoE.

Full % of Top Conditions

Fig 2A.

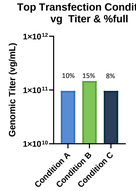


Fig 2B.

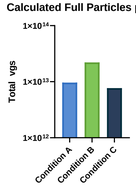


Fig 2C.

Condition B AUC	
Empty	63.5%
Partial	21.0%
Full	15.5%

Fig 2. Transfection Optimization Scale Up. (A) Top 3 conditions were scaled up to 125mL shake flasks and batch purified to read %full via the Stunner. (B) Projection of total vgs per liter to identify top condition. (C) AUC data from affinity purified 2L STR material of Condition B.

Plasmid Ratio Optimization Increases vg Titer and %Full

Fig 3A.

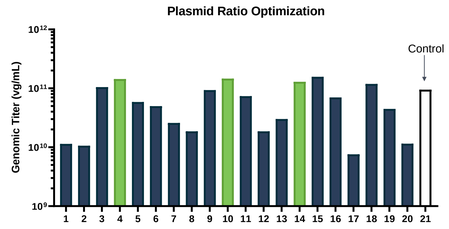


Fig 3B.

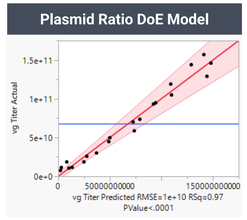


Fig 3C.

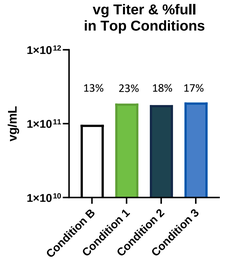


Fig 3D.

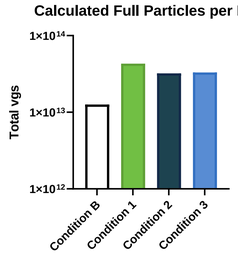


Fig 3. Plasmid Ratio Optimization Process. (A) Shake flask DoE performed looking at plasmid ratios (B) Model from plasmid ratio DoE. (C) Top conditions were scaled up to 125mL productions to be batch purified. (D) Stunner data from batch purified material from 125mL productions (E) Projections for total vgs produced per Liter to determine top conditions to move forward for scaling up.

2L Scale Up of Top Conditions

Fig 4A.

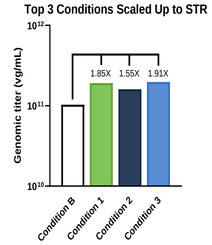


Fig 4B.

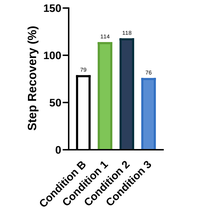


Fig 4C.

Affinity AUC			
Sample	Empty	Partial	Full
Condition 1	67.3	5.5	27.2
Condition 2	71.1	6.4	22.5
Condition 3	64.3	10.7	25.0

Fig 4. 2L Scale Up and Purification. (A) Top conditions were scaled up to 2L STR for downstream to purify through Affinity and IEX(B)) Step recoveries from 2L STR Affinity material.(C) 2L STR Affinity material was tested for %fulls via AUC.

Condition 1 Results in Most %Full Improvement

Fig 5A.

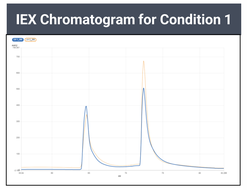


Fig 5B.

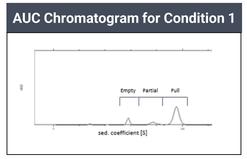


Fig 5C.

IEX AUC			
Sample	Empty	Partial	Full
Condition B	21.8	22.0	56.2
Condition 1	14.7	11.8	73.5
Condition 2	15.1	20.4	64.5

Fig 5. IEX Purification. (A) IEX chromatogram for Condition 1 from purified 2L STR starting material . (B) AUC chromatogram for Condition 1 from purified 2L STR starting material. (C) IEX material was tested for %fulls via AUC.

CONCLUSION

- The top transfection conditions and plasmid ratio was successfully scaled up from shake flask to 2L Stirred Tank Bioreactors (STR)
- The use of Stunner (DLS) as a quick and inexpensive method for assessment of full/empty ratios was successfully implemented to screen upstream conditions by analysis of semi-purified lysates
- A reduction of the number of upstream conditions analyzed by AUC also helped accelerate downstream process development activities
- Using optimized plasmid ratio conditions, the percent of full capsids increase from 15% to 27% in upstream production as well as reduce partials
- AAV6 yields were successfully increased >15X by through upstream process optimization
- The percent of full capsids in AAV6 preps was further improved by Ion Exchange Chromatography (IEX) development reaching over 70% Full capsids.

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