A Robust and Scalable Research Grade Platform Process for AAV6 Production and Purification

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INTRODUCTION

Adeno-associated virus (AAV) is the most used vector in the field of gene therapy, with applications to treat and cure monogenetic disorders. There are currently hundreds of clinical trials underway for recombinant AAV (rAAV) therapeutics, in addition to a several clinically (EMA and FDA) approved products. Despite these clinical advancements in the gene therapy space, manufacturing of these vectors continues to be an ever-evolving challenge. Moreover, there is a clear need for robust, scalable, and cost-effective production of research grade material for indicative proof-of-concept work that is highly representative of process development platform material.

Many variables influence the success of rAAV vector production, including, but not limited to, production cell line, cell density, plasmid systems, including plasmid ratios and total plasmid DNA, media composition, and plasmid sequence composition. In the downstream process additional variables can include harvest and lysis conditions, filtration, stabilization and storage, purification method, and post-purification processing.

Here we describe research grade and process development productions of rAAV6, both based on transient transfection of serum-free cells grown in suspension. We demonstrate the progression of our well-established methods, including modifications to transfection process and purification method. Using a rAAV packaging genome sizes of 4.2kb- 4.7 kb, containing representative therapeutics , we have been able to consistently achieve titers of >7x10^{10} vector genomes (vg)/mL from crude production, with on average 55% and 65% yield after affinity batch purification in our research grade platform referred to as ElevateBio (EB) Batch, and AKTA capture chromatography in our process development (PD) platform, respectively.

The goal of aligning our research grade and process development productions of AAV6 is for ease of transition when scaling and streamlining products. This seamless transition will allow for acceleration of proof-of-concept work to Chemistry, Manufacturing and Controls (CMC) activities. Additionally, it allows R&D to stage gate and optimize the following aspects in AAV6 products; selecting the superior gene of interest, testing target-specific capsid, and development of validated analytics.

R&D VS. PD AAV6 PRODUCTION METHODS

Fig 1A.

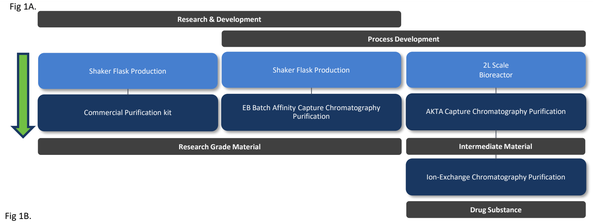


Fig 1B.

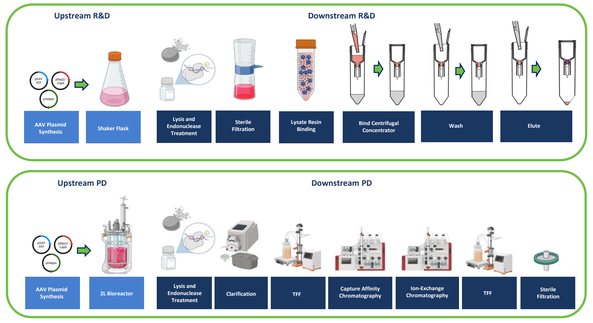


Fig 1. Process workflow for research grade AAV6 material and process development-made AAV6 material. A) Schematic for variable production and purification methods across the two methods of AAV6 production. B) Breakdown of processes for AAV6 production between research grade and process development material. Both include triple transfection production into serum-free suspension cells, followed by lysis harvest, as well as variable purification methods. Additional processing can be implemented into process development material depending on intended use of AAV6.

IMPROVEMENTS TO R&D AAV6 UPSTREAM & DOWNSTREAM PROCESS

Fig 2A.

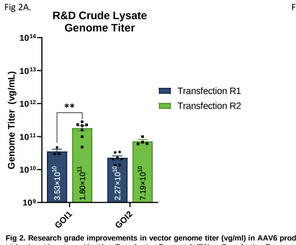


Fig 2B.

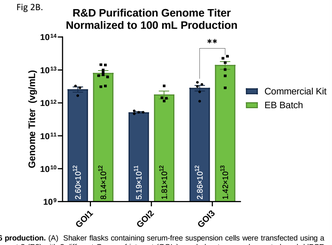


Fig 2. Research grade improvements in vector genome titer (vg/ml) in AAV6 production. (A) Shaker flasks containing serum-free suspension cells were transfected using a triple plasmid system with either Transfection Reagent 1 (R1) or Transfection Reagent 2 (R2) with 2 different Genes of interest (GOIs), crude lysates were harvested, and ddPCR was performed to compare genome titers (vector genomes (vg)/mL) of upstream processes. (B) Research grade AAV6 crude lysate containing different GOIs were purified with either a Commercial kit or ElevateBio (EB) Batch purification method. Genome titers were normalized to 100 mL production volumes and 1mL final elution volumes.

TRANSDUCTION EFFICIENY: COMPARING AAV6 FROM DIFFERENT PURIFICATION METHODS

Fig 3A.

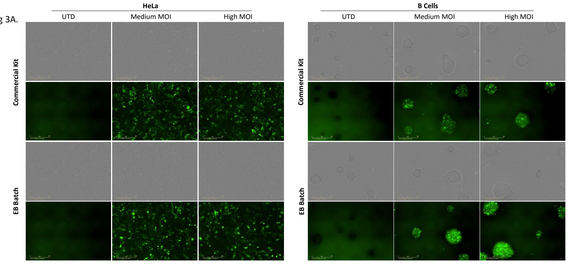


Fig 3B.

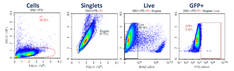


Fig 3C.

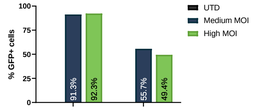


Fig 3D.

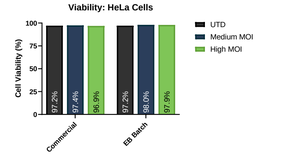


Fig 3E.

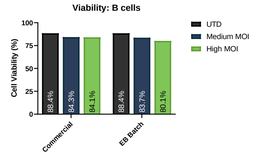
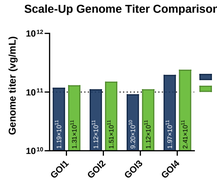


Fig 3. Potency assay at 48 hours post AAV6-GFP transduction in HeLa and B cells. A) Incucyte images were taken to show bright field for cell morphology and GFP expression in transduced cells. Protein expression was also analyzed by flow cytometry. B) gating strategy is shown for C) percent GFP positive cells and D-E) viability of cells from AAV6-GFP purified from a Commercial Kit or using our EB Batch method at different MOIs (Medium and High) compared to untransduced (UTD). No differences in cell viability was seen between Commercial Kit vs EB Batch in either HeLa or B Cells.

RESULTS

R&D VS. PD PRODUCTION COMPARISONS

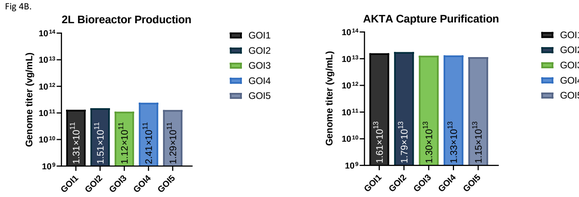
Fig 4A.



	GOI 1	GOI 2	GOI 3	GOI 4
Shaker flask (vg/mL)	1.19x10^{11}	1.12x10^{11}	9.20x10^{10}	1.97x10^{11}
2L Bioreactor (vg/mL)	1.31x10^{11}	1.51x10^{11}	1.12x10^{11}	2.41x10^{11}
2L Titer Comparability	109.2%	126.8%	125%	122.3%

Fig 4A. Crude harvest titers comparison between 30mL shaker flask and PD bioreactor crude productions. Titers >8x10^{10} vg/mL in a 30mL flask generally indicates >1x10^{11} vg/mL in a 2L bioreactor and an overall increase in titers due to ability to monitor and control the environment in a bioreactor.

Fig 4B.



	GOI 1	GOI 2	GOI 3	GOI 4	GOI 5
Crude (vg/mL)	1.31x10^{11}	1.51x10^{11}	1.12x10^{11}	2.41x10^{11}	1.29x10^{11}
Eluate (vg/mL)	1.61x10^{13}	1.79x10^{13}	1.3x10^{13}	1.33x10^{13}	1.15x10^{13}
% Recovered	71	62	45	47	43

Fig 4B. Production process from PD showing crude harvest titers from a 2L bioreactor production and elution titers following an AKTA capture chromatography purification (vg/mL), as well as percent recovery. Recovery from process development generally yields rAAV6 crude titers >9x10^{10} vg/mL in a 2L bioreactor and elution titers > 1x10^{13} vg/mL with 40-70% recovery.

TRANSDUCTION EFFICIECNY OF EB BATCH AAV6 MATERIAL

Fig 5A.

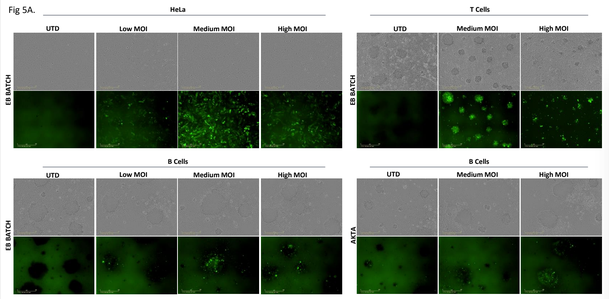


Fig5A. Potency assay at 48 hours post AAV6-GFP transduction in HeLa and B cells of HeLa, T cells, B cells at 48 hours post AAV6-GFP transduction produced by EB Batch, as well as B cells transduced by AKTA purified AAV6-GFP, at low, medium or high MOIs (vg/cell) compared to UTD. Strong GFP expression can be seen in the clumped B cells and T cells.

Fig 5B.

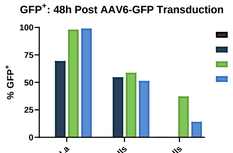


Fig 5C.

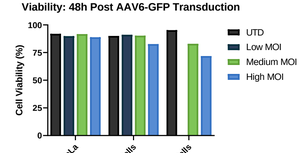
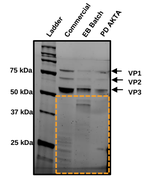


Fig 5. Dose dependent GFP expression at 48 hours post AAV6-GFP transduction in HeLa, B cells and T cells. Flow cytometry comparing B) % GFP positive cells, as well as C) viability using AAV6-GFP purified by Commercial Kit vs EB Batch at different MOIs (Low, Medium and High) compared to UTD. Some toxicity was seen at high MOIs in T cells, but generally well tolerated in HeLa and B cells.



Method	Hands on Time (hours)	Input volume (mL)	Eluate Volume (mL)	Total AAV Titer (vg)	Average Recovery (%)	Titer for 100 mL productio n (vg/mL)	Serotype agnostic	Scalable
Commercial Kit	5-6	300	1	7.5x10^{12}	20	7.5x10^{11}	Yes	No
EB Batch	2	100	1	7.5x10^{12}	58	7.5x10^{12}	Requires optimization	Yes, using an AKTA
PD AKTA	2	2L	5	2.5x10^{14}	65	1.3x10^{13}	Requires optimization	Yes

Table 3. Inputs and Outputs of AAV6 Purification Methods used in R&D and PD. Commercial kit requires around 3x as much crude input in order to achieve similar total VGs compared to the EB Batch method of purification. Additionally, commercial kit requires more hands-on time, as well as reagents such as plasmids, cells, media, etc. Because EB Batch uses similar resin, it can be scaled to an AKTA purification method and optimized for different serotypes.

Fig 6. SDS-PAGE comparison of residual process impurities between purification methods. AAV6 material produced via RD method, purified via commercial kit, EB Batch, or PD AKTA. Purified AAV6 was equally loaded at 2.2x10^{10} VG. AAV VP1, VP2, and VP3 have molecular masses of around 87, 72, and 62 kDa, respectively. Process impurities can be seen at lower molecular weights that can be found in the dotted rectangle.

		Commercial Kit	EB Batch	PD AKTA
	Proprietary Kit	Resin based affinity chromatography	Resin based affinity chromatography
Benefits	All serotypes	Higher purity final product	High purity final product
Benefits	Faster than ultracentrifugation methods (5-6 hrs)	Scalable; requires optimization	Scalable; requires optimization
Benefits	Minimal expertise/equipment	2-hour process	Setup required, automated process
Drawbacks	Higher residual contaminants eg. HCP	Low Throughput (100mL)	Expertise/equipment required
Drawbacks	No separation of empty vs full particles	Required optimization for each serotype	Required optimization for each serotype
Drawbacks	Does not include supernatant (10-15% of AAV)	Total lysis	Total lysis
Drawbacks	Not scalable		

Table 4. Benefits and Drawbacks comparing AAV6 purification methods used in R&D and PD.

CONCLUSIONS

- Upstream and downstream processes have been established and optimized for alignment and scalability between the small-scale production of research and development (RD) and large-scale production of process development (PD).
- Improvements to RD upstream AAV6 production process allowed for 2-3x increase in crude harvest titers (vg/mL) using a 4.7kb therapeutically relevant gene of interest and scalability to a bioreactor production.
- EB Batch purification process shows 3-4x downstream yield improvements in vector genome titer compared to a commercially available kit for RD material.
- The purified, high-titer, AAV6-GFP demonstrates efficient and potent transduction of B cells and HeLa cells, denoted by GFP expression, with no overt signs of toxicity and minor reduction in viability with our AAV6 products. Preliminary T Cell data shows a drop in GFP expression at high MOIs, as well as viability, additional studies with other donors will be conducted to confirm these results.
- Next steps are to further characterize AAV6 with percent (%) full analytics, VP ratios, intact GOI

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