Rescuing lost titer: identifying and eliminating transfection-driven failure modes at scale

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1. Abstract

Introduction
Epicrispr Biotechnologies is a clinical-stage company that is developing EPI-321, an investigational, single-dose gene-modulating therapy designed to silence aberrant DUX4 expression in skeletal muscle, which drives progressive muscle degeneration in patients with FSHD. Delivered intravenously via a clinically validated AAV vector targeting muscle tissue, EPI-321 has shown early signals of biological activity and clinical benefit, including improvements in strength and function, increased lean muscle volume on whole-body MRI, and reductions in circulating DUX4 biomarkers. FSHD predominantly manifests in adulthood, and all skeletal muscle is vulnerable to disease in affected individuals. Thus, gene therapy approaches must employ myotropic delivery systems for broad muscle distribution. This, coupled with relatively high prevalence of FSHD compared to other neuromuscular disorders which may be amenable to gene therapy, drives manufacturing demand, showcasing the need for efficient and scalable processes.

Methods
Bench-scale experiments were conducted to characterize critical process parameters (CPPs) associated with the transfection unit operation. Transfection Reagent A (TFXN-A), selected for the initial version of our EPI-321 manufacturing process (v1.0) based on favorable small-scale performance, was evaluated across complexation time and transfer flow rate. These parameters were ranged to define acceptable operating ranges and provide context for impact on productivity at large-scale. Following the 1000 L production, an exact scaledown model was created to support root-cause analysis. This model was established to isolate the impact of complexation time, with agitation, operating conditions, and cell density aligned to large-scale. Based on these diagnostic studies, limitations in the scalability of TFXN-A were identified, providing motivation for evaluation of an alternative transfection reagent. Using the same experimental framework, Transfection Reagent B (TFXN-B) was evaluated for improved robustness across complexation time and transfer flow rate.

Results
Extended complexation time and non-optimized transfer flow rates using TFXN-A resulted in 45% and 30% reduction in titer, respectively. Despite these losses, the characterization of these CPPs enabled selection of most acceptable flow rate and complexation time for large-scale application of TFXN-A. Data from the 1000 L scaledown model were consistent with bench-scale findings, confirming a 50% reduction in titer due to complexation time, which was necessitated by transfer flow rate limitations. Collectively, these results define a scaling ceiling for TFXN-A. In contrast, TFXN-B demonstrated significantly less sensitivity relative to TFXN-A, showing a positive correlation between transfer turbulence and titer. Additionally, while TFXN-A requires a double-bolus transfection strategy due to constraints, TFXN-B has optionality for a single-bolus approach, reducing operational complexity and risk.

Conclusions
The results here demonstrate that transfection reagent selection is a key determinant of scale suitability, robustness, and commercial readiness. These strides in manufacturing, together with early data from the ongoing first-in-human study showing favorable performance across multiple functional measures, strongly support the promise of advancing epigenetic editing therapies.

2. Platform

GEMS: ***G**ene **E**xpression **M**odulation **S**ystem*

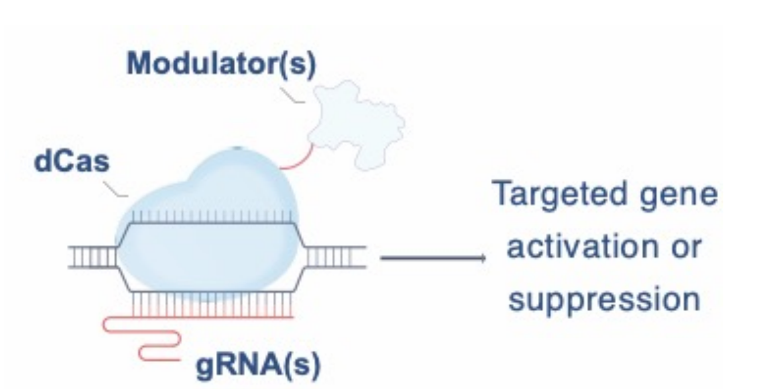


Figure 1: Epicrispr Biotechnologies' GEMS Platform. System comprised of gRNA, dCas, and modulator proteins. Compactness allows GEMS to be delivered therapeutically by any single delivery vector.

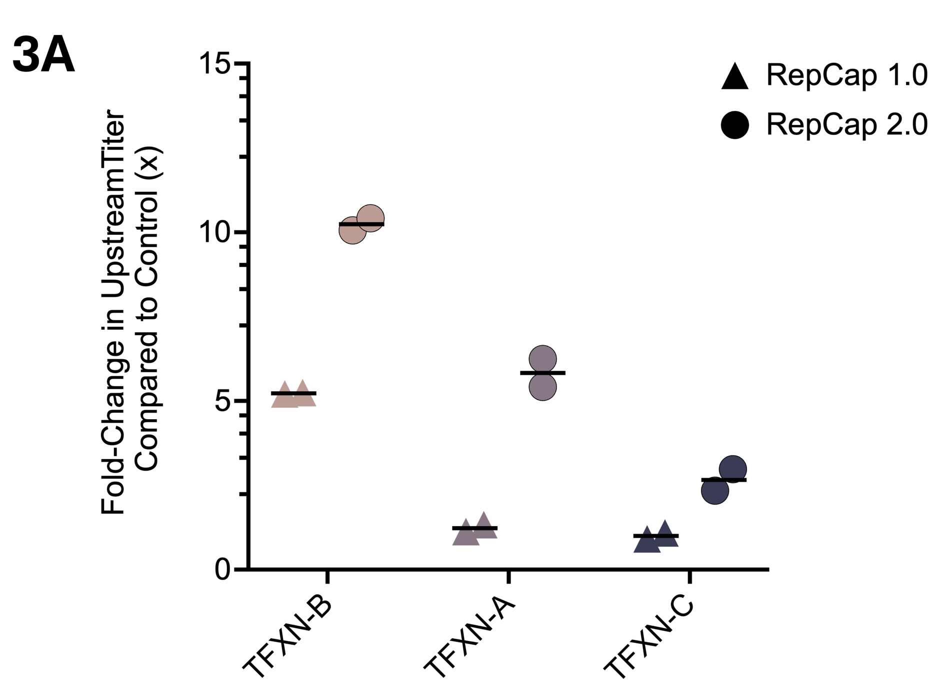
Epicrispr holds the first and only open epigenetic editing IND authorization in the US for EPI-321, which utilizes their GEMS technology.

Early strong clinical data and a clean safety profile from their FIH study reinforces the potential of epigenetic editing as a fundamentally new therapeutic approach.

3. Background

Historical EPI-321 Process 1A titers in E+10 vg/mL range made it challenging to support doses for systemic administration while minimizing COGs. Insufficient scaling from bench to production-scale was observed.

3A



3B

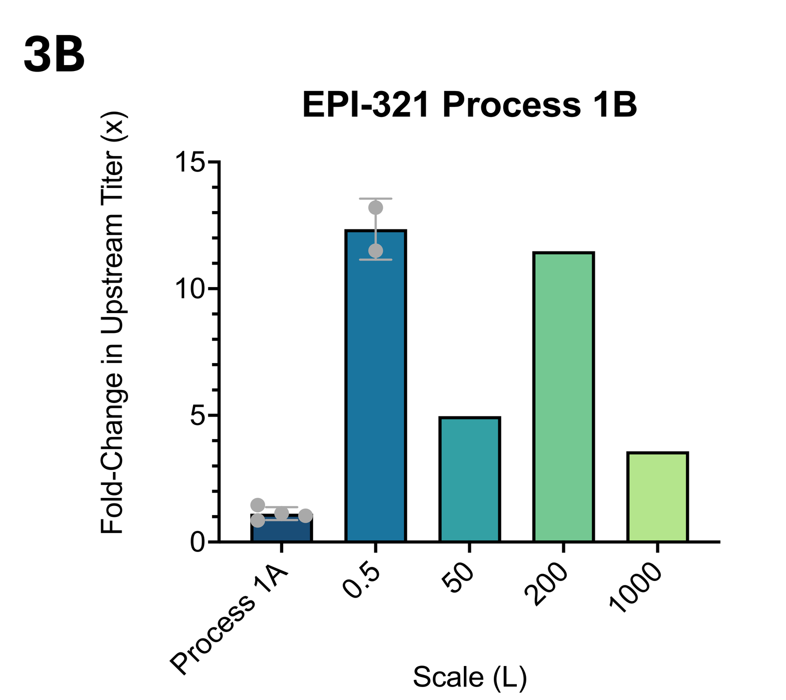
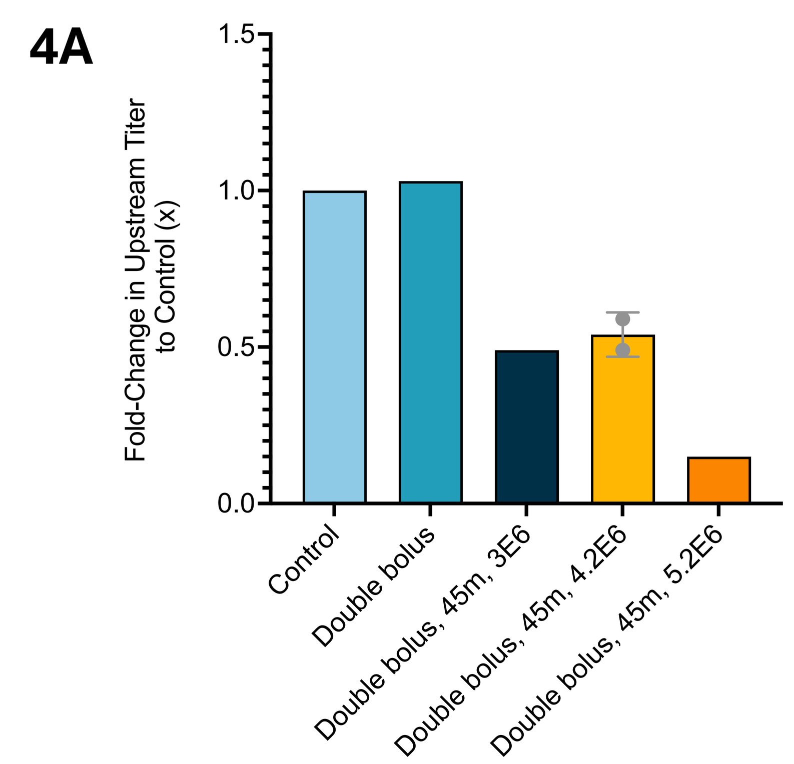


Figure 3: EPI-321 transfection reagent selection and historical scaling data. A. TFXN-A and RepCap 2.0 yielded upwards of ~5x increase compared to control titer and ~20x compared to historical titer. Small scale purification to drug substance completed for each condition using RepCap 2.0 identified TFXN-A as the superior reagent. **B.** Upstream titers across increasing scale show similarities between flask and 200 L scales.

4. Process 1B diagnostic study

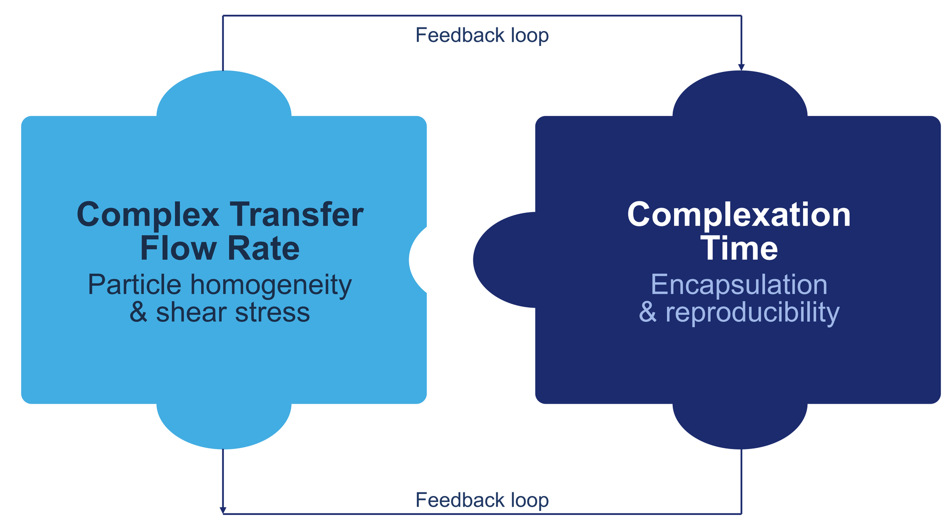
4A



- **Negligible** — Double bolus transfection method did not impact titer
- **↓ 50%** — Titer reduced by half when complexation window is prolonged
- **↓ 90%** — Combined stressors result in near-total loss

Figure 4: Additive 1000 L diagnostic study using TFXN-A. A. Stepwise experimental design used, starting with a control condition representing optimal operation and sequentially adding one parameter at a time. Design allows assessment of individual contribution and any synergistic detrimental effects. Results indicate that extended complexation time reduces titer by ~50%. A defined transfection density window of 3 to 4.2E6 cells/mL was identified, beyond which cell growth led to productivity losses up to 90%. Experiment executed at 5 L scale.

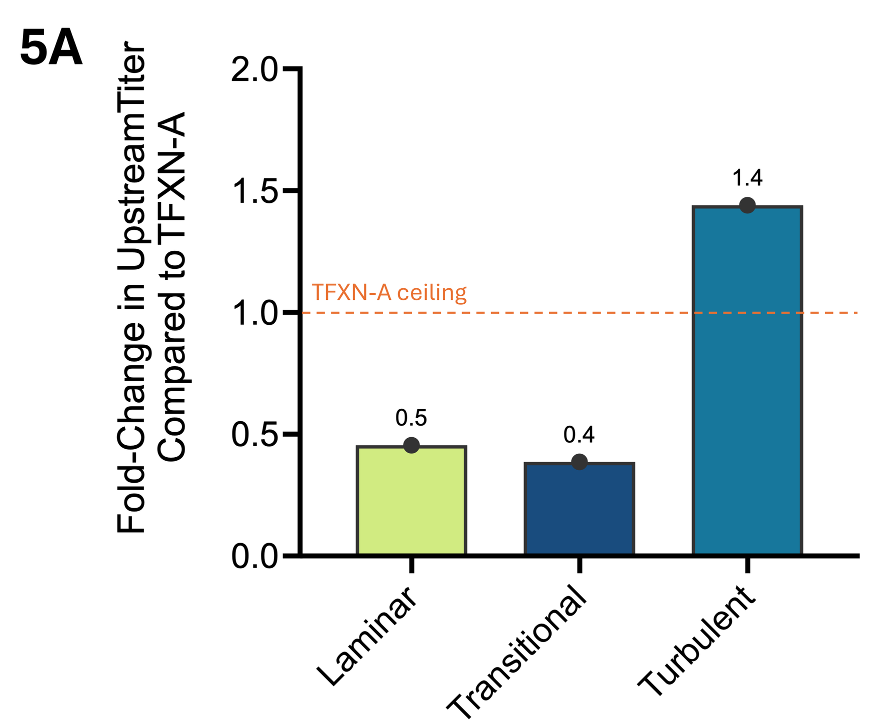
5. Transfer robustness and complexation time



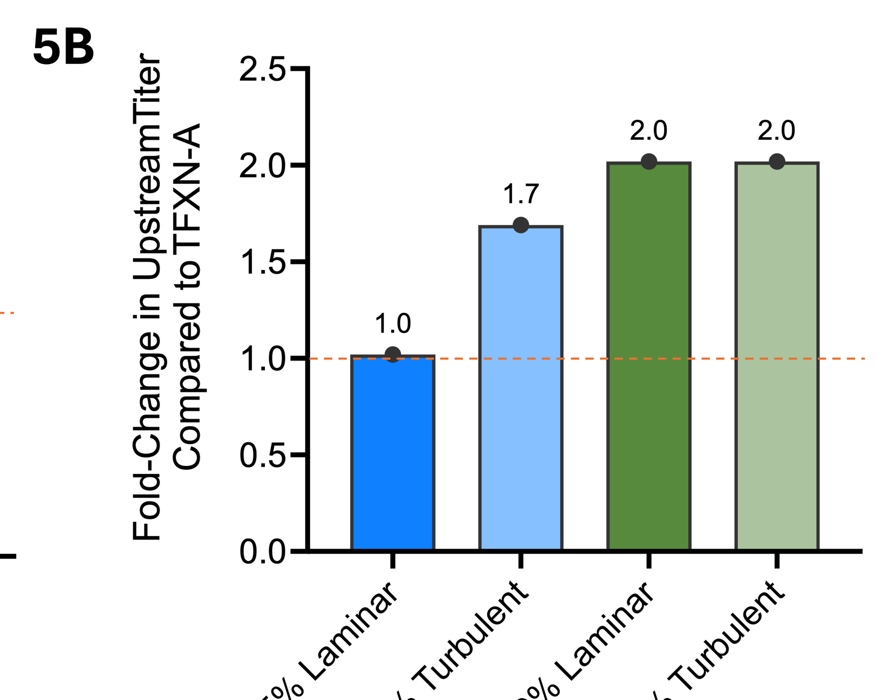
Manufacturing scalability in transfection-based production is fundamentally governed by two parameters: complex transfer flow rate and complexation time. The flow rate determines how the DNA-PEI complexes behave under shear or varying turbulence. Deviations in rate can create heterogeneous particle size distributions. Complexation time controls how condensed and encapsulated the DNA is by PEI. Together, these variables create a feedback loop where neither can be optimized in isolation.

Robust scalability depends on co-optimization and a well-defined transfection strategy.

5A



5B



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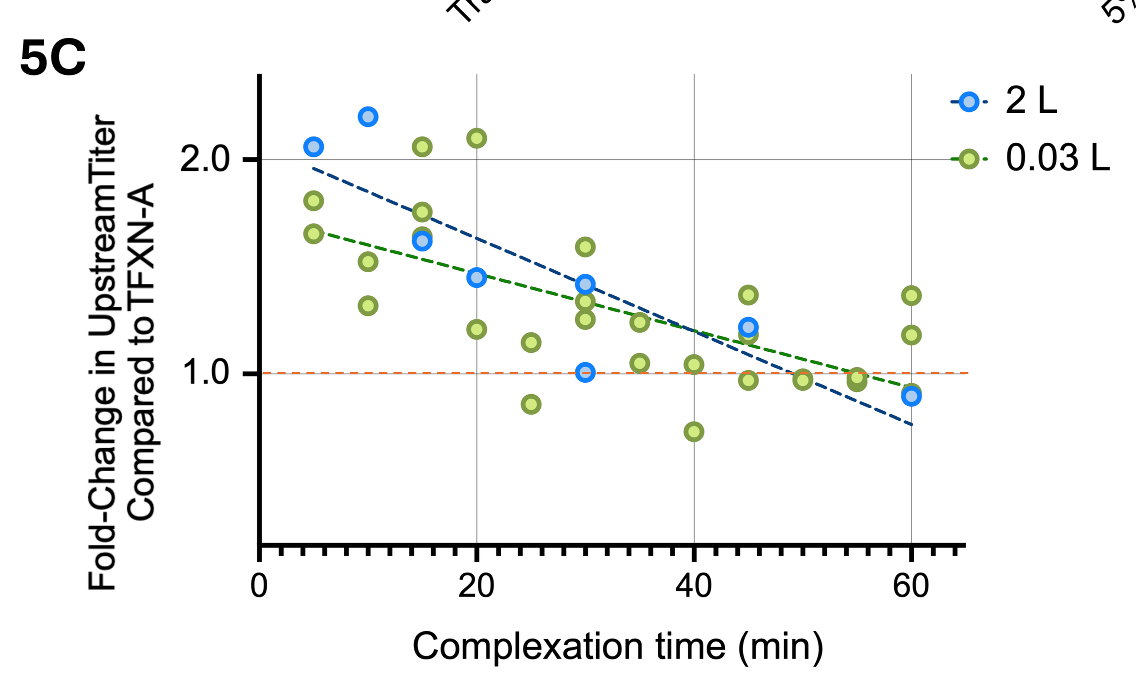
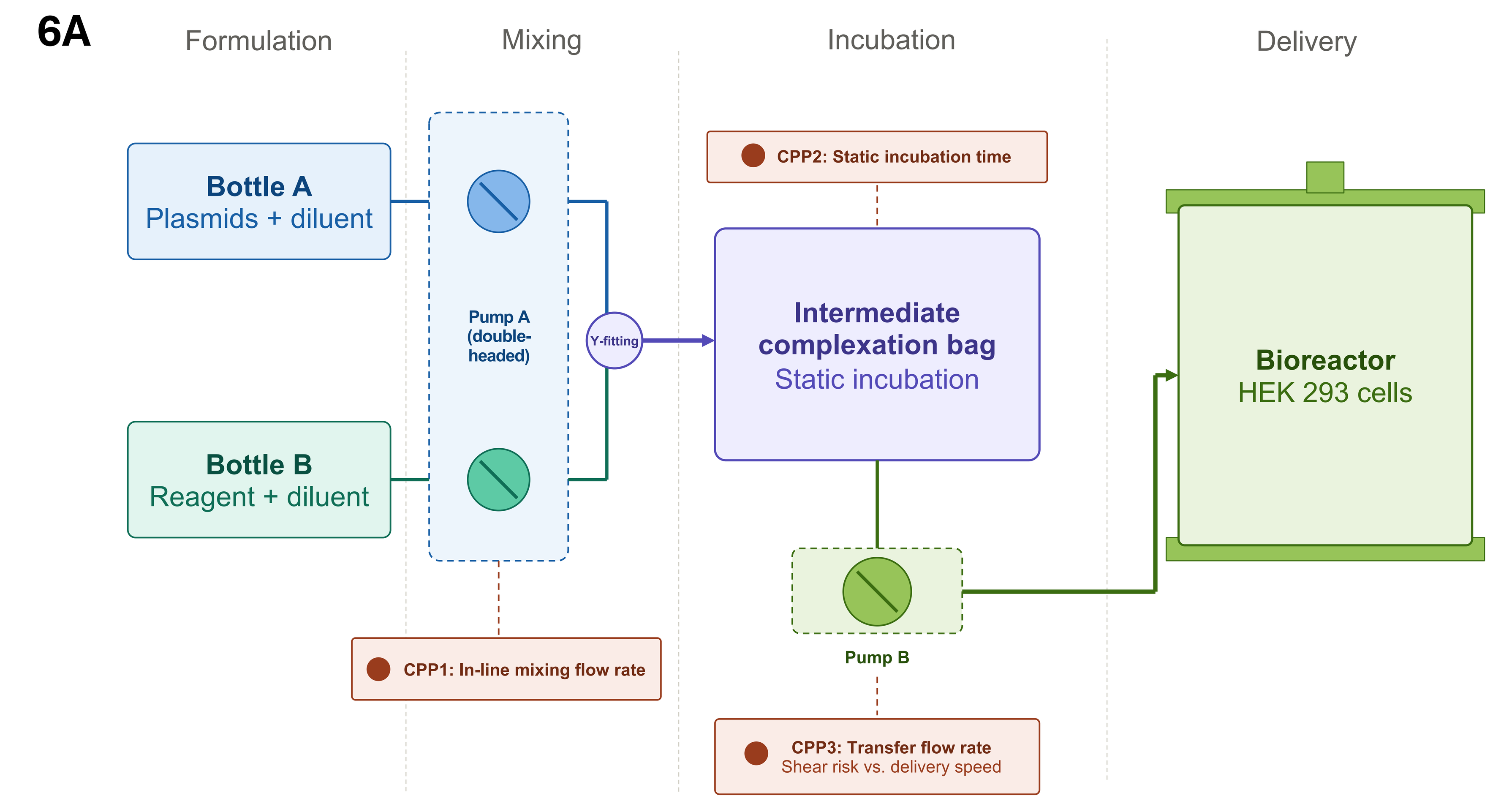


- Given sufficient time, complexes continue to form, which creates a population of oversized aggregates
- Higher transfer rates may introduce hydrodynamic shear that disrupts loose clusters back into uptake-competent complexes
- TFXN-B likely over-aggregates under normal conditions, but because the complex bonds are stronger, moderate shear disrupts aggregates rather than destroying the complex's integrity

Figure 5: TFXN-B robustness data. A. Turbulent delivery of complex to cells resulted in almost 4x increase of upstream titer. **B.** Larger complex volume appears to rescue upstream titer when delivered under laminar conditions. No impact of delivery speed at 10% CV. **C.** Direct and negative correlation between complexation time and titer. Highest productivity observed within 10 minutes of mixing DNA with PEI.

6. Transfection strategy

6A

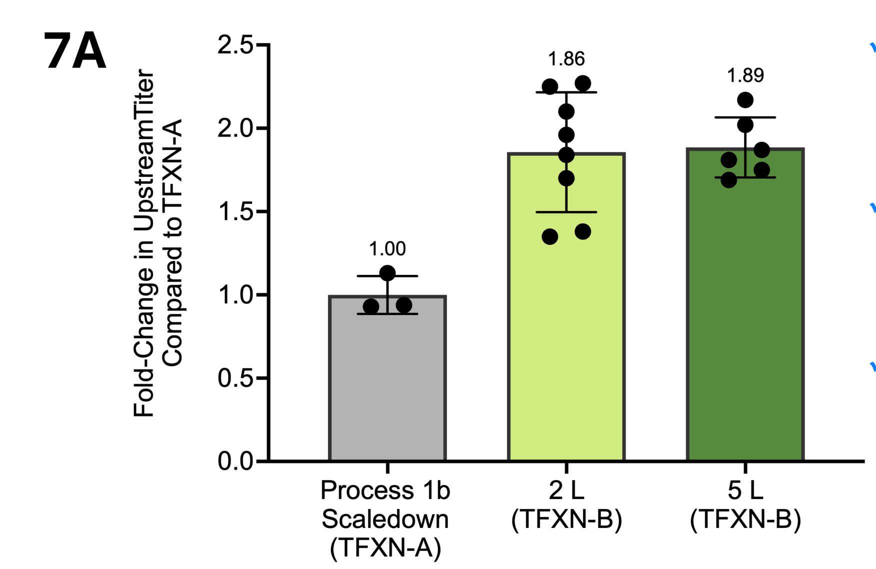


Scale (L)	Additions (#)	Complex volume (L)	Y-line transfer Re	Y-line transfer time (m)	Static incubation (m)	Cocktail transfer Re	Cocktail transfer time (m)	Minimum complex residence time (m)	Average complex residence time (m)	Maximum complex residence time (m)
50	2	2.5	6179	1	14	11169	0.5	14	15	21
1000	2	50	6179	9	5	11169	10	5	15	24

Figure 6: Schematic overview of EPI-321 transfection unit operation. A. Plasmids and transfection reagent are combined to form the transfection complex and subsequently delivered to cells with CPPs governing flow rates and incubation time. A residence-time-based strategy is used for scaling between 50 and 1000 L. Ensuring average residence time is constant across scales maintains the cellular environment during transfection best.

7. Scaledown data

7A



- ✓ **Nearly 90% improvement in upstream yield with TFXN-B across bench scales. Substantial gains over baseline condition observed.**
- ✓ **Tolerance to high transfer rates maintained even at small-scale. Scaledown system is not uniquely sensitive to high flow rate at reduced production volumes.**
- ✓ **Reproducible, consistent and high-performing dataset is being generated thus far. This demonstrates enhanced control over critical process parameters.**

Figure 7: EPI-321 process 2 data. A. TFXN-B demonstrates consistent results at 2-5 L scales. Turbulent processing rates used across all runs.

8. Summary and next steps

TFXN-B demonstrates improved process robustness relative to TFXN-A, with greater tolerance to transfer rate and complexation time. The observed preference for shear during transfer suggests inter-complex affinity may be more important than conventional lipid/polymer behavior, enabling greater manufacturing flexibility. This has informed a residence-time-based scale-up strategy in which average complex residence time is held constant across scales, even as transfer times vary. Scale-down data suggests an improvement of up to **10x** in upstream yield with the implementation of TFXN-B at 1000 L scale.

Next steps for TFXN-B include:
- Scaling to 50 L using residence-time-based strategy
- Complex sizing analysis to better understand particle size properties best suited for cellular uptake
- Manufacturing-relevant studies to support stability

9. Contact

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Oral Presentation
Epigenetic Editing Restores Frataxin Expression to Physiological Levels in Friedreich's Ataxia Cells
Kaitlin Pensabene, Jackson Powell
Ballroom AB, May 14, 4:45 PM

Poster #2360
APPRAISR: A model for predicting guide RNA efficacy for a hypercompact CRISPR-Cas epigenetic editor using machine learning
Tyler Borrman
Poster Hall, May 12, 5 PM

Poster #2315
Breaking Size and Silencing Barriers: A Compact CRISPR Epigenetic Activator for Durable AAV-Compatible Gene Activation
Zaki Jawaid
Poster Hall, May 13, 5 PM

Poster #3318
Epigenetic editing activates SORLA (Sortilin-related receptor with A-type repeats) to reduce soluble beta amyloid (Aβ) and Tau in sporadic Alzheimer's disease patient neurons
Melanie Silvis
Poster Hall, May 14, 5 PM

Poster #3313
EPI-331: A CRISPR-based epigenome editing as a mutation-agnostic treatment of Duchenne muscular dystrophy (DMD)
Abhinav Adhikari
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