

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

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AFFILIATIONS: 1. Solid Biosciences Inc.

SESSION: Scale-up, scale-out, formulation, supply chains, quality, and logistics I



Disclosures

- I am a full-time employee at Solid Biosciences

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Platform Development Strategy, Goals, & Approach

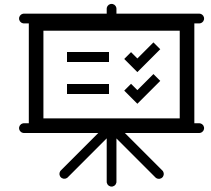
Development Strategy

- Follow manufacturability principles
- Establish deep process knowledge & robustness around key parameters
- Adjust development complexity based on criticality of operation/parameter
- Consider inter-relationships between unit-ops
- Leverage HT or orthogonal analytics to inform performance/optimization



Performance Goals

1. > 5E14 vg/L USP, > 40% DSP Recovery
2. Compatible with multiple capsid/GOI combinations
3. Achieve product quality requirements of a high-dose GT program

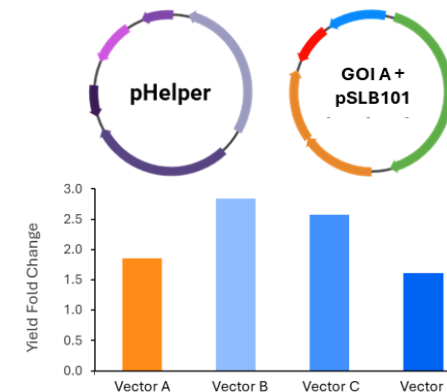


Approach

- Baseline Platform development performed using Solid's POLARIS-101™ capsid & a transgene from a clinical program:
 - Strong set of historical product quality data to benchmark performance
 - Established & robust set of analytical methods available
 - POLARIS-101™ capsid being used in multiple pipeline programs at Solid

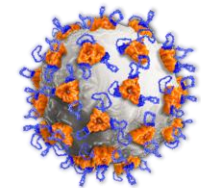
Solid's Dual Plasmid

Up to 2.8x yield improvement over triple plasmid independent of capsid/GOI



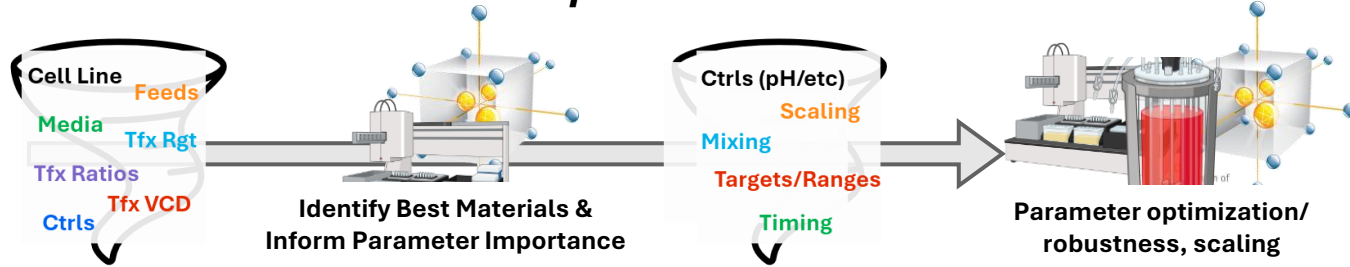
POLARIS-101™ Novel Capsid

Next-generation muscle topic AAV

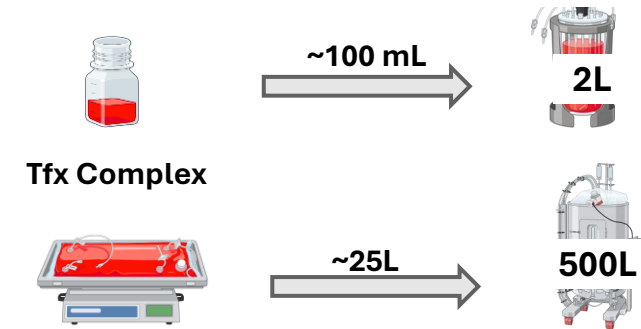


Building Foundational Knowledge at Small-Scale is Critical for Successful Performance Between Programs & Scales

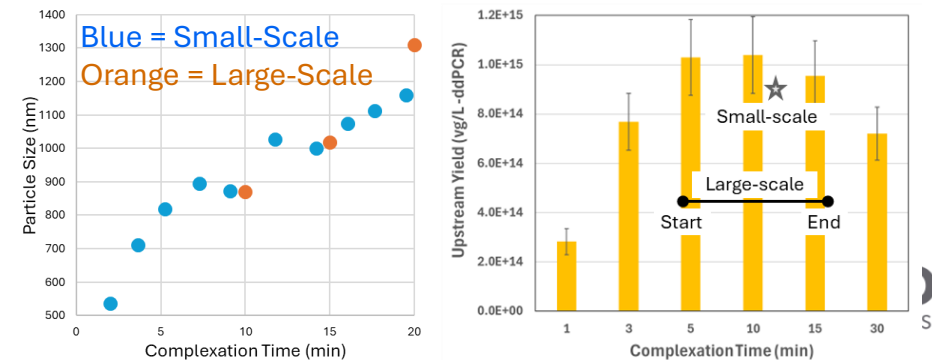
Baseline Platform Development Tools & Workflow



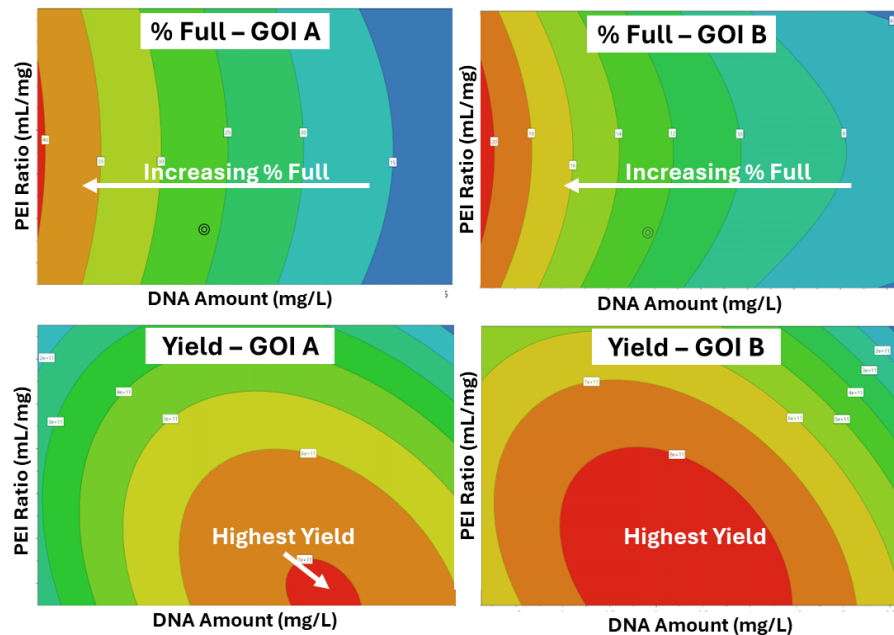
Transfection Scaleup – Ensuring Success



- ✓ 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



Understanding Levers & Building Knowledge Around Critical Upstream Operations



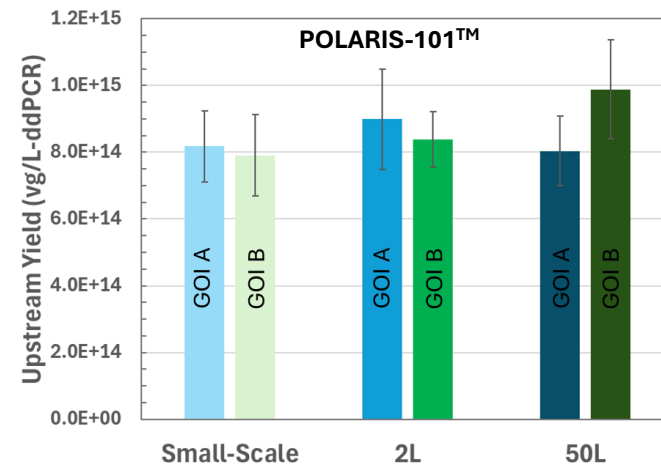
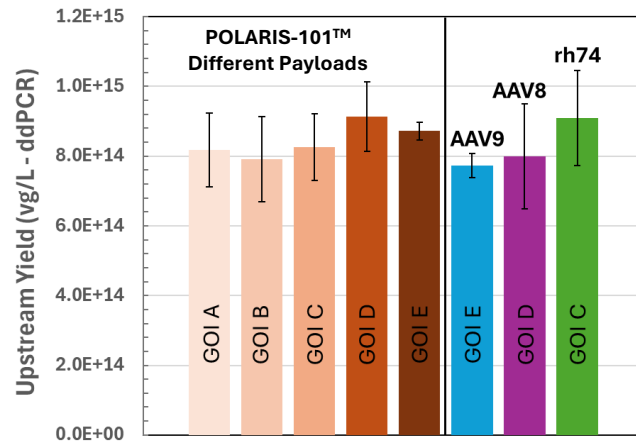
- Optimal quantities/ratios & magnitude of output differ from one GOI to another – need to tweak for each program
- Yield & % Full are generally at odds with each other – need to pick one

Established a High Yielding, Scalable, & Flexible Upstream Process

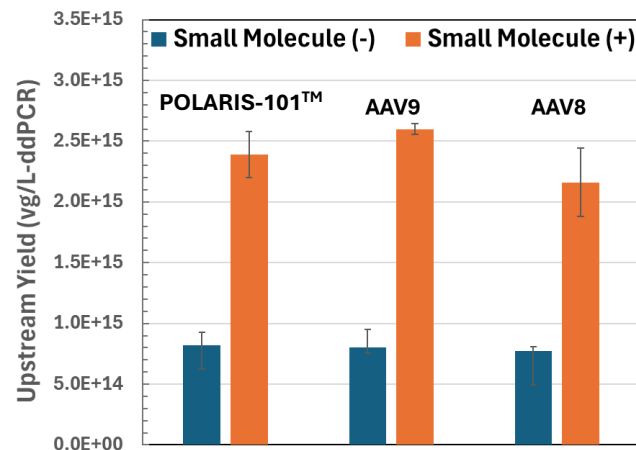
Leverage historical knowledge & innovation to improve performance & understanding

High Yielding & Scalable Upstream Process Compatible with Numerous Capsids & GOIs

Platform Version 1

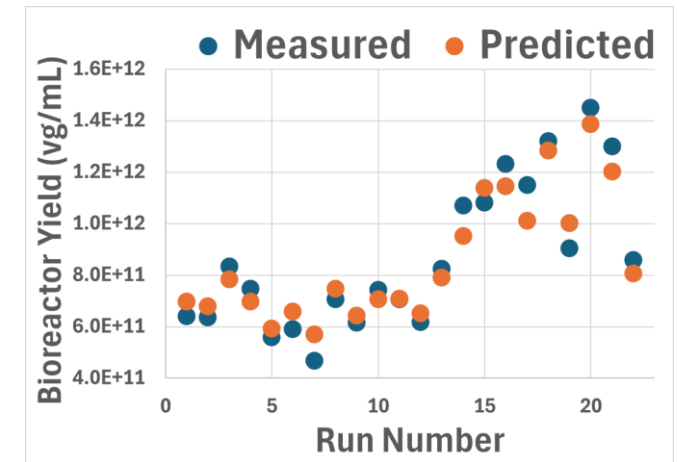


Platform Version 2 - >3x yield improvement over Version 1



- Platform Version 2 designed for seamless integration into established process

Using Historical Data to Improve Knowledge & Performance*



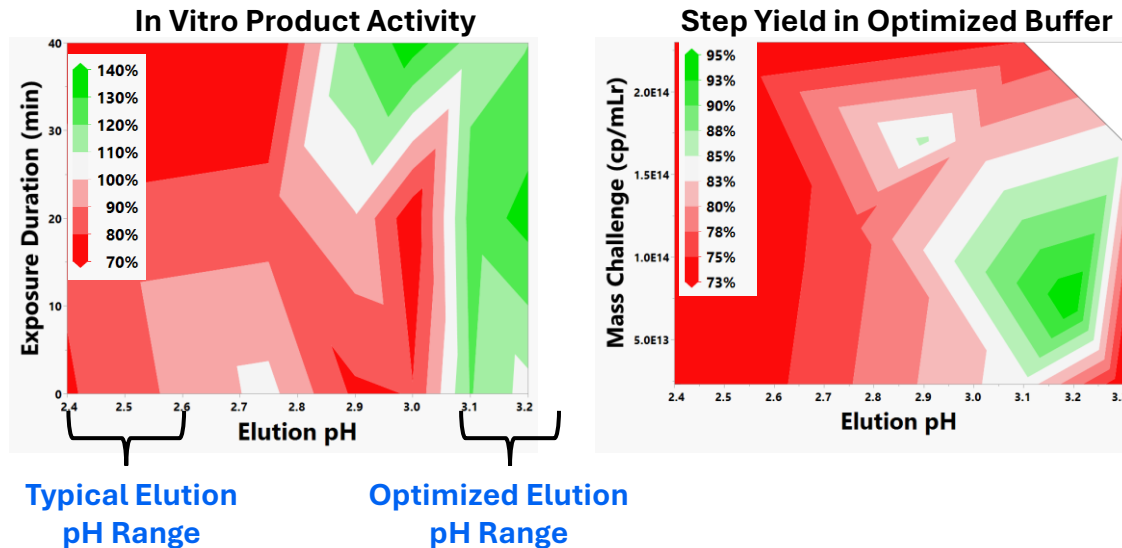
- Machine Learning & Predictive Modeling efforts towards optimizing parameters for improved yield/quality using historical data

* For more info, see oral talk titled “Utilizing machine learning and mechanistic understanding to appreciate the impact of pH, DO, and pCO₂ on upstream AAV yield & product quality”

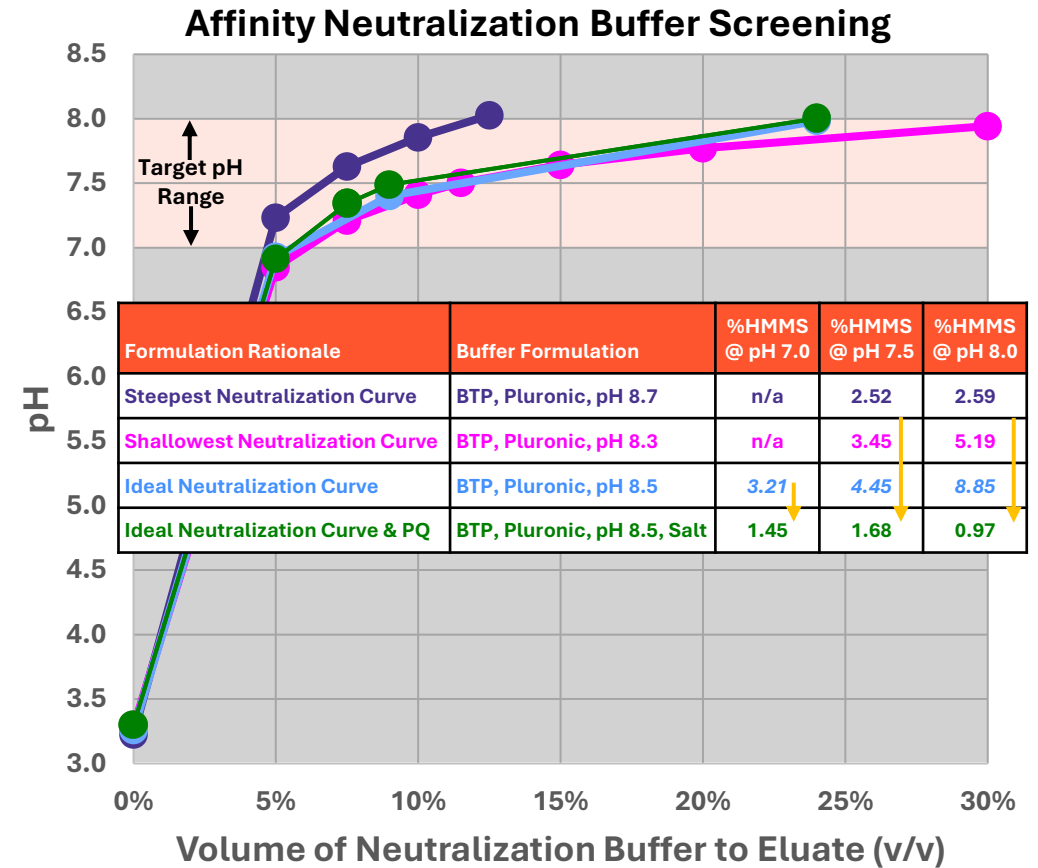
Building Upfront Robustness, Understanding, & Quality into the Process

Affinity Elution & Affinity Neutralization - Examples

Maximizing Product Recovery & Product Activity through Elution Buffer Optimization



- Buffer formulation is key to improving Affinity recovery at an elution pH that doesn't degrade product activity



Product Quality, Manufacturability, and Robustness Driven Development

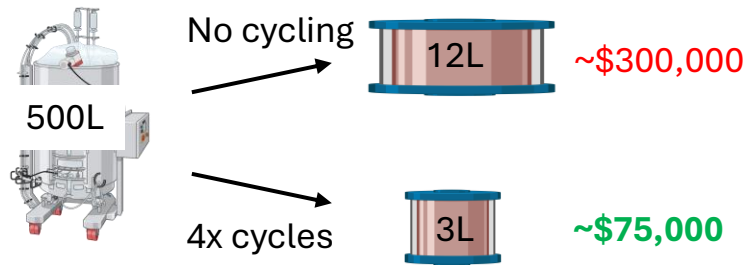
Significant Improvements to Process CoGs & Efficiency with Affinity Resin Cycling & Ultracentrifugation Load Volume Reduction

Building a downstream process that doesn't act as choke point for high upstream yields

Resin Cycling Study Design

		Cycle Number								
		1	2	3	4	5	6	7	8	9
Measurement	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

* Upfront development work performed to optimize cleaning procedure to prevent product carryover. Confirmed at several points throughout cycling study

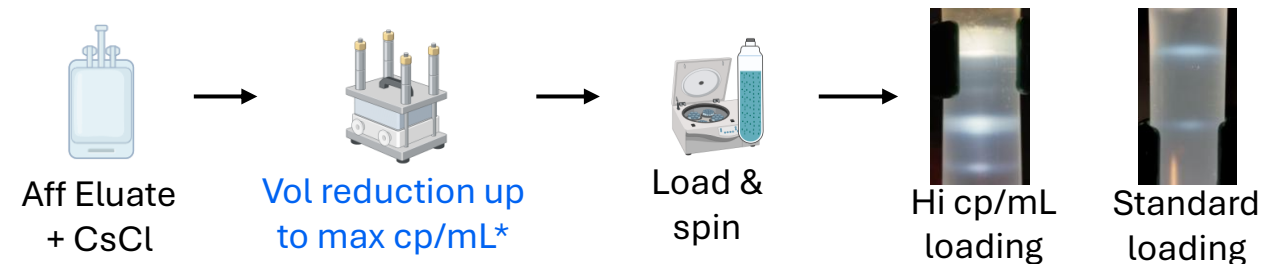


Significant Benefit to CoGs & Column Sizing Flexibility with Resin Cycling

Reducing Load Volume & Maximizing Flexibility at the Ultracentrifugation Step

Approach to UC Volume Reduction

- ✓ 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



* Actual concentration target determined based on facility and/or process volume needs

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

Platform Performance Assessment Workflow

Stage 1: HT Optimization *SGT-601 Candidate*



Activities

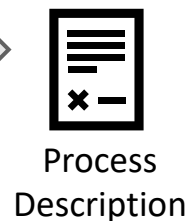
- Tfx optimize, yield estimate
- MS & Affinity recovery red flags
- Affinity EL PQ as necessary

Stage 2: E2E Processing



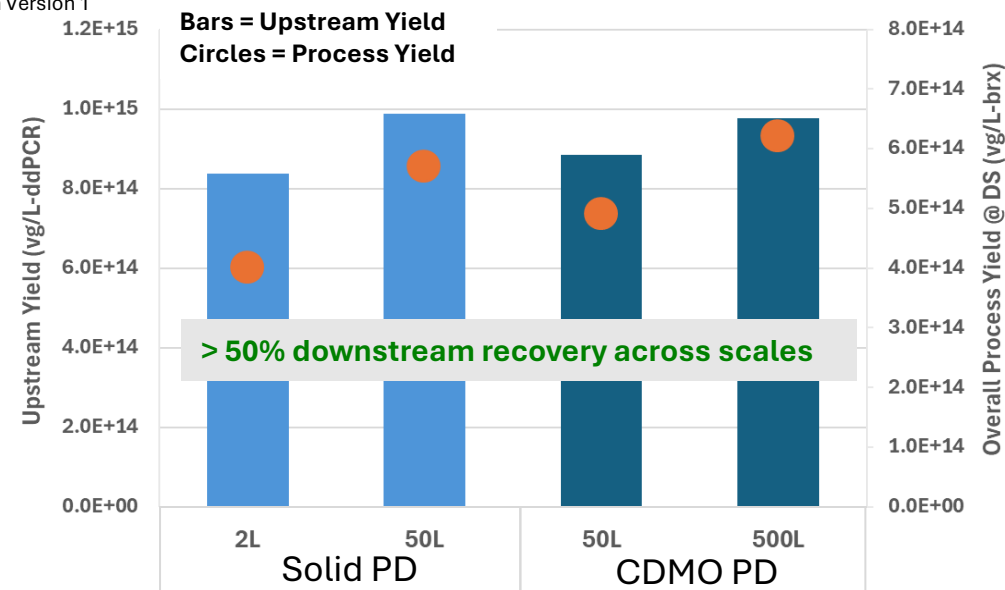
Activities:

- Material generation (AD, etc)
- Yields/recovery, impurity removal
- Targeted IP pool & DS stability
- As-needed tuning/optimization



SGT-601 Transfer & Scaleup

*Platform Version 1



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

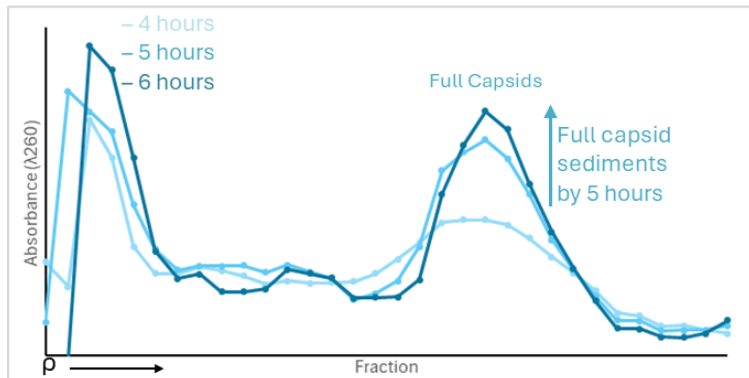
*Consistency challenges with manual UC band extraction



Next Generation Ultracentrifugation

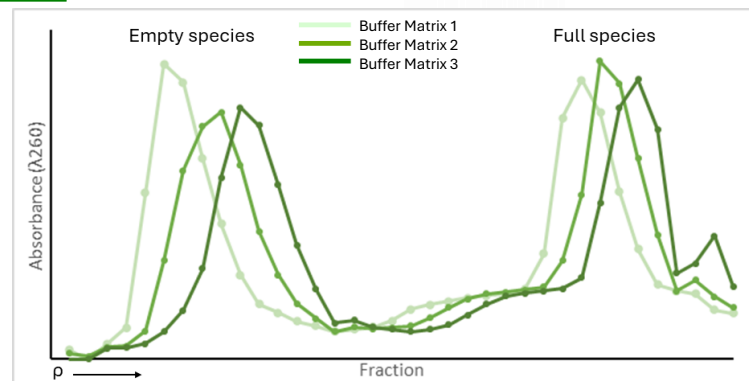
Solving bottlenecks while building in Precision, Throughput, and Accuracy

Throughput



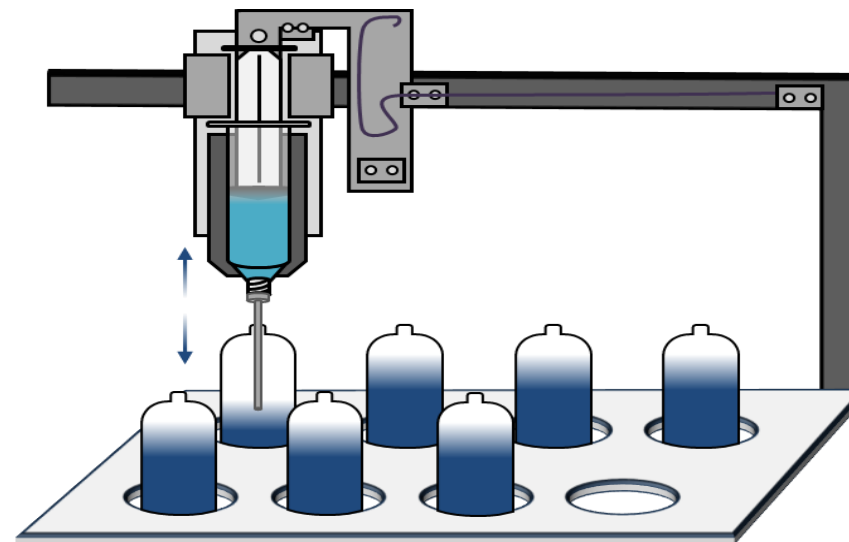
Optimized tube design & spin strategy reduces spin time from 18 to 6 hours

Precision

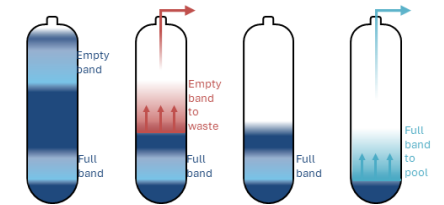


Controlling the UC matrix components results in highly precise & reproducible band migration

Accuracy



Purpose built liquid handler for automated band extraction of UC tubes



Waste fraction z-level: 44 mm
Waste fraction volume: 22 mL
Full fraction z-level: 68 mm
Full fraction volume: 10 mL
Aspirate speed: X $\mu\text{L}/\text{sec}$
Dispense speed: Y $\mu\text{L}/\text{sec}$
Illustrative numbers only.

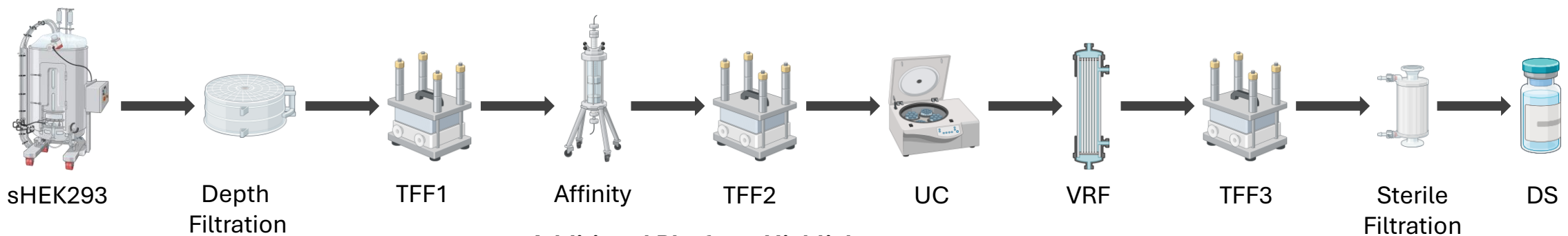
Load Volume Reduction + Improved Tube/Spin Strategy + Automated Band Extraction = Significant Opportunity to Make Ultracentrifugation a Scalable, Robust, and Consistent Enrichment Method

A High-Performance Platform Process that Delivers Superior Yield & Quality to Meet Therapeutic Demand

- Scalable & efficient AAV production Platform capable of high-quality purified process yields of 1.3E15 vg/L-bioreactor
 - 500L = 6.3E17 total vg's; > 100 adult patients @ 8E13 vg/kg dose
- Robust, efficient, consistent, and well-understood process due to intense front-loaded development effort
 - > 200 process parameters experimentally defined, 30 – 40 with extensive knowledge & robustness established for consistent yield & quality
 - Extensive process knowledge may be leveraged for more efficient late-stage activities
- Proprietary formulation buffer with excellent stability profiles

Plug-n-play & direct process knowledge application for programs using POLARIS-101™ capsids

Process Flow



Additional Platform Highlights

- P_{Max} & V_{Max} for DSP membranes, TMP excursions for TFF
- Stability established downstream hold steps
- Nominal titer DS processing strategy
- Optional hold step at TFF1 to freeze/pool multiple USP lots

Thank you!

