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Optimization of a scalable upstream process for production of novel AAV capsids

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Presented at the American Society of Gene and Cell Therapy (ASGCT) Annual Meeting, 16–19 May 2022, Washington DC, USA

Introduction

The development of novel capsids possessing more efficient gene delivery capabilities is the next evolution in recombinant adeno-associated virus (rAAV)-based therapeutics.

To meet clinical demands, scalable production processes are needed. The work presented here focuses on developing a scalable transient transfection process using HEK293 cells in suspension:

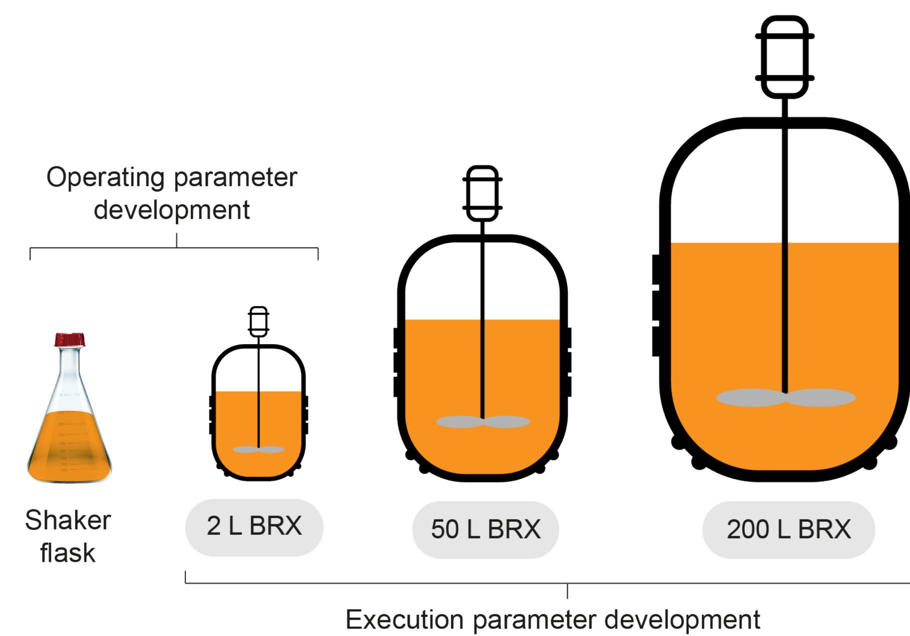
- Transfection conditions were screened in shaker flasks
- Conditions that achieved higher titer were selected for further testing
- Process was scaled to 200 L bioreactors

Objectives

The objectives of this study include:

- To develop a scalable transient transfection process for producing rAAV
- To confirm the process parameters do not reside near an edge of failure
- Explore parameters likely impacted by scale (Figure 1)

Figure 1. Different scales used in transient transfection development and where parameters were developed



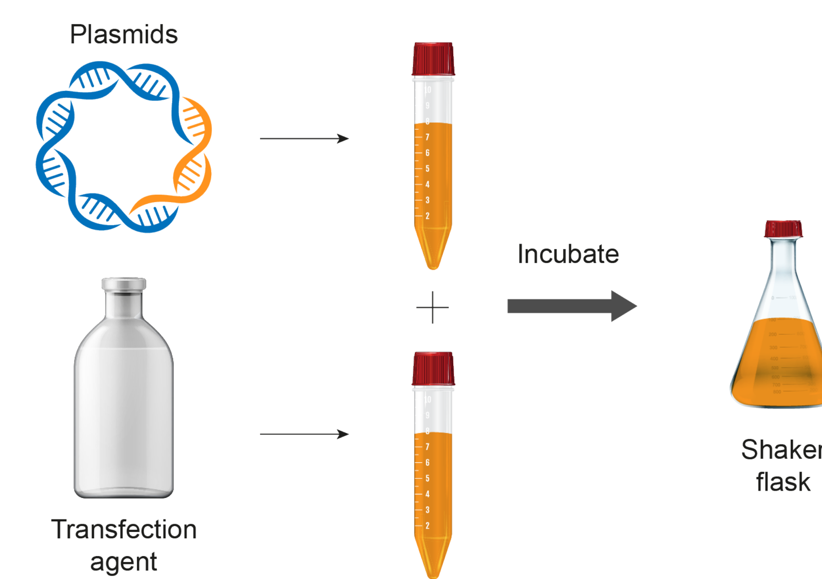
The transient transfection process involves the scale-independent and scale-dependent process parameters listed below. Flask-based studies allow faster screening of operating conditions and identification of ranges.

- Plasmid ratio
- Complexation media
- DNA amount
- Transfection reagent
- Ratio of transfection reagent to DNA amount
- Complexation time
- Mixing time
- Transfer time
- Transfer flowrates
- Temperature ranges
- Batch length
- Storage times

Flask transfection method

- Flasks are seeded at the target density on Day 0
- Transfection is performed on Day 1 with cells at a density of 1.5–3.0 x 10^6 c/mL
- Transfection process is schematically shown in Figure 2

Figure 2. Flask transfection process schematic

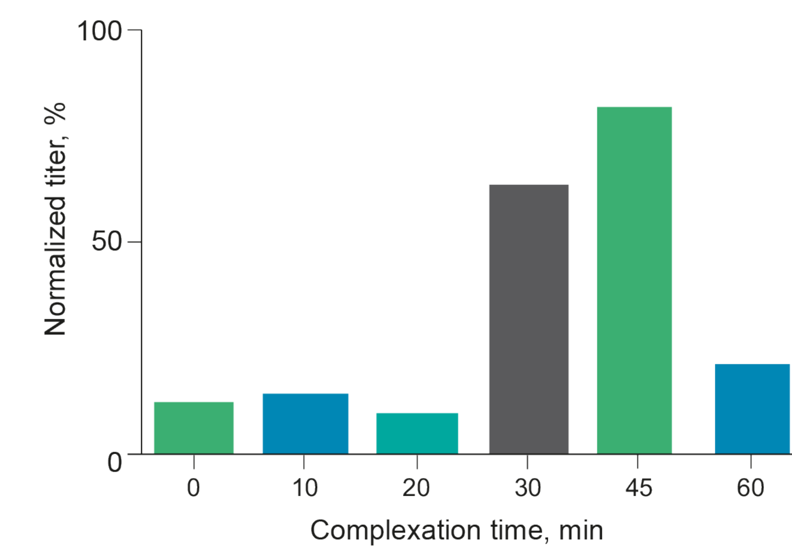


- Transfected cells are incubated for a target duration of 72 hours
- Cell culture is lysed and treated with endonuclease prior to harvest
- The titer of vector genome copies in the supernatant was determined

Results

- The shaker flask screening process identified acceptable transfection conditions, eg 2 x 5% complexation volume in Media A using FectoPro, with a complexation time of 30 minutes, targeting 2.2 x 10^6 c/mL
- Conditions were further developed at 2 L and 50 L bioreactor scales
- Titer at the 200 L scale was consistent with the expected titer (±15%) based on shaker flask screening

Figure 3. Titer as a function of complexation time



- The effects of complexation time on productivity were screened in shaker flasks
- The time required for transfection execution may increase as the process scales
- The same source culture was transfected at different complexation durations (Figure 3). Complexation volume was mixed at Time 0 and incubated
- The titer was reported as normalized by the 45-minute time point. Target complexation time is 30–45 minutes

Figure 4. Titer as a function of complexation media and transfection agent to DNA ratio

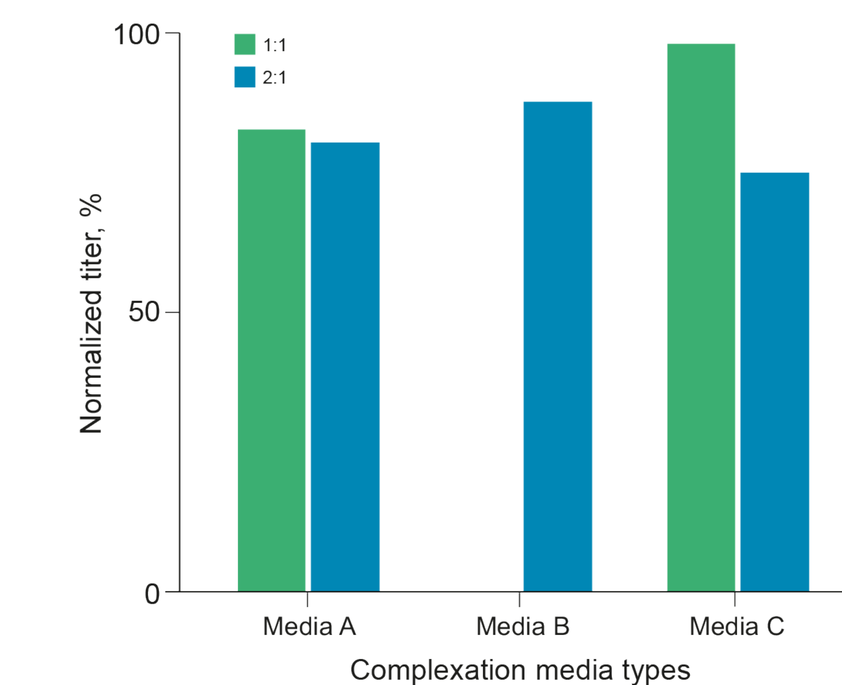
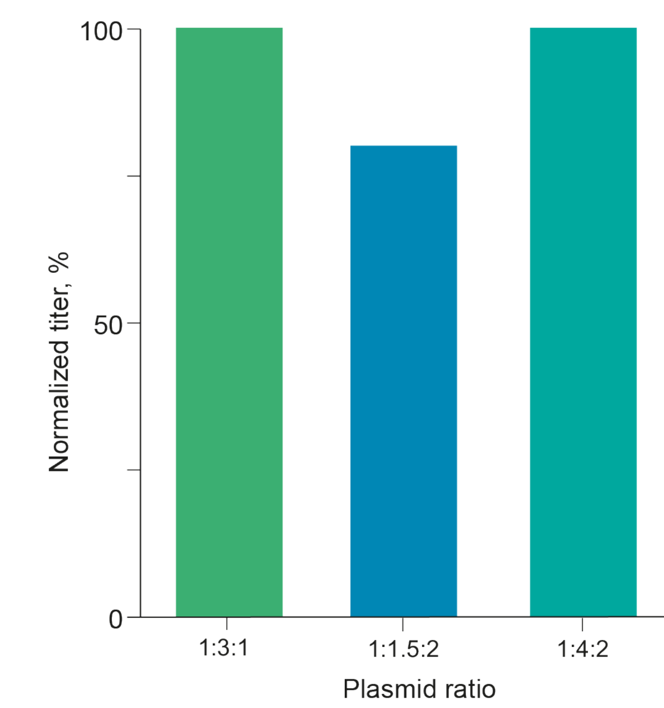


Figure 5. Titer as a function of plasmid ratio



- Complexation media and ratio of transfection agent to DNA were screened in shaker flasks (Figure 4). Media B is recommended by the manufacturer; however, the highest titer was obtained with Media C
- Media B required twice the transfection agent to achieve a comparable titer. Higher amounts of transfection agent increase production costs
- Plasmid ratio was also screened to determine optimal amounts of helper, gene of interest and RepCap plasmids (Figure 5). High-producing conditions were identified, with the highest titer condition achieved with FectoPro

Figure 6. Titer and transfection agent selection

- Multiple transfection agents (TFX) were screened for compatibility with our process (Figure 6), the objective being to minimize the amount of plasmid DNA used
- The highest titer was achieved with FectoPro
- TFX B and TFX C could not achieve a similar titer after multiple DOE-based optimization rounds

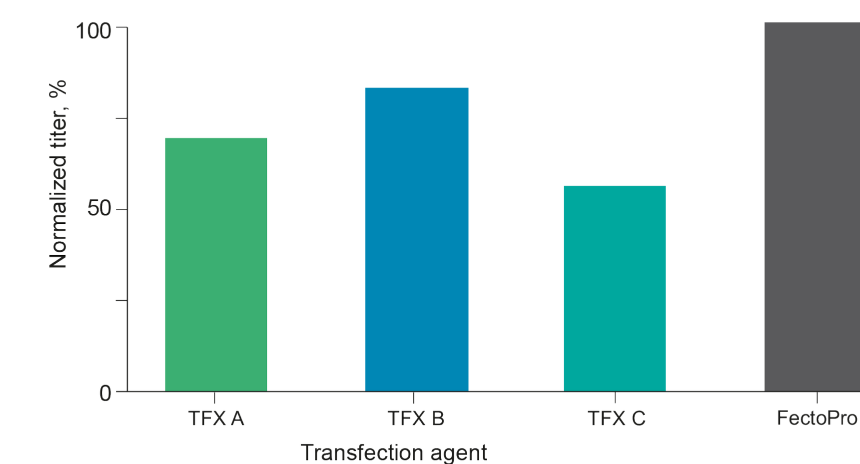


Figure 7. Effect of antifoam on titer

- Foam is a concern in bioreactors because of the addition of sparging. Typically, antifoam is added to prevent foaming; however, antifoam as a surfactant may interfere with the transfection process
- The addition of antifoam up to 100 ppm was screened in shaker flasks using the transfection agent FectoPro (Figure 7)
- Titer was normalized to the 0 ppm value
- Normalized titer was in line at the two concentrations tested, within 10% of 0 ppm

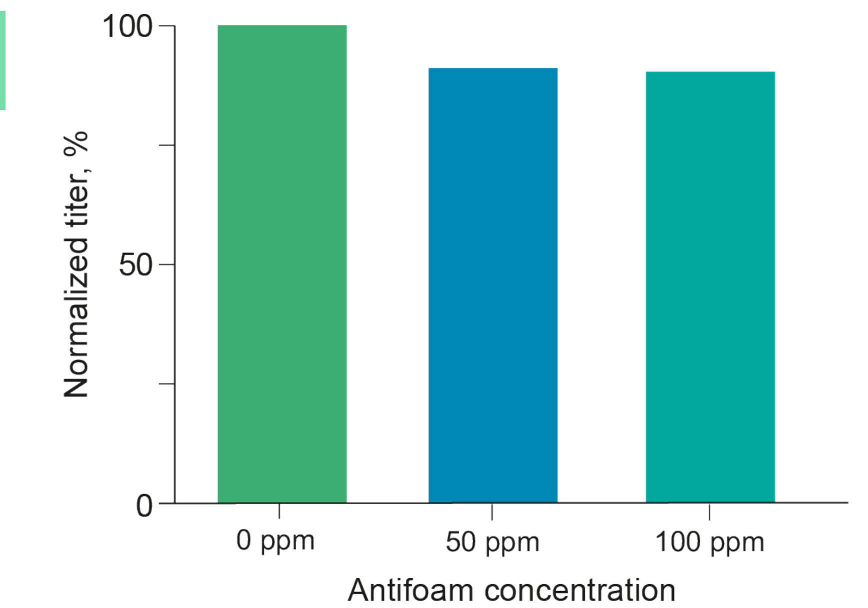
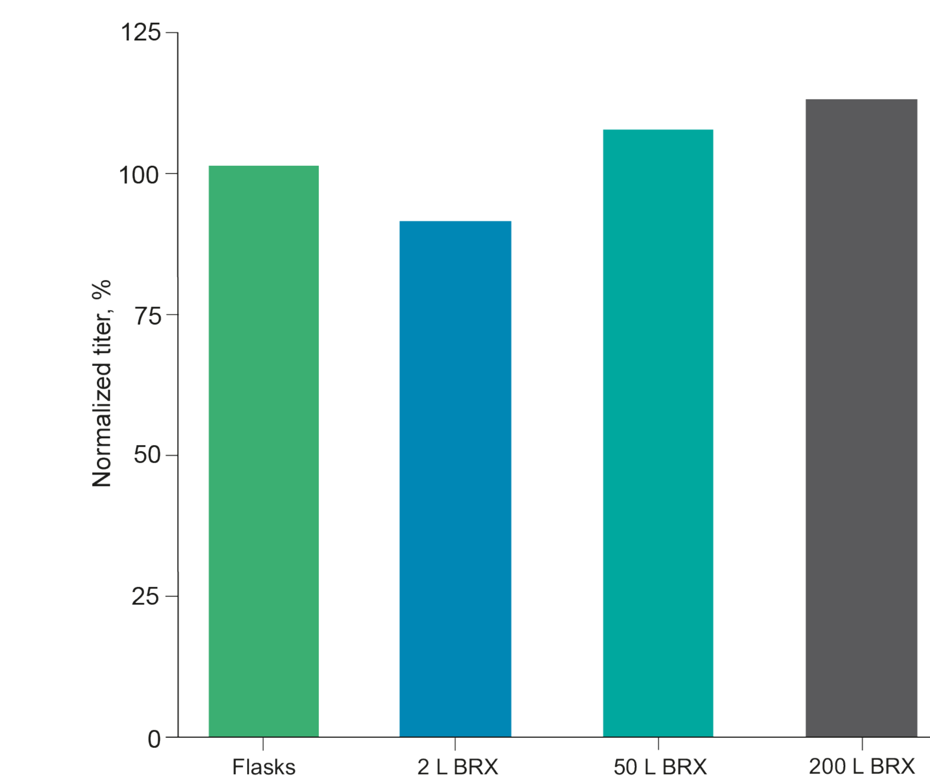


Figure 8. Production of Capsid A across scales



- Production of Capsid A, a novel capsid from Capsida Biotherapeutics' platform, using suspension-based process scales
- Titer is consistent from a 30 mL shaker flask to 200 L bioreactors (BRX) when using the same transfection conditions (DNA amount, transfection reagent, complexation time etc [Figure 8])
- Titer is normalized to the values from screening studies in shaker flasks
- The 200 L process was executed in a manufacturing setting. The step was completed in the expected duration

Conclusions

- Shaker flasks are a viable method for screening transfection parameters and establishing ranges
- User guides and literature are a starting point for transfection processes.[1–3] However, screening work can identify conditions and reagents that yield higher titers
- Scaling studies should focus on items related to execution
- The Capsida production process scales to 200 L

Acknowledgments

We would like to thank the Manufacturing and Analytical Development groups at Capsida Biotherapeutics. We would also like to thank PolyPlus for their helpful discussions during screening work.

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Video

References

1. Protocol: FectoPRO *in vitro* DNA transfection reagent
2. Protocol: FectoVIR-AAV DNA transfection reagent for virus production
3. LV-MAX Lentiviral Production System User Guide

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