Achieving AAV vector productivity >1E15vg/L and scaling suspension transfection to 2,000L through upstream process optimization

M Stanvick, R Gilmore, D Tsiros, K Szymczak, E Wiberg, S Wallace, J Yin

Oxford Biomedica Solutions, Bedford, MA

ABSTRACT

Transient transfection of plasmids into suspension HEK293 cells for producing AAV provides great flexibility and speed to move drug candidates into clinical trials. Having a platform model allows for efficient implementation of continuous innovation. Traditionally, a batch process transfected with three plasmids at 2E6 viable cells/mL is used. To improve bioreactor vector genome (vg) productivity and percentage intact capsids, OXB Solutions has developed a high cell density process with a novel transfection method and cutting-edge dual plasmid transfection. This new process demonstrates substantial increases in bioreactor vg titer by about 10 fold and increases the percentage of intact capsids in the affinity product by about 2 fold. The new process is highly reproducible and allows for plug-and-play use, now tested in over 9 different capsid serotypes (clades A, B, E & F), in most cases resulting in 2L bioreactor vg titer close to or above 1E15vg/L and intact capsids in the affinity product close to or above 50%. Furthermore, we successfully scaled up the new platform process from 2L to 50L through to 2,000L bioreactor with consistent vg productivity and Drug Substance product quality, confirming 90% fully intact vector. To push the bar even higher, we have been developing our next-generation high cell density transfection process and are reporting a bioreactor vg titer of over 2.5E15vg/L from our preliminary 2L

CONTACT

Oxford Biomedica Solutions
solutions.partnering@oxb.com

INTRODUCTION

The Upstream Process Development team developed a new platform process to increase the vg productivity and AAV packaging in the bioreactor. The targeted development approach focused on:

1. Increasing cell specific productivity through plasmid design and process additives
2. Increasing total productivity through an increase in transfection cell density

These process improvements aimed to be linearly scalable to 2,000L bioreactors and broadly applicable across different capsid serotypes. This plug-and-play process can achieve upwards of 1E15 vg/L out of the bioreactor.

RESULTS

DUAL PLASMID

We evaluated the three combinations that the components of triple transfection could be arranged onto two plasmids. The combination of GOI+RepCap along with a separate pHelper resulted in substantial improvement in VG titers as well as an increase in the percentage of calculated full vectors.

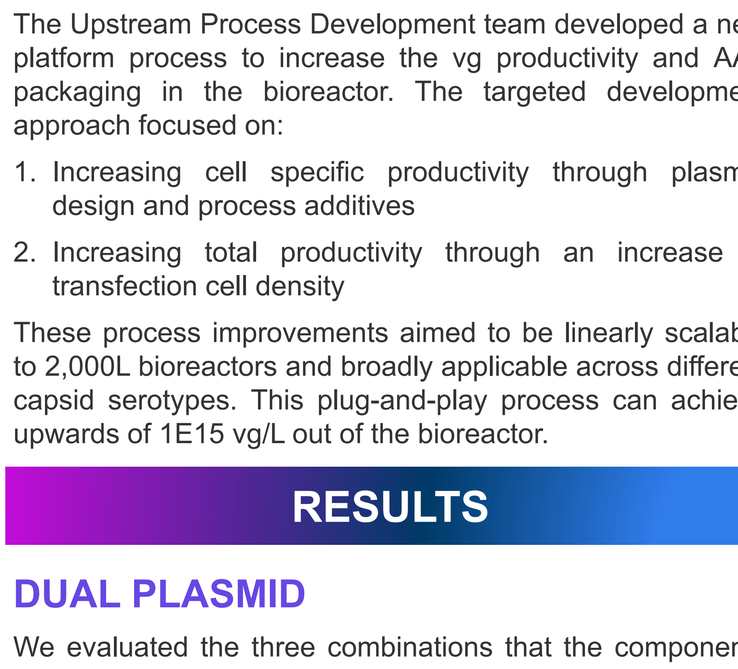

Figure 1: Productivity Assessment of Three Possible Dual Plasmid Configurations and Triple Transfection. 125mL shake flasks were transfected with GOI+RepCap & Helper, GOI & RepCap+Helper, GOI+Helper & RepCap or standard triple transfection. Crude lysate samples were quantified for VG productivity, capsid productivity (not shown), and the percentage of calculated full vectors

RESULTS

UPSTREAM PROCESS DEVELOPMENT

A novel productivity additive was identified that increased productivity over 2-fold. By increasing the transfection density from 2E6 viable cells per mL to 4E6, the bioreactor productivity increased an additional 2.7-fold.

In combination, the following resulted in over 10-fold increase in bioreactor productivity and an increase in percent full capsids:

- dual plasmid transfection
- productivity additive
- increase in transfection density
- transfection preparation control strategy

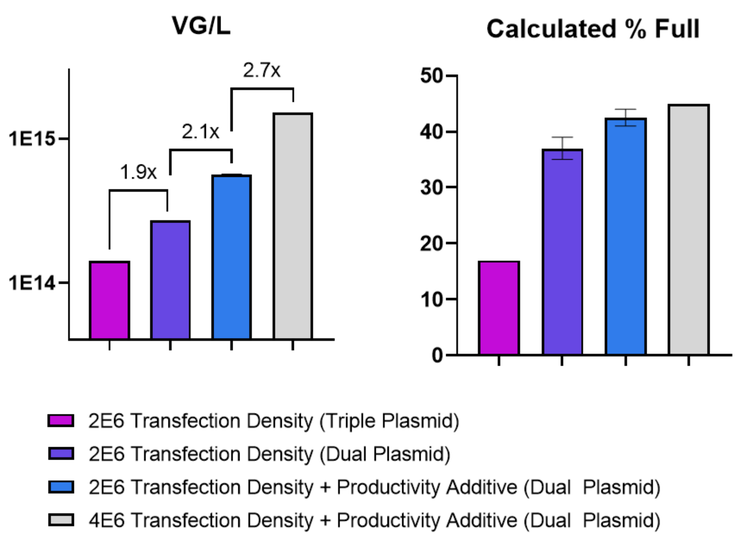

Figure 2: Productivity Assessment of Plasmid Design, Transfection Density, and Productivity Additive. 125mL shake flasks were transfected with dual plasmids at 2E6 or 4E6 transfection density. Crude lysate samples were quantified for VG productivity and the percentage of calculated full vectors.

ALTERNATIVE SEROTYPE EVALUATION

The new platform process allows for plug-and-play use across greater than 9 diverse AAV capsids (clades A, B, E & F). All serotypes demonstrated an increase in VG productivity using dual rather than triple transfection. Additionally, equivalent or increased calculated full vectors was observed for all serotypes tested. Triple plasmid transfection resulted in an average productivity of 5.7E14 VG/L. Dual plasmid transfection resulted in an average productivity of 9.6E14 VG/L.

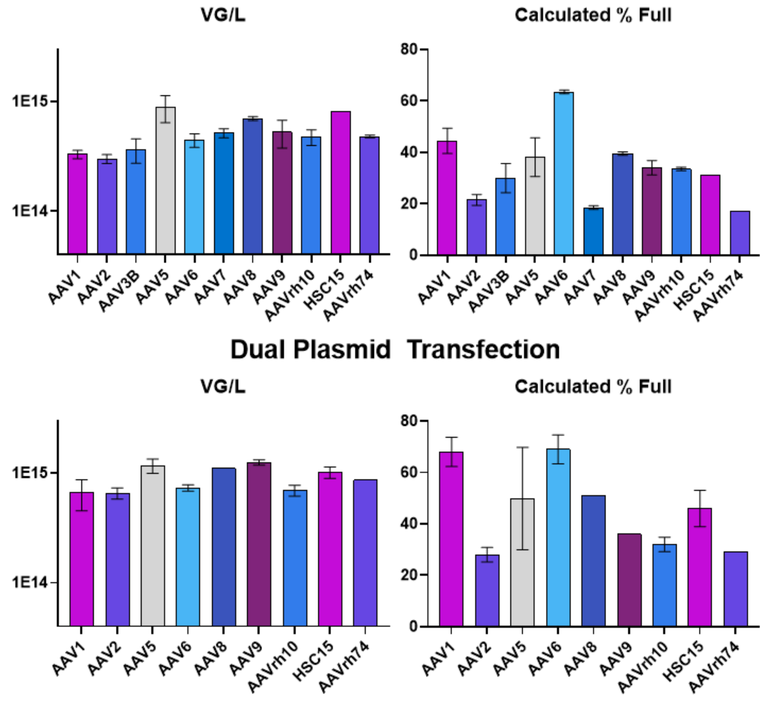

Figure 3: Productivity Assessment of Eleven AAV Serotypes with Triple Transfection and Nine AAV Serotypes with Dual Transfection. Transfections were performed in 2L bioreactors. Crude lysate samples were quantified for VG production and calculated percent full vectors.

SCALE UP

Process scale-up was evaluated at the 50L, 500L, and 2,000L scale. The major areas of focus during scale up are:

- Transfection Volume, Process Timing, and Control
- Bioreactor Control Strategy

By tightly controlling all these areas, we successfully scaled 3 constructs and 2 platforms to the 500L and 2,000L bioreactors with comparable VG productivity and no change in product quality.

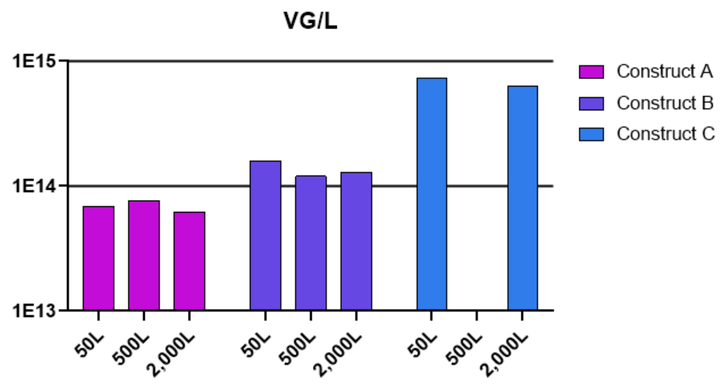

Figure 4: Bioreactor AAV Productivity Comparisons at 50L, 500L, and 2,000L Scales. Transfections were performed with for each AAV capsid serotype in 50L, 500L, and/or 2,000L bioreactors. Construct A and B utilized triple plasmid transfection. Construct C utilized dual plasmid transfection. Crude lysate samples were quantified for VG production and the percentage of calculated full vectors.

PRODUCT QUALITY COMPARISON ACROSS SCALES

	Construct A		Construct B		Construct C	
	500L	2,000L	500L	2,000L	50L	2,000L
% Purity	99.0	95.4	99.8	99.1	98.8	99.3
% Aggregates	1.6	1.5	1.0	0.7	1.9	1.0
VP ratio	meet	meet	meet	meet	meet	meet
% Full	80	79	90	90	89	88
Infectivity	meet	meet	meet	meet	meet	meet
HCP	BLOQ	BLOQ	1.7	6.3	BLOQ	BLOQ
HC DNA	98	159	11	52	11	14

NEXT GENERATION HIGH CELL DENSITY TRANSFECTION

To improve bioreactor productivity further, the Upstream Process Development team is investigating transfection at even higher cell densities using a perfusion process. To maintain the transfection efficiency at higher cell densities, the team optimized the method of transfection. This high density perfusion process can reach productivities of >2.5E15 vg/L.

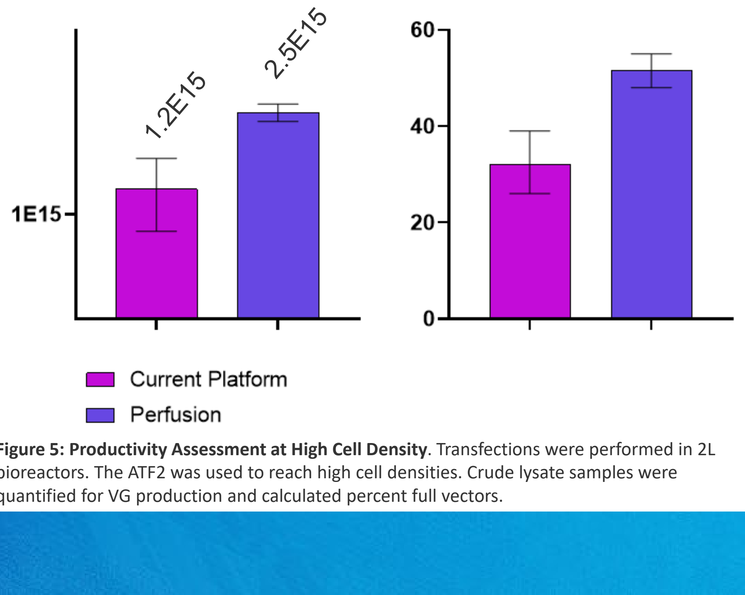

Figure 5: Productivity Assessment at High Cell Density. Transfections were performed in 2L bioreactors. The ATF2 was used to reach high cell densities. Crude lysate samples were quantified for VG production and calculated percent full vectors.

CONCLUSIONS

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

1) Highly productive with >1E15 vg/L from the bioreactor
2) Scalable to 2,000L
3) Reproducible across eleven capsid serotypes

This novel system enables production of significantly more patient doses per batch and therefore can help to greatly reduce the number of batches needed while driving down the overall manufacturing cost for both clinical and commercial product.

To continue to stay on the cutting edge of AAV production, the Upstream Process Development team is developing our next generation high cell density transfection utilizing a perfusion process which can reach 2.5E15 vg/L in a 2L bioreactor.

Presented at ESGCT, October 2022, Edinburgh, Scotland