EPI-321 Scaling Challenges: Considerations Required for a Robust AAVrh74 Upstream Manufacturing Process

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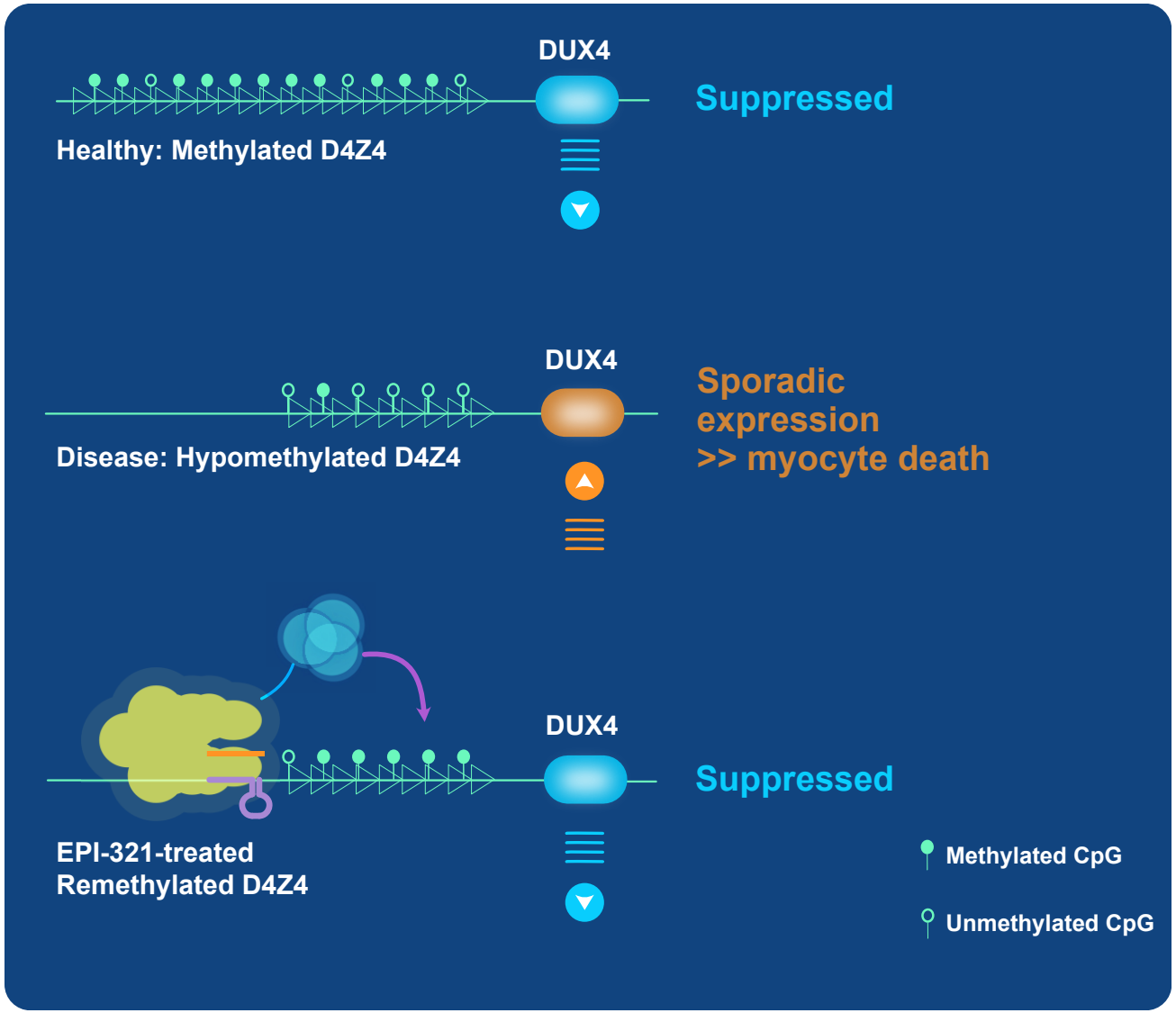
EPI-321

Treatment for Facioscapulohumeral Muscular Dystrophy (FSHD)

On track to enter the clinic in 2025

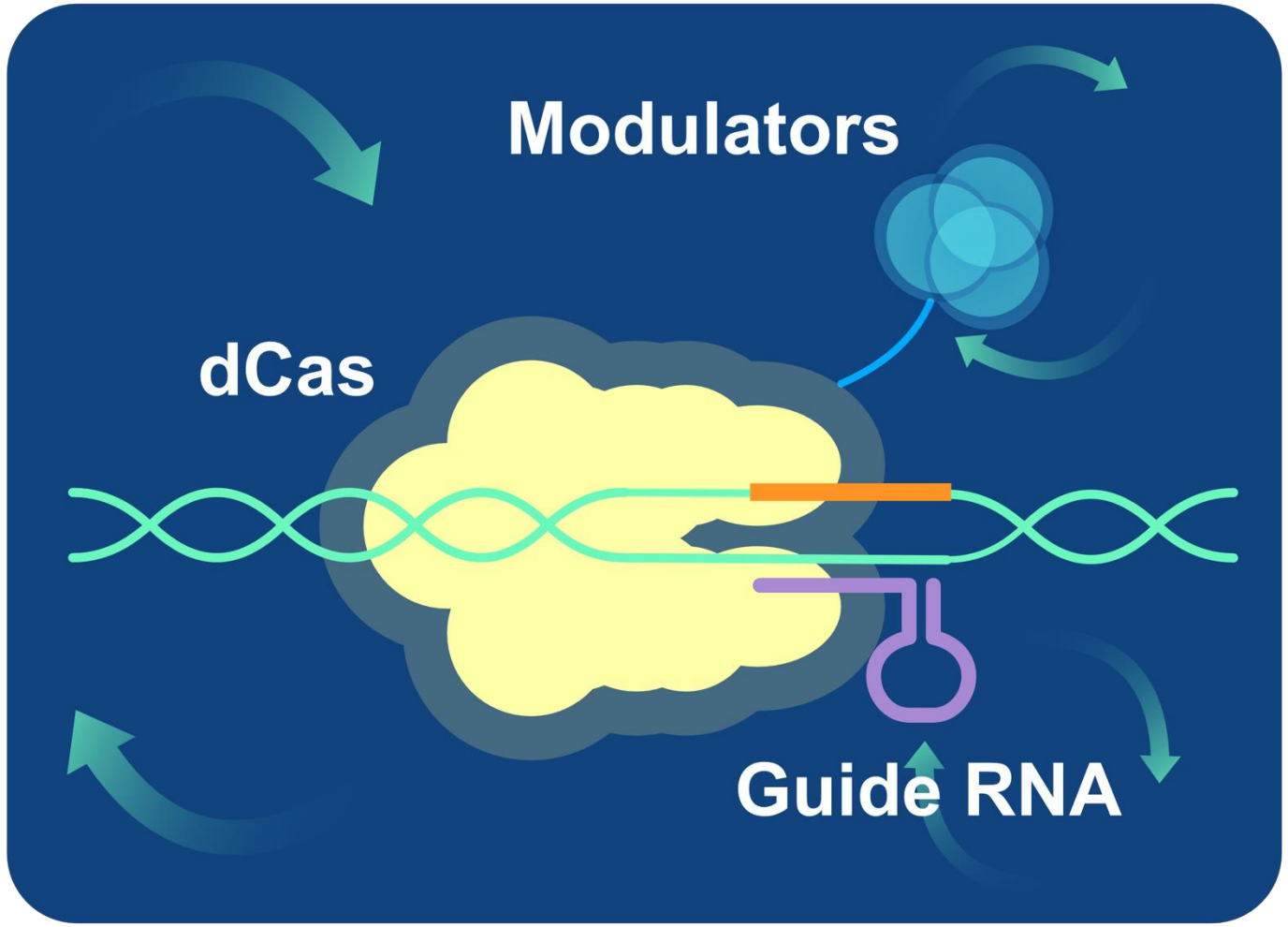
epicrispr biotechnologies

EPI-321 addresses the root cause of FSHD by methylating the D4Z4 region to prevent toxic DUX4 expression



- MUTATION: Less that 10 repeats of D4Z4 region
- LEADS to hypomethylation near the DUX4 gene region and DUX4 leaking out stochastically and transiently
- DUX4 is toxic to skeletal muscles

How Do We Do It? - Epic Bio's Proprietary Platform:
GEMS: Gene Expression Modulation System



- The **guide RNA** (gRNA, purple), provides specificity for the epigenetic editing. It's the "genome GPS" for the GEMS system
- The **nuclease "dead" Cas protein** (CRISPR-associated protein, yellow), fused to the modulator, binds to the gRNA at the target site. It does not cut the DNA
- The **modulator proteins** (blue) are engineered to modify the epigenome and either activate or repress the targeted, nearby gene

EPI-321 Manufacturing Scalability

epicrispr biotechnologies

EPI-321 Scaling Challenges

- Historical EPI-321 titers in E+10 vg/mL range made it challenging to support doses for systemic administration in adults while minimizing COGs
  - Insufficient scaling from bench to production-scale observed
- Titer optimization was essential to ensure commercial success and mitigate scaling losses

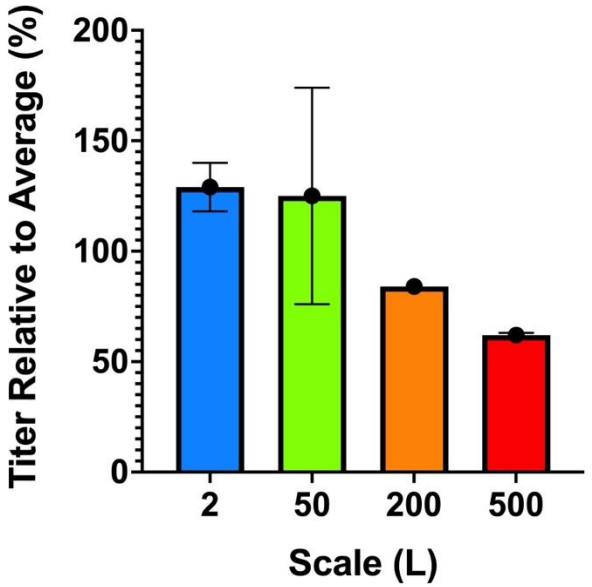
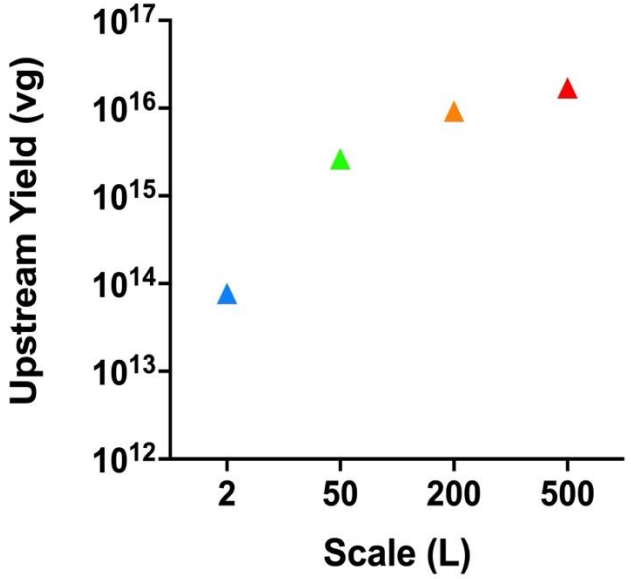


Figure 1: Clarified Lysate Upstream GOI Titer by Scale. Historical EPI-321 upstream titers show a direct, negative relationship between productivity and scale.



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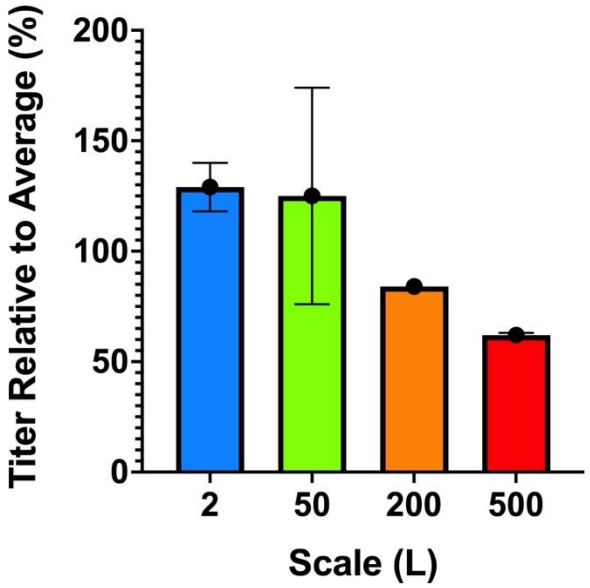

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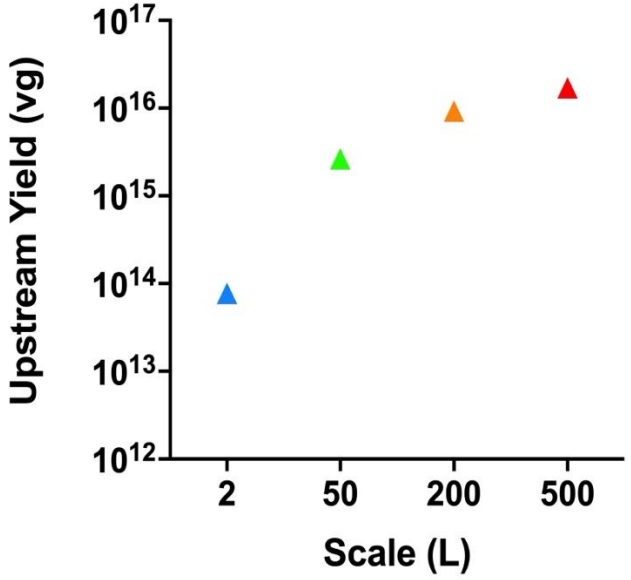

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Manufacturing Considerations

#1: Maximizing Productivity

For commercial viability, high titers are necessary to meet dosage demands

#2: Agitation

Increased tip speeds at higher volumes can impact cell productivity

#3: Turbulence Effects

Higher transfer flow rates can lead to “shredding” of transfection complex

Manufacturing Consideration #1: Maximizing Titer

Improving yield often requires optimizing the transfection step, with two common approaches being plasmid engineering and transfection reagent parameter optimization.

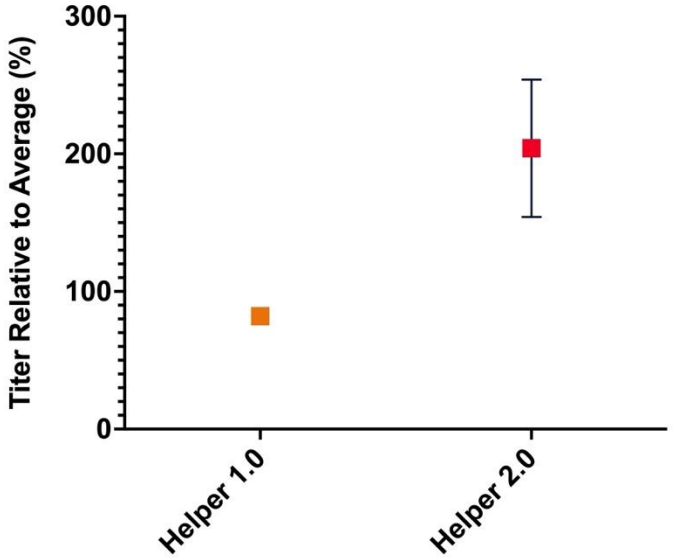

Figure 3: Engineered Helper Plasmid Evaluation. Implementation of Helper 2.0 resulted in a ~2-fold increase in clarified lysate upstream GOI titer when compared to Helper 1.0. Experiment executed at 500 mL scale.

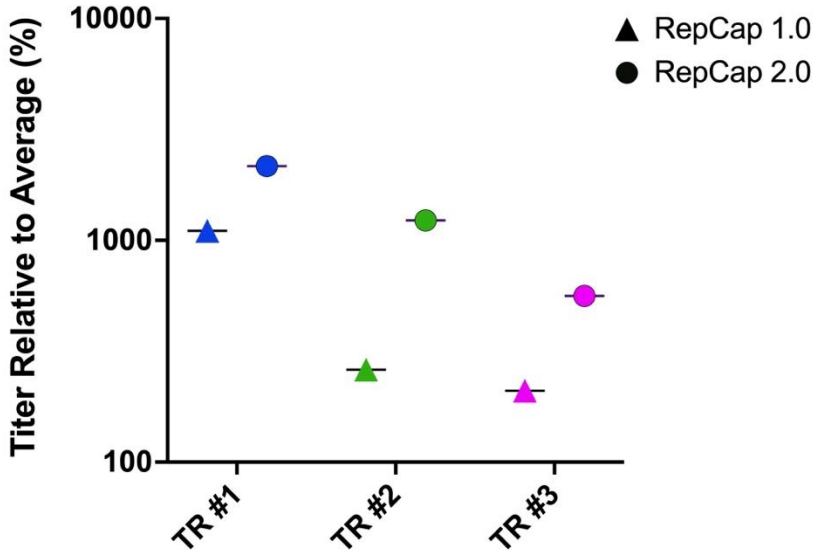

Figure 4: Engineered RepCap Plasmid and Transfection Reagent Screening. TR #2 and RepCap 2.0 yielded upwards of ~20-fold increase in productivity. Helper 2.0 used for all conditions. High clarified lysate titers may suggest synergistic effects between engineered plasmids and optimized reagent. Experiment executed at 500 mL scale.

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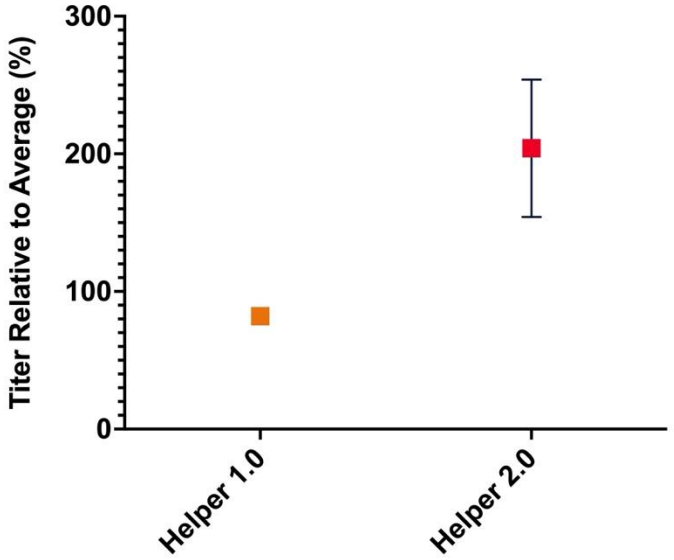

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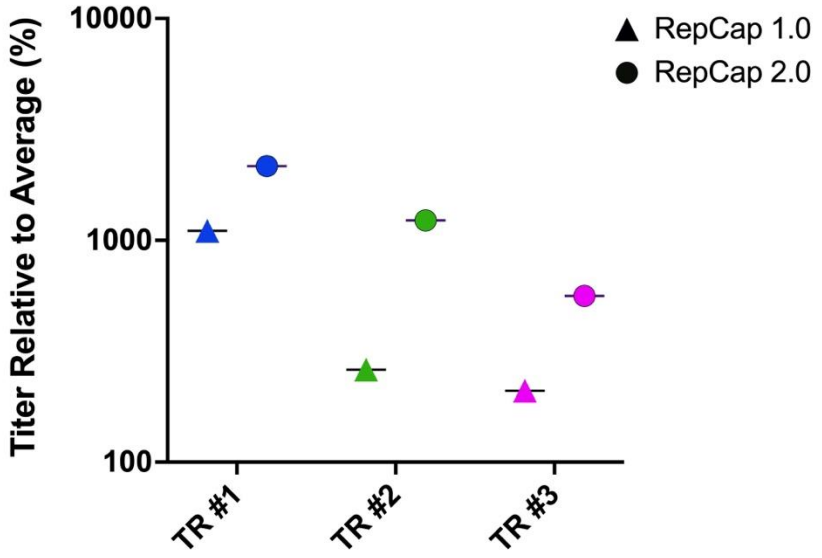

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Transfection Reagent	Yield Recovery (%)	Total Purity (%)
#1	26.51	83.93
#2	45.14	98.36
#3	20.29	98.1

Table 1: Engineered RepCap Plasmid and Transfection Reagent Screening Purity. Small-scale purification completed for each condition using RepCap 2.0. Drug substance % recovery and total purity show that TR #2 is the superior condition.

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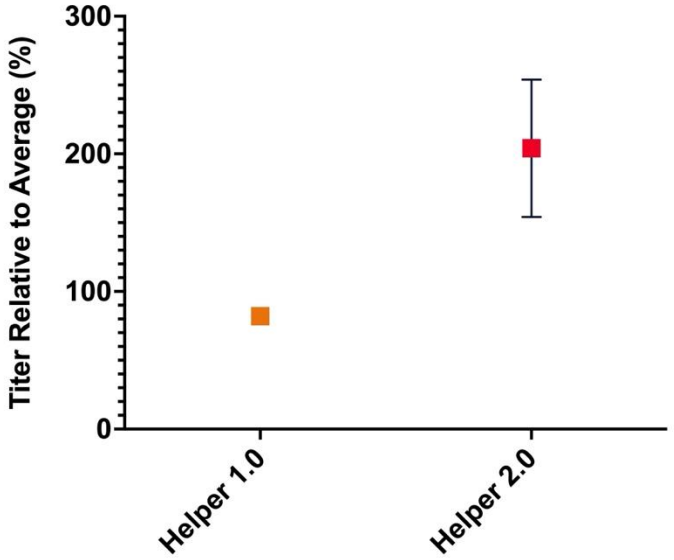

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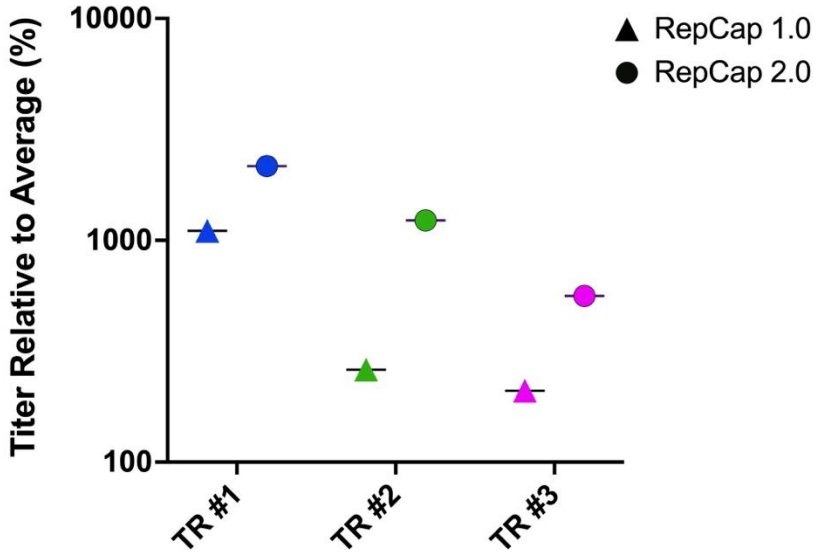

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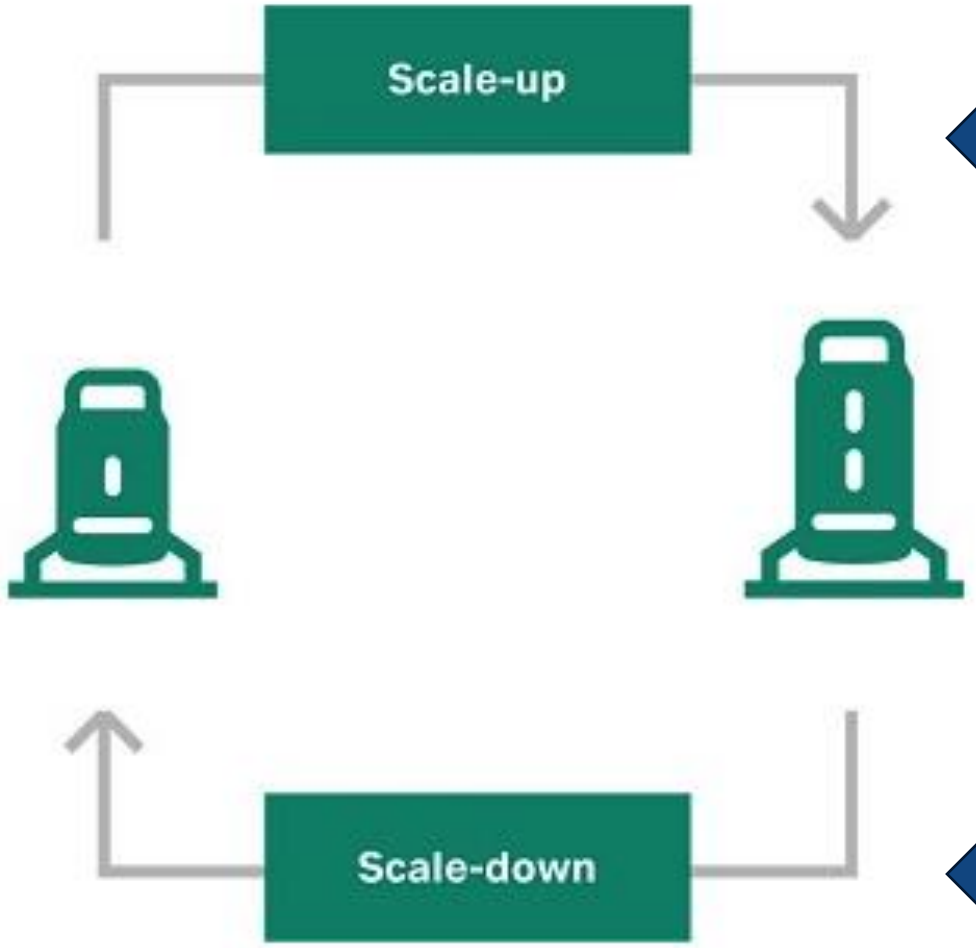
To better meet pre-clinical and commercial demands, Helper 2.0, RepCap 2.0, and Transfection Reagent #2 were integrated into the EPI-321 process.

Scaling Considerations



Running a bench-scale process at larger production volumes

Mimicking a production-scale process at bench-scale

A scale-down approach is preferred, as it is critical within PD to create a "scaledown" model that can predict product quality at a lower price point

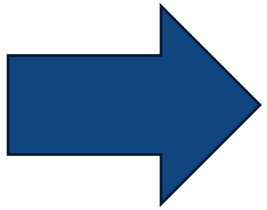
Non-linear Scaling

Volume-independent parameters:

Temperature, pH, dissolved oxygen (DO), media composition

Volume-dependent parameters:

Agitation rate, aeration rate, impeller diameter



Cell culture consistency:

Oxygen consumption, shear stress, sparge stress

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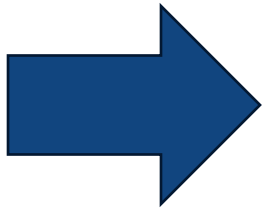

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Example: Common strategy in upstream bioprocessing is to scale by power input (P/V)

- If power input is held constant while vessel size increases, both RPM and impeller diameter (D) will increase
- Higher tip speeds >> shear stress >> "sub-lethal" effects

$$TS = pi * D * RPM/60$$

Manufacturing Consideration #2: Agitation

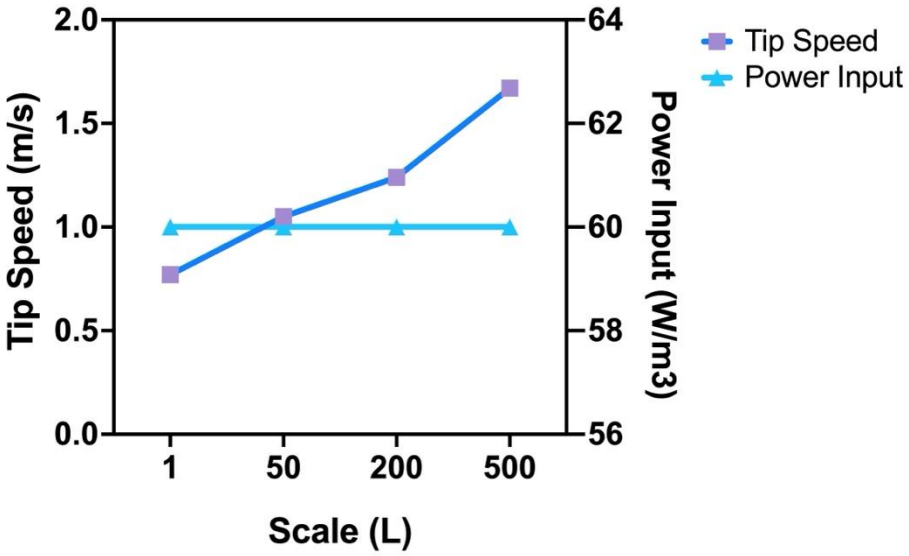

Figure 5: Tip Speed vs. Power Input by Scale. When power input is held constant across increasing scales, tip speeds also increase. >2-fold change in tip speed from bench to production scale. Determining tip speed limits is crucial for characterizing shear stress on cell culture.

- While newer cell lines are more resilient to hydrodynamic stress, it is generally recommended to minimize tip speeds as much as possible (Godoy-Silva et al., Sieck et al.)
- However, certain bioreactor designs can require a higher P/V to ensure homogenous mixing

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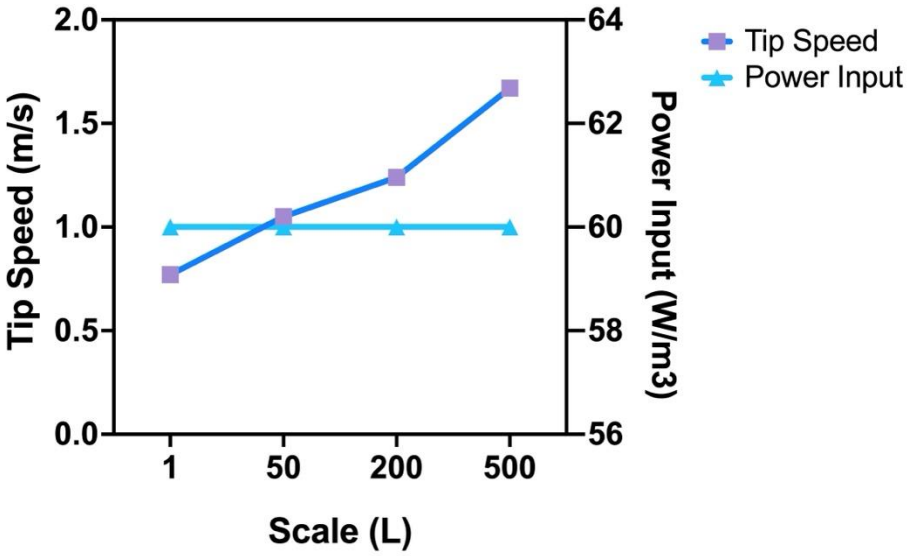

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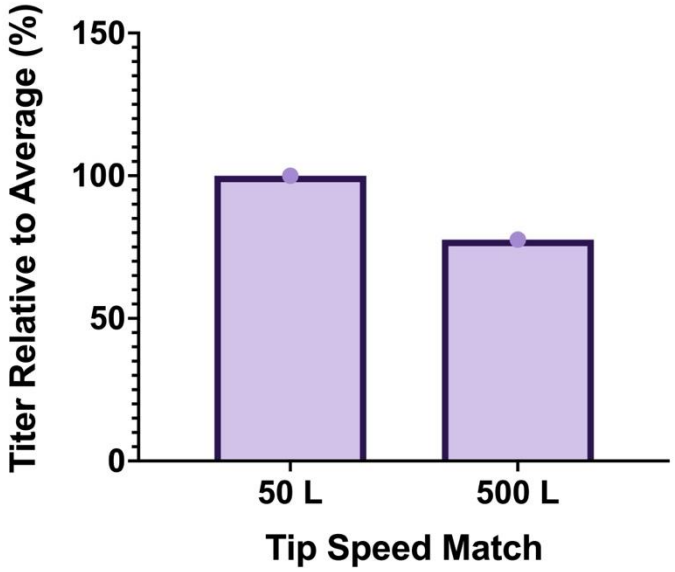

Figure 6: Tip Speed Evaluation. Study comparing 50 L match (1.0 m/s) vs. 500 L match (1.5 m/s) using 5 L scaledown model. ~25% decrease in clarified lysate titer was observed, validating the presence of shear stress on cells.

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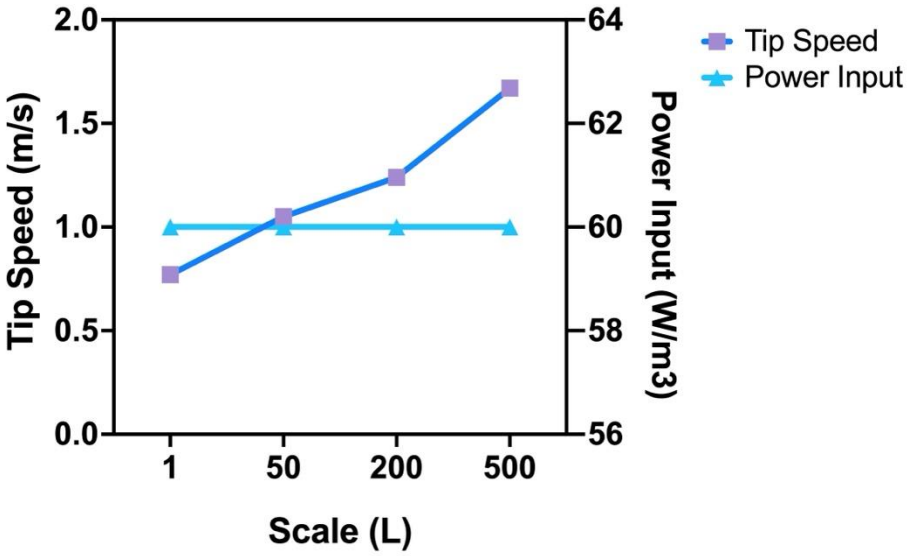

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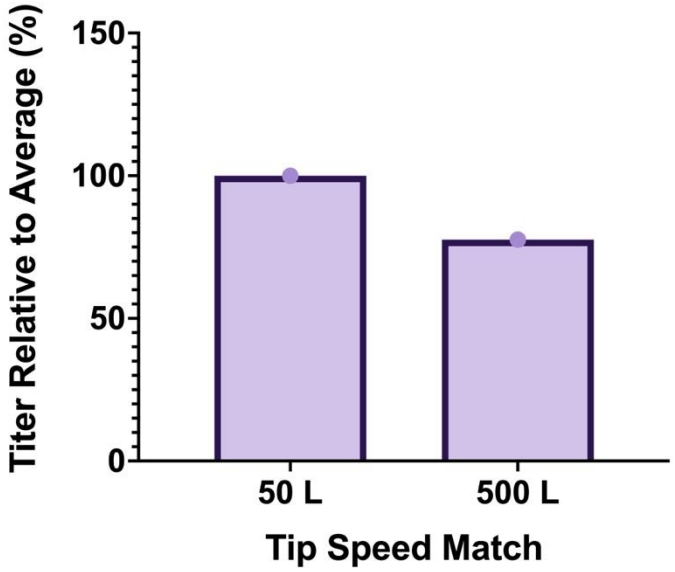

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- However, certain bioreactor designs can require a higher P/V to ensure homogenous mixing

Due to inherent geometry of the bioreactor system, a high P/V was necessary for adequate mixing and aeration. As a result, the titer losses associated with tip speeds present a risk moving forward.

Manufacturing Consideration #3: Turbulence Effects

- New transfection reagents are less robust than PEIpro
- As batch volumes increase, complex volumes will too; however, certain limitations, such as complexation time, remain unchanged
    - Complexation time must be characterized, as it is the limiting factor in scalability of the transfection transfer step
    - Determined acceptable ranges will allow for flexibility in transfer flow rate(s)

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Scale (L)	Complex Volume* (L)	Transfer Flow Rate (LPM)	Re	Static Complexation Time* (m)	Transfer Time (m)	Total Complexation Time (m)
50	2.5	1	2234	30	2.5	32.5
200	10				10	40
500	25				25	55
1000	50				50	80

*Assuming 5% complex volume, 30-minute incubation time, and constant tubing diameter for purpose of example

Reynolds number is a dimensionless quantity that predicts flow patterns in a pipe and can serve as a rough metric to gauge turbulence effects in tubing.

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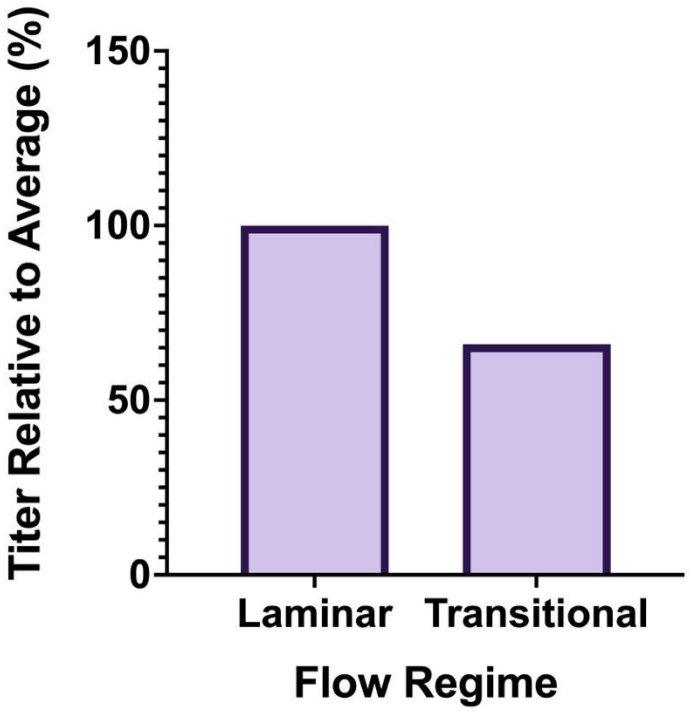

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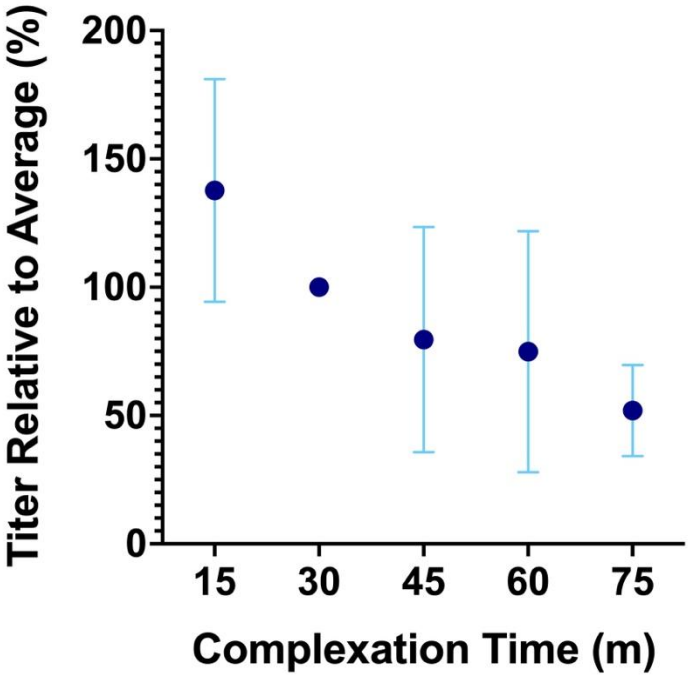

Figure 8: Transfection Complexation Time Ranging Study. Characterization of TR #2 static complexation time. Data suggests that a shorter complexation time is superior, and detrimental effects to crude lysate titer are observed at >45m. Experiment was executed at 30 mL SF scale.

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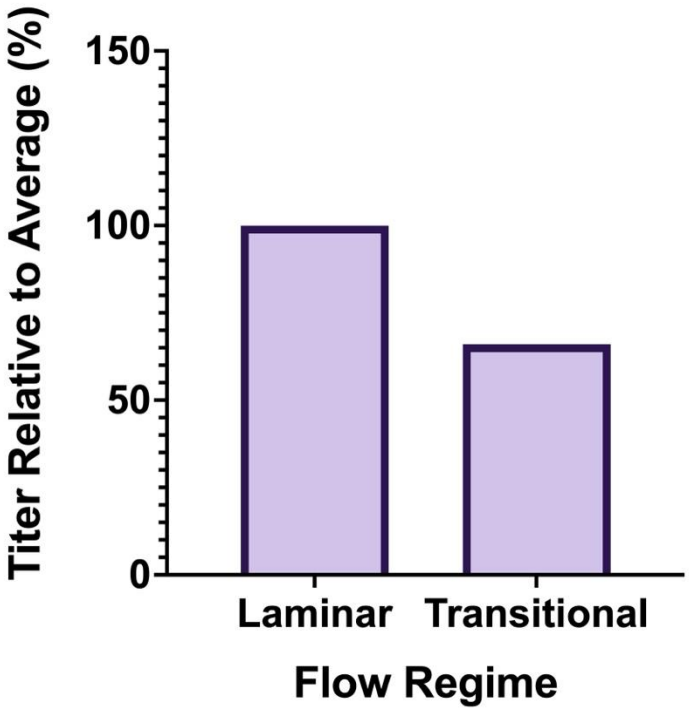

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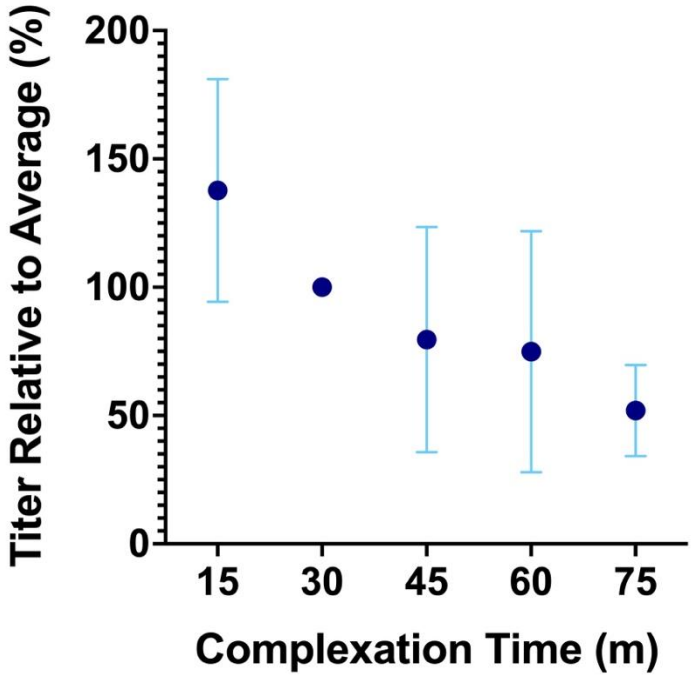

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Using data from these studies, an optimal flow rate was selected to ensure the average complexation time remained under 45 minutes without sacrificing titer to turbulence effects.

v2.0 Scaleup: 50 L

Goal:
To assess scalability of EPI-321 process with implementation of engineered plasmids (Helper 2.0, RepCap 2.0) and a new transfection reagent (TR #2).

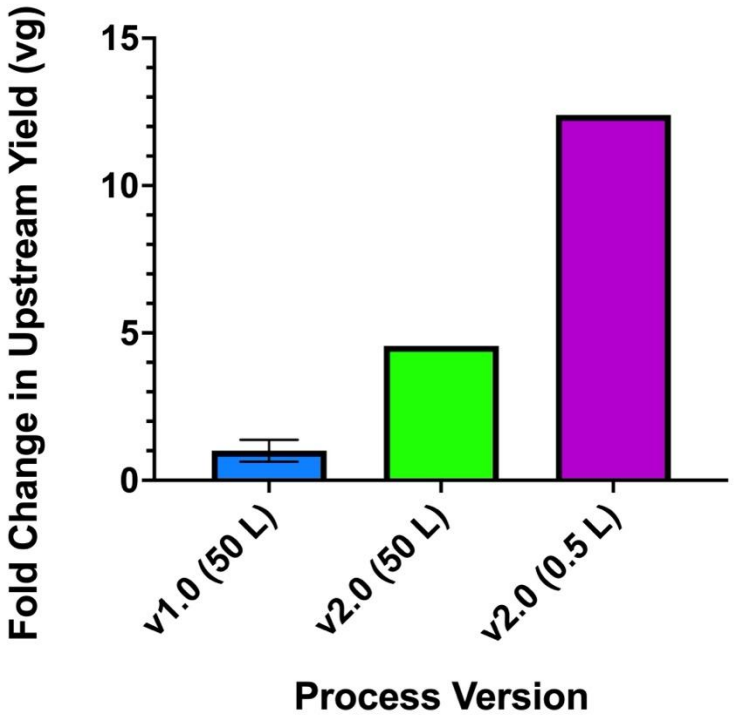

Figure 9: Optimized 50 L Clarified Lysate Upstream GOI Titer. Optimized 50 L v2.0 process improved overall upstream GOI titer by 4-fold. Scaling losses still observed between bioreactor and satellite shake flask, suggesting potential for scaleup loss.

Vessel ID	Scale (L)	Time Aliquot Pulled	Fold Change in Yield (x)
v2.0 (50 L)/ 50L-A	50	N/A	4.03
v2.0 (0.5 L)/ 50L-B	0.5	0.5 hrs pre-transfection	10.94

Figure 10: Optimized 50 L Design. Scaleup run evaluated main bioreactor condition as well as a satellite shake flask pulled from main vessel 0.5 hrs pre-transfection. Purpose of this control was to see if pumping the complex influences titer.

Satellite shake flask (SSF) performed almost 11x higher than v1.0 process; however, it also performed >2x higher than the bioreactor it was pulled from

- SSF did not undergo transfection in bioreactor; complex was not exposed to potential turbulence effects from pumping
- Next steps will include evaluating a pre- and post-transfection SSF

v2.0 Scaleup: 200 L

Goal:
To assess scalability of EPI-321 v2.0 process at manufacturing scale and understand how each unit operation affects upstream titer.

Vessel ID	Scale	Stage, Cell Source	Time Aliquot Pulled
200L-A	200	N-1, production vessel	N/A
200L-B	0.5	N-2, seed vessel	Pre-inoculation of 200 L
200L-C	0.5	N-1, pre-feed	48 hrs pre-transfection
200L-D	0.5	N, pre-transfection	0.5 hrs pre-transfection
200L-E	0.5	N, post-transfection	0.5 hrs post-transfection
200L-F	0.5	N-3, seed train	N/A

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200L-B	Pre-inoculation
200L-C	48 hrs pre-transfection
200L-D	0.5 hrs pre-transfection
200L-E	0.5 hrs post-transfection
200L-F	N/A

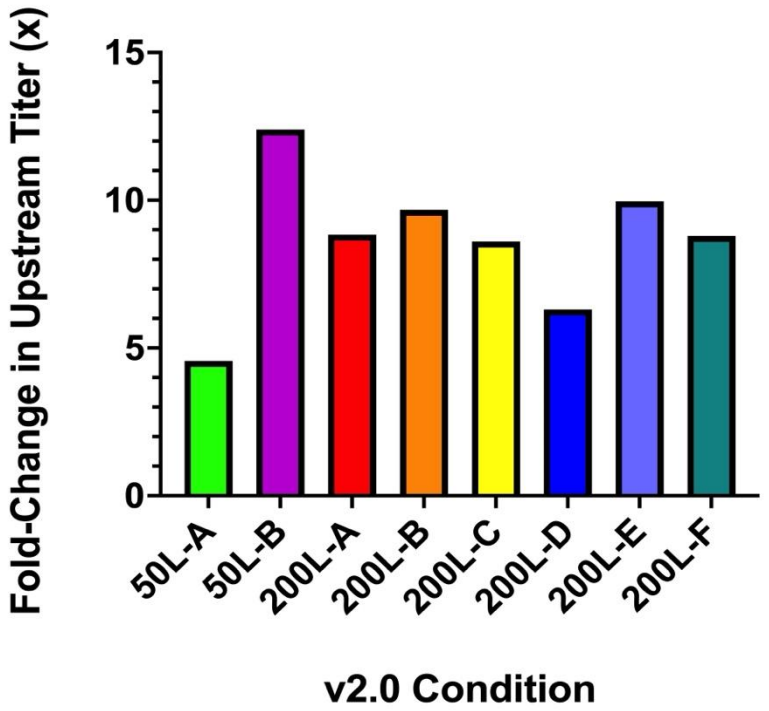


Figure 10: v2.0 Process Scalability. 200 L clarified lysate titers performed ~2x higher than 50 L vessel. 200 L pre-transfection satellite shake flasks (200L-D) did not outperform main bioreactor (200L-A), which supports safety of complex pumping. 200L-A titers may have been rescued by shorter complexation time.

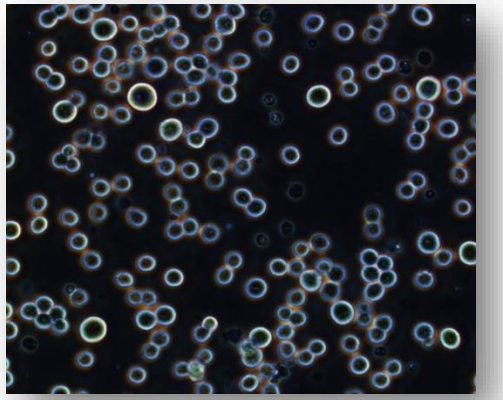
v2.0 EPI-321 Takeaways:

- Demonstrated scalability from bench- to production-scale
- Increased historical 50 L yield by 4-fold
- Increased historical 200 L yield by 8-fold

Avenues for Further Optimization

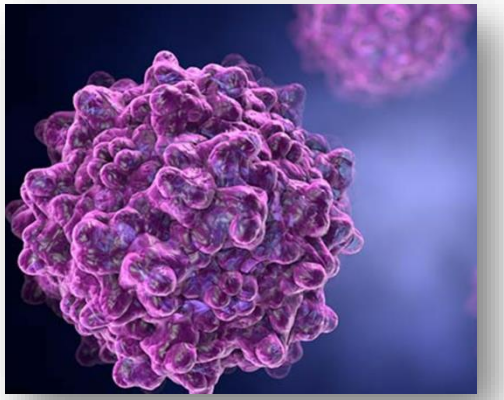
Cell Line Engineering

Improved quality
Improved stability
Operational feasibility



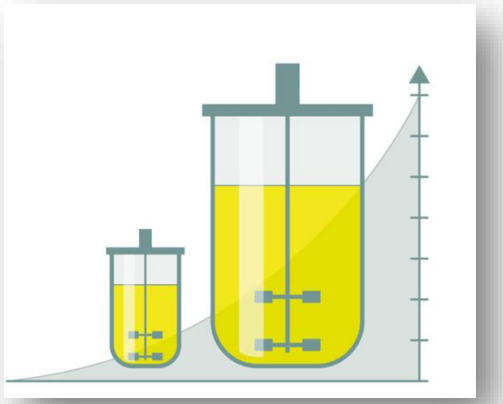
Transfection

Next-gen reagents
SM enhancers
Plasmid engineering



Scaling

Gassing strategy
Agitation strategy
Geometry/bag design



Characteriziation

Data mining
Predictive modeling

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