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Simplifying Progress

Development of a High-Yield AAV Production Platform Using DOE and Automated Bioreactors

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Abstract

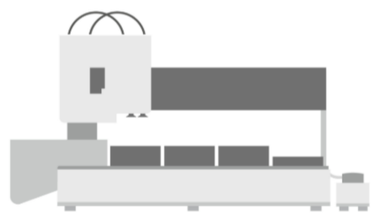
Recombinant adeno-associated virus vectors (AAV) are increasingly adopted for clinical gene therapy applications due to their relative safety, broad tropism, and durable transgene expression. As regulatory approvals and clinical trials continue to expand, scalable and cost-effective manufacturing methods are imperative to meet growing demand. We report here the development of a robust, high-throughput production platform that integrates Design-of-experiments (DOE) methodology with automated, single-use micro bioreactors to optimize AAV2 production yield in Expi293F HEK 293 suspension cells. Using the triple-plasmid transfection system, we conducted five multifactorial experimental runs in the Sartorius Ambr® 15 micro bioreactor system, systematically varying key process parameters including pH, culture media, feeding strategy, agitation rate, transfection cell density, plasmid DNA load, FectoVIR®-AAV to DNA ratio, and plasmid stoichiometry. Concurrently, we implemented pPLUS® AAV-Helper, a novel helper plasmid designed to enhance the efficiency of viral particle packaging.

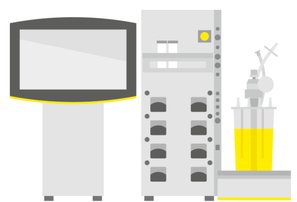
Process performance was evaluated by measuring vector genome titer via quantitative and digital PCR methods, total capsid particle concentration by ELISA, and % full capsids by mass photometry, alongside cell viability and growth kinetics. DOE analysis with Sartorius MODDE® Pro software revealed critical interactions between transfection reagent ratio and cell density and identified an optimal operating window that delivered up to 8.8x1011 vg/mL, a 70-fold improvement over baseline conditions, while achieving a favorable % full capsids of ~25%. To demonstrate scalability, the optimized conditions were translated to the UniVessel® 2L SU bioreactor system. Preliminary scale-up results from three runs indicate comparable productivity and product quality, confirming the robustness of the Ambr® 15-derived scale-down model. This approach not only accelerates process development by enabling simultaneous, data-rich screening of multiple variables, but also reduces reliance on traditional shake flask workflows, offering significant time and resource savings. Our findings underscore the utility of combining DOE strategies with automated, single-use bioreactors to establish a scalable, high-yield AAV manufacturing platform suitable for preclinical and clinical supply.

Project Overview

Media Screening (Run 1)	New Helper plasmid (Run 2)	Plasmid ratios (Run 3)	New RepCap plasmid (Run 4)
Factors	**Factors**	**Factors**	**Factors**
1) pH Before TFX (Low, Middle, High) After TFX (Low, Middle, High)	**1) Seeding VCD** 5 different seedings	**1) Seeding VCD** 1.5E+6 cells/mL	**1) Seeding VCD** 1.5E+6 cells/mL
2) Stirring At seeding (High) Post-Transfection (Low, High)	**2) DNA (µg/E+6 cells)** 5 different ratios	**2) DNA** 1.75µg / E+6 cells/mL	**2) DNA** 1.75µg / E+6 cells/mL
3) Media Media 1; Media 2; Media 3; Control	**3) FectoVIR®-AAV : DNA ratio** 8 different ratios	**3) FectoVIR®-AAV /DNA ratio** 1:1	**3) Mass plasmid ratios (Help pl./RC pl./Vector pl.)** 0.45/0.45/0.1; 0.33/0.56/0.1; 0.25 / 0.5 / 0.25
Responses	**Responses**	**4) Mass plasmid ratios** 17 different ratios	**4) FectoVIR®-AAV /DNA ratio** 1:1
1) Genome Titer (qPCR/ddPCR) (Vg/mL) **2) Viral Capsid Titer** (ELISA) **3) Full capsids %** (Vg/Total Capsid) **4)** AAV protein identity (Western blot) **5)** TFX efficiency (Flow cytometry at 24HPT)	Same as Screening (Run 1)	**Responses** Same as Screening (Run 1)	**Responses** Same as Screening (Run 1)

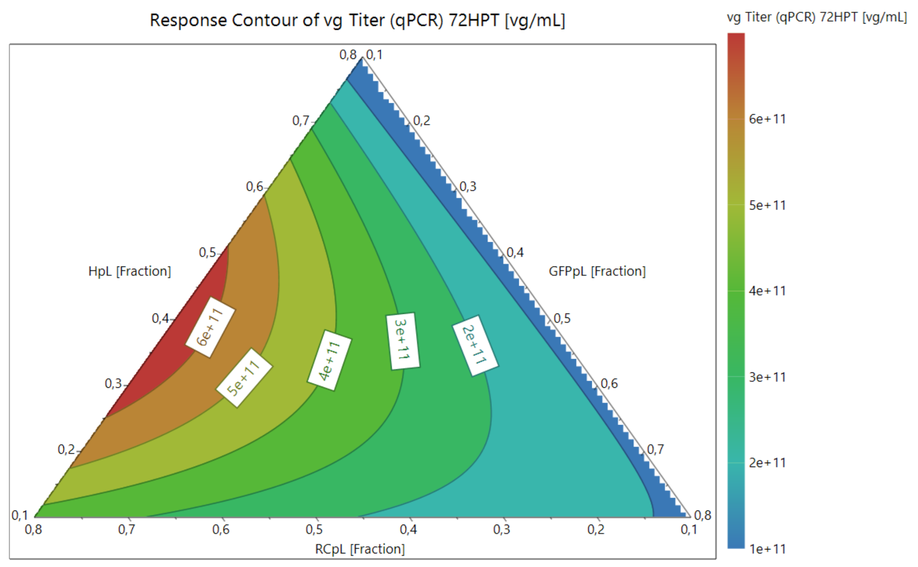
DOE in Sartorius Ambr® 15





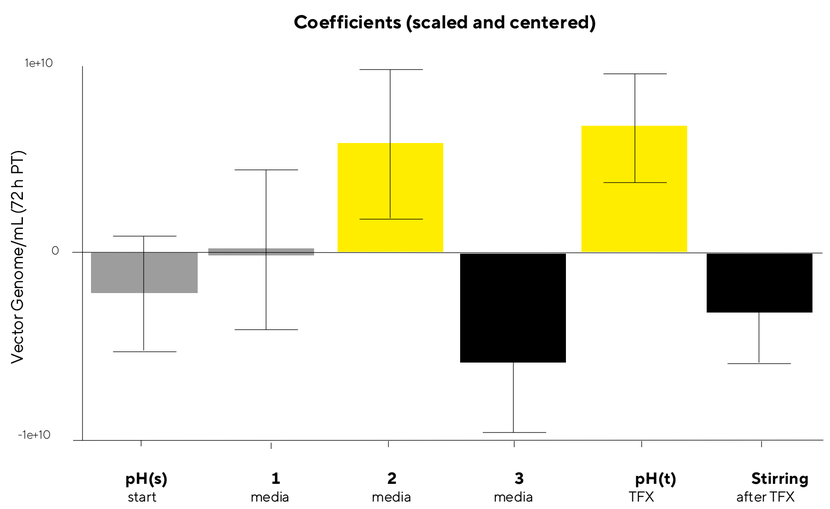
Scale-up in Biostat® B DCU + UniVessel® SU 2L

Optimizing Transfection Plasmid Ratios



In Run 4, plasmid ratios were tested to identify optimal levels of plasmids in triple transfection for maximizing both vg titer and % full capsids. The contour plot displays the ratio region with the highest vg titer. The following ratios are optimal for maximizing vg titer: pPLUS®-AAV Helper 45%, RepCap 45%, GFP 10%.

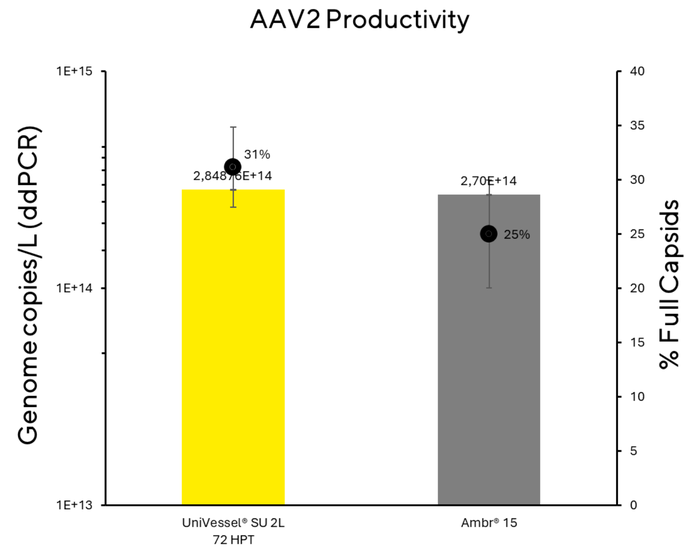
Optimizing Bioreactor Operating Parameters



Key Findings from bioreactor process optimization in Ambr® 15:

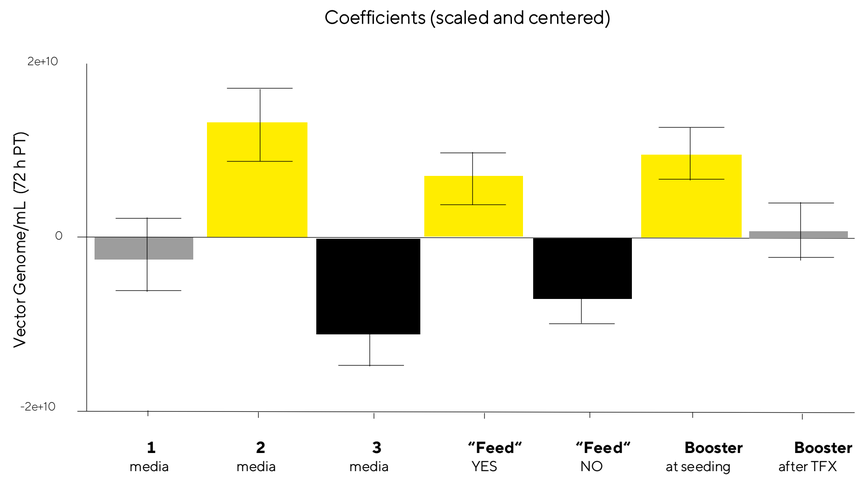
- Media 2 significantly improves vg titer
- Increased stirring speed at transfection reduce vg titer
- Higher pH during transfection correlates with increased vg titer
- Fresh medium addition at time of seeding positively impact vg titer

Scale-Up to 2L Bioreactors



The process was scaled up to the 2 L UniVessel® SU operated by the Biostat® B-DCU using Sartorius Process Insights® software. Titers measured by qPCR (not shown) and ddPCR at 48 and 72 hours post-transfection were comparable across scales (15 mL to 2 L), confirming successful scale-up. Notably, in the 2 L scale, yields and % full capsids at 48 hours were similar to 72 hours, supporting the potential for an earlier and more cost-efficient harvest.

Optimizing Transfection Conditions



Key Findings from transfection parameter optimization in Ambr® 15:

- Media 2 has a notable effect upon vg titer
- Media 3 has a negative effect upon vg titer
- Feed addition at transfection has a positive effect upon vg titer
- Booster addition at seeding has a positive effect upon vg titer but no effect when delivered after transfection

Summary

This project successfully created and optimized a new AAV2 process using next gen raw materials. Using DOE methodology and high-throughput micro bioreactors, four experiments were run to optimize bioreactor operating parameters, cell culture parameters, transfection conditions, and plasmid ratios. The process was then scaled-up to a 2L bioreactor and process performance confirmed.