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End-to-end process scalability of HEK 293 media for high titer AAV production

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As adeno-associated virus (AAV) gene therapies move from early development into clinical trials and commercial phases, manufacturing scalability becomes a defining measure of success. The question evolves from "does the platform work" to "how reliably does the processes translate into robust, end-to-end manufacturing". In this context, the transition from shake flasks to stirred tank bioreactors represents a critical milestone in derisking AAV programs across the production lifecycle.

Introduction

Shake flasks are valuable workhorses during early AAV platform/process development, and enable rapid assessment of process components and conditions. However, small scale data does not always accurately represent performance once processes enter controlled bioreactor systems. Changes to parameters such as pH, dissolved oxygen (DO), and agitation can adversely affect the critical quality attributes (CQAs) of viral titer and percent full capsids when processes are scaled to bioreactors; these changes can unearth issues that cascade into downstream challenges and overall program concerns if not addressed early[1,2].

Benchtop stirred tank bioreactors at the 3 L scale act as a practical bridge between process development and manufacturing. Provided that scalable operating ranges are established early, AAV production in suspension HEK 293-based cell lines can be successfully transitioned from shake flasks into these systems while maintaining or improving on small scale platform performance[3,4]. Just as importantly, bioreactors allow upstream results to be aligned with downstream processing such as clarification capacity, chromatography column loading, and impurity control well before processes are locked for clinical use.

As an important inflection point in the progression of AAV-based gene therapy programs, production scalability into a bioreactor uniquely connects upstream and downstream workflows. Having confidence to answer the question "how reliably does the process translate into robust, end-to-end manufacturing" streamlines technology transfer and reduces late-stage process re-engineering, driving cost and labor benefits across a therapeutic program.

Materials and methods

Cell culture and scale-up process

Commercial Human Embryonic Kidney (HEK) 293 cells were thawed directly into TheraPEAK 293-GT® Medium in 125 mL shake flasks. Cells were maintained according to TheraPEAK 293-GT® Media System Instruction for Use and expanded to larger flasks after 3 passages. Three days prior to transfection, cells were seeded into two identical single-use 3 L stirred tank bioreactors in such a way to achieve a viable cell density between 2.5 x 10^6 to 3.5 x 10^6 cells/mL at the time of transfection. For both bioreactors, agitation speed was maintained at 220 rpm, air overlay was maintained at 25 mL/min and gas sparging rate was set at 7.5 mL/min. pH and dissolved oxygen level were controlled by gas sparging at 7.3 ± 0.1 and 50%, respectively.

Transfection and lysate harvest

TheraPEAK 293-GT® AAV Supplement was added to cell culture within one hour prior to transfection at a ratio of 1 : 30 v/v (83.3 mL for 2.5 L culture volume). Transfection was performed with a commercially available reduced serum medium as the complexation buffer, following a complexation volume ratio of 10% v/v (250 mL added to 2.5 L of culture). Lonza Xcite® AAV Transient Transfection Plasmids were used with a molar ratio of 1 : 1 : 1 for Helper : RepCap : GOI (GFP) plasmids. Commercially available transfection reagent and AAV enhancer were acquired from respective vendors. Vendor's recommendations on DNA amount per unit of cell, DNA to transfection ratio, enhancer addition volume and complexation time were followed. 72 ± 2 hours after transfection, cell culture was lysed by adding lysis buffer directly to the vessels.

AAV genome titer quantification

AAV titer was assessed with droplet-based digital PCR using Lonza's protocols through quantification of encapsulated copy numbers of gene of interest (GFP). Harvested crude lysate samples were processed to remove free-floating DNA and then digest capsid proteins. Reaction mixtures were prepared and droplet-based digital PCR was then performed following manufacturer's protocol.

Capsid full/empty ratio analysis

Crude lysate samples were first purified using small scale affinity purification. Purified lysate was then diluted with Dulbecco's Phosphate Buffered Saline (DPBS) and capsid full to empty ratio was analyzed by mass photometry. Final purified products were analyzed similarly after appropriate dilution.

Downstream purification process

After filtration, 25% of total lysate from both bioreactors were pooled for downstream purification. Pooled sample was first purified by affinity chromatography, followed by anion exchange and high-resolution chromatography for polishing. Finally, the collected material was concentrated by tangential flow filtration.

Results

Shake flask AAV production screening in TheraPEAK 293-GT® Media System

A previous study with TheraPEAK 293-GT® Media System conducted at the scale of 125 mL shake flask was reviewed first. Two commercial transfection reagents and AAV enhancers were tested and performance was evaluated by the titer and full/empty capsid ratio of three serotypes AAV2, AAV8 and AAV9. Transfection reagent A with enhancer 1 together produced the highest AAV titer across all serotypes, amongst which AAV9 shows titer beyond 1.50E+12 vg/mL (Figure 1). In order to demonstrate scalability of optimal productivity, subsequent 3 L bioreactor runs were designed for the production of AAV9 with transfection reagent A and enhancer 1.

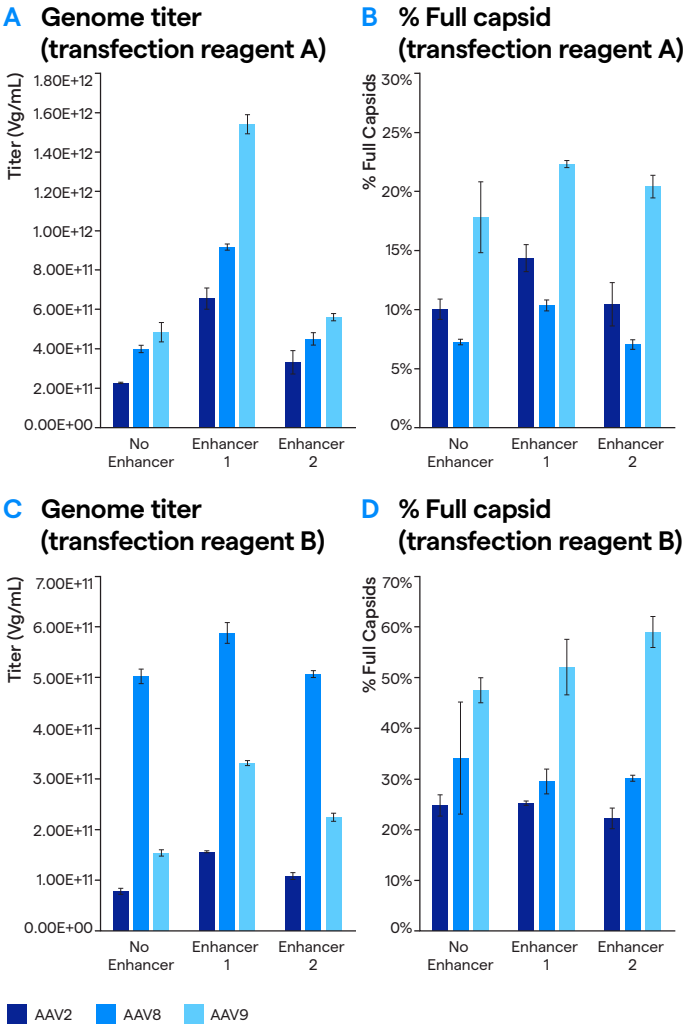

Figure 1.
Flexibility of TheraPEAK 293-GT® Media System demonstrated by AAV genome titer and % full capsid with the usage of commercial transfection reagent A (A and B) and transfection reagent B (C and D) with 2 different commercial and AAV enhancers. Lonza Xcite® AAV Transient Transfection Plasmids were used for transfection.

Successful process scale-up from shake flasks to 3 L bioreactors

Comparable AAV9 titer and capsid full/empty capsid ratio were observed for the bioreactor duplicates, with Bioreactor 1 demonstrating slightly higher AAV9 titer and full/empty ratio (Figure 2). The results were compared against averaged results from the corresponding previous shake flask outcomes mentioned above. The same reagents were used and same transfection parameters were applied in the shake flask process. One notable difference in the shake flask process is that cells were diluted with fresh TheraPEAK 293-GT® Medium to 3.0 x 10^6 cells/mL from a higher cell density on the day of transfection. Comparable results obtained from both small and large scales demonstrates a successful scale-up AAV production process and suggests robust scalability of TheraPEAK 293-GT® Media System.

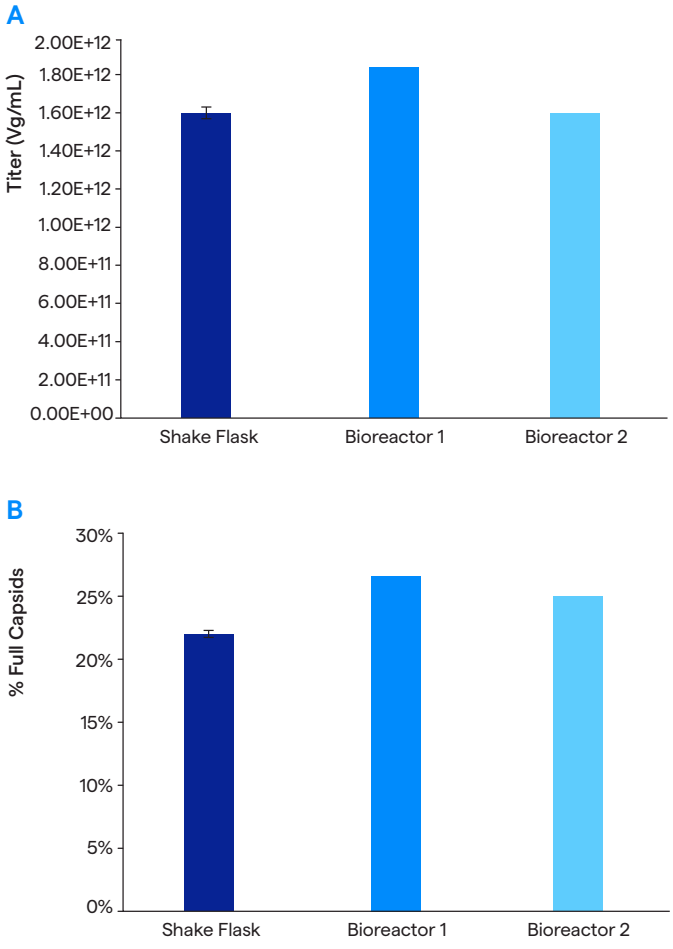

Figure 2.
AAV9 genome titer (A) and % full capsids (B) for shake flasks and 3 L bioreactor 1 and 2 with TheraPEAK 293-GT® Media System used together with the same commercial transfection reagent and enhancer. Error bars for shake flask results represent standard deviation of the triplicates.

Successful downstream purification of AAV9 produced in 3 L bioreactor

Table 1 lists AAV9 titer and % full capsids quantified for crude lysate harvest as well as final purified product. Final AAV9 titer above 5.5E+13 and full capsid over 75% indicates that with a standard, non-optimized downstream process, high yield and quality can be achieved through scale-up AAV production with TheraPEAK 293-GT® Media System.

	AAV(Titer (vg/mL)	% Full Capsids
Bioreactor 1 crude lysate	1.86E+12	26.9%
Bioreactor 2 crude lysate	1.55E+12	24.9%
Purified product	5.87E+13	75.4%

Table 1.
AAV9 titer and % full capsid before and after downstream processing

Conclusion

The flexible and scalable high-performance of TheraPEAK 293-GT® Media System supports end-to-end acceleration of AAV development programs. For vector development and small scale workflows, the media system compatibility across process variables delivers increased titer and favorable full capsid ratios. For process development, these CQAs are conserved with comparable performance, making Lonza's TheraPEAK 293-GT® Media System both an R&D and manufacturing advantage for cGMP-compliant commercial gene therapy programs.

References:

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