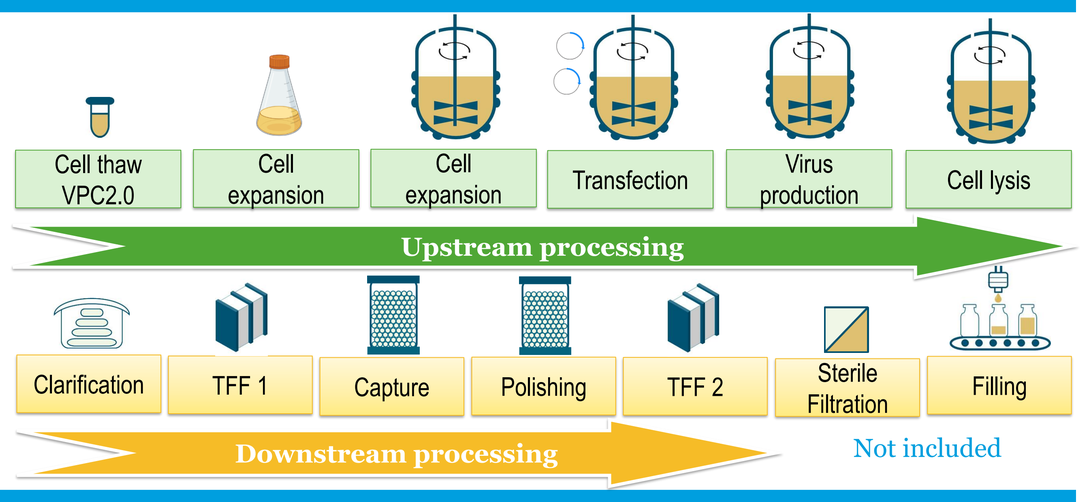
Adeno-associated virus production platform performance for 2L and 50L scales

Amna Anwar, Bilal Ozdoganoglu, Helen Chen, Katerina Farukshina, Juline Guenat, Laura Giner Robles, Peter Dashwood, Nathan Sweeney, Daria Marsh

CATAPULT Cell and Gene Therapy

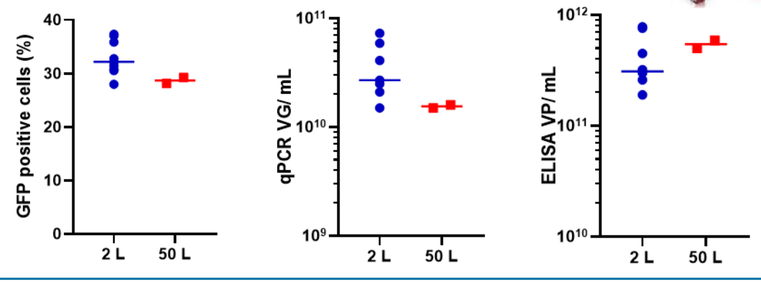
Introduction

Gene therapy has the potential to provide one-time curative treatment for many diseases with unmet need. Adeno-associated virus (AAV) based gene therapy has made major advances over the last decade, with options of multiple serotypes allowing for targeted therapy. The detection, production, purification, and delivery of AAV requires dedicated development to ensure cost efficient and effective manufacture of next generation ATMPs. One of the challenges associated with the production of rAAV is the formation of empty, partially full or mispackaged AAV particles that do not contain the full therapeutic gene. Further to this, only a small percentage of these particles are infectious and would achieve clinical output *in vivo*. AAV manufacturing processes require optimisation to meet clinical demand. CGT Catapult has developed an end-end scalable AAV2 production and purification platform which has been demonstrated at a 2 L and 50 L production scale. AAV2 total particle titre, full AAV2 particle titre and impurity levels are critical quality attributes (CQAs) in the production and purification of these vectors. The process has been well characterised through internal and external analytical assays which measure process performance and CQAs throughout the purification process.



Upstream process

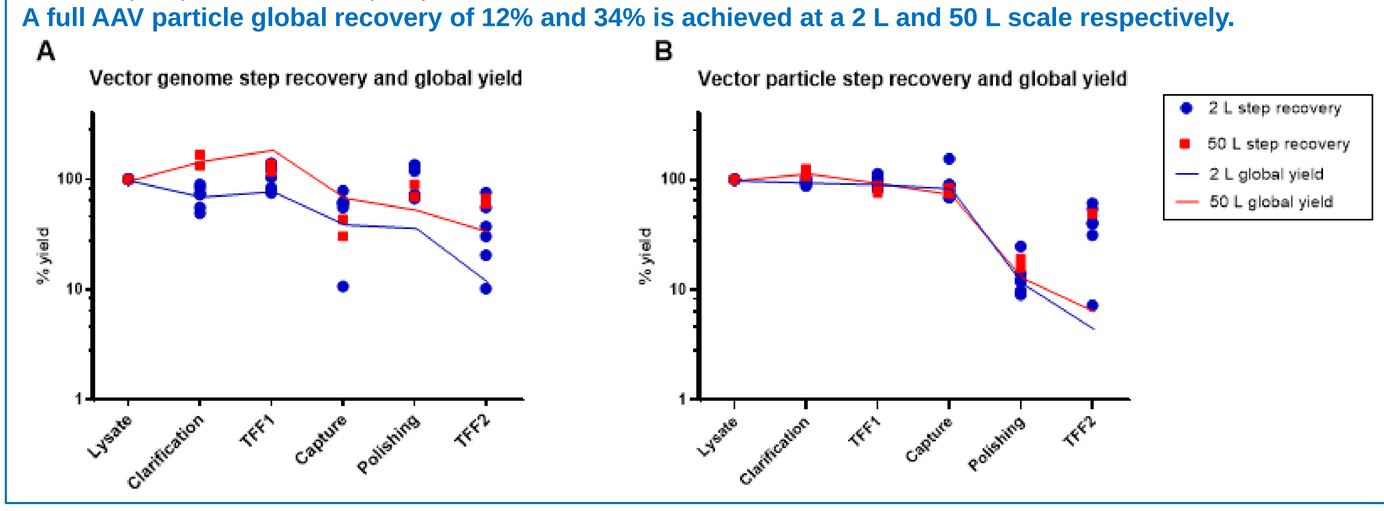
AAV production was developed in Sartorius Univessel® SU 2 L (N=9) using a commercially available HEK293 line and chemically transfected with a two-plasmid system (GFP as reporter gene). Process was scaled-up to ThermoFisher DynaDrive SU 50 L (N=2). Performance was assessed by measuring transfection efficiency (GFP positive cells at 24 h), physical (VP/ mL) and genomic (VG/ mL) titration at 72 h.



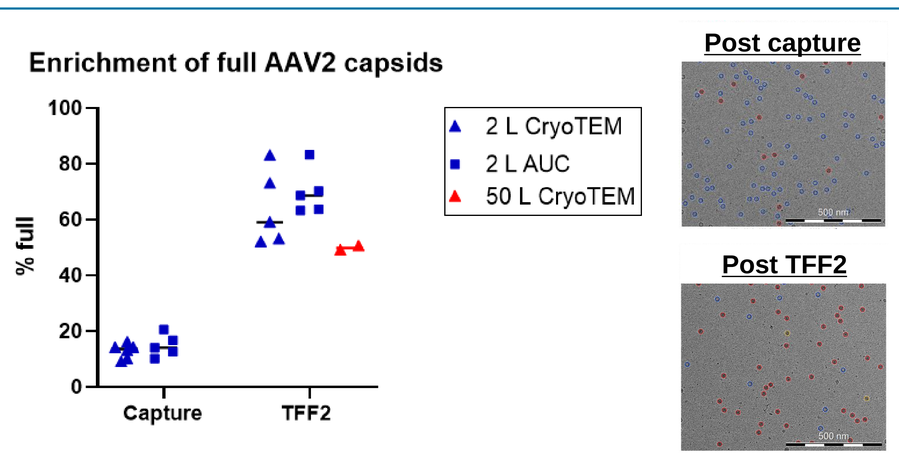
Downstream process recovery

Downstream platform consists of five steps that were directly scaled up from a 2 L to 50 L scale. Several changes to Polishing and TFF2 steps were applied to address limitations including supply chain, facility and scalability issues. A comparison of full particle step recovery and global yield (A) and total particle step recovery and global yield (B) at a 2L scale (N=6) and 50L scale (N=2) is shown.

A full AAV particle global recovery of 12% and 34% is achieved at a 2 L and 50 L scale respectively.



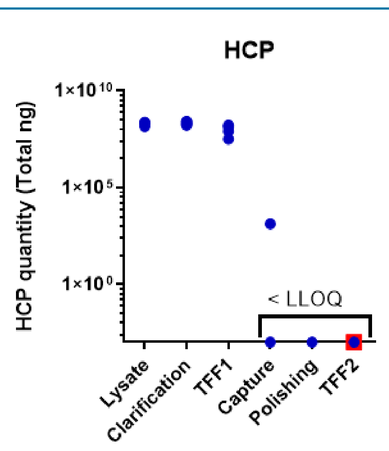
Enrichment



Enrichment of full AAV2 particles was measured using CryoTEM and AUC on post capture and post TFF2 pools for the 2 L runs and percentage of full AAV2 particles was measured only in TFF2 pools for the 50 L runs using CryoTEM.

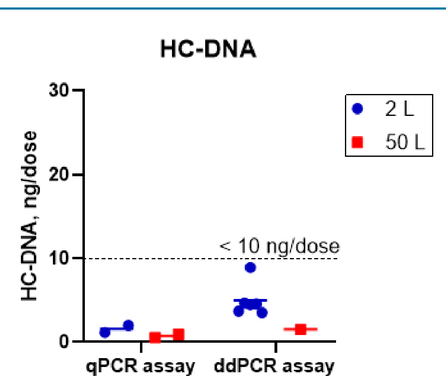
A 5 fold enrichment was achieved.

Impurity clearance



The HCP levels were measured throughout the DSP at a 2 L scale and only post-TFF2 at a 50L scale

Samples from Polishing and TFF2 steps are below level of quantification (10 ng/mL).



The HC-DNA levels were measured post-TFF2 with two assays qPCR (Thermo) and ddPCR (BioRad), some samples were measured using only one assay. Dose used in calculation was 1.5×10^{11} vg matching Luxturna.

All samples are below the required 10 ng/dose.

Summary

An AAV2 downstream platform has been developed at CGT Catapult's development site to follow the 2 L upstream production, robustness was demonstrated across six runs. This downstream platform has been successfully transferred to the CGTC's MS&T team, scaled up to 50 L and robustness was demonstrated across two runs. The end-end downstream process provides:

1. **A full AAV2 particle global recovery of 12% at a 2 L scale and 34% at a 50 L scale.** Differences between scales: upstream process (different bioreactor systems), downstream methods (namely polishing and TFF2) and volume to filter surface area ratios.
2. **5-fold enrichment allowing 49–83% of full capsids in Drug Substance based on CryoTEM and AUC analysis.** Initial percentage of full capsids is robust, however, the variability post-TFF2 may be caused by peak collection challenges in the polishing step.
3. **Host Cell Protein (HCP) content is below LLOQ in Drug Substance at both scales.**
4. **Host Cell DNA (HC-DNA) concentration is below the target of 10 ng/dose when Luxturna dose is used for calculation.**

Scale up process challenges

- **Upstream process transfer** between 2 L and 50 L scales using a different production system, impacted the downstream process with a difference in AAV2 titre and impurity levels.
- **Different peak collection strategies** during the chromatography steps.
- **Supply chain issues** caused a difference in the TFF2 membrane used.

Analytical challenges:

- Sensitivity and robustness of analytical assays with challenging in process sample matrices; particularly, in the HC-DNA assays where there is a low throughput using either method and a difference of 3-4 fold in the same sample using different assays. In house method development is ongoing.

Achievements

- CGTC has tech transferred this process to multiple collaborators, and trained operators on each individual unit operations, differing from the CMO tech transfer approach.

Future work

- Process intensification work ongoing to improve yields.
- Adaptive control and implementation of Process Analytical Technologies to guarantee product quality.
- Assay improvement including the development of new methods such as HPLC and SEC-MALS to characterise full and total AAV particle concentration.

UKRI Innovate UK

Cell and Gene Therapy Catapult
12th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
+44 (0) 203 728 9500 | info@ct.catapult.org.uk | ct.catapult.org.uk

Acknowledgments: Ana Sergijenko, Antonia Karachaliou, Augustin Barna, Chathyan Pararasa, Ele Zucchelli, Fathema Chowdhury, Francesco Rossi, Giuseppa Piras, Jane Balsham, Joe Harvey, Marta Bagnati, Mateusz Filipiak, Misbah Fayyaz, Nafisa Rahman, Nicola Haines, Nishanthi Weeratunge, Omaymah Belhaj-Fahsi, Roksana Tarnowska, Rolando Matos, Salvador Perez, Toluwani Alade, Wilson Li