# Development of a scalable upstream platform process with 1E15 vg/L bioreactor titer for AAV

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## ABSTRACT

High productivity and product quality have been crucial in developing novel AAV-based drug candidates, not only to fuel clinical trials, but also to reduce the high manufacturing cost per patient dose. The pursuit of high AAV productivity has led to an increased focus on the upstream processing. To improve bioreactor titer, OXB has developed a new and improved upstream process showing up to a 10-fold increase in bioreactor productivity, up to a 2-fold increase in percent full capsids, and yields exceeding 1E15 AAV vector genomes (vg) per L of culture. This new platform has resulted in up to 90% decreases in manufacturing costs per individual dose, which will prove essential to meet high patient demands.

Process improvements leading to the large increase in titers were the result of a combination of factors across the upstream process, all while maintaining, or even improving, the high level of product quality. Raw material evaluations were performed across a wide range of media, transfection reagents, and post-transfection additives. These evaluations confirmed the high performance of OXB process reagents and surprisingly identified an additive that resulted in up to a 3-fold titer increase. Integration of a dual-plasmid transfection system resulted in another 2-fold increases in vg titer and consistently higher percent full capsids. Additionally, the team overcame challenges around increased transfection cell density to successfully move from 2E6 to 4E6 cells/mL which led to an additional nearly 2-fold titer increase. Lastly, the team focused on bioreactor parameters for a further boost in productivity. This new high-performance upstream platform has proven to produce bioreactor titers close to or above 1E15 vg/L and, in most cases, over 50% full capsids in affinity-purified product across all tested constructs, including 9 different AAV serotypes spanning 4 different Clades, as well as 10 different genes of interest (GOIs).

Another common challenge in the field of AAV process development is demonstrating scalability and reproducibility. The OXB upstream process delivers a robust and desirable product quality profile across manufacturing-friendly operating ranges. The new upstream process has demonstrated comparable titers and consistent product quality across multiple batches at 2L, 50L, and 500L scale for different constructs and AAV capsid serotypes. Successful scale-up of this process is enabled by critical control of our high-performing transient transfection.

The benefits of a highly productive upstream process may not be realized through to drug substance without a similar effort and focus on the downstream side. At OXB, we have combined our new 1E15 vg/L upstream process with a comparably intensified downstream process that allows us to manufacture over 1E17 vector genomes of drug substance per 500L bioreactor.

## CONTACT

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## INTRODUCTION

Upstream process development focused on increasing vg productivity and AAV packaging in the bioreactor through:

- Screening cell growth media, transfection reagents, and post-transfection additives
- Plasmid design, increasing transfection cell density, and optimization of bioreactor parameters

These process improvements aimed to be linearly scalable to 500L bioreactors and broadly applicable across different capsid serotypes and GOIs to achieve upwards of 1E15 vg/L out of the bioreactor.

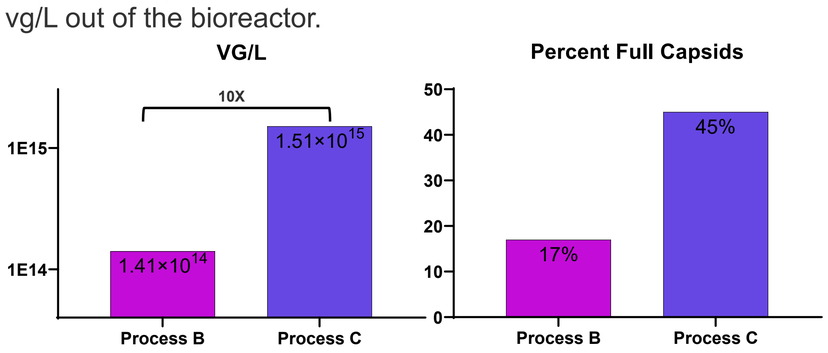



**Figure 1: Productivity and packaging comparison of Process B and Process C.** 125mL shake flasks were run with platform Process B or Process C. Transfection density was increased from 2E6 to 4E6 cells/mL, dual plasmid transfection was utilized, and a post-transfection additive was incorporated for the Process C shake flask conditions. Crude lysate samples were quantified for VG (ddPCR) and capsid (ELISA) and then the percentage of full vectors was calculated.

## RESULTS

### RAW MATERIAL SCREENING

Multiple screening studies were executed integrating several media, transfection reagents, and post-transfection additives to ensure that optimal raw materials were being utilized in the platform to boost vg titer. The best performing production additive was incorporated into Process C

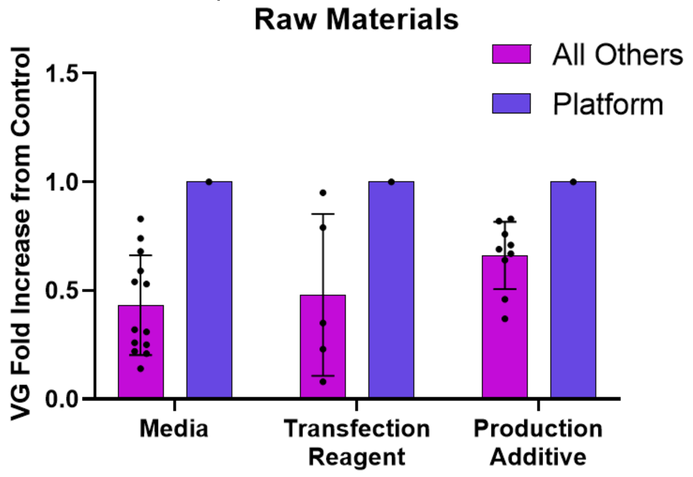
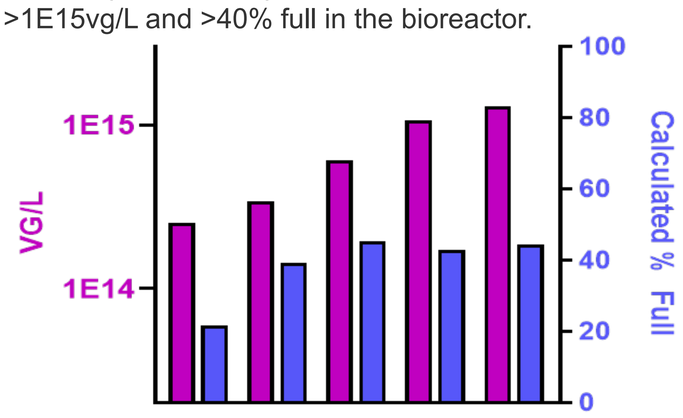



**Figure 2: Raw Material Screening for Media, Transfection Reagent, and Production Additive.** Multiple 125mL shake flasks were run using various media, transfection reagents, and production additives. Crude lysate was quantified for VG productivity.

### UPSTREAM PROCESS INTENSIFICATION

Upstream Process Development focused on improving titer through the integration of a dual plasmid transfection system (shown below), increasing the transfection cell density to 4E6 cells/mL, incorporation of a post-transfection additive, and bioreactor parameter optimization. Combined efforts have led to >1E15vg/L and >40% full in the bioreactor.




| Construct | Triple | Dual | Dual | Dual | Dual |
|---|---|---|---|---|---|
| TFX Density | 2E6 | 2E6 | 2E6 | 4E6 | 4E6 |
| Additive | No | No | Yes | Yes | Yes |
| Bioreactor Parameters | A | A | A | A | B |

**Figure 3: 2L Bioreactor Productivity Assessment of Platform Process Changes.** 2L bioreactors were transfected with triple or dual transfection, 2E6 or 4E6 transfection density, and with or without the production additive. Crude lysate samples were quantified for VG (ddPCR) and capsid (ELISA) and then the percentage of full vectors was calculated.

### CROSS-SEROTYPE AND GOI FIT

To ensure that the bioreactor parameters were optimal across multiple serotypes, further evaluation was performed at 2L scale for AAV2, AAV5, AAV6, and AAV8 and confirmed the results. The platform 2L process has also achieved over 1E15 vg/L titer in the bioreactor for 10 separate GOIs.

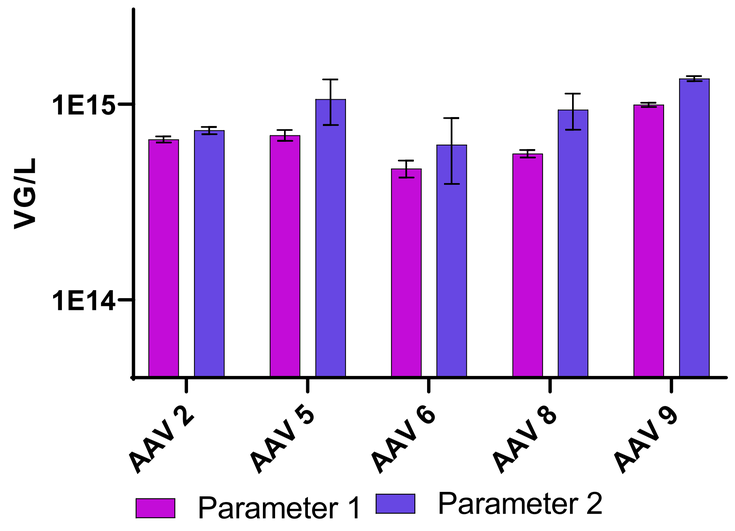



**Figure 4: Cross-Serotype Productivity Assessment of Optimized Bioreactor Parameters**. Dual plasmid transfections were performed in 2L bioreactors for AAV2, AAV5, AAV6, AAV8, and AAV9 (GFP GOI) with and without optimized bioreactor parameters. Crude lysate samples were quantified for VG production.11

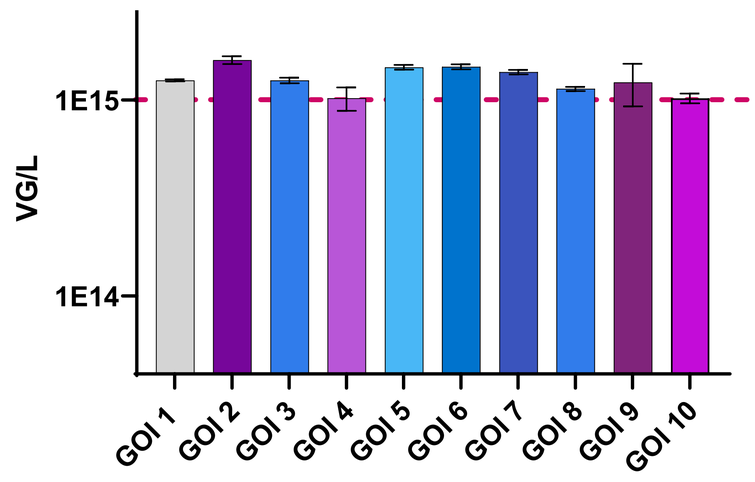
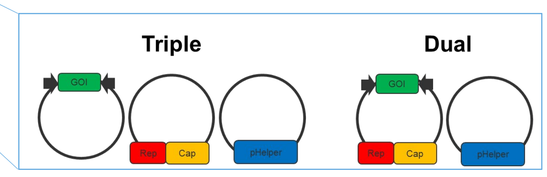
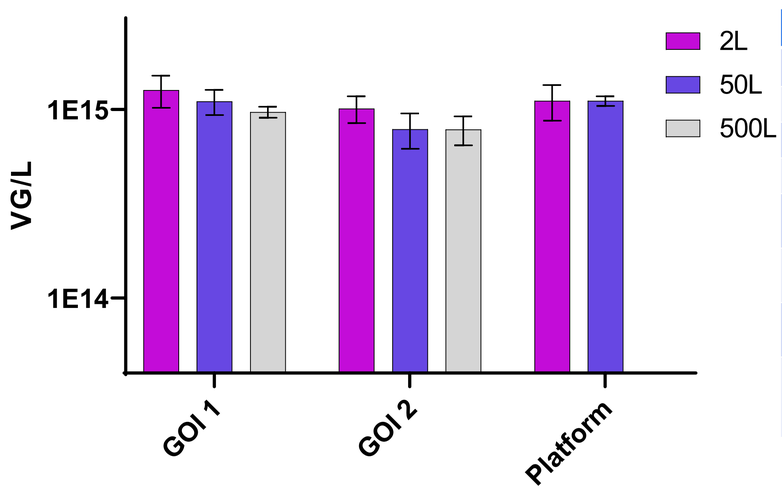



**Figure 5: Productivity Assessment of Platform Process Across Multiple GOIs**. Multiple GOIs across the Clade F serotype were evaluated in the platform 2L dual transfection bioreactor process. Crude lysate samples were quantified for VG production.




### SCALE UP AND PRODUCT QUALITY

Process scale-up was evaluated at the 50L and 500L scale. Primary focus of scale up operations were preparation and addition of the transfection mix as well as bioreactor mixing and gassing. Comparable titer and product quality in the platform process has been demonstrated for two separate GOI constructs at the 500L scale.




| | Construct 1 | | Construct 2 | |
|---|---|---|---|---|
| | 50L | 500L | 50L | 500L |
| Purity, % | 100 | 99 | 99 | *(in process)* |
| Aggregates, % | 0.46 | 0 | 0.70 | *(in process)* |
| % Empty | 12 | 11 | 14 | 18 |
| Infectivity (TCID50) | meet | meet | meet | meet |
| HCP (ng/1E13 VGs) | BLoQ | BLoQ | BLoQ | BLoQ |
| HC DNA (ng/1E13 VGs) | 2.8 | 0.47 | 3.4 | 2.0 |
| **Total VG per Batch** | **>1E16** | **>1E17** | **>1E16** | **>1E17** |

**Figures 6 and 7: Process Scale-Up Performance**. Three separate GOIs have been evaluated in platform Process C at the 2L and 50L bioreactor scale, and two have been further assessed up to the 500L bioreactor scale. Crude lysate vector genome titer is compared as well as drug substance product quality and yield.

## CONCLUSIONS

The Oxford Biomedica team has developed a proprietary plug-and-play transient transfection system that can be:

1) Highly productive with >1E15 vg/L from the bioreactor
2) Easily integrated across various capsid serotypes and GOIs
3) Scalable to 50L and 500L scale while maintaining high yield and product quality

The novel AAV production system can significantly increase the number of patient doses per batch, reducing the overall number of batches needed for clinical and commercial manufacture of therapeutics.

Multiple therapeutic targets are within reach for any potential partner due to seamless integration into the Upstream platform, applicable to a multitude of capsid serotypes and/or genes of interest, ultimately resulting in a robustly productive process that yields a high-quality product.

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