Orthogonal Purification Strategies for AAV: Bridging Rapid Feasibility via Ultracentrifugation with Scalable Chromatography-Based Manufacturing to Support Diverse Therapeutic Timelines.

P0066

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Abstract

The rapid expansion of the adeno-associated virus (AAV) therapeutic landscape has created a growing need for versatile downstream processing (DSP) workflows. Developers often face a trade-off between speed and scalability: early-stage feasibility studies require high-throughput material generation at minimal scale, while late-stage clinical programs demand robust, scalable processes for regulatory compliance.

To address this need, we developed a bi-modal DSP platform that bridges candidate discovery and CMC readiness.

- **Workflow A** (accelerated feasibility): Uses iodixanol-gradient ultracentrifugation (UC) coupled with automated parallel buffer exchange. This route was optimized for small-scale parallel processing (<50 mL), prioritizing rapid turnaround and high material recovery for in vitro screening.
- **Workflow B** (scalable platform): Utilizes classical chromatography-based purification, including affinity chromatography (AC) and anion-exchange chromatography (AEX) for full-to-empty capsid enrichment, followed by Tangential Flow Filtration (TFF) for large-scale material generation (>2 L).

Why it Matters:

- **Rapid evaluation of new AAV candidates**
- **Scalable purification route for development programs**
- **Flexible workflow selection based on project stage and timeline**
- **Accelerated transition from research to CMC-ready process**

Workflow A

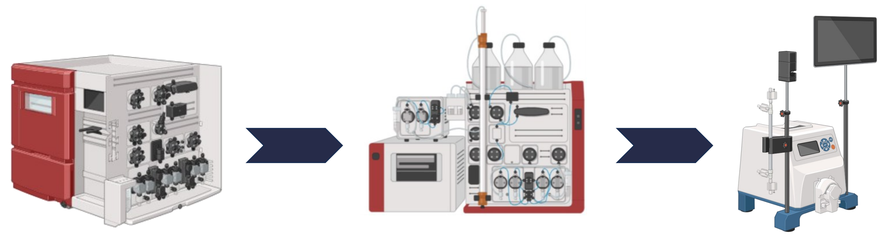
Workflow A* = Rapid Feasibility / Early Development

Volume reduction (pressurized UF/DF) → **Iodixanol UC**** → **Buffer exchange & concentration (pressurized UF/DF)** → **Formulation & final filtration**

*Downstream processing starting point = Nuclease treated and clarified lysate.
**Capacity testing performed: 3E+12 – 3E+15 caps/UC tube.

Workflow B

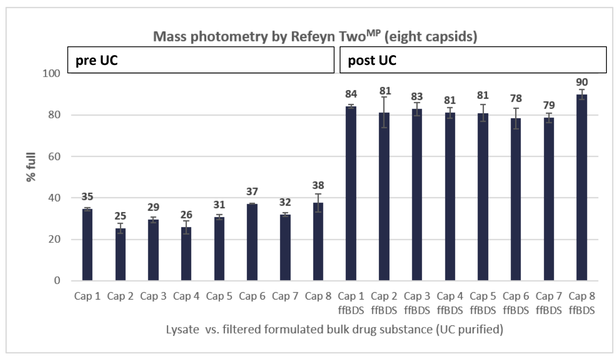
Workflow B* = Scalable / Manufacturing-Oriented Development



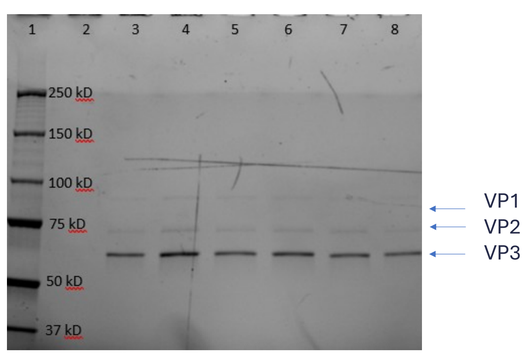
AC → **Ion-exchange chromatography (AEX)** → **TFF (UF/DF)** → **Formulation & final filtration**

*Downstream processing starting point = Nuclease treated and clarified lysate.

Workflow A* results summary



% full pre and post purification (eight constructs shown).

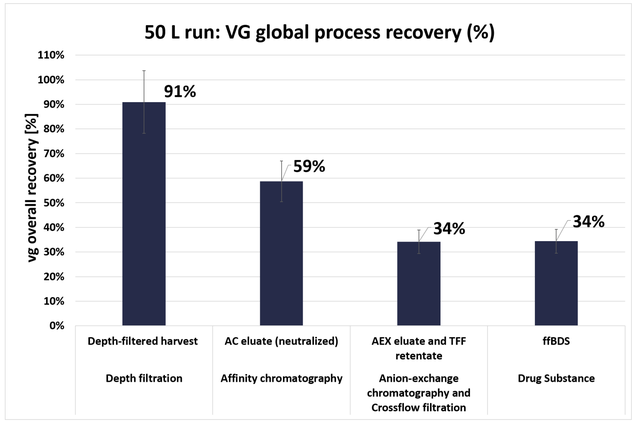


SDS-PAGE of filtered, formulated bulk drug substance (ffBDS) (six constructs shown).

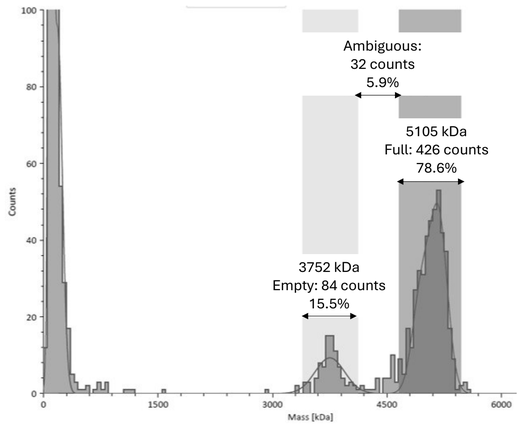
Workflow A	Client request	Result (filled ffBDS)	
VG titer	≥ 1.0E+12 vg/mL	>1.1E+12 vg/mL	✓
VG recovery (lysate to ffBDS)	N/A (focus on target vg titer and volume)	26%-45%	✓
Full-to-empty capsid ratio and enrichment factor	> 70% full capsids after purification	71%-90% full capsids in ffBDS; **2.1- to 3.2-fold enrichment** (25%-38% to 71%-90% full capsids)	✓
Formulation and total product volume	Iodixanol removed and formulated in 0.001% Poloxamer 188; volume ~ 1 mL	≥ 1 mL (total volumetric concentration factor: 48)	✓
Purification strategy	Parallel processing of different constructs in USP/DSP, 12 constructs in total	Up to 8 Ambr250 in parallel (USP), six constructs per DSP cycle (buffer exchange or UC): 2 cycles = 12 constructs purified	✓

* Data taken from representative Ambr®250 feasibility runs for a client.

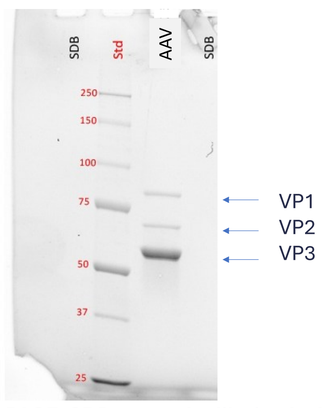
Workflow B* results summary



Vector genome global process recovery.



Mass photometry histogram of ffBDS.



SDS-PAGE: SDB = sample dilution buffer, Std = (molecular weight) standard (kDa).

Workflow B	Result (filled ffBDS)
VG recovery (lysate to ffBDS)	34%
Full-to-empty capsid enrichment factor	**1.8-fold enrichment** (44% post AC to 79% ffBDS)
VG titer	2.6E+13 vg/mL
Total vg (ITR-ddPCR)	6.0E+14 vg total
Endotoxin	0.074 EU/mL
Host-cell DNA (18S-ddPCR)	2.0E+05 18S copies/1E+12vg

* Data taken from a representative 50 L in-house TOX run for a client.

	Workflow A	Workflow B
Benefits	- Serotype agnostic – minimal optimization needed to prepare enriched full capsid material. - Robust separation with high purity. - Parallel processing of up to six preparations/constructs in one UC rotor. - Automated pre-UC volume reduction and post-UC buffer exchange and concentration. - Separation is driven by density differences between full and empty AAV capsids. - Workflow A can support design of Workflow B (e.g., buffer screening).	- Scalable, with potential for linear scale-up. - GMP-compatible. - Opportunity for adventitious viral clearance. - Basic purification (“one-step purification”) is feasible (AC only). - Various AC and AEX resins and membranes available (range: from scale down model with 0.2 mL columns and 50 cm^2 membranes to manufacturing scale). - Surface charge difference between full and empty AAV capsids results in separation.
Challenges	- Scale-out, rather than scale-up. - Laborious operation: Manual product collection (UC harvest). - Iodixanol absorbs in UV range (buffer exchange recommended).	- Serotype dependent – need to optimize construct-specific elution and peak collection strategy. - May be challenging to sufficiently enrich feeds with very low full capsid content (<10%). - AEX generally requires low ionic strength at unit operation start; buffer exchange or dilution is mandatory.

Summary

Comparative analysis across multiple serotypes demonstrated that the parallel UC workflow provides a reliable assessment of vector quality and full-to-empty capsid ratios. Workflow A enabled the simultaneous processing of up to six variants while substantially reducing turnaround times.

The two workflows are complementary and can be selected according to project requirements and timelines, allowing flexible support of client programs without compromising data quality or process robustness.

Furthermore, the integration of automated buffer exchange in Workflow A supported rapid formulation screening for product stability and helped inform the final TFF parameters used in Workflow B.

By offering orthogonal purification pathways, we provide a de-risked transition from early feasibility studies to large-scale manufacturing. This flexible framework helps meet therapy development timelines without compromising on the vector quality attributes, ultimately accelerating the delivery of AAV-based gene therapies to the clinic.

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