Development and Implementation of a High Yield and Scalable Manufacturing Process for Adeno-Associated Virus (AAV) Production

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BACKGROUND

Adeno-associated virus (AAV) vectors are widely used in gene therapy but remain challenging to manufacture at scale due to limitations in yield, robustness, process consistency, and cost. To address these challenges, we developed a high-yield AAV manufacturing platform centered on Solid Biosciences' proprietary dual-plasmid system and the POLARIS-101™ capsid (formerly known as AAV-SLB101).

Development Strategy

- Follow manufacturability principles
- Establish deep process knowledge & robustness around key parameters
- Consider inter-relationships between unit-ops
- Leverage HT or orthogonal analytics to inform performance/optimization

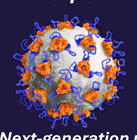
Approach

- Baseline development performed using a proprietary dual plasmid design & Solid's POLARIS-101 capsid with a transgene from a clinical program

Dual Plasmid

- Dual plasmid delivers up to 2.8x improvement in yield compared to triple for numerous capsid/GOI combinations

POLARIS-101 Novel Capsid



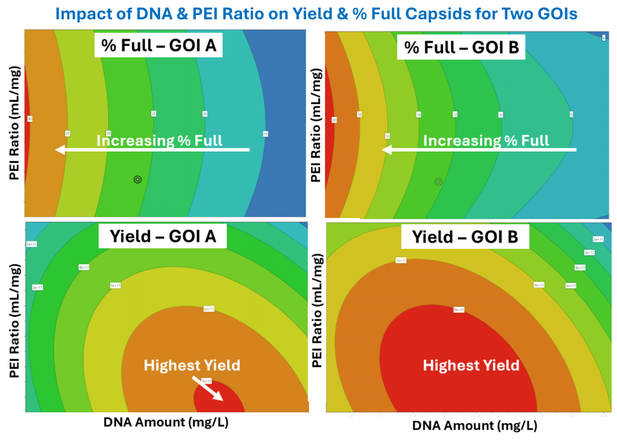
Next-generation muscle tropic AAV

- Deep & robust set of product quality data to benchmark performance
- Established & robust set of analytical methods available
- POLARIS-101 capsid being used in multiple pipeline programs at Solid

UPSTREAM DEVELOPMENT

Understanding Levers & Knowledge Around Critical Upstream Operations

Impact of DNA & PEI Ratio on Yield & % Full Capsids for Two GOIs

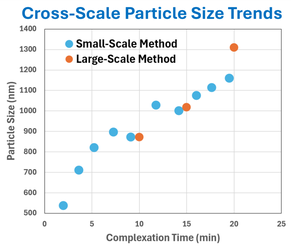


✓Yield and % full capsids are at odds – need to sacrifice one for the other

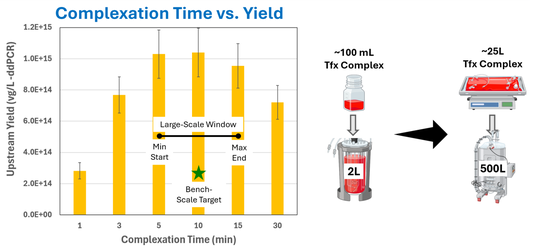
✓Relationships between DNA concentration & PEI ratio are similar, but optimal ranges are different. These ratios will need to be determined for each program

Leveraging HT Analytics to Ensure Successful Transfection Scaleup

Cross-Scale Particle Size Trends



Complexation Time vs. Yield



✓Optimized at-scale complexation to match particle size trends

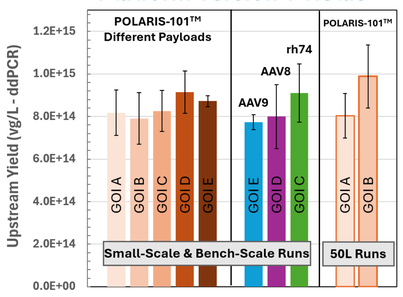
✓Determined addition start time & flow rates based on pre-defined optimal complexation windows

✓Confirmed at-scale shear rates didn't influence particle size at flow rates that deliver complex within optimal time window

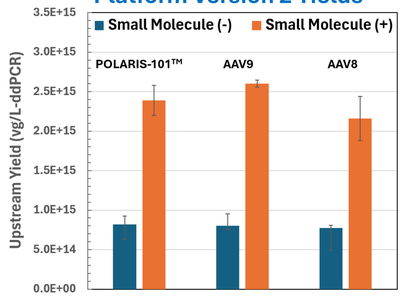
✓Established defined ranges around temperature of reagents (diluent, DNA, PEI) & complexation conditions

Established a High Yielding, Scalable, and Flexible Upstream Process

Platform Version 1 Yields



Platform Version 2 Yields



✓Upstream performance demonstrates consistent & high yields independent of scale, capsid, or payload

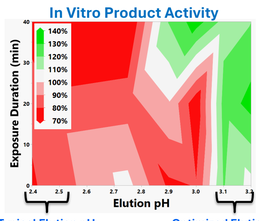
✓Platform Version 2 delivered yield improvements of 2 – >3x and seamlessly integrates into upstream/downstream process

DOWNSTREAM DEVELOPMENT

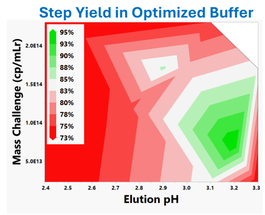
Using Product Quality, Robustness, and Manufacturability to Guide Development

✓Optimized affinity elution buffer enables high recoveries at an elution pH range that doesn't impact product activity

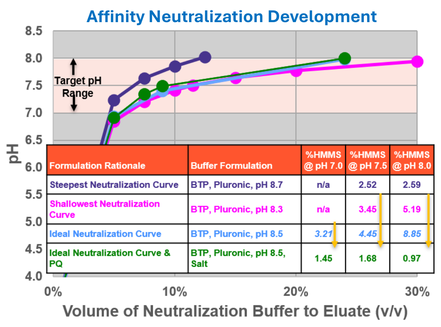
In Vitro Product Activity



Step Yield in Optimized Buffer



Affinity Neutralization Development



Formulation Rationale	Buffer Formulation	%HMMS @ pH 7.0	%HMMS @ pH 7.5	%HMMS @ pH 8.0
Steepest Neutralization Curve	BTP, Pluronic, pH 8.7	n/a	2.52	2.59
Shallowest Neutralization Curve	BTP, Pluronic, pH 8.3	n/a	3.45	5.19
Ideal Neutralization Curve	BTP, Pluronic, pH 8.5	*3.21*	*4.45*	*8.85*
Ideal Neutralization Curve & PQ	BTP, Pluronic, pH 8.5, Salt	1.45	1.68	0.97

✓Direct eluate collection into neutralization buffer minimizes low pH exposure

✓Shallow neutralization curve enables flexibility on manufacturing floor

✓Buffer components optimized to limit formation of high-molecular-weight species

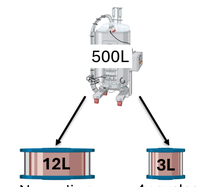
Significant Improvements to Process CoGs & Efficiency with Affinity Resin Cycling & Ultracentrifugation Load Volume Reduction

✓Benefit to CoGs & Sizing Flexibility with Resin Cycling

Affinity Resin Cycling Study Design

NT = Not tested	Cycle Number								
	1	2	3	4	5	6	7	8	9
Product Recovery	X	X	X	NT	X	NT	X	X	X
Impurity Clearance	X	NT	X	NT	X	NT	X	NT	X
Pressure vs. Flow	NT	NT	X	NT	NT	X	NT	NT	X
Binding Capacity	NT	NT	NT	NT	NT	NT	NT	NT	X

*Development work performed to optimize cleaning procedure to prevent product carryover & confirmed at several points throughout cycling study



✓Reduced UC Spin Volume via HD Tube Loading

Standard density UC tube loading

High density UC tube loading

✓Pushed the limit on TFF retentate capsid titer to determine max concentration

✓Identified optimal TFF membrane MWCO

✓Confirmed no impact to PQ, stability, follow-on unit ops at pre-defined capsid titer limit

✓Performed TMP excursion studies to determine robust operating ranges & ensure scalability

Innovations in Ultracentrifugation to Improve Efficiency, Accuracy, Consistency, and Quality

✓Significant improvements to efficiency & throughput of UC operations with optimized spin conditions

Comparison of UC Spin Methods

	Manual	Automated
Spin Time/Rotor	≥18 hrs	≥6 hrs
Tube Volume	39 mL	36.2 mL
Max Rotor Slots	12	12
Total Volume/Rotor	468 mL	434 mL
Extraction Time per Tube	~5 min	~30 sec

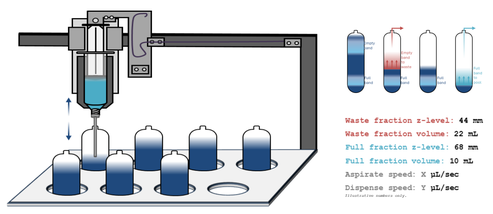
Estimated Mfg Suite Operational Time*

Total Spin Time	180 hrs	60 hrs
Total Collection Time**	9 hrs	1 hrs

*500L bioreactor at 2.5E12 vg/mL & 25% full capsids

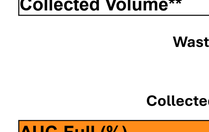
**Total duration for full band extraction & collection

✓Purpose built liquid handler enables precise, accurate, and consistent collection of full capsids from UC tubes



✓Automated collection allows for highly controllable band extraction location and volume to maximize product quality.

CsCl RI*	1.3685		1.3690	
Collected Volume**	9	11	9	11
AUC Full (%)	89.6	83.5	93.9	88.3
AUC Partial (%)	9.1	14.4	4.2	9.0
HEK DNA (pg/1E9 vg)	8.6	12.1	11.4	10.5



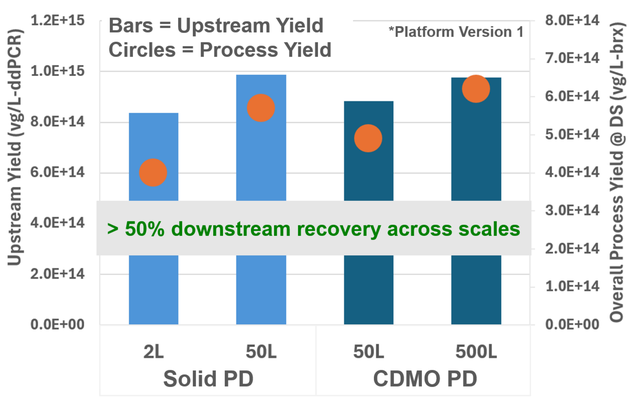
*Refractive Index; **Extracted full band volume

PROGRAM IMPLEMENTATION, TRANSFER, & SCALEUP

Successful Transfer, Scaleup, and Implementation of Platform Process for Solid's SGT-601 Program (POLARIS-101) capsid with TNNT2 transgene

✓Equivalent upstream yields, downstream recoveries, and drug substance product quality across scales & sites

Cross-Scale & Cross-Site Process Yields



Product Quality at Drug Substance

Assay	Unit	2L	50L	50L	500L
HEK HCP	ng/mL	< 4	< 4	< 4	< 4
HEK DNA	pg/1E9 vg	7	10	18	17
AUC	% Full	89.2	77.7	84.1	73.6
	% Partial	6	15.5	11.8	19.4
	% Empty	1.3	1.9	1.1	2.1
In Vitro Activity	% Relative to Control	NT	100	80	100

**Cross-scale/site consistency challenges due to manual band extraction*

CONCLUSIONS

Solid has established a high-performance AAV manufacturing platform for POLARIS-101 that delivers robust yield, quality, and scalability to meet therapeutic demand

- Capable of achieving purified process yields up to 1.35E15 vg/L-brx at 500L scale, enough for >100 adult patient doses at 8E13 vg/kg
- >200 process parameters have been experimentally defined, with extensive process knowledge and robustness established for 30–40 key parameters
- Deep process understanding drives consistent performance and enables more efficient late-stage development
- Proprietary formulation provides excellent product stability
- Plug-and-play platform knowledge enables rapid deployment across programs using the POLARIS-101 capsid

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