Enhancing Upstream Processes for High-Yield, High-Quality AAV Vector Production Using a Novel In-House Cell Line

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Presentation Overview

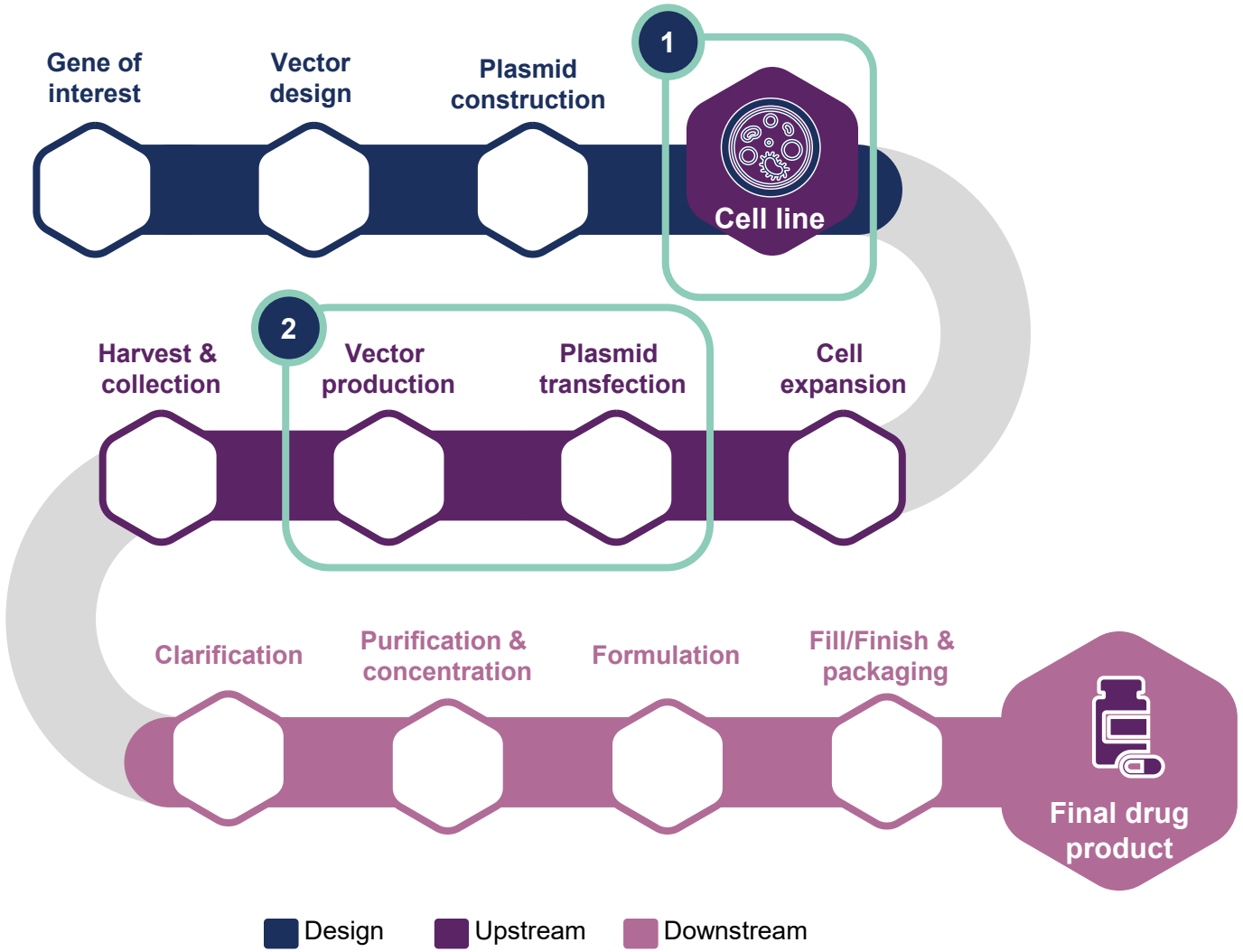
1. OXB's AAV Platform
2. Approach to Upstream Process Enhancement
3. Developing an In-House Cell Line for AAV
4. 'Plug-n-Playability' of new Upstream Platform

Unique competitive positioning

- **Best-in-class capabilities** across AAV, lentivirus & other vector types
- **Trusted by global industry leaders** – successful collaborations with big pharma, established biotech and emerging biotech
- **State-of-the-art facilities & scalable production capabilities** designed to meet the growing demand for C&GTs
- **Deep scientific know-how** – a team of world-leading specialists in viral vector optimisation
- **Cutting-edge technology** – leveraging 30 years of insights to enhance speed, efficacy, quality and safety in new therapies
- **Global reach & strategic positioning** with manufacturing facilities located in key biotech hubs

30	960+	45+	30+	65+
Years of manufacturing experience	Successful GMP batches since 2014	Client programmes	IND submissions	Successful audits

OXB's AAV Platform



Upstream Optimization Focuses

1 Cell Line

- Current platform uses commercially available HEK293 cell line for highly robust, high-titer AAV production
- **Goal: develop in-house cell line with equivalent productivity and product quality**

2 Transfection and Production

- Always evaluating new materials and methods to remain best-in-class
- Transfection practices and parameters are robust, cost-effective, and scalable
- ***Plug and Play!*** Platform must be optimized to fit both triple and dual transfection across all capsid serotypes

Creating an Enhanced, Next-Generation Upstream Process

Conducted process development work on multiple Upstream factors, notably:

Transfection Reagent

Bench-scale screening to identify best commercially available reagent

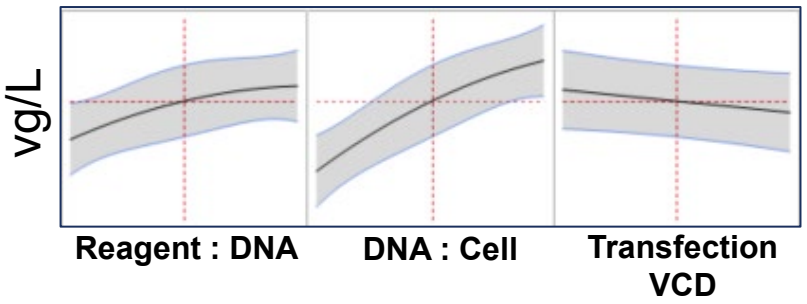
Top candidate selected for **titer, percent full capsids, reduced batch costs, and transfection complex stability**

Transfection Parameters

Full factorial DOE to optimize:

- **Transfection reagent : DNA ratio**
- **DNA : Cell ratio**
- **Transfection Cell Density**

Ensured setpoints and ranges appropriate for large scale MFG



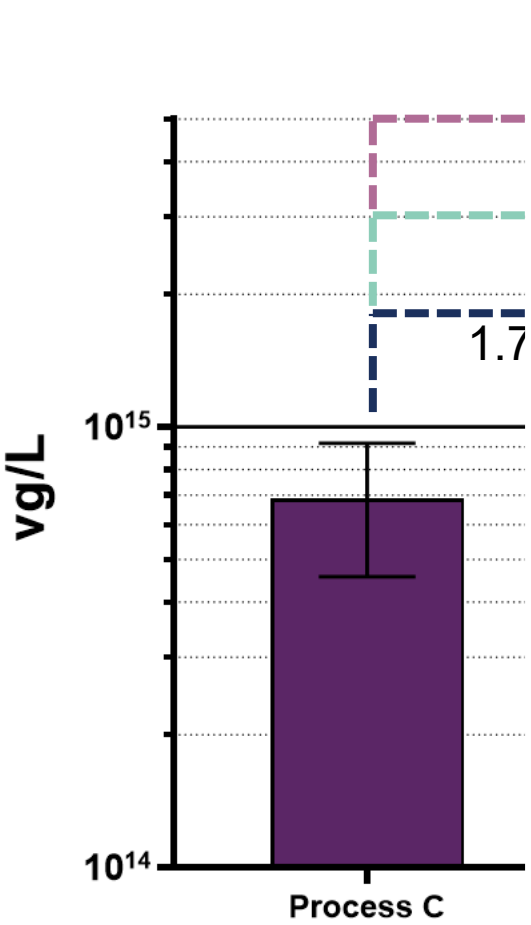
Process Additives

Identified commercially available additive to boost vg productivity

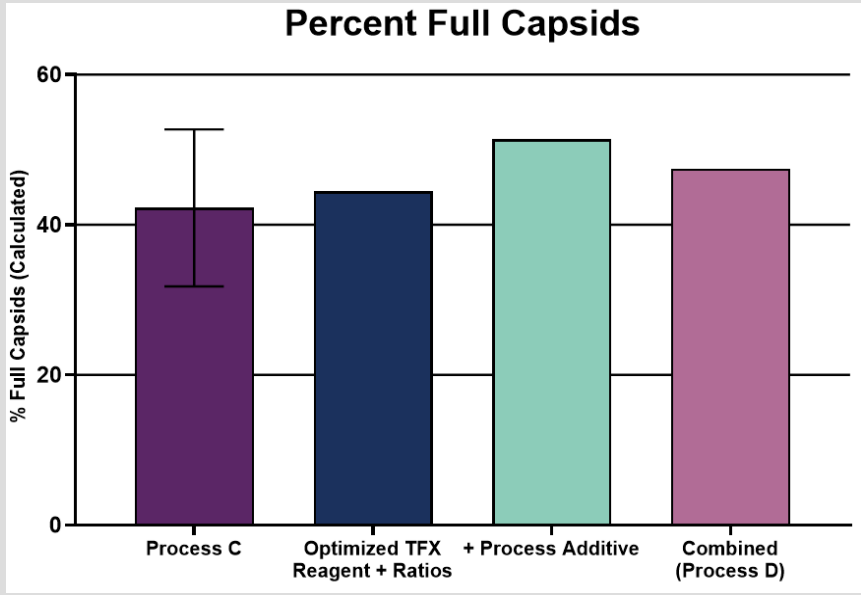
Optimized **addition amount** and **addition timing** for maximum yield and product quality

3.2x Titer Improvement from Upstream Process Optimization

AAV9 Dual Transfection; 2L Bioreactor Scale



Comparable packaging seen with each process improvement

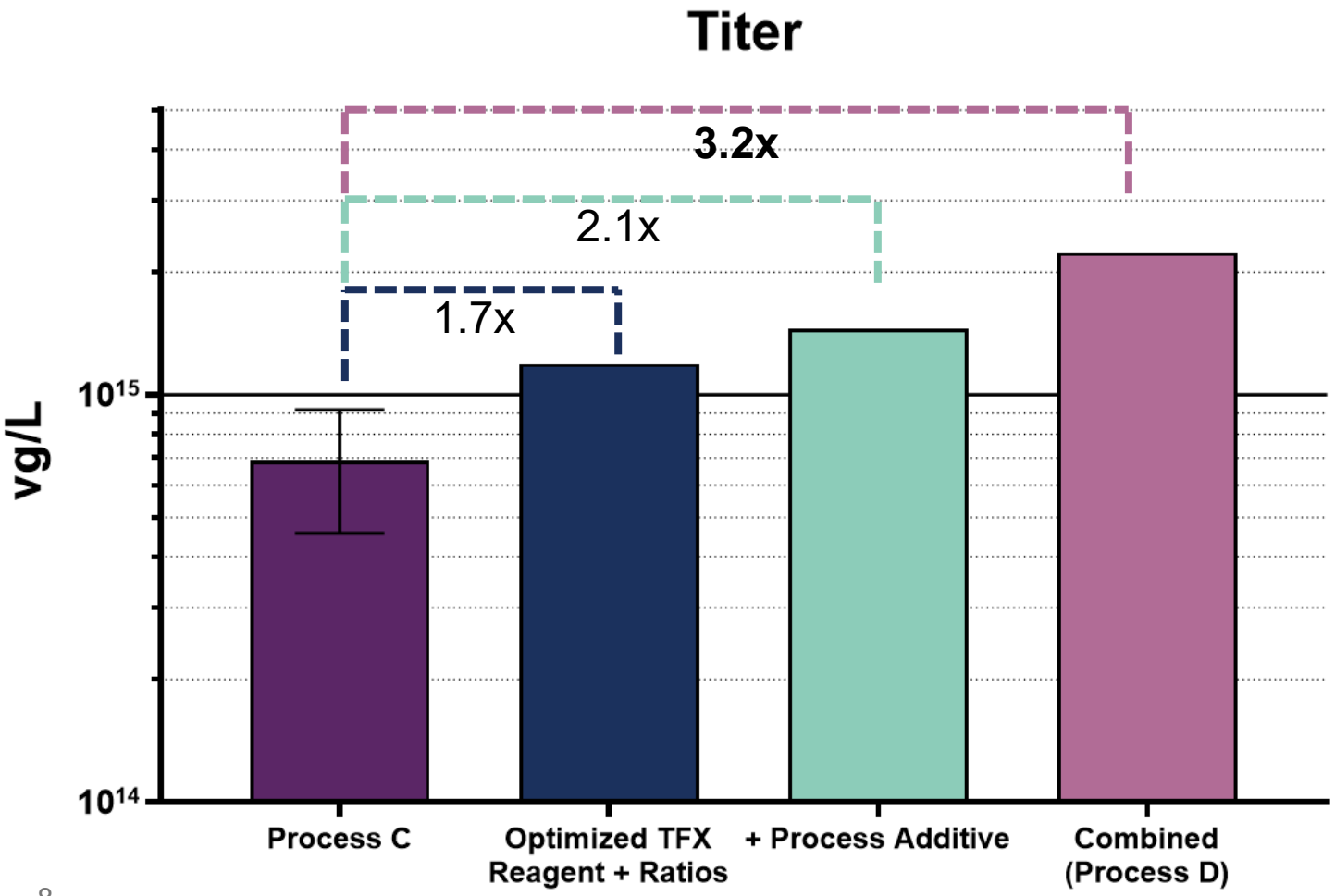


Plug-and-Playability

Will this new Upstream platform fit multiple cell lines and serotypes?

3.2x Titer Improvement from Upstream Process Optimization

AAV9 Dual Transfection; 2L Bioreactor Scale



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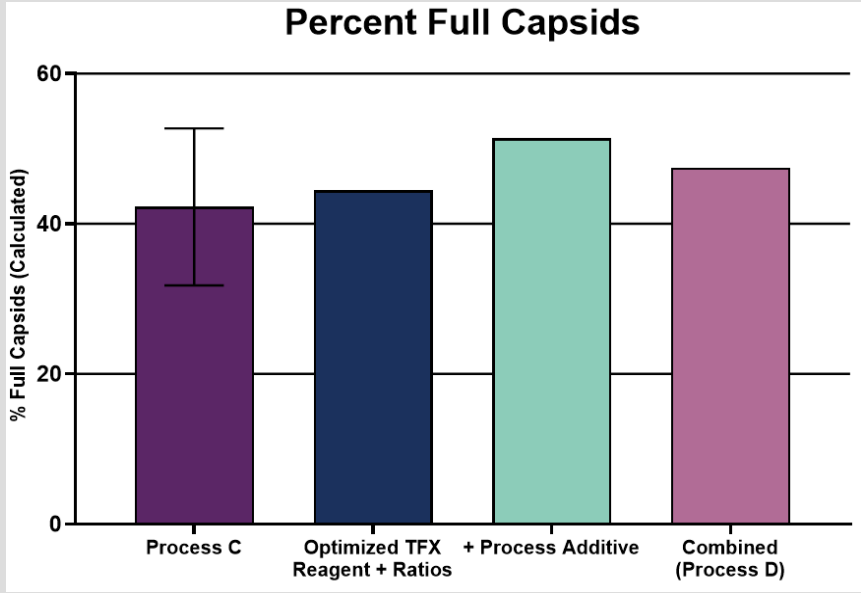


Plug-and-Playability

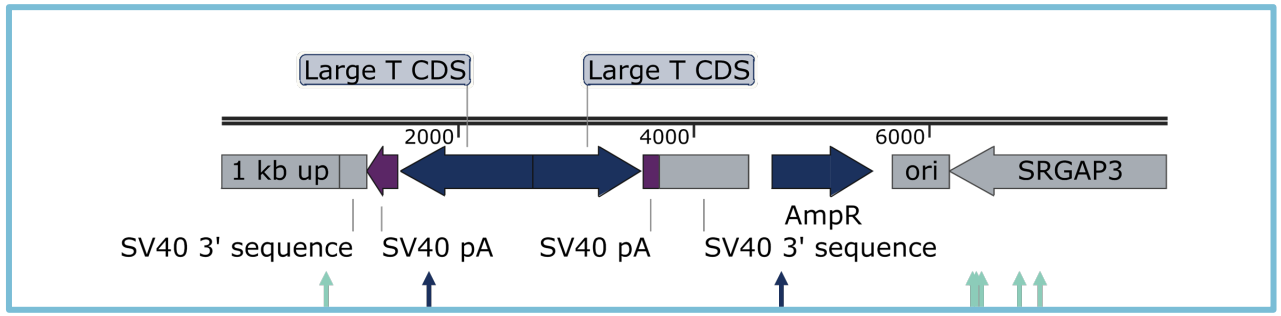
Will this new Upstream platform fit multiple cell lines and serotypes?

Creation of an Internal OXB Cell Line for AAV Production

Parental HEK293T Cell Line

HEX1S.86

Used in OXB's Lentivirus platform



SV40T Removal and Screening

Mad7 and crRNA Plasmid Delivery
Transfected into OXBHEX1S.86

Single Cell Printing
Clone monitoring and monoclonality screening

HTP Clone Screening
AAV production & SV40T PCR

Top Candidates Screening
6DWP Evaluation & targeted sequencing

Mass Spec & DNA Sequencing
SV40T sequence removal confirmation

Lead Clone Selection:
HEX2s52

Final OXB Cell Line

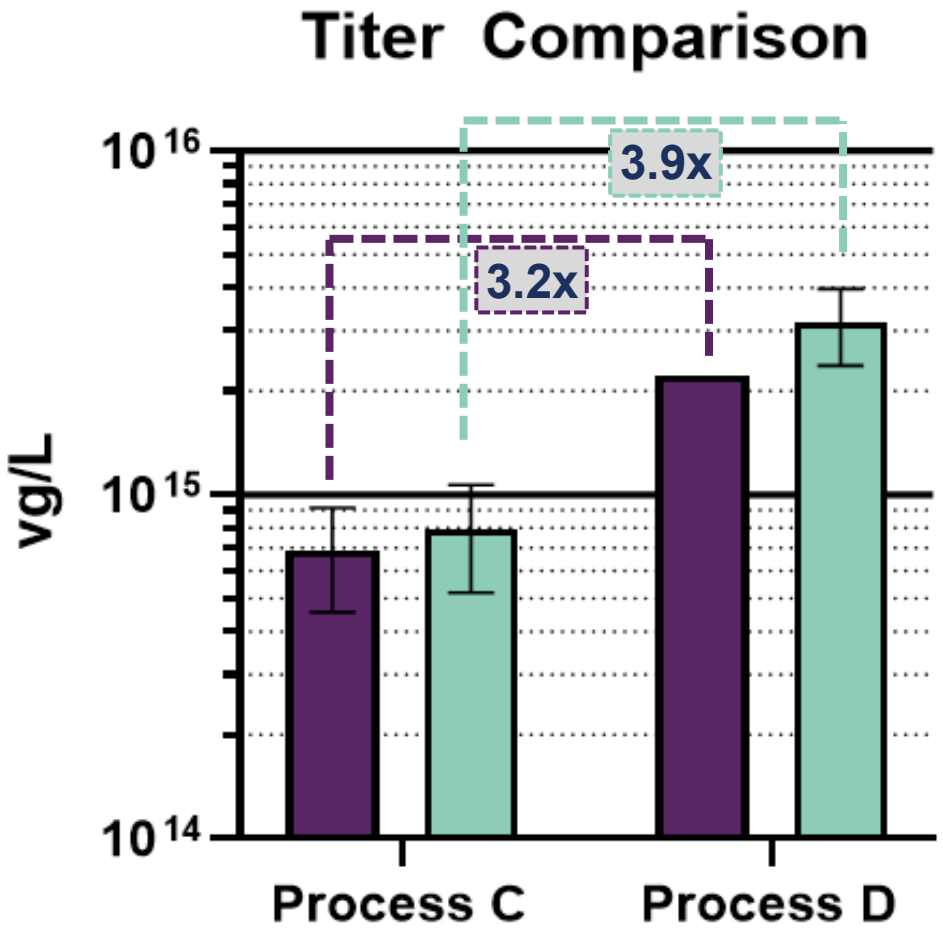
HEX2s52

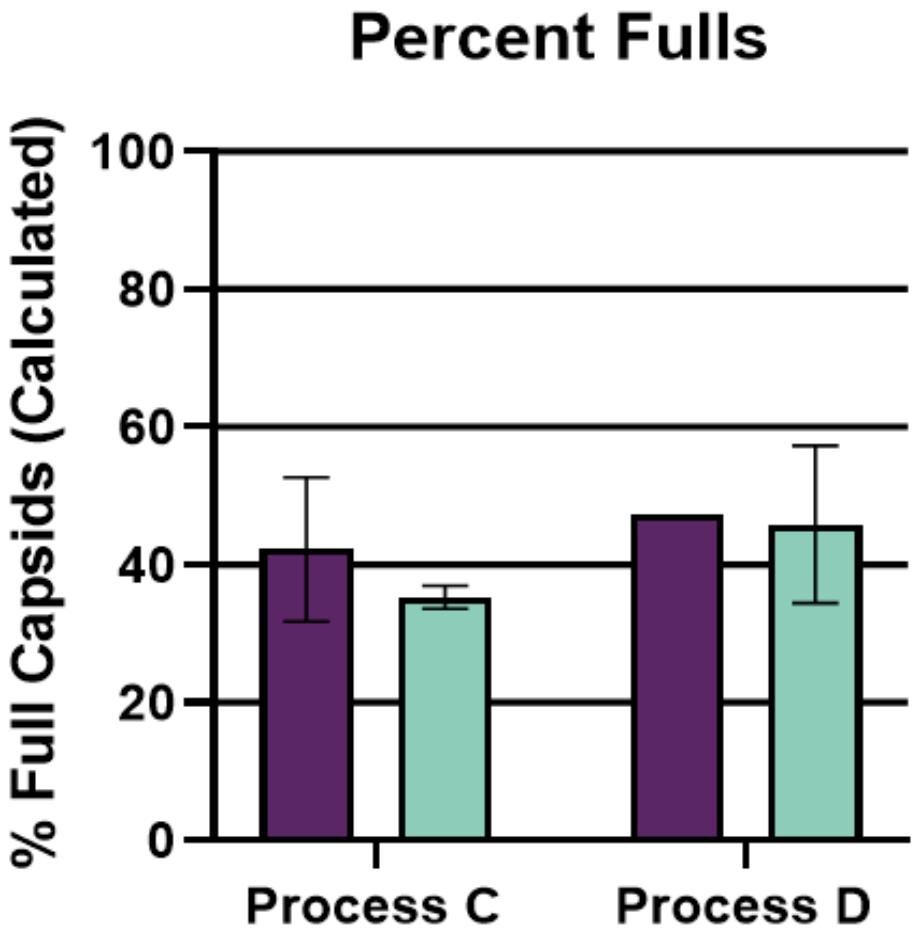
Whole Genome Sequencing

No SV40T or Mad7 Detection

Enhanced Upstream Process Improves Titer in Both Cell Lines

AAV9 Dual Transfection; 2L Bioreactor Scale



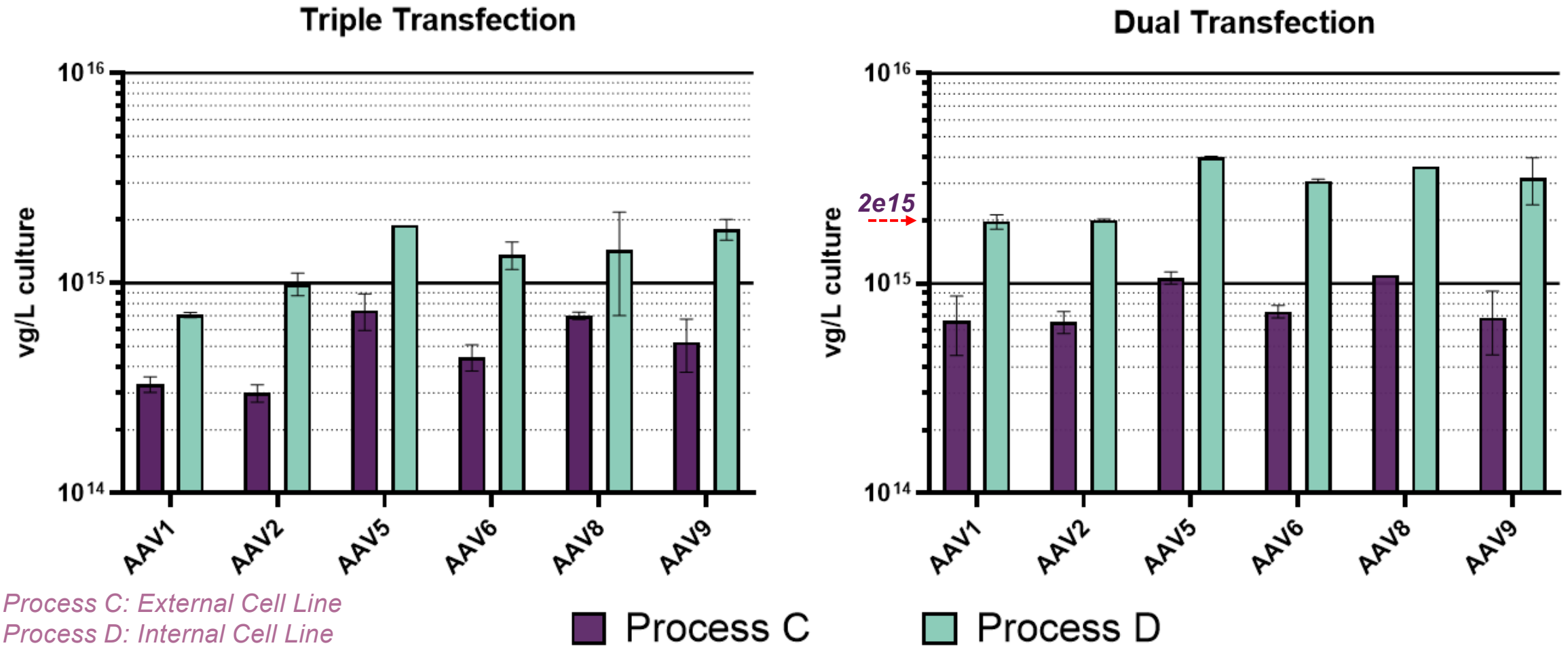


External Cell Line

Internal Cell Line

Enhanced Upstream Process Improves Titer In Multiple Serotypes

Increased performance from dual transfection seen in **both cell lines and platforms**



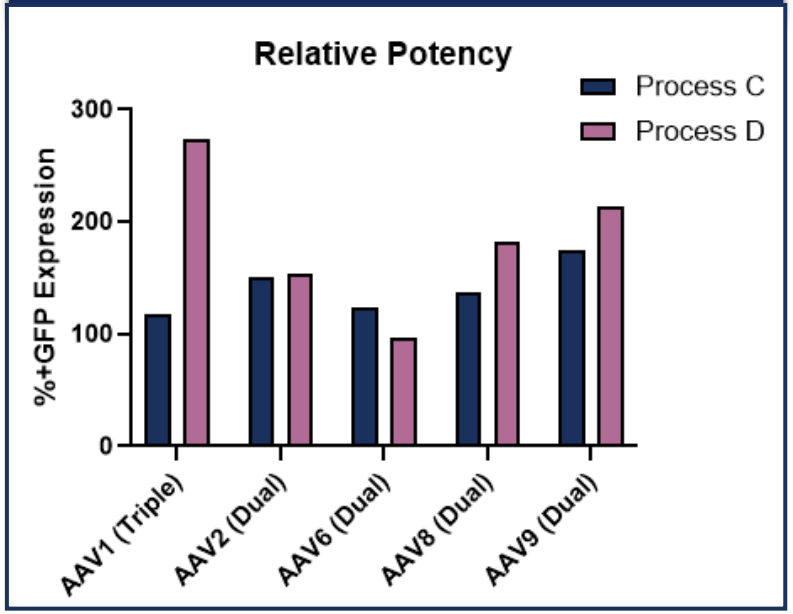
Process C: External Cell Line
Process D: Internal Cell Line
All 2L Bioreactor Scale Data

2e15 vg/L or higher in all 6 tested serotypes for dual transfection

Good Product Quality Maintained Regardless of Process or Cell

Final DS		Process C			Process D
		External Cell Line	HEX2s52		HEX2s52
VP Ratio		1:1:10	1:1:8		1:1:8
AUC	%Empty	13.5%	7.2%		10.0%
	%Partial	7.2%	12.1%		10.0%
	%Full	**79.3%**	**80.7%**		**80.0%**
Aggregation		0.8%	0.2%		0.4%
Host-Cell Protein (ng/1E13 vg)		BLOQ	BLOQ		BLOQ
hcDNA (ng/1E13 vg)		6.4	25.7		4.6
Residual RepCap DNA (copies/1E13 vg)		4.3E9	BLOQ		5.2E9
Residual pHelper DNA (copies/1E13 vg)		5.0E8	4.5E8		2.1E9
Residual Ad E1A DNA (copies/1E13 vg)		BLOQ	BLOQ		BLOQ

Potency comparable or improved in all serotypes for Process D



Summary

1. Upstream platform enhanced via new transfection materials and parameter optimization

2. In-house cell line engineered to fit upstream process and produce equivalent titer and PQ

3. Optimized Upstream “Process D” shown to be plug-and-play with multiple cell lines and serotypes, consistently achieving >2e15 vg/L

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