Increasing AAV Productivity: Upstream Process Optimization for a Robust, Plug-and-Play Platform

Shannon Frost*[1], Dicky Gilmore[1], Diane Golebiowski[1], Laura Van Lieshout[1], Marissa Stanvick[1], Chase Rebidue[1], Hannah Stewart[2], Louis Frost[2], Magda Reis[2], Rebecca Astley[2], Jeff Gagnon[1], Serena Dollive[1], Ify Iwuchukwu[1]

*: Presenting Author
1: Oxford Biomedica (US), LLC, 1 Patriots Park, Bedford, MA
2: Oxford Biomedica (UK) Limited, Windrush Court, Transport Way, Oxford, OX4 6LT

Introduction

Creating a highly productive AAV process is important for delivering the best possible drug product to our clients.

The Upstream process is important to creating high-yield, high-quality vector.

One way to improve productivity in your Upstream process is to optimize parameters and raw materials.

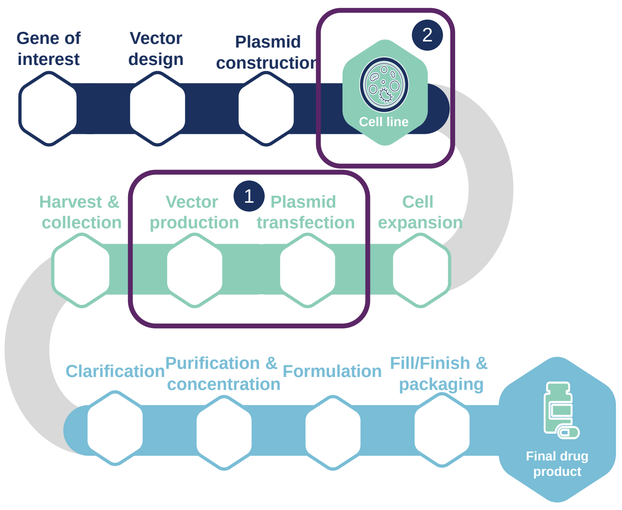
Three Upstream process improvements that were evaluated were transfection parameters, a new transfection reagent, and a process additive.

The goal when optimizing OXB's AAV process was to create a plug and play platform that can work for both triple and dual transfection across all serotypes and cell lines. To demonstrate this, an internal cell line was generated to achieve similar performance to commercially available AAV production cell lines.

When creating the next gen Process the goal was to have a highly productive, robust, and cost-effective option for our Partners.

AAV Process

Below is an overview of OXB's end-to-end AAV process workflow. The first section in navy highlights vector engineering and optimization, if desired by our Partners. Upstream (teal) covers cell line development and thaw through bioreactor production and harvest and is the focus of this poster. The Downstream process (blue) entails clarification, purification, full capsid enrichment, and fill/finish.



When optimizing the Upstream process to be highly productive, flexible, and robust, there were two sections that were focused on from this AAV workflow.

1

Transfection and Production
- It's important to evaluate new materials and methods to ensure our process is best-in-class.
- The goal is to create a next gen Process that can be Plug-and-Play for any cell line, serotype, or transfection.

2

Cell Line
- OXB's current platform uses a commercially available HEK293 cell line.
- The goal is to develop an in-house HEK293 cell line with comparable AAV productivity and product quality to show upstream process robustness

Optimization of Transfection

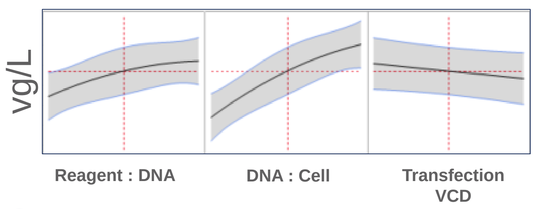
When optimizing the transfection process, we looked at multiple Upstream factors, especially:

A Transfection Reagent

A shake flask screening was performed to compare commercially available transfection reagents to OXB's current transfection reagent. These screenings were done at the shake flask scale and the top transfection reagent was selected based on titer, perfect full capsids, reduced batch cost, and transfection complex stability.

B Transfection Parameters

A full factorial DOE was run to optimize our transfection reagent:DNA ratio, DNA:cell ratio, and transfection cell density. The optimal set points for ratios were chosen based on titer. We ensured the setpoints and ranges are applicable and robust for large scale manufacturing. The results of the DOE for Vg/L can be seen here:



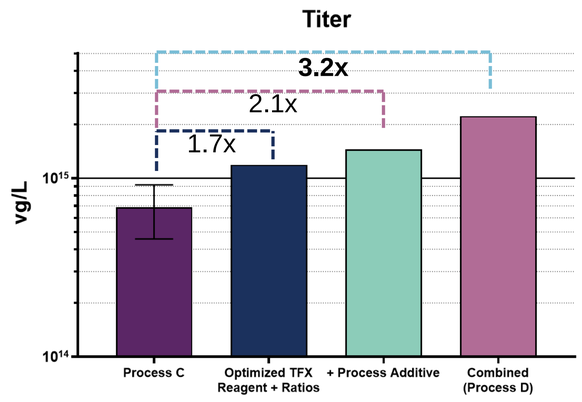
C Process Additives

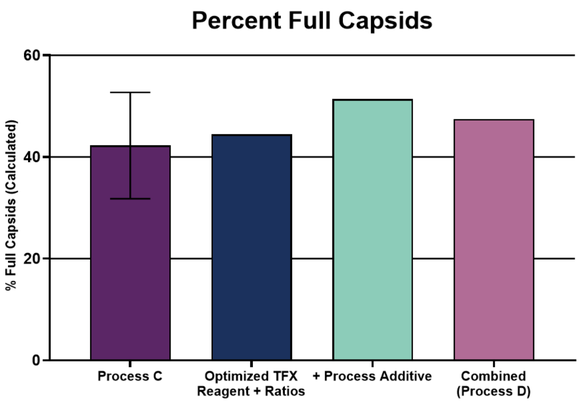
A commercially available additive was identified that can boost titer. This reagent was evaluated at shake flask scale by testing a range of concentrations and addition timing. The optimal concentration and timing of the addition were chosen based on titer productivity.

Process "D"

After choosing a new transfection reagent, transfection parameters, and process additive we now have our new next-gen process, Process D.

The next step is to run Process D in a 2L Bioreactor (AAV9 dual transfection) to see how it compares to Process C.



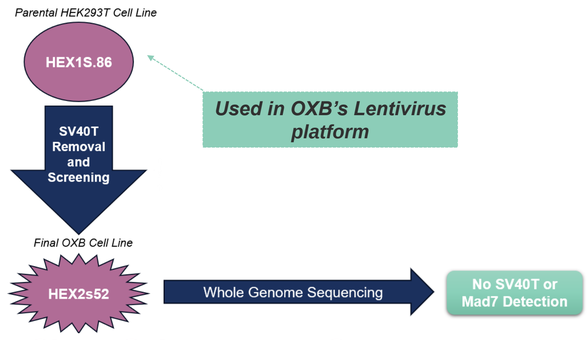


Process D gave a 3.2x Vg/L increase over Process C when run with AAV9 Dual plasmid transfection at 2L bioreactor scale.
The calculated % full is comparable with each process improvement.
At OXB we want a platform that we can use as a Plug-and-Play process. Will this new Upstream platform fit multiple serotypes and cell lines?

Creating an In-House Cell Line

Process C uses a commercially available HEK293 cell line. We wanted to explore creating an in-house cell line for our next-gen process that could expand our offerings to our Partners and also provide a tool to show that process improvements are not cell-line specific.

This cell line was created by removing the SV40 large T antigen from our Lentivirus cell line to make it safe for AAV use. The clones were screened for vector genome productivity at shake flask scale and the removal of the SV40 large T loci was confirmed via targeted nanopore sequencing. The top choice clone was then confirmed for full SV40 large T antigen and associate NeoR cassette removal via PacBio whole genome sequencing and lack of expression of large T antigen and NeoR with mass spectrometry. After this confirmation we chose our new HEK293 cell line with our top clone.



Clone Selection and Screening

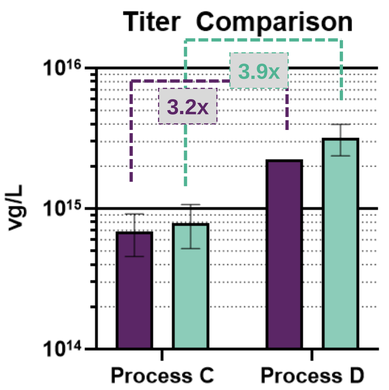
HEX2s52 was chosen as our top clone after confirming at 2L bioreactor scale after first evaluating at shake flask scale. This 2L bioreactor confirmation evaluation was run with AAV9 dual transfection and with our Process C.

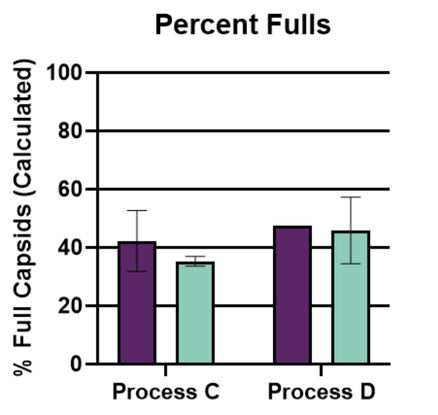
After optimizing next-gen platform Process D and confirming performance with our commercially available cell line, the next step was to demonstrate effectiveness with our in-house cell line, HEX2s52.

External vs Internal Cell line with Upstream Process Improvements

The Internal cell line performs comparably to the commercially available cell line in both Upstream platforms, both for productivity and percent full capsids.

The commercially available cell line saw a 3.2x increase in titer when switching from Process C to Process D. OXB's new in-house cell line saw a 3.9x increase in titer when switching from Process C to Process D with no decrease observed in calculated percent fulls.



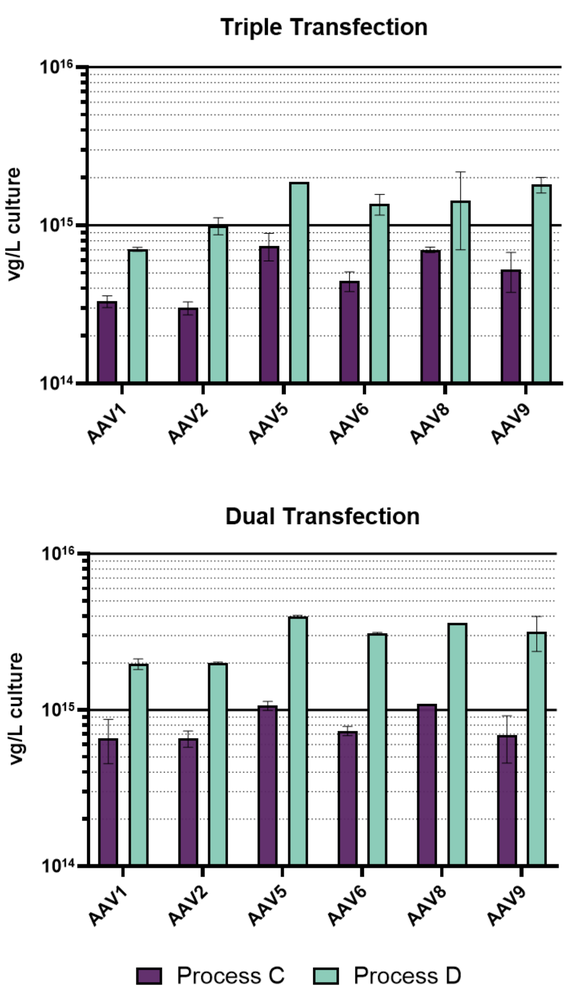


External Cell Line | Internal Cell Line

Process C vs Process D Across Serotypes

As previously mentioned, it's important to ensure this new Process D would work across multiple serotypes so OXB can have a plug-and-play process for our Partners.

The most common serotypes we see with our Partners were evaluated at 2L bioreactor scale with both Triple and Dual plasmid transfections.



Process D outperformed Process C at every serotype for both dual and triple transfection. Dual transfection gave a boost in titer with both Process C and Process D. We achieved >2e15 vg/L in Dual transfection for all 6 tested serotypes.

Conclusions

Exploring alternative transfection reagents, parameters, and process additives can boost vg titer by >3x.

A novel in-house cell line was developed that shows comparable productivity to the commercially available cell line. We hope to be able to offer this cell line to our Partners after further evaluation.

OXB's newly optimized platform, "Process D", can boost titer to as high as **4e15 vg/L.**

Oxford Biomedica (US), LLC, 1 Patriots Park, Bedford, MA

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