Scalable Production of an AAV Manufacturing Platform: Transitioning from Adherent to Suspension-Based Triple Transfection

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Abstract

Adeno-associated virus (AAV) gene therapy holds immense potential for addressing genetic causes of frontotemporal dementia (FTD). Clinical supply of Passage Bio's PBFT02, an AAV1-based gene therapy candidate for treating FTD-*GRN* and FTD-*C9orf72*, has been produced to date using a triple transfection adherent process in HEK293 cells (Process A). A high-productivity, suspension-based process (Process B) was developed to improve manufacturing scalability and efficiency.

This presentation describes the output of development activities that enabled the adaptation of HEK293 cells to a suspension process under serum and animal component-free conditions and highlights the optimization of key process parameters to maximize yield, including cell density at transfection, DNA ratios, transfection reagents, and harvest time.

The suspension process conducted at a 200-liter scale demonstrated a significant increase in vector yield per cell (vg/cell) and more than a 20-fold increase in total batch yield compared to the adherent process. Furthermore, key quality attributes were observed to be comparable or improved.

The successful demonstration of this suspension-based triple transfection process is a significant milestone in the development of PBFT02, as it enables a commercially viable process that is both robust and efficient. Additionally, this highly productive and adaptable suspension process represents a significant step towards establishing a scalable platform for the manufacturing of future AAV products.

Process B Dev. Goals

- Process changes can be evaluated within an analytical comparability protocol
- Process controls are suitable for a robust commercial process
- Meet or exceed the following performance goals:

Attribute	Proc A	Proc B Goal	Results
Batch Yield (Doses)[1]	50	> 228	> 1,000
Capsid Purity (%)	> 80	≥ 90	≥ 95
Full Capsids (%)	58	> 70	> 74

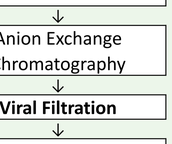
[1] *Based on PBFT02 Dose 2 (2.2E13 Total GC)*

Background/Approach

Process B development was executed across three (3) phases; Feasibility, Process Optimization, and Scale-up. At the end of each phase, the selected process parameters were evaluated in confirmation runs, which ultimately culminated in a production scale batch.

Feasibility

- Dual vs Triple Plasmid Platform evaluated @2L
  - ➢Demonstrated of the suitability of Passage Bio's triple plasmid platform in a suspension model

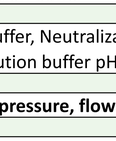


Process Optimization

- Shake flask DOEs evaluated productivity, residual profile, purity, and potency
- Studied multiple cell lines, medias, transfection reagents, enhancers & operating conditions
- 2L BRX studied feed/harvest control strategy
- 10L BRX supported harvest studies and scale dependent parameters
  - ➢Establishment of operating parameters

Scale-up

- Confirmed seed train scaling parameters
- Evaluated at-scale feed/harvest control strategy
  - ➢Demonstration of a production scale batch (200L) at commercial manufacturing site

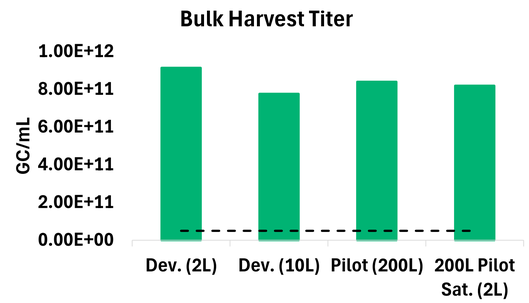


Process Flow Diagram

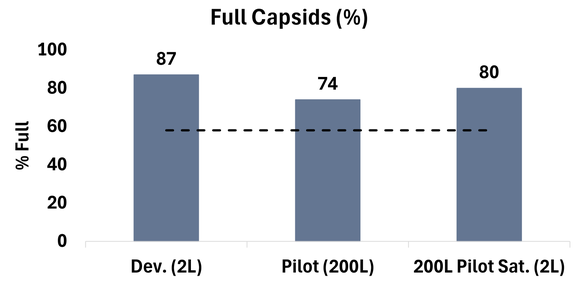
Unit Operation	Process Inputs/Controls
Vial Thaw	***Master Cell Bank***, Thaw temperature, Thaw time, Temperature, %CO_2, Agitation, Duration
Seed Expansion	***Growth Medium***, ***Growth Vessel***, Temperature, %CO_2, Agitation, Duration, volume
Seed Expansion at N-1	***Growth Medium***, ***Growth Vessel***, Temperature, pH, DO, Agitation, Duration
Production Bioreactor (N)	Stage 1 – Cell Growth ***Production Vessel***, ***Growth Medium***, Temperature, pH, ***DO***, Agitation, ***Duration***
	Stage2 – Transient Transfection ***Production Vessel***, ***Growth Medium***, ***Glucose Feed***, Plasmids (cis, trans & helper), ***Plasmid Source***, ***Transfection Reagent***, ***Transfection Enhancer***, ***Plasmid ratio***, ***Transfection complex ratio***, Temperature, pH, ***DO***, Agitation
Harvest - Lysis and Clarification	LDAO, Lysis buffer, **Nuclease Reagent**, Temperature, Agitation, Duration
TFF1 - Concentration and Buffer Exchange	Equilibration buffer, Load hold time, diafiltration volumes, 0.2um filtration, Intermediate Storage
Affinity Chromatography	0.2um filtration, Equilibration buffer, ***Wash 1 buffer***, Wash 2 buffer, Wash 3 (Equilibration buffer), Elution buffer, Neutralization buffer
Anion Exchange Chromatography	0.2um filtration, ***Dilution buffer***, Wash buffer, Elution buffer, Neutralization buffer, Load hold time, Column load, Bed height, Elution buffer pH
Viral Filtration	**Equilibration Buffer, Filtration pore size, flush volume, pressure, flow rate**
TFF 2 - Final Formulation	Formulation Buffer, Load hold time, diafiltration volumes
Drug Substance Filtration and Storage	Formulation Buffer, ***0.2 µm filter***, DS Storage

Bold = Parameter modified from Process A (adherent) to B (suspension)

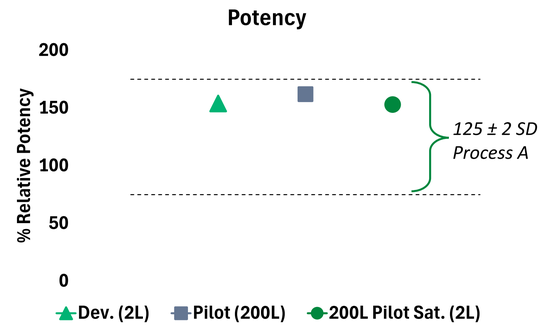
Process B Results

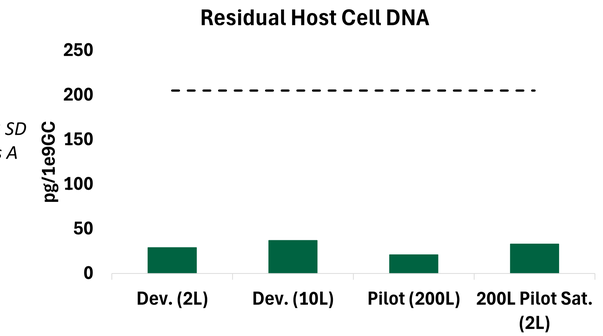


--- Historic Process A Titer (5.0E+10 GC/mL)



--- Historic Process A %Full (58%)





--- Historic Process A Residual HC DNA (205 pg/1e9GC)

Batch Key

Batch Name	Scale (L)	Description	Location
Dev. (2L)	2	Bench scale stirred tank reactor (STR)	Passage Bio Lab, Pennington, NJ
Dev. (10L)[1]	10	Bench scale STR	Passage Bio Lab, Pennington, NJ
Pilot (200L)	200	Production scale STR	Catalent, Baltimore, MD
200 L Pilot Sat. (2L)	2	Bench scale STR control of Pilot 200L	Catalent, Baltimore, MD

[1]*Dev. (10L) run was only processed through AAVX as the study was focused on bioreactor parameters. No values reported for % Full and Potency at Drug Substance.*

Learnings

1. All Process B development goals were met or exceeded.
2. Overall control strategy is robust at 2L, 10L, and 200L scales. Minor adjustments were required for bioreactors during scale-up.
3. Productivity, residual profile, transfection efficiency (full capsids %) are impacted by many factors, including plasmid ratio, plasmid quantity, transfection reagent, cell culture media, and use of enhancer reagent.
4. Transfection complex stability is critical for robust bioreactor performance at large-scale. Some transfection reagent conditions showed better performance during small-scale studies but underperformed at large-scale.
5. Harvest time post-transfection had an inverse impact on AAV vector potency (extended harvest time reduced relative potency).

Future Direction

1. Expand characterization of harvest time impact on AAV vector potency and refine in-process controls
2. Demonstrate reproducibility at 200 L manufacturing scale (comparability campaign)

Passage Bio

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