Optimized Transfection Platform with Improved Productivity and Transgene Packaging for Scalable rAAV Production

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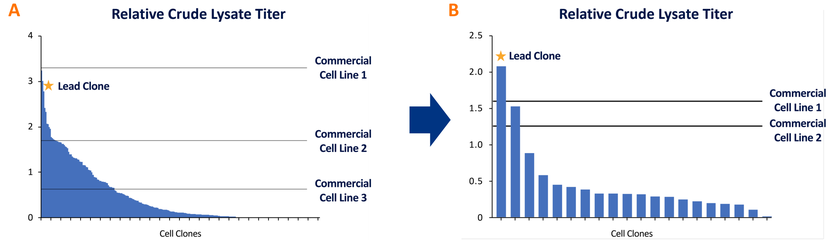
EXECUTIVE SUMMARY

Production of adeno-associated virus (AAV) gene therapies is a complex endeavor requiring the consideration of multiple factors to develop a scalable, robust process with optimal productivity and a favorable product quality profile. Here, we outline development conducted on the different components of our AAV production platform and the implementation of this platform to drive a program from candidate selection to GMP manufacturing.

Figure 1. **Strategy Used to Build the Optimized Transfection Platform**

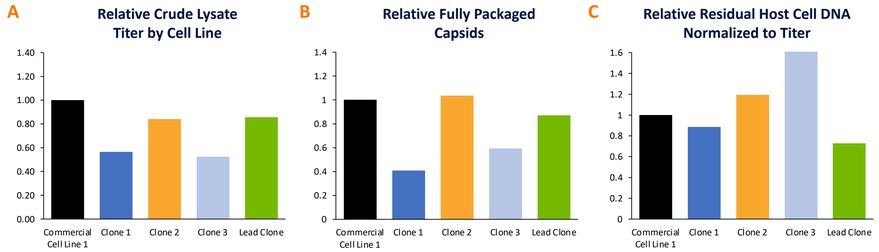
CELL LINE DEVELOPMENT

Figure 2. **Single Cell Cloning Produced > 500 Distinct Clones**



(**A**) Single cell cloning produced hundreds of unique clones that were expanded and transfected using a generic plasmid system. These clones observed a wide range of productivities with top candidates outproducing commercial cell lines. (**B**) The top performing clones from the previous screening study were run again with an optimized plasmid system to investigate program specific productivity. Top candidates were selected for further characterization.

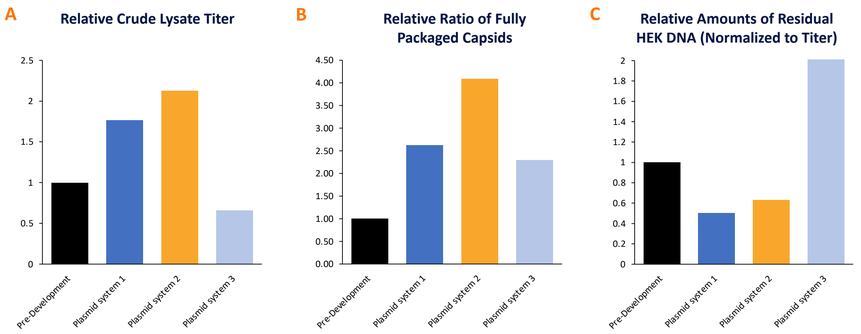
Figure 3. **Final Selection to Pick a Clone with High Productivity and Favorable Product Quality Attributes**



(**A**) Top clones were produced in 2L bioreactors alongside the commercially available cell line. (**B**) Capsid occupancy profiles were measured with AUC for all cell lines. The same two clones had comparable proportions of fully packaged capsids as the commercial lines. (**C**) Measurements of residual HEK DNA shows lead clone had significantly lower residual DNA. Lead clones is selected since it had comparable upstream productivities and capsid occupancy profiles with lower amount of residual DNA.

PLASMID DESIGN

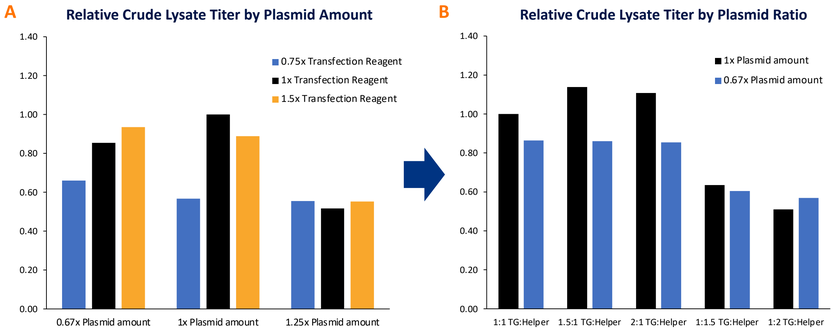
Figure 4. **Plasmid Design Improved Upstream Yield and Capsid Packaging**



(**A**) Improvements to the design of the plasmid led to three final candidates that significantly improved upstream yields out of the bioreactor, with system 2 yielding the largest improvement at over a 4x increase in titer from the pre-development system. This material was then purified via affinity chromatography to assess product quality. (**B**) Measurements of capsid occupancy by AUC reveal plasmid system 1 and system 2 greatly improved the proportion of fully packaged capsids with an over 2x increase presented by transfection system 2. (**C**) Analysis of residual HEK DNA also shows a favorable reduction in systems 1 and 2 compared to the pre-development plasmid system. Ultimately, based on the significant increases to productivity and favorable product quality profiles, system 2 was chosen for further development.

TRANSFECTION OPTIMIZATION

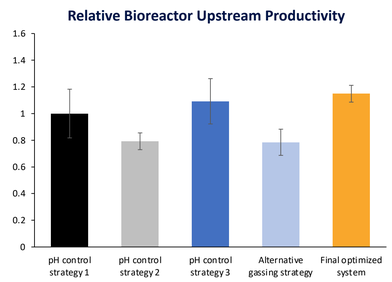
Figure 5. **Optimizations to Key Transfection Parameters Such as Plasmid Ratios, Plasmid Amounts, and Reagent Amounts Ensured the Most Efficient Production of rAAV**



(**A**) Optimizations to the transfection parameters first started by with a multi-level study investigating the total amount of plasmid used during transfection and the associated amounts of transfection reagent. Increasing the amount of transfection reagent provided little benefit, but decreasing the amount observed significant productivity loss so the 1x concentration was used moving forward. Decreased amounts of plasmid did not experience significant drops in titer, so this was tested again in the subsequent study. (**B**) The ratio of the transgene plasmid to the helper plasmid was investigated in the second study using the two possible plasmid amounts from the previous experiment. It was determined that a 1.5:1 plasmid ratio was optimal regardless of the total amount of plasmid used.

BIOREACTOR CONTROL STRATEGY

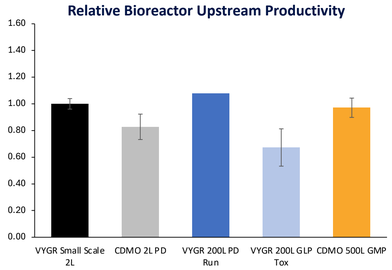
Figure 6. **Introduction of an Updated Bioreactor Control Strategy Resulted in More Consistent Upstream Productivity**



Development of a bioreactor control scheme had two major goals. The first was to identify areas that may be limiting the overall productivity at harvest and the second was to ensure consistent reproducible results before scaling up for manufacturing. The optimized bioreactor process employed a strategy that controlled the gas profiles while also maintaining the culture within an appropriate pH deadband.

SCALE-UP AND IMPLEMENT

Figure 7. **Process Development Scale Down Models Accurately Predicts Upstream Productivity of Large-scale Runs**



Finalized bioreactor parameters were transferred internally to our Pilot Plant and externally to a CDMO using a constant power per volume (P/V) across scales. These small-scale models accurately predicted the upstream productivity of internally-produced GLP tox and CDMO-produced GMP batches.

CONCLUSIONS

- Voyager produced a proprietary cell line that yields good productivity with a favorable product quality profile.
- Alterations to plasmid design significantly increases productivity while also yielding more full capsids and less residual DNA.
- Optimizations to the transfection parameters and implementation of a proper bioreactor control scheme ensures a robust process with efficient use of raw materials.
- Comparable performance across sites and scales (2L, 200L, and 500L) demonstrates a robust process where manufacturing scale performance can be accurately modeled with small scale bench-top bioreactors.