Large Scale Suspension Production of AAV Capsid Variant Libraries from Stable Recombinant HEK293 Cell Banks

 # 437

Gueorgui Todorov, Alex Tai, Steven Long, Alissa Lindquist, Ngoc Nguyen, Mehdi Gasmi, Diana Cepeda

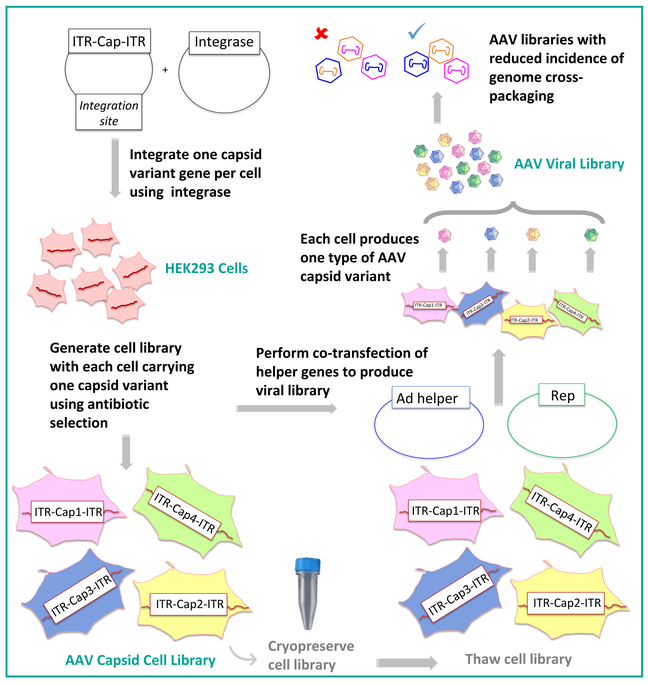
Adverum Biotechnologies Inc., Redwood City, CA, USA

ADVERUM BIOTECHNOLOGIES

Background

- Directed evolution is an established process for selecting novel AAV variants with improved interactions and immuno-neutralization profiles, which requires generating libraries of AAV with a high diversity of capsid mutants.
- Capsid libraries are typically made in adherent HEK293 cell systems utilizing limiting-dilution transient transfection of the plasmid encoding the capsid gene to limit genome cross-packaging, which reduces AAV capsid output and produces single-use AAV libraries that are not feasibly scalable.
- To address the limitations of single-use library systems, we first created HEK293 library cell lines in which each cell carries a single capsid variant coding sequence stably integrated into its genome.
- Once created, these stable producer cells can be used immediately to generate AAV viral libraries with generic AAV replicase and adenovirus helper plasmids, or cryo-preserved for future use.
- Low AAV viral library yields observed with two-dimensional adherent methods limit screening to in-vitro or small animal formats, highlighting the need for developing scalable suspension platforms.

Generating AAV Capsid Cell Libraries Limits Cross-packaging and Preserves Valuable Cell Banks for Future Use



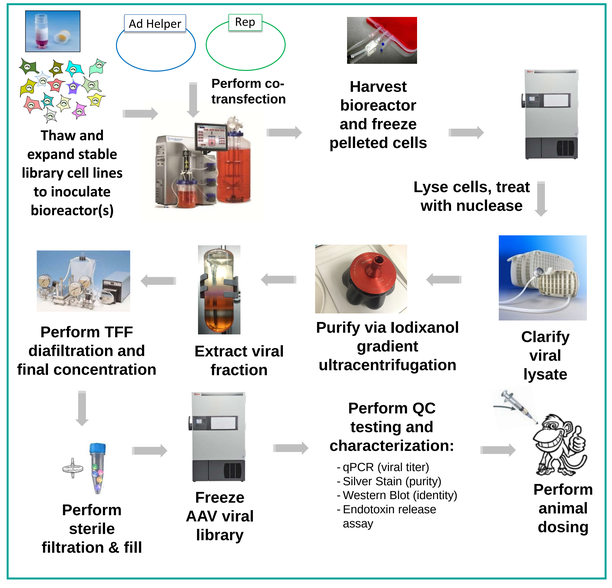
Purpose

- The goal of this work was to create a **scalable suspension production platform** using our stable recombinant HEK293 AAV capsid library cells capable of meeting the increased vector requirements of **large animal library screening studies.**
- To enable scalability in bioreactors, we established our AAV capsid cell library platform in a HEK293 suspension format.

Study Design and Methods

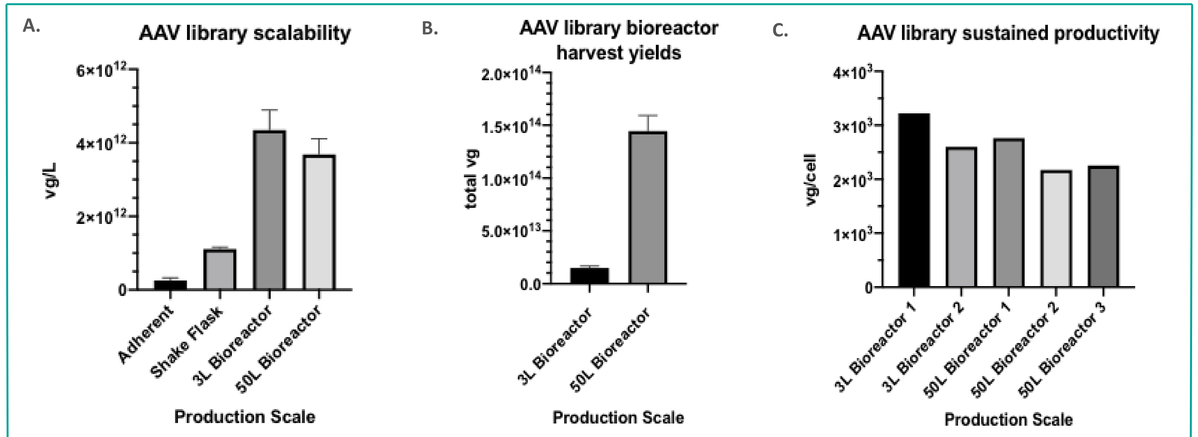
- Stable AAV capsid cell libraries were created in HEK293H cells, which were adapted to suspension culture conditions.
- Transfection conditions were adapted for optimal yield in suspension culture, maintaining a constant concentration of polyethylenimine transfection reagent and DNA per mL of culture.
- To rapidly expand from established 3-liter (3L) bioreactor parameters to 50-liter (50L), we adjusted agitation setpoints to maintain a constant volumetric mass transfer coefficient (k_La) and scaled aeration rates by maintaining a constant vessel volume per minute (VVM).
- To enable purification of one 50L vessel in a single working day, we modified our existing downstream purification method to accommodate higher volumes of cell lysate.

Overview of AAV Viral Library Production Process

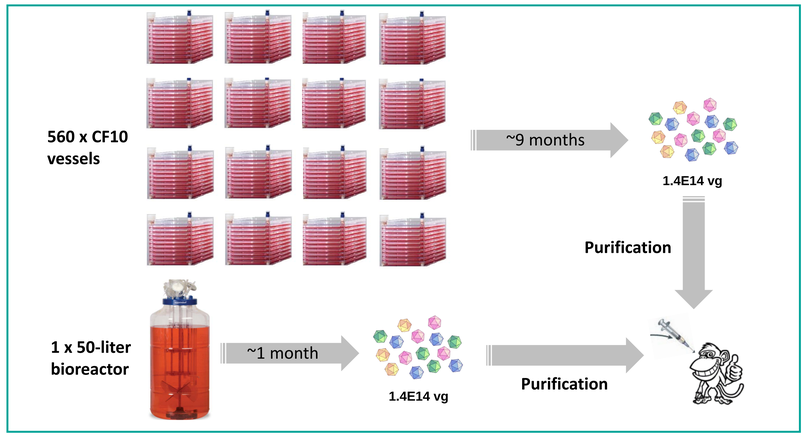


Results

AAV Viral Library Lysate Yield Improvement Across Production Scale



Scalable Suspension Platform Expands and Expedites Vector Discovery Capability



A. *Representative viral harvest lysate yields across production scales. Vector genome (vg) titers determined by qPCR assay and normalized to liters of culture media. From left to right: CF10 (Adherent), 30-mL shake flask, 3-liter and 50-liter bioreactor vessels. Error bars: SEM.*

B. *Bioreactor reproducibility across production scales. Total vg titers of viral harvest lysates determined by qPCR assay. Data represents five consecutive production runs (2x 3-liter and 3x 50-liter bioreactors) inoculated with cells from a single vial thaw representing the total capsid variant population. Error bars: SEM.*

C. *Sustained viral productivity across consecutive productions in graph **B**. Vg titers determined by qPCR assay and normalized to cell count at time of transfection.*

Conclusion and Future Directions

- We have demonstrated successful scale-up of our **AAV capsid library suspension HEK293 platform** to 50L bioreactors with **14X higher vg/L** harvest yield than our adherent methods and **consistent productivity** across consecutive batches.
- We are currently developing a 200L scale production process to meet the increased material demand of large animal systemic library screening studies.

Disclosures

AT, SL, AL, NN, and DC: Employees of Adverum Biotechnologies, Inc. GT and MG: Consultants of Adverum Biotechnologies, Inc

Contact Information

atai@adverum.com