Exploring upstream process conditions to improve productivity for novel AAV Capsids

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INTRODUCTION

Recombinant adeno-associated virus (rAAV) has emerged as a promising technology for the delivery of gene therapies. As novel capsids with improved transduction efficiency are developed, opportunities to treat larger patient populations and prevalent indications arise. To this end, scalable, higher-producing processes are needed to meet the material demands. Suspension-based processes are more amenable to scale-up and are the basis for the work presented. Using HEK293 cells in suspension and a triple transfection process, upstream process conditions are tested. Conditions are identified that:

- improve titer,
- reduce residual impurity levels,
- improve harvest performance.

Collectively, the work here shows bioreactor conditions can be optimized to improve titer and process robustness for the production of novel capsids.

Bioreactor vs. Shaker Flasks Controlled Parameters

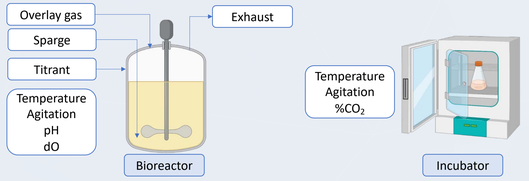
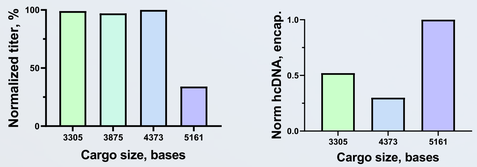


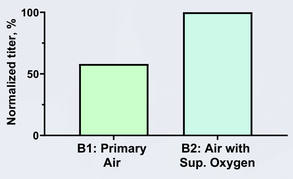
Figure 1: Bioreactor and incubator schematics and controlled parameters. Bioreactors offer setpoint control around pH, dissolved oxygen, agitation, and temperature. Bioreactors can support higher cell densities compared to shaker flasks. Directional screening of process parameters in shaker flasks is helpful. Certain parameters require optimization in the bioreactor.

Effects of cargo size on productivity and residual DNA



Figures 2 and 3: Effects of cargo size on titer and residual hcDNA packaging were assessed. Titer was not affected by cargo sizes below 4300 bases. Residual hcDNA was more dependent on cargo size. Residual hcDNA was higher for both the smaller (3305 bases) and larger (5161 bases) cargos.

Effects of dissolved oxygen (dO) control schemes on productivity



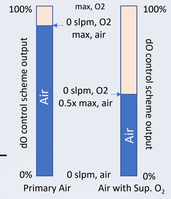
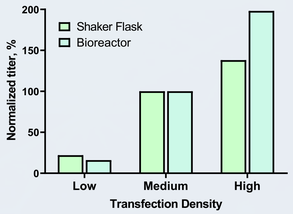


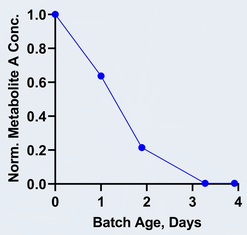
Figure 4: Two liter bioreactors were run under different dissolved oxygen (dO) control schemes, bioreactor B1 primarily with air and B2 air with supplemental oxygen. Titer was 40% higher, and pCO2 levels were approximately 70% higher in B2.

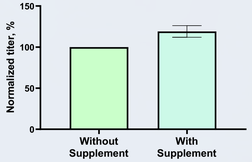
Effect of transfection density on productivity

Figure 5: Effects of transfection density on productivity were screened in shaker flasks and trends subsequently confirmed in 2L bioreactors. Titer normalized to the medium value is reported. HEK293 cells were grown in suspension. Shaker flasks and bioreactors were seeded at low, medium, and high cell densities. The same plasmid ratio, plasmid amounts, transfection reagent (FectoPro) were used in all experiments. Increased titer was observed when transfecting at higher cell densities. The increase was larger in bioreactors compared to shaker flasks. Higher plasmid amounts were tested at the higher transfection density and did not result in increased productivity (data not shown).



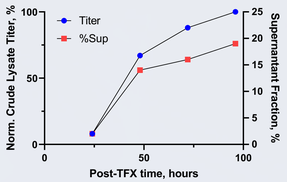
Effect of metabolite supplements on productivity



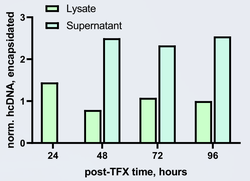


Figures 6 and 7: A metabolite panel was monitored across multiple batches. It was observed Metabolite A is depleted through the course of a batch and runs out around Day 3. Supplementing Metabolite A at various amounts and time points was tested. Optimized supplementation of Metabolite A increased titer around 20%.

Effects of post-transfection duration on productivity and AAV partitioning in the supernatant

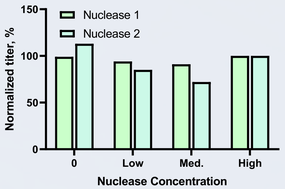


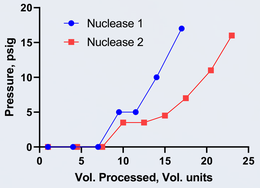
Time Post-TFX	$\%Full_{CL}$ -$\%Full_{Sup}$
24 hr	3%
48 hr	12%
72 hr	10%
96 hr	13%



Figures 8 and 9: Transfection duration was assessed in a time course study. Bioreactors were transfected and sampled over 96 hours post-transfection. Titer and residual hcDNA of the supernatant and crude lysate were measured to assess partition fraction and residual levels. Literature and user guides have typically used 72-hour transfection durations.[2,3] We observed a 20% increase in titer extending the harvest time to 96 hours. Most of the vector, approximately 80%, resides in the cell. The difference in %fulls (cell vs. supernatant) is larger after 24 hours, *i.e.*, the cell pellet has more full capsids. Residual hcDNA levels are 2-3 times higher in supernatant material. This trend is consistent across the different timepoints. Note, our 24-hour supernatant sample did not have enough material to measure hcDNA. No value is shown in the graph.

Screening different nucleases, effects on titer and filtration





Figures 10 and 11: Nucleases are used in the cell culture lysis step. Two nucleases were screened for effects on crude lysate titer, Figure 10, and filtration, Filter 11. Nucleases were added to the lysis buffer and incubated for a set duration. Both nucleases from 0 to a high concentration had no effect on titer. The same source cell culture was lysed with Nuclease 1 or Nuclease 2 at the same concentration (high AU/ml). Lysed cell culture was subsequently filtered. Pressure profiles pre-primary filter are shown. Filter capacity is approximately 30% higher when using Nuclease 2. Nuclease 2 also costs less.

Crude lysate harvest filtration train schematic

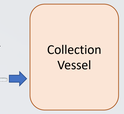


Figure 12: Our crude lysate is clarified with a depth filtration train consisting of primary and secondary depth filters followed by a 0.2 µm filter. Filtrate is collected for further downstream processing. Pressure profiles prior to bioreactor process changes are shown. The primary depth filter is fouling. The bioreactor process changes increased the debris load and required additional work on the filtration train.

Filter screening: Identifying a more effective secondary depth filter

Test Train	Primary depth filter	Secondary depth filter	0.2 µm filter
T1	Model A (model not varied)	Model 1	Model B (model not varied)
T2		Model 2	
T3		Model 3	

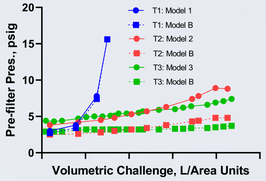


Figure 13: Secondary depth filters were screened, and pressure profile are provided (pre-secondary and pre-0.2 µm filters). Model 1 was part of the early process but fouled at lower loading (blue lines) when bioreactor process changes were made. Model 2 and Model 3 filters were better at removing debris and protecting the 0.2 µm filter. Process robustness was improved by switching the secondary filter model.

GEA single-use centrifuge, a more robust method for harvesting: Successful proof of concept run!

Operating Parameters
Bowl Speed
Flowrate
Solids Concentration

Figures 14 and 15: Culture is dying post-transfection creating a more variable debris load on harvest day, a challenge for filtration. Either filters are significantly oversized, or back-up trains are maintained. Single-use centrifugation is a new option to replace the primary depth filter, reducing total depth filter area by at least half. Results with a GEA unit are promising. The needs minimal setup. Centrate turbidity was inline with crude lysate spindown. The centrifugation train, initial clarification with the GEA centrifuge, showed improved 0.2 µm filter capacity compared to our standard depth filtration train.

CONCLUSIONS

Optimization of bioreactor operating parameters and conditions can improve productivity and other product quality attributes. Individually the process changes highlighted led to 20-50% improvements in titer. Collectively, the optimization work led to a 3-6x improvements in titer. ***Similar improvement trends were observed across multiple cargo-novel capsid combinations.***

Noteworthy improvements came from

- Evaluation of dO control schemes
- Range finding around transfection density and duration
- Identifying depleted metabolites

Harvest filtration is an important step because of the cost and the potential of product loss. We found screening different nucleases, filter types, and technologies improved process robustness.

Contact

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References

1. Schematic diagrams made using Biorender
2. AAV-MAX Helper-Free AAV Production System User Guide
3. Protocol: FectoVIR-AAV DNA transfection reagent for virus production

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