AAV manufacturing

Accelerated upstream process development of adeno-associated viruses from high-throughput systems to clinical scale

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Abstract

This poster presents a case study demonstrating the use of AMBR™15 for high-throughput Design of Experiment (DoE) development of an AAV9 production process and its direct scale-up to a 2,000 L Thermo Scientific™ HyPerforma™ single-use bioreactor (S.U.B.). It outlines methodologies and best practices for enabling successful upstream scale-up, including strategies to maintain critical transfection parameters.

Introduction

Adeno-associated virus (AAV) is a leading gene delivery platform widely used in gene therapies for chronic diseases such as hemophilia and cancer, with over 200 clinical trials conducted as of 2022. Despite its therapeutic promise, AAV manufacturing faces major challenges—particularly in scaling up production and reducing costs. The most significant barrier is the high cost of goods (COGs) per dose, which can exceed $1 million per treatment. These costs stem from low product yields, expensive raw materials (particularly plasmids), high proportions of empty capsids requiring extensive purification, and substantial labor and facility overhead. Additionally, long development and manufacturing timelines (12–24 months) further increase complexity and expense. Scaling triple transfection–based AAV production is especially difficult. Maintaining critical parameters, such as plasmid DNA–transfection reagent complexation time, during scale-up is operationally challenging. [1] Differences in hydrodynamics and mass transfer between small-scale development systems (e.g., Ambr™ systems) and large-scale bioreactors complicate process translation. Although Quality by Design (QbD) approaches are increasingly adopted in gene therapy, there is limited published research on using high-throughput systems like Ambr systems to accelerate design of experiments (DoE) and successfully scale AAV production.

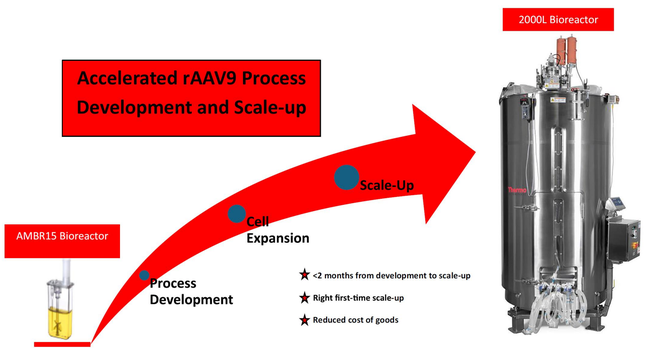


Figure 1. Rapid development of rAAV9. The goal of this study is for the rapid development and scale-up of rAAV9 production to address challenges with long manufacturing timelines and high cost of goods.

Materials and methods

Cell culture

Gibco™ Viral Production Cells 2.0 (HEK293F-derived) were expanded in shake flasks using LV-MAX™ Production Medium and maintained at 37˚C with 5% CO_2. Cell density and viability were routinely monitored. For process development, cultures were grown in Ambr™ 15 bioreactors under controlled conditions (37˚C, pH 6.8–7.2, DO 30%). For scale-up, cells were expanded sequentially into 250 L and 2,000 L HyPerforma S.U.B.s under similar temperature and pH control strategies.

AAV9 production

A Design of Experiments (DoE) in AMBR15 evaluated (figure 1):

- Total DNA: 0.25–1.50 µg DNA per 10^6 cells
- Viable cell density (VCD): 1–5 × 10^6 cells/mL

Other parameters (plasmid ratios, DNA:reagent ratio 1:1, 30-min complexation time) were held constant. Three plasmids were used: Rep/Cap9, helper, and transgene plasmids. Each condition was run in duplicate.

For 2,000 L production:

- Cells were expanded to target density (~2.4 × 10^6 cells/mL at transfection).
- DNA and FectoVir™-AAV transfection reagent were complexed in a 50 L single-use mixer.
- Plasmids were filtered into the mixer, gently mixed, incubated 30 minutes, and transferred into the bioreactor using a low-shear Quattroflow™ pump to preserve complex integrity.

Harvest

Three days post-transfection, cultures were lysed using $MgCl_2$, Polysorbate-80, and Benzonase™ Nuclease to release vector and degrade host DNA. Salt was added to adjust conditions, samples were centrifuged, and supernatant was collected for analysis.

Analytical testing

- Vector genome titer: Measured by ddPCR targeting the CK8 promoter.
- Capsid titer: Determined by AAV9-specific ELISA.
- Residual host cell DNA: Quantified via magnetic bead extraction and qPCR.

Statistical analysis

JMP software was used for DoE and response surface modeling (RSM) to optimize transfection and bioreactor parameters. A third-order polynomial model and a COGs/dose calculator were applied to identify optimal operating conditions balancing productivity and cost.

Results

HEK293F triple transfection optimization in Ambr 15 system

A Design of Experiments (DoE) study in the Ambr 15 system evaluated the effects of DNA amount and viable cell density (VCD) on AAV9 production. Higher DNA and transfection reagent levels reduced cell viability, likely due to cytotoxicity and nutrient depletion. Increasing cell density improved viral genome (vg) titers—especially at ≥0.65 µg DNA per 10^6 cells—but often lowered the percentage of full capsids. Because downstream purification only modestly enriches full capsids (~2.5-fold), an upstream minimum of 20% full capsids was required to meet the final bulk drug substance target (>50%). The optimal condition selected for scale-up was:

- 0.25 µg DNA per 10^6 cells
- VCD of 2.3 × 10^6 cells/mL

This condition delivered the best balance of specific productivity and ≥20% full capsids, while achieving a 60% reduction in cost of goods per dose (COGs) compared to industry benchmarks (figure 2). COGs were primarily driven by plasmid/transfection reagent usage and upstream genome titer.

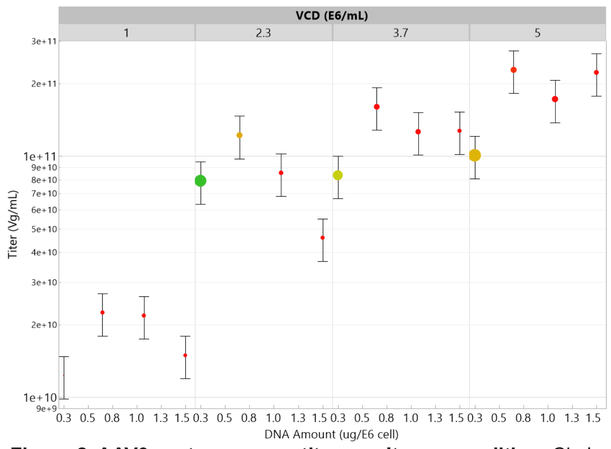
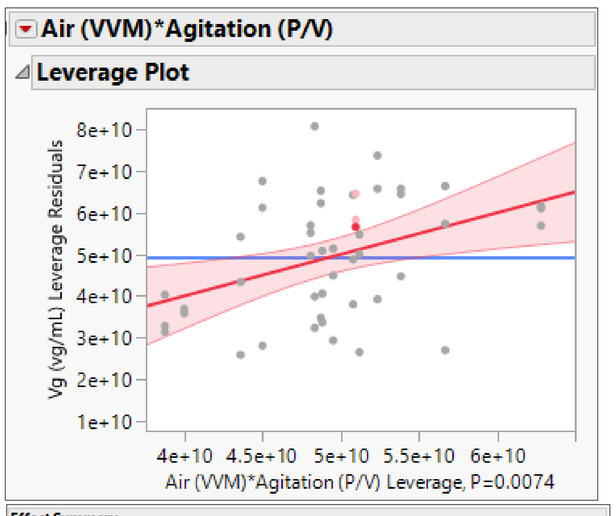


Figure 2. AAV9 vector genome titer results per condition. Circle size indicates % full capsids estimate based on vg:capsid ratio. Color indicates cost of goods per dose with green being up to 60% reduction and red being 0%.

Agitation and gas input bridging study

Because Ambr 15 systems operate differently from large-scale bioreactors, agitation and gas flow required careful bridging. Viral genome titer significantly correlated with the combined effect of agitation (power per volume, P/V) and air flow rate due to their impact on mass transfer (figure 3). Testing identified operating ranges in Ambr 15 systems that maintained productivity. For scale-up, a constant P/V of 20 W/m³ and 0.004 VVM air flow was selected for the 2,000 L bioreactor, aligning with historical large-scale operating ranges. This approach demonstrated that maintaining constant P/V and controlled gassing can successfully translate performance across scales.



Effect Summary

Source	Logworth	PValue
Air (VVM)*Air (VVM)	3.101	0.00079
Air (VVM)*Air (VVM)*Air (VVM)	3.032	0.00093
Air (VVM)	2.194	0.00640 ^
Air (VVM)*Agitation (P/V)	2.133	0.00736
Agitation (P/V)*Agitation (P/V)	1.794	0.01606
Agitation (P/V)	1.634	0.02324 ^
Agitation (P/V)*Agitation (P/V)*Agitation (P/V)	1.567	0.02713

Figure 3. Significant interactions (p < 0.05) between air, agitation, and a combination of air and agitation rates are found. P/V range of 8–160 w/m³ with air VVM of 0.0038–0.02 were assessed in the Ambr 15 system.

Results (continued)

Scale-up from Ambr 15 system to 2,000 L

An accelerated development timeline was achieved by maintaining a continuous seed train (up to 16 passages) rather than repeatedly thawing cells. Stable viability (>90%) and product quality were maintained. Key scale-up strategies included (figure 4):

- Direct scale-up of optimized transfection conditions from Ambr 15 system to a 2,000 L HyPerforma™ single-use bioreactor (S.U.B.)
- Use of a 50 L single-use mixing vessel (S.U.M.) for controlled transfection complex preparation
- Low-shear diaphragm pump transfer to preserve complex integrity
- Plasmid filtration to reduce contamination risk

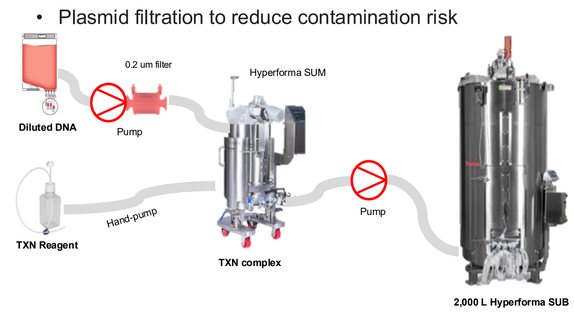


Figure 4. Process flow diagram of the transfection process. Large scale transfection process flow diagram that shows the fluid pathway of the transfection complex components and the transfection complex into the bioreactor. A peristaltic pump was used to filter diluted DNA into a Hyperforma S.U.B. A Quattroflow single-use pump was used to pump the transfection complex into the bioreactor.

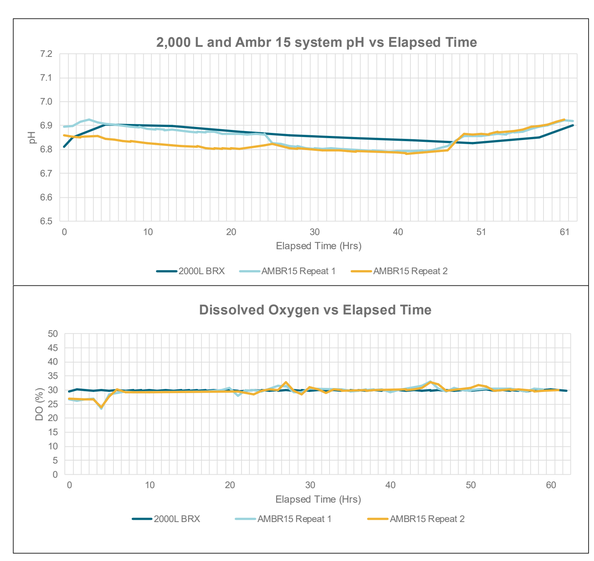


Figure 5. Comparison of Ambr 15 system to 2,000 L BRX. Comparison of on-line bioreactor pH and DO trends between Ambr 15 and 2,000 L bioreactor. DO was controlled at 20–40% while pH was controlled at 6.8 ± 0.1 during production.

Matching shear conditions to maintain transfection performance pH and dissolved oxygen profiles were tightly controlled and comparable between scales (figure 5). Viral genome titers were:

7.9 × 10^{10} vg/mL (Ambr 15 system)

6.2 × 10^{10} vg/mL (2,000 L)

These results were statistically comparable (within assay variability), demonstrating successful scale-up over a >100,000-fold increase in volume. Full capsid percentage at 2,000 L (22%) matched Ambr 15 system performance, while shake flask studies overestimated full capsid content, highlighting their limited scalability relevance. Residual host cell DNA levels were consistent across scales, and reproducibility testing in Ambr 15 system showed expected biological and process variability (37%), with 2,000 L performance falling within this range.

Table 1. Quality attribute summary at harvest for 2,000 L HyPerforma SUB, Ambr 15 system and shake flask.

Quality attribute	Shake flask	AMBR®15	2000 L
Viral genome titer (vg/mL)	6.5 ± 1.3 ×1010	7.9 ± 1.6 ×1010	6.2 ± 1.2 ×1010
Capsid titer (cp/mL)	1.4 ± 0.3 ×1011	3.6 ± 0.7 ×1011	3.0 ± 0.6 ×1011
Vg-to-capsid ratio (%)	46 ± 4[a]	22	21
Residual host cell DNA (ng/mL)	N/A	836	848

[a]Average of triplicate flasks.

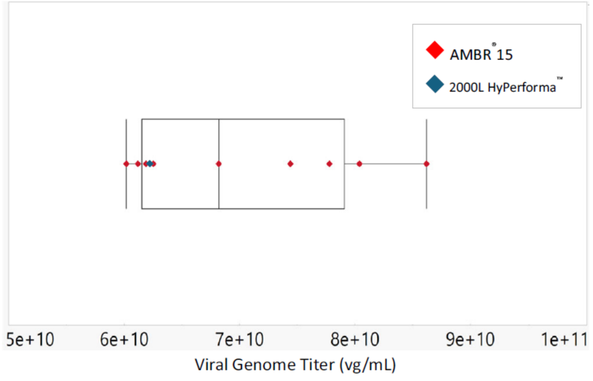


Figure 6 Summary of Ambr 15 system and 2,000 L bioreactor data. 9 runs in Ambr 15 system demonstrate reproducibility of viral genome titer to titer obtained in 2,000 L bioreactor. Error bars represent 1 standard deviation.

Conclusions

This poster addresses key challenges in AAV manufacturing via transient triple transfection, including high cost of goods (COGs), scalability limitations, long timelines, and impurities such as empty capsids. Using high-throughput screening in the Ambr 15 system, the team rapidly identified an optimized transfection condition within two months that maximized productivity while minimizing COGs. Results showed that carefully balancing DNA/transfection reagent levels and cell density is essential to achieve high productivity with low cytotoxicity. A bridging study demonstrated that viral genome titer correlates with the combined effect of agitation and airflow, enabling identification of scalable operating conditions. Scale-up to a 2,000 L HyPerforma bioreactor was achieved by incorporating a single-use mixer and low-shear pump to properly control transfection complex formation and transfer. The optimized process successfully translated to 2,000 L, maintaining comparable viral genome titers, percent full capsids, and residual host cell DNA levels. Overall, combining high-throughput process development with thoughtful engineering controls enabled faster development timelines, consistent product quality, and reduced COGs at commercial scale.

References

1. Andaluz, A., Monteverde, B., Vera, K., Tse, B., Gajic, I., Froelich, C. and Motevalian, S.P. (2025), Accelerated Adeno Associated Virus Upstream Process Development From High-Throughput Systems to Clinical Scale. Biotechnology and Bioengineering, 122: 3051-3060. https://doi.org/10.1002/bit.70052

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