Development of a Novel High-Yielding Scalable Sf9-Baculovirus Platform to Produce Quality AAV at 200L Scale

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INTRODUCTION

Significant cost of goods (COGS), low yields, and limitations in scaling all pose challenges to manufacturing of recombinant Adeno-associated Virus (rAAV), especially with mammalian cell platforms utilizing transient plasmid transfection. Large patient populations and/or high dose demands amplify these risks, delaying clinical supply and hindering potential commercialization. In response, we present our novel Sf9-baculovirus process that produces commercially viable yields of high quality AAVrh.10 at the 200L stirred tank bioreactor (STR) scale and currently supports two Lexeo Therapeutics clinical stage programs.

PROCESS OVERVIEW

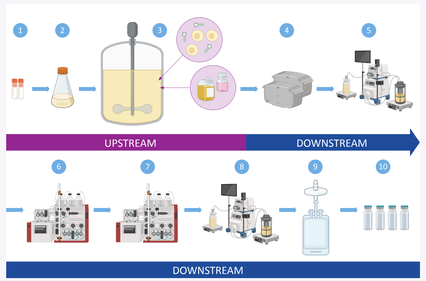
Upstream

- A suspension Sf9 insect cell line is cultured in an animal component free chemically defined insect cell media.
- Two GLP recombinant baculovirus (rBV) constructs are utilized: one containing the *Rep* and *Cap* genes for the adeno-associated virus serotype rh.10 (AAVrh.10) capsid, and the other containing the gene of interest (GOI): GOI-A or GOI-B.
- The Sf9 cell line is infected with rBV to generate AAVrh.10, GOI-A, and GOI-B baculovirus-infected insect cell (BIIC) banks.
- For rAAV production, the Sf9 master cell bank (MCB) is thawed and expanded in serial shake flasks, followed by inoculation of the STR and infection at low multiplicity of infection (MOI) with BIICs.

Downstream

- The harvest is lysed, then unwanted protein and cellular debris are removed by depth filtration, followed by Tangential Flow. Filtration (TFF)-1 for concentration and buffer exchange.
- A 2-step chromatography purification is utilized, 1) Affinity Chromatography (AFF) to enrich for AAVrh.10 capsids followed by 2) Anion Exchange (AEX) to remove empty capsids.
- TFF-2 is employed to diafilter into final formulation, followed by sterile filtering and filling of the Bulk Drug Substance (BDS) prior to Drug Product (DP) vialing.

Figure 1. Upstream and Downstream AAV Platform Process



(1) Sf9 MCB Thaw (2) Cell Expansion (3) STR Inoculation, Infection, and Feed Addition (4) Harvest and Clarification (5) TFF-1 (6) Affinity and (7) Anion Exchange Chromatography (8) TFF-2 (9) Filtration & Bulk Drug Substance Fill (10) Final Drug Product

PROCESS DEVELOPMENT

Upstream

- Improvements to upstream vector genome (VG) yield and productivity (measured by ddPCR) and full capsid percentage (measured by an AAVrh.10 capsid ELISA) were evaluated at the shake flask scale.
- Design of experiment (DoE) studies were performed to optimize process parameters such as MOI, ratio of AAVrh.10 and GOI BIICs, supplementation and timing of nutrient feeds.
- Scalability was demonstrated with benchtop STRs at the 250mL and 3.5L scale.

Downstream

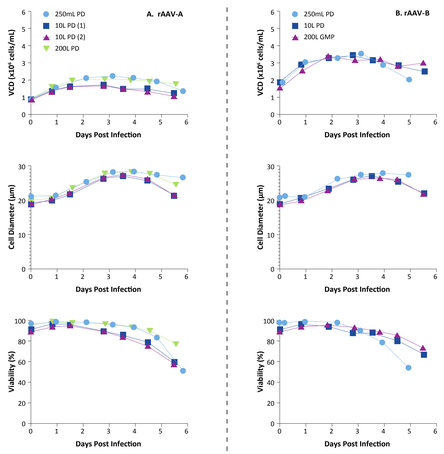
- Depth filter media and filters for TFF-1/TFF-2 were tested selecting for optimal pressure, flux, unit operation time and total VG recovery (measured by ddPCR).
- AFF Chromatography resin screening and buffer optimization were performed selecting for total VG recovery (ddPCR) and full capsid % (Mass Photometry (MP) and capsid ELISA).
- AEX buffer optimization and resin screening led to the development of a novel AEX method which allows for higher VG recovery and fewer empty capsids than traditional methods.

RESULTS

Upstream Cell Culture and Infection

- Daily cell growth characteristics of the Sf9 cell culture following BIIC infection are reported from 250mL to 200L STR scales (Figure 2).
- Characteristics such as viable cell density (VCD), viability, and cell diameter were comparable across both programs and across scales.

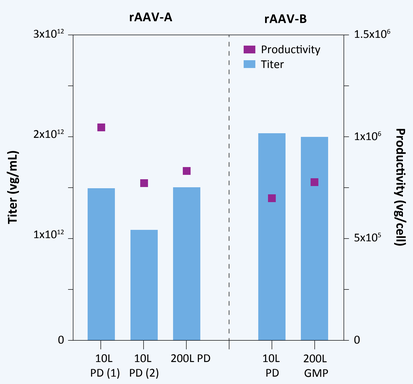
Figure 2. Upstream cell culture trends during bioreactor scale up for rAAV-A and rAAV-B



Upstream Productivity

- VG titers and productivities of 1.1-2.0E+12 vg/mL and 0.6-1.0E+06 vg/cell respectively were reported across all scales and both clinical programs (Figure 3).

Figure 3. Upstream yield and productivity during bioreactor scale up for rAAV-A and rAAV-B

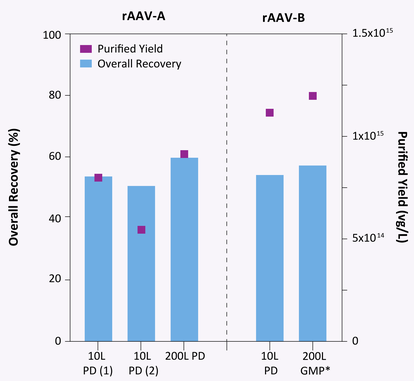


Downstream Process Yield

- The performance of the optimized downstream process is consistent across different production scales and GOI, achieving overall VG recovery from harvest lysate above 50% in 5 batches and greater than 60% at the 200L scale.
- Harvest titers exceeding 1E+12vg/mL, combined with the purification platform, result in over 1E+15vg/L purified material for rAAV-B at 10L and 200L scales, and 9.2E+14 vg/L for rAAV-A at the 200L scale.

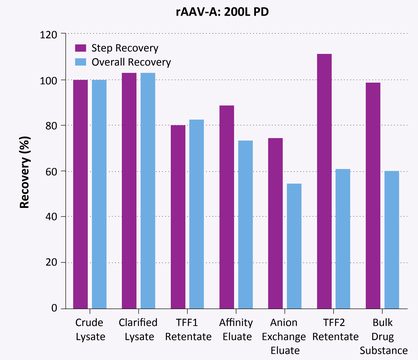
Figure 4. Downstream purification recovery and purified yield measured by ddPCR

a. Two clinical rAAV constructs at multiple PD batch scales



*Harvest Lysate titer used to calculate overall recovery was estimated based on historical cell culture data and AEX chromatogram UV area.

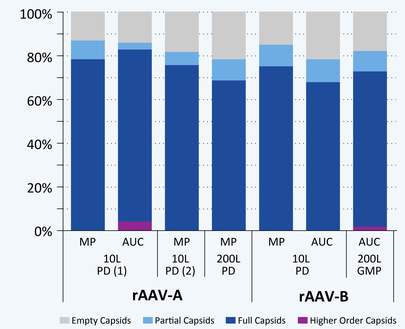
b. Step and overall recovery for each unit operation in a 200L PD batch



Downstream Process Full Capsid Enrichment

- Consistent separation of full and empty capsids was observed across scales and GOI using a proprietary AEX method that results in ≤25% empties.
- Current GMP process shows <20% empty capsids.

Figure 5. rAAV full/empty capsid ratio in two clinical stage constructs



PRODUCT QUALITY

Table 1. Product quality characteristics from 200L scale runs in PD and GMP

Assay	rAAV-A (200L PD)	rAAV-B (200L GMP)
Harvest Endotoxin (EU/mL)	0.14	0.05
Bioburden (CFU/mL)	<0.1	<0.1
Residual Sf9 Protein (ng/mL)	Pending	<7
Capsid Purity	98%	97%
VP1:VP2:VP3*	2.0:1.2:10	2.0:1.4:10
% Empty Capsids (AUC)	Pending	18.55%

*Reported viral protein (VP) ratios have been normalized to a VP3 of 10. For a detailed description of the characterization of the critical quality attributes (CQAs) for batches manufactured with the Lexeo Sf9-baculovirus platform process, please refer to the QR code in **More Information**.

KEY TAKEAWAYS

1. The primary drivers of the high cost of manufacturing of gene therapies are the large amounts of starting materials – typically plasmid and transfection reagent – needed to inoculate a bioreactor, coupled with low yields and scalability challenges. As a result, existing rAAV manufacturing processes are unable to support high dose demands and large patient populations.
2. Breakthroughs at Lexeo in process development have established a novel platform at the 200L scale and demonstrated across two clinical programs: >1E+15 vg/L of purified rAAV yield, >55% overall recovery, and <25% empty capsids, with minimal starting material demand, ease of scaling and low overall complexity in the process.
3. Lexeo Therapeutics' Sf9-baculovirus process addresses the limitations of plasmid-based rAAV manufacturing while enabling robust commercialization and greater patient access to gene therapies.
4. Based on significant advancements to upstream yield and downstream recovery alongside demonstrated scalability at the 200L GMP manufacturing scale, our platform technology process is poised to deliver an industry leading and transformational COGS profile.

MORE INFORMATION

To **learn more**, visit: **www.lexeotx.com**

For more insight into characterization of critical quality attributes, visit our **Publications** page

ACKNOWLEDGMENTS

We acknowledge the technical support and feedback provided by the Lexeo Therapeutics CMC team, the analytical support provided by our CMC Analytical team, and poster support from Nilomi Shah from the Medical Affairs team. We acknowledge technical collaboration and support provided by our colleagues at Millipore and Virovek, and our manufacturing partners at Fujifilm.