Scale-up of an ELEVECTA™ transient rAAV production process in the Xcellerex™ X-platform single-use bioreactor

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Abstract

The delivery of therapeutic genes using recombinant adeno-associated viruses (rAAV) has emerged as a viable treatment option for various diseases, as shown by the approval of multiple rAAV-based *in vivo* gene therapies. However, the development of scalable, high-titer GMP-compatible production processes remains a significant challenge for the industry.

Previous work has shown that the ELEVECTA™ transient cell line grows in a single-cell suspension, reaches high cell density, and supports high-titer rAAV production for multiple serotypes with significantly reduced levels of hcDNA (including encapsidated) compared to another commercially available HEK293 cell line. To further this work, the previously established process has been scaled to 50 L and 200 L in the Xcellerex™ X-platform single-use bioreactor. Scalability is shown up to 200 L scale with comparable cell culture performance and titer.

The next-generation Xcellerex X-platform single-use bioreactor, in tandem with the ELEVECTA transient cell line and optimized, GMP-grade HyClone™ prime expression medium, effectively addresses the market demands for scalable, high-titer manufacturing of high-quality rAAV products from R&D through to commercial manufacturing.

Process parameters

Table 1. Process parameters and targets for 50 L and 200 L scale ELEVECTA process

Parameter	Target
Agitation	50 w/m^3
Temperature	37°C
Seeding density	0.25 × 10^6 cells/mL
pH	7.3 ± 0.1, controlled with CO_2 and sodium bicarbonate
DO	40%
Plasmids	pALD-HELP, pALD-LUC-GFP, pALD-AAV9 (Aldevron)
Transfection Reagent	PEI MAX (Kyfora Bio)
Target Transfection density	3.3 × 10^6 cells/mL (Acceptable range: 3.0–3.6 × 10^6 cells/mL)
Plasmid DNA/cell	0.67 µg DNA/10^6 cells
Plasmid DNA to Transfection Reagent Ratio	1:2
Transfection volume	10%
Growth medium	Prime expression medium
Complexation medium	Prime expression medium
Complexation Time	15 minutes
Day of Transfection	Day 3
Production Phase	Harvest 72 hours post-transfection
Enhancer	RevIT AAV enhancer (Mirus Bio)

Materials and methods

Production performance for rAAV9 was initially analyzed in 15 mL microbioreactor format (n = 6) and then scaled up to a 50 L and 200 L final harvest volume. The process utilized HyClone prime expression medium throughout and included a shake flask to ReadyToProcess WAVE™ 25 bioreactor seed train. The X-platform 50 L and 200 L bioreactors with fed-batch II bag was seeded at 0.25 × 10^6 cells/mL and transfected upon reaching 3.3 × 10^6 cells/mL. Three days post-transfection, the culture was endonuclease-treated and lysed before undergoing downstream processing.

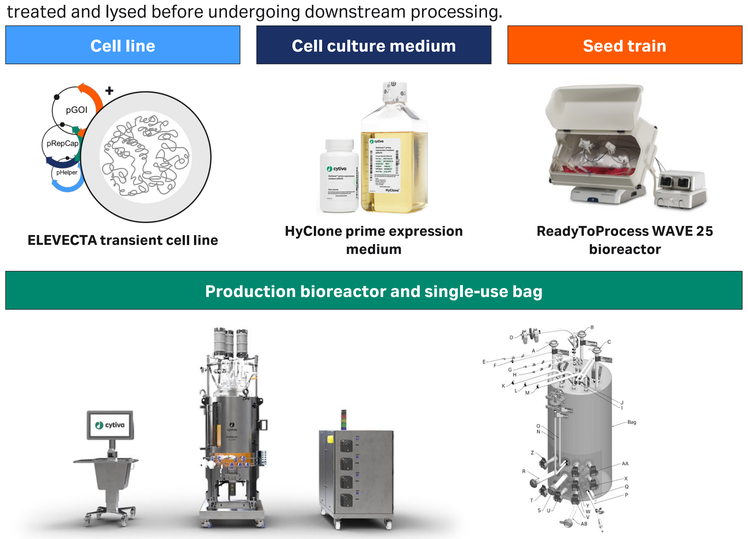


Fig 1. The rAAV production process utilizes the ELEVECTA transient cell line grown in HyClone prime expression medium. The ReadyToProcess WAVE 25 bioreactor was used for the N-1 step and the Xcellerex X-platform single-use bioreactor was used for viral production. The single use bag that was selected for this process possesses a macrosparger with a 1 mm pore size.

Product quality performance

To assess vector quality attributes, rAAV bioreactor clarified lysates were subjected to affinity chromatography and analyzed for hcDNA levels (including encapsidated). In contrast to the Freestyle 293-F cell line material, similar low-level amounts of 6-18 ng hcDNA/10^{14} vg (100-fold difference) were evident for all ELEVECTA transient cell line samples independent of scale.

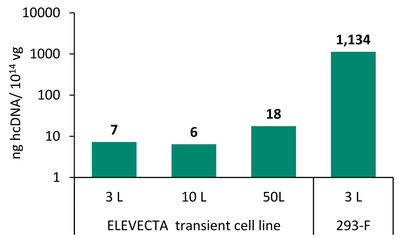


Fig 2. Comparison of hcDNA levels (ng per 10^{14} viral genomes [vg]), including encapsidated, after purification of viral vectors via affinity chromatography and analysis with a commercially available residual hcDNA qPCR assay.

Bioreactor scale-up performance

To evaluate process scalability, rAAV9 production with RevIT AAV Enhancer addition was executed in 50 L and 200 L stirred-tank bioreactors and compared to 15 mL microbioreactor performance. Robust and consistent performance in terms of growth, viability, and titer was observed throughout all production scales (Fig 3 and 4).

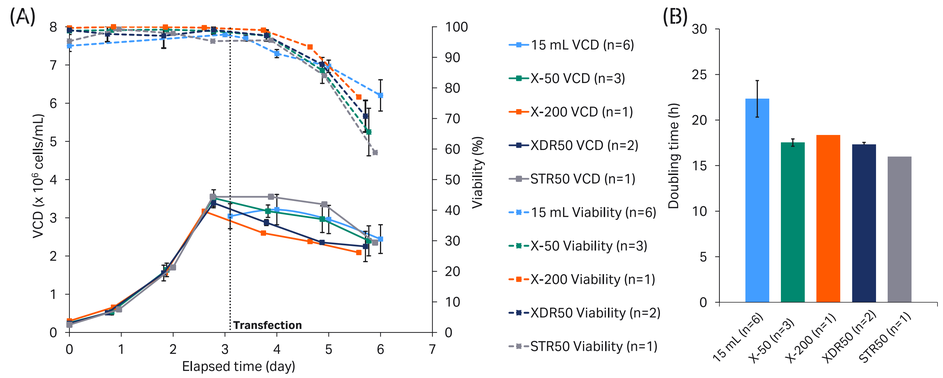


Fig 3. (A) Growth characteristics and viability of the ELEVECTA transient cell line in 15 mL Ambr (n = 6), X-50 (n = 3), X-200 (n = 1), 50 L Xcellerex XDR (n = 2), and 50 L Allegro™ STR (n = 1) bioreactors in presence of RevIT AAV Enhancer. Ambr15 cultures were seeded at 0.5 × 10^6 cells/mL and diluted to 3.3 × 10^6 cells/mL on day 3 prior to transfection (data not shown), while 50 L and 200 L cultures were seeded at 0.25 × 10^6 cells/mL to target the same transfection density without dilution. (B) Doubling time in hours for Day 0 to day 3 (day of transfection) for the bioreactor systems. Cultures grew slightly faster and more consistently in X-platform, XDR, and STR potentially due to scaling parameter differences in the microbioreactor system.

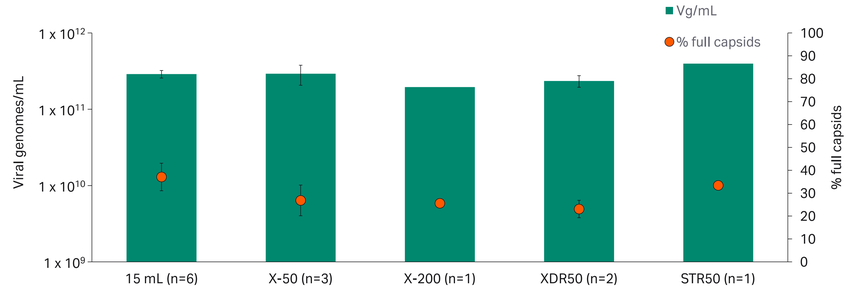


Fig 4. Comparison of rAAV9 titers and fullness in 15 mL Ambr (n = 6), X-50 (n = 3), X-200 (n = 1), 50 L Xcellerex XDR (n = 2), and 50 L Allegro STR (n = 1) bioreactors in presence of RevIT AAV Enhancer. Titers of rAAV were analyzed by qPCR and ELISA and used for calculation of percent full capsids.

Conclusions

We have shown that the ELEVECTA transient cell line has:

- Strong and consistent growth performance in HyClone prime expression medium across multiple Cytiva bioreactor systems including the Xcellerex X-platform single-use bioreactor
- High productivity for rAAV9 and low hcDNA levels (including encapsidated)
- Robust scalability with highly reproducible bioreactor process performance (15 mL to 200 L scale)

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Experimental work in Ambr15 was performed from Nov 2023 to March 2024 and data is held at Cytiva Cologne. Experimental work in X-platform, XDR, and STR was performed from Dec 2024 to March 2025 and data is held at Cytiva Westborough and Cytiva Uppsala.

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