Small Scale AAV Bioreactor Optimization Demonstrates Iterative Titer Gains of rAAVrh74 Serotype EPI-321, a CRISPR-mediated Epigenetic Therapy

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EPICRISPR- WHO WE ARE

We have engineered compact yet highly effective DNA methylation modulators, DNA demethylases, ultracompact transcriptional modulators as well as compact deactivated Cas effectors with altered PAM recognition. We demonstrate that these compact protein domains are functional in human cells and at endogenous human genes. In addition, when combining these domains as CRISPR-mediated epigenetic editors, the size of these compact epigenetic activators or suppressors enables single AAV packaging for in vivo epigenome engineering.

Our engineered compact epigenetic editors provide a versatile and potent platform for gene expression modulation, thereby showing substantial potential for use in future epigenetic editing therapeutic payloads.

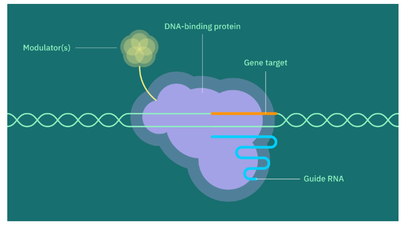


Figure 1 | GEMS Up Close
Epicrispr Biotechnology's Gene Expression Modulation System (GEMS) is a modular system comprising highly compact "nuclease dead" Cas effector, transcriptional and epigenetic modulators, and engineered compact guide RNAs.

ABSTRACT

Although recombinant AAV is a viable option for delivering gene therapies with a high degree of tropism, high cost of goods remains a significant obstacle in the field. At Epicrispr Biotechnologies, we have developed EPI-321, an epigenetic therapy to treat Facioscapulohumeral muscular dystrophy (FSHD), the most common adult muscular dystrophy caused by the misexpression of DUX4 gene in skeletal muscle. It works by encoding an ssDNA sequence of a small transcriptional repressor tethered to a miniaturized Cas molecule and a unique sgRNA sequence within an rAAVrh74 capsid. Our technology can target the hypomethylated D4Z4 locus of DUX4 gene, thereby inhibiting its ability to activate downstream signaling cascades which cause rapid cell death and subsequent muscular wasting. The discovery and engineering of such a medicine is challenging; generating patient-ready rAAV poses further obstacles, including, but not limited to, process flexibility, productivity, and scalability. rAAV production can be triggered through the introduction of essential AAV genes and the gene therapy cassette of interest through a triple transfection system in mammalian HEK293 cells, the most widely executed method today largely due to its process flexibility. However, scalability remains a pain point as HEK293 culture requires thousands of liters to manufacture, thus confounding matters of rAAV titer productivity and reproducibility. To address these reservations, small scale bioreactor production of rAAV offers insight into scaling. Furthermore, increasing productivity from conception is a boon since it tends to drop at each stage of scaling. One way to enhance productivity in the triple transfection system is by selecting the transfection reagent that stably delivers plasmids into host cells; often, commercial versions of a cationic polymer known as polyethylenimine (PEI) are used. For each reagent screened, parameter optimizations including, but not limited to, PEI volume, DNA level (ug/mL), plasmid ratios, PEI to DNA ratios, and transfection complex formation times, are often required. Here, we first screened Reagents A-D at a smaller 125 mL shake-flask scale to identify the best-performing system (Note: Reagent B is our control transfection method). Then, we executed DoE using our top performing Reagent A to evaluate the effects of adjusting the parameters previously described. Furthermore, we evaluated the impact of the addition of a small molecule Enhancer X with both Reagents A and B and after observing comparable results, decided to move forward with Enhancer X alongside Reagent A only. Following our shake flask studies, we scaled the use of Reagents A and B to 2 L and 5 L bioreactors. Next, we screened for the optimal AAV harvest timepoint for Reagent A and assessed that 3-days post transfection with Reagent A works best. With all iterations of testing considered, we conclude that Reagent A mediated transfection with addition of Enhancer X increases titer (vg/mL) by nearly 1 order of magnitude compared to using Reagent B alone. These gains in productivity can lead to significant cost savings when scaled up appropriately. Our current framework provides us with a process to efficiently generate EPI-321 but also leaves room for further growth until full tech transfer to large scale manufacturing is achieved.

GEMS

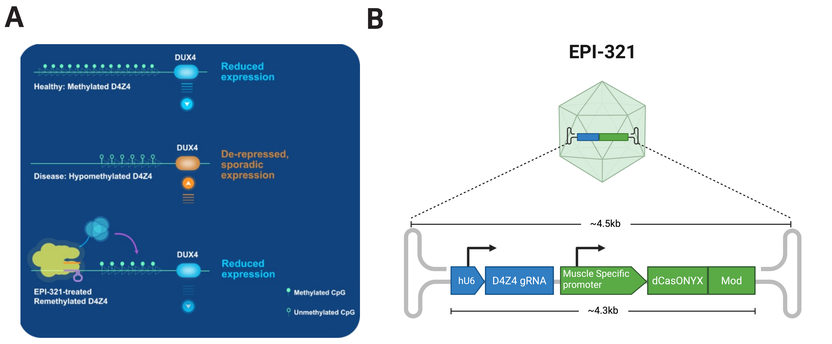


Figure 2 | GEMS In Action
A GEMS supressing DUX4 expression via methylation. **B** Schematic showing the compactness of EP-321. This compactness enables configuration inside an rAAV molecule.

PEI Transfection Reagent Screening

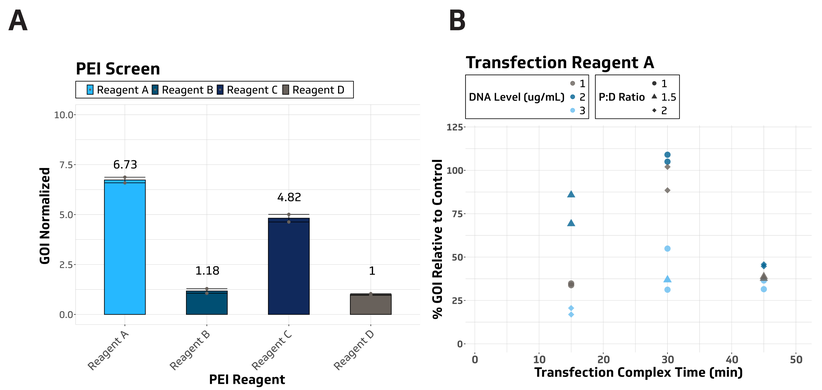


Figure 3 | Identification of optimal transfection reagent and parameters by basic titer screening
A Barplot comparing normalized titer results post screening 4 different transfection reagents, all PEI based (n = 2). Reagent A outperforms Reagent B, a reagent used by our CDMO, by nearly 6 fold. **B** Scatterplot comparing transfection complex time (min) versus titer showing a parameter screen of plasmid DNA level (μg mL^{-1}) and PEI:DNA ratios. Results suggest that fine tuning to a DNA level of 2 μg mL^{-1} and a PEI:DNA ratio between 1 and 2 is most effective to achieve high titer. Increasing transfection complex time may decrease titer.

Small Scale 2 L & 5 L Benchtop Bioreactor Testing

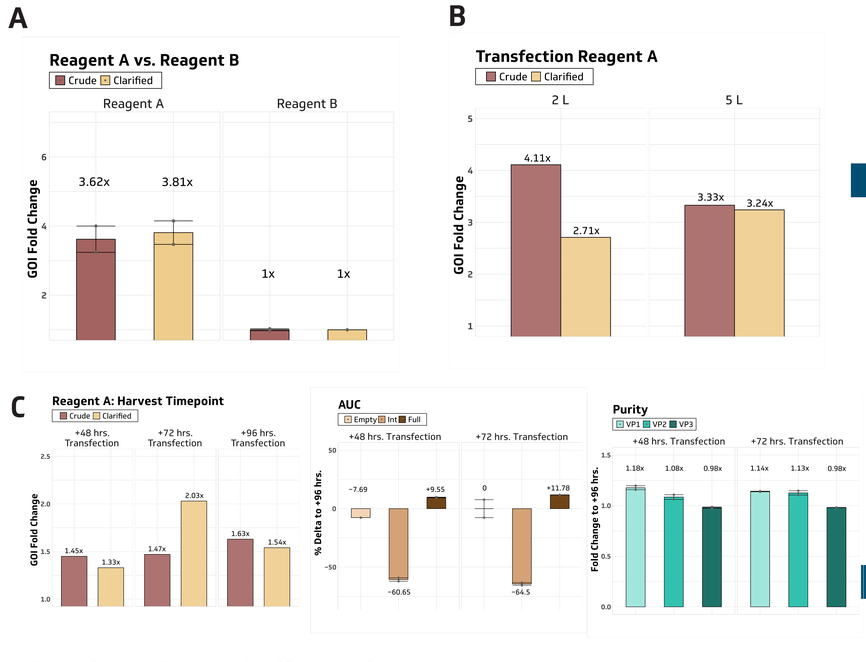


Figure 4 | Benchtop bioreactor testing of Transfection Reagent A
A Barplot comparing titer results between Reagents A (n = 3) and B (n = 2) at both crude and clarified lysates. **B** Titer results comparing 2 L and 5 L scales. **C** Study examining titer outcomes based on the AAV harvest timepoint. Harvesting 72 hrs. post transfection can lead to ~12% gain in full capsids relative to 96 hrs. Purity may remain constant at all harvest timepoints tested.

Continual Process Improvements & MSAT Considerations

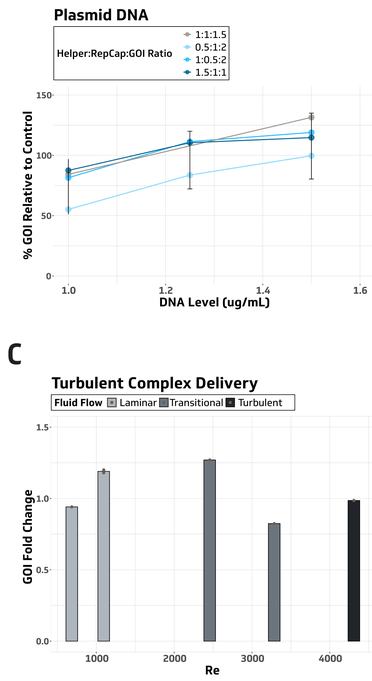
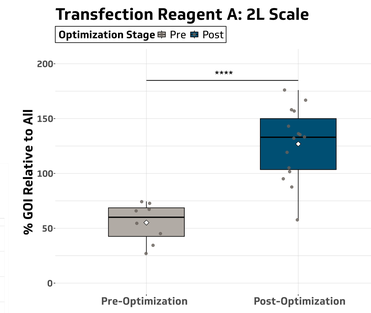


Figure 5 | Additional parameter screening and optimizations
A Lineplot comparing titers post plasmid ratios and DNA levels adjustments (minimum n =2 at every combination). Study was performed at 30 mL shake flask scale. **B** Titer results from screening various complexation times with Reagent A and plasmids. Study was performed at 30 mL scale. **C** Determining the stability of complexes as the flow rate used to deliver into cells increases. Fold change is equal to the ratio of the test condition to the control. A score of 1 represents no difference between the two conditions. One point on the barplot is one experimental run comprising 2 bioreactors.

CONCLUSION



Our screening and optimization efforts at Epicrispr demonstrate successful scale up from shake flask to 2 L bioreactor with our best performing transfection reagent A. Current ongoing efforts at 5 L bioreactor scale will further boost our confidence in developing high titer EPI-321. This work will enable us to move towards larger scale reactors, allowing for therapeutically relevant product to be transferred to patients in the near future.

Figure 6 | Small scale bioreactor optimizations demonstrate increased titer results
Post-optimization (n = 15) efforts at 2L result in a significant increase in titer versus the pre-optimization (n = 8) bioreactor runs (p-value <0.0001, student's t-test) .

CONTACT AND REFERENCES

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Poster No. 617
Directed Evolution and Characterization of Cas Effectors in Mammalian Cells for Expanded Epigenome Editing Space
Courtney Klappenbach
Poster Hall I2, May 13 2025, 6:00 PM - 7:30 PM

Poster No. 1939
EPI-321 Development: Strategies to Establish a Scalable and Robust rAAVrh74 Upstream Manufacturing Process from 0.5 L to 1000 L Scale
Surabhi Godbole
Poster Hall I2, May 15 2025, 5:30 PM - 7:00 PM

Poster No. 1110
Compact DNA Demethylase-Activator combination Modulators for CRISPR-Mediated Epigenetic Gene Activation
Dan Hart
Poster Hall I2, May 14 2025, 5:30 PM - 7:00 PM

Presentation
Non-Human Primate (NHP) Safety Study of High-Dose EPI-321: A Novel AAV-Delivered Epigenetic Editing Gene Therapy for the Treatment of FSHD
Sid Boregowda
NOLA Theater B, May 14 2025, 4:45 PM - 5:00 PM