Advancing High-Yield Suspension HEK293 rAAV Manufacturing in Single-Use Bioreactors

Steven Wesel, Danielle Sexton, Rebecca Raig, Justin Auerbach, Jessie Chua, Frank Agbogbo, David Dismuke

FORGE BIOLOGICS
A member of Ajinomoto Bio-Pharma Services

BACKGROUND

Abstract

- Scientific progress, positive clinical outcomes, and increasing commercial approvals continue to fuel a growing demand for rAAV vectors for gene therapy. As programs mature, scalable and well-understood upstream platforms are required to balance productivity, robustness, and process control.
- Forge Biologics has established a platform process for scalable rAAV manufacturing based on transient transfection of Ignition™ HEK293 cells. The FUEL™ platform builds upon established processes, resulting in higher vector yields through iterative and innovative optimization to enhance upstream productivity and downstream recovery to support programs across clinical milestones. With high rAAV yields, more patients can be dosed per batch, thereby fulfilling Forge's mission to enable access to life-changing gene therapies by bringing them from concept to reality.
- This presentation describes a structured, data-driven approach to upstream process optimization for AAV production enabled by the FUEL™ platform. The platform integrates Forge's advanced technologies including Ignition™ cell line, pEMBR™ 2.0 Ad-helper plasmid, and FUEL™ modified RepCap plasmids and be combined with product-specific optimization packages. A transfection design of experiments (DoE) is presented to enable targeted evaluation of transfection parameters and upstream process conditions, thereby further improving productivity while maintaining consistent, robust process performance. Within this framework, transfection enhancers and feed strategies were evaluated as integrated components of the product-specific optimization package, working synergistically to enhance productivity while preserving process robustness. One-factor-at-a-time (OFAT) and DoE methodologies have been applied to optimize critical transfection parameters—including capsid plasmid design, plasmid ratios, total DNA, reagent-to-DNA ratios, and transfection reagents—ensuring robustness and reproducibility of the process.
- These improvements work cooperatively to achieve impressive increases in yield and scalability, demonstrating Forge's transformative impact on rAAV manufacturing. Furthermore, this foundational optimization work for the platform and program-specific cases creates understanding by leveraging data from screening and scale-up experiments for late-stage risk assessments and ultimately process characterization efforts.

Contact

Steven Wesel, Director of Upstream Process Development
Swesel@forgebiologics.com
Forge Biologics, 3900 Gantz Road, Grove City OH 43123

INTRODUCTION

The FUEL™ Platform

FUEL FORGE BIOLOGICS

Enhanced Starting Materials

Ignition™ HEK293 Cells
A well-documented Forge suspension cell line.

pEMBR 2.0 Helper
consistently improve yields by 2-fold.

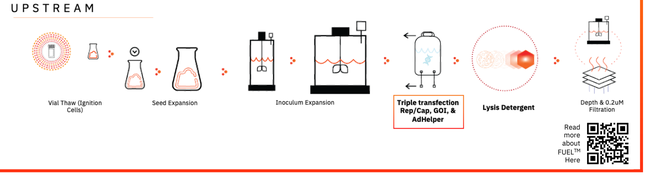
Read our research paper on pEMBR 2.0 here

FUEL RepCap
plasmids combined with pEMBR™ 2.0 improved titers by up to 15-fold.

Product-Specific Optimizations

Transfection DoEs help identify product-specific optimized process parameters to improve yields. These parameters include: **Plasmid Ratios, Transfection Reagents and Enhancers, Supplementation and Feeds.**

Forge's Platform is a Strong Foundation with Flexibility for **Product-Specific Optimization**



Plasmids, Feed, Enhancers

Forge Biologics employs a systematic approach to process development by which optimal parameters, unique to each program, are identified. Process optimization may include parameter optimization via DoE or OFAT, selection of pEMBR™ 2.0 helper and FUEL™ RepCap plasmids, transfection enhancer screening, and tailored feed strategies. This approach consistently results in a set of parameters that achieve improved yields relative to historical process (Figure 3).

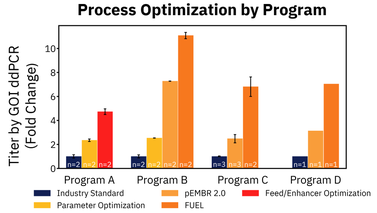
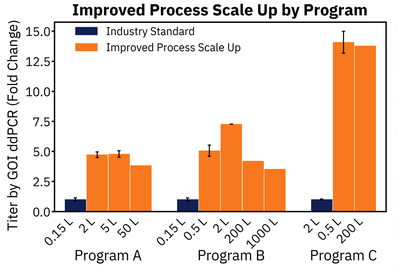


Figure 3. Programs and resulting titer improvements.

Scale Up



Process scalability is a primary goal of Forge's FUEL™ platform, ensuring that conditions optimized for maximum rAAV production translate robustly to cGMP-relevant manufacturing scales. Three client programs were successfully scaled up to ≥50 L, demonstrating a 4 to 14-fold increase in clarified lysate titer relative to each program's historical process (Figure 6). These results highlighted the reproducibility and scalability of process improvements identified through process development.

Figure 6. Process scalability from shake flasks or small-scale bioreactors to manufacturing scale (≥ 50 L SUBs).

One program proceeded on an accelerated path to cGMP manufacturing, bypassing process development to meet clinical timeline needs. Following eight cGMP batches, the program aimed to optimize their manufacturing process before resumption of cGMP manufacturing. Following process development, a 9-fold titer improvement over historical was achieved (Figure 7). Changes included DoE optimization, implementation of pEMBR™-2.0, and transfection enhancer. The final process (v2) included optimization of the seed vessel, which pushed yields even higher.

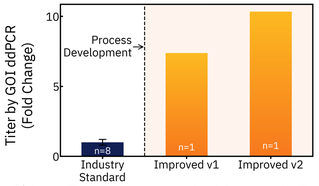


Figure 7. Clarified lysate titer of GMP batches before and after process development.

RESULTS

Design of Experiments

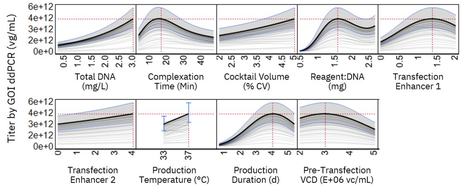


Figure 1. Prediction profiler set to maximize clarified lysate titer.

Robust statistical methods are key part of Forge's FUEL™ platform. DoE is used to identify process parameters with the greatest influence on rAAV yield while minimizing cost of development resources. Some of the transfection parameters identified in Figure 1 were either incorporated into Forge's platform process or identified as opportunities for optimization in client programs.

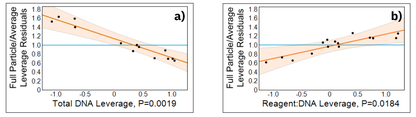


Figure 2. Full particle versus Total DNA (**a.**) and reagent:DNA ratio (**b.**).

In another experiment, full particle % was explored as a secondary response-variable in a limited-scope analysis of clarified lysate. This provided early process insights, isolating upstream process from downstream purification. Total DNA was negatively correlated with full particle %, while the ratio of transfection reagent:DNA was positively correlated with full particle % (Figure 2).

Robustness Studies

To accelerate timeline to cGMP, process robustness can be confirmed during scale up through use of shake flask controls (Figures 4 and 5). Parameters like complexation time, pre-transfection viable cell density, production duration, and feed strategy commonly influence upstream rAAV yield. As such, results of these shake flask productions can be used to inform acceptable ranges for the final manufacturing process.

When key process variables (e.g., transfection reagent, DNA concentration, cocktail volume, etc.) are optimized during early process optimization, it is important to confirm acceptable ranges. These shake flask robustness assessments are an optional but informative service clients may elect to leverage alongside scale up, providing an additional layer of process understanding prior to cGMP manufacturing.

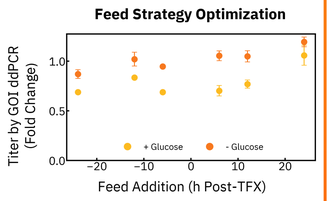


Figure 4. Feed optimization formulated with and without glucose. Fold change is calculated relative to control.

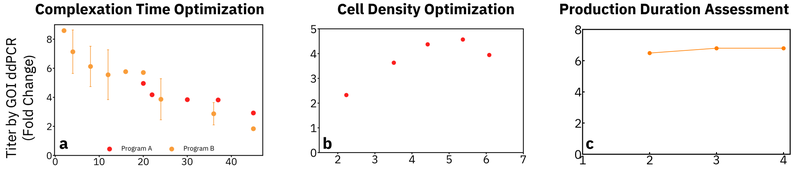


Figure 5. Example shake flask process screen for complexation time (**a.**), pre-transfection viable cell density optimization (**b.**), and production duration assessment (**c.**) where fold change is calculated relative to each program's historical process.

CONCLUSIONS

Summary

AAV manufacturability is established early and driven by the integration of vector design and process development. This work demonstrates that thoughtful plasmid design, combined with production-relevant screening, enables identification of high-performing constructs while eliminating those with inherent limitations. Implementation of a product specific optimizations within Forge's FUEL™ platform such as a transfection DoEs, implementation of feed, and addition of transfection enhancers further refines upstream conditions by systematically defining key parameters that impact yield, quality, and reproducibility. Performing robustness studies with Ambr250 and shake flask controls builds an understanding of parameter robustness for tech transfer to cGMP and informs late-stage activities as well.

Together, these strategies form a cohesive approach to maximize yields, improve robustness, and ensure scalability. By aligning molecular design with optimized upstream and downstream processes, the FUEL™ platform enables efficient translation from discovery-stage constructs to robust, cGMP-ready manufacturing while building a foundation for late-stage process characterization.