Evolution of a High Performing, Modular Platform for AAV Manufacturing

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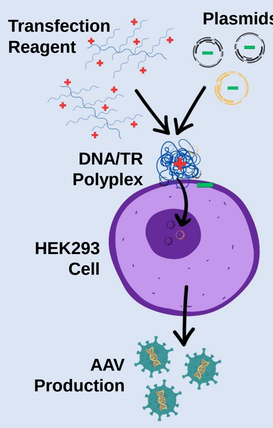
Introduction

MeiraGTx is continuously developing its adeno-associated viral vector (AAV) upstream manufacturing platform to maximize yield and minimize the generation of non-therapeutic AAV particles and other process-related contaminants.

The work presented here shows the evolution of MeiraGTx's upstream manufacturing platform.



AAV5
Size ITR-ITR: 4159 bp

AAV2
Size ITR-ITR: 4546 bp

AAV8
Size ITR-ITR: 3972 bp

Methods

General

Suspension HEK293 cells were cultured in 250mL STRs to 2.0-3.5x10^6 VC/mL and triple-transfected with two commercially available transfection reagents, with or without AAV production enhancers, to generate AAV5, AAV2, and AAV8. Product was harvested 3 to 4 days post-TFX. Harvest culture was clarified and purified via affinity chromatography (AAVX). Viral genome (VG) titre and residual pDNA (KanR) were determined in lysate using qPCR. % full capsids and hcDNA were measured in AAVX eluate by AUC and qPCR, respectively.

Perfusion Culture

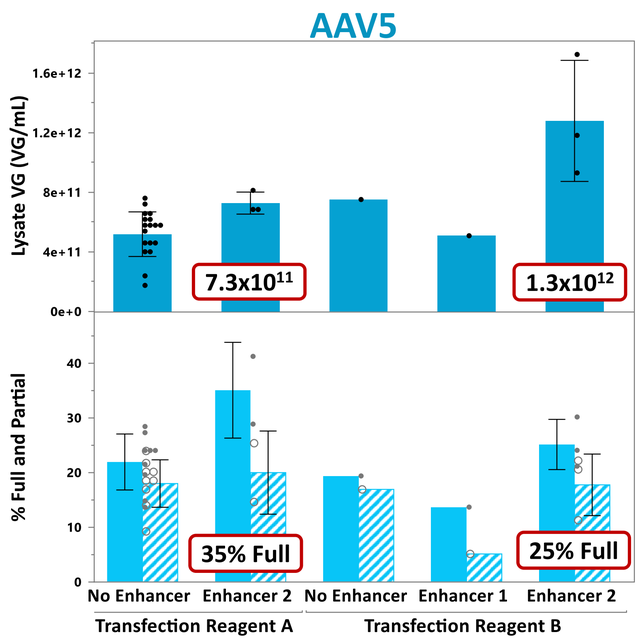
For cultures ran in perfusion mode, cells were recirculated through a hollow fibre filter at constant flow rate using a centrifugal pump (Levitronix, Switzerland). A total of 2 vessel volumes of medium was perfused over ~10h prior to transfection. HEK293 cells were transfected at a target VCD range of 4.5-5.0x10^6 VC/mL.

Conclusions

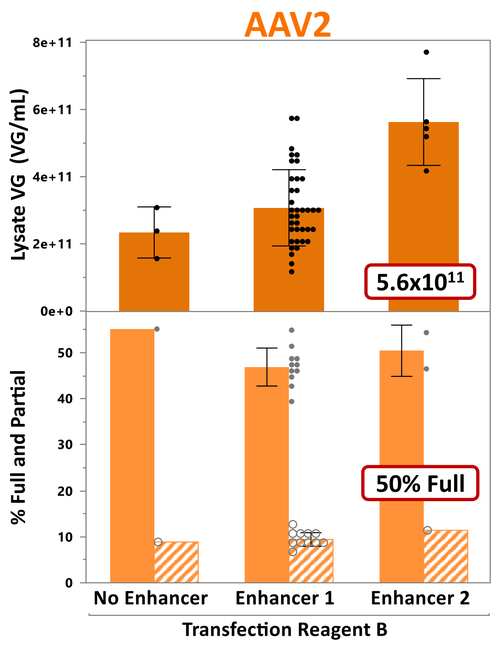
- A modular upstream manufacturing process was developed and optimized internally at MeiraGTx, using different commercially available transfection reagents, AAV production enhancers, and finely-tuned transfection parameters.
- Fed-batch process optimization resulted in harvest AAV titres of up to **1.3x10^{12} VG/mL** and up to **50% full capsids** prior to polishing steps, for multiple combinations of serotype and therapeutic DNA cassette.
- Residual DNA levels were demonstrated to be controllable and adequate for patient safety.
- Enhancing the platform process using an optimized perfusion method has resulted in up to a **2.2X increase in harvest VG titre**, reaching **4.4x10^{12} VG/mL**, without compromising on product quality, whilst **reducing pDNA usage by up to 50% and COGs per dose by 2.2-fold**.

High Performing HEK293 Triple-Transfection Fed-Batch Platform

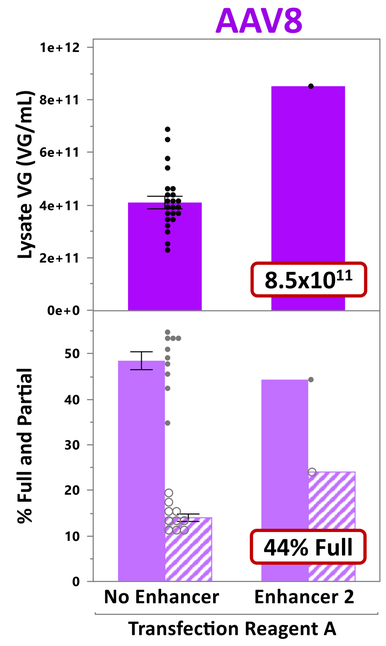
High Titre | **High % Full** | **Low Residual pDNA** | **Low Residual hcDNA**



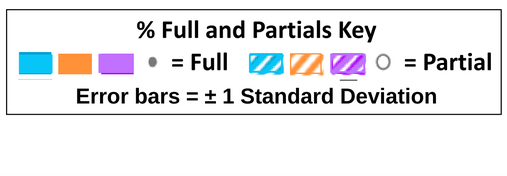
- Residual hcDNA ≤ 16 ng/ 10^{12} VG with Transfection Reagent A
- Residual hcDNA ≤ 31 ng/ 10^{12} VG with Transfection Reagent B + Enhancer 2



- Residual hcDNA ≤ 8 ng/ 10^{12} VG

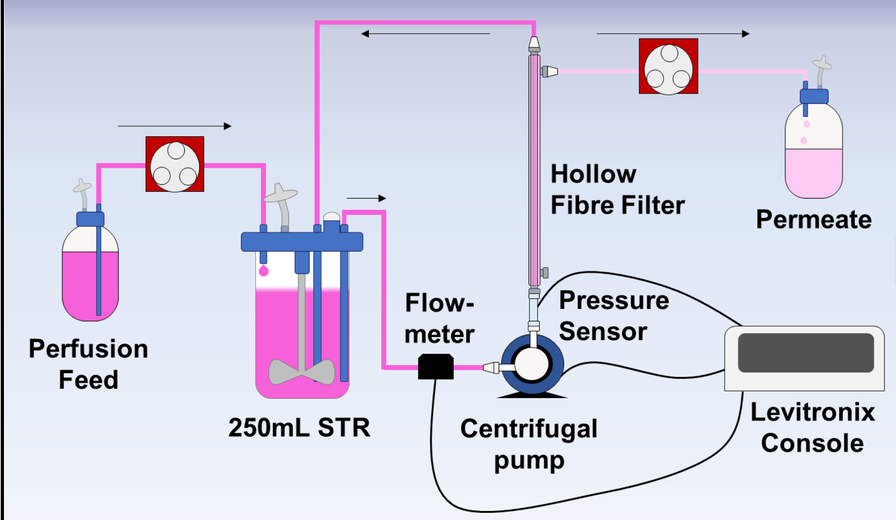


- Residual hcDNA < 5 ng/ 10^{12} VG



- All residual pDNA < 6% (KanR qPCR).
- Harvest VG titre > 5x10^{11} VG/mL across multiple AAV serotypes.
- Use of Enhancer 2 resulted in a 1.4 to 5.2-fold increase in VG titre and comparable or improved % full capsids compared to No Enhancer.
- Enhancer 2 is currently the enhancer of choice for the fed-batch platform.

Perfusion-Enhanced HEK293 Triple-Transfection Platform

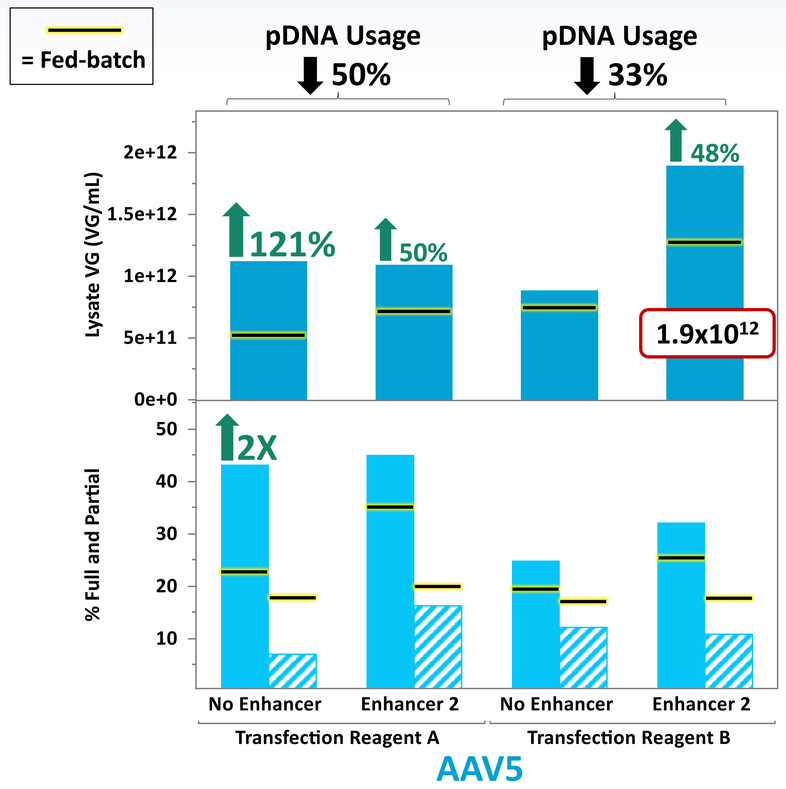


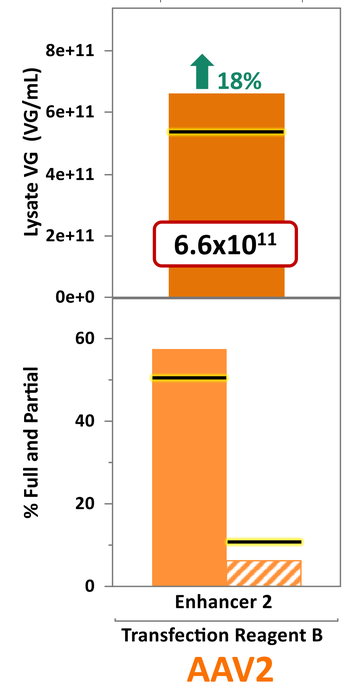
Up to 2.2X Increase in VG Titre

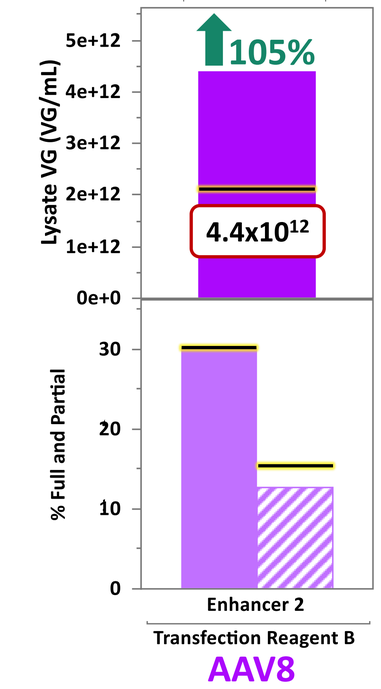
Improved Quality Profile

33-50% Reduction in pDNA Usage

Up to 2.2X Reduction in COGs per Dose







- An optimized perfusion process resulted in a 1.2 to 2.2-fold increase in harvest VG titre, as well as improved or comparable % full and partial capsid profiles compared to fed-batch.
- pDNA usage was decreased by 33% to 50% and cost of goods (COGs) per dose was reduced by up to 2.2-fold.
- Residual hcDNA was reduced 1.4 to 2.4-fold versus fed-batch.
- All conditions showed residual pDNA ≤ 4%.