High-titer rAAV production in bioreactors using ELEVECTA™ stable producer cell lines

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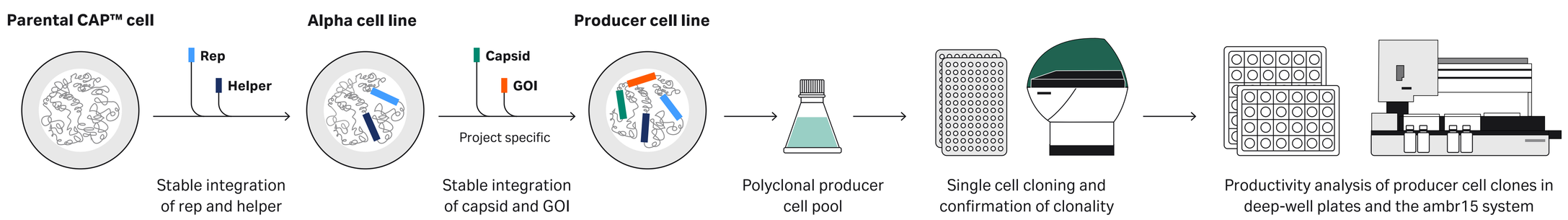
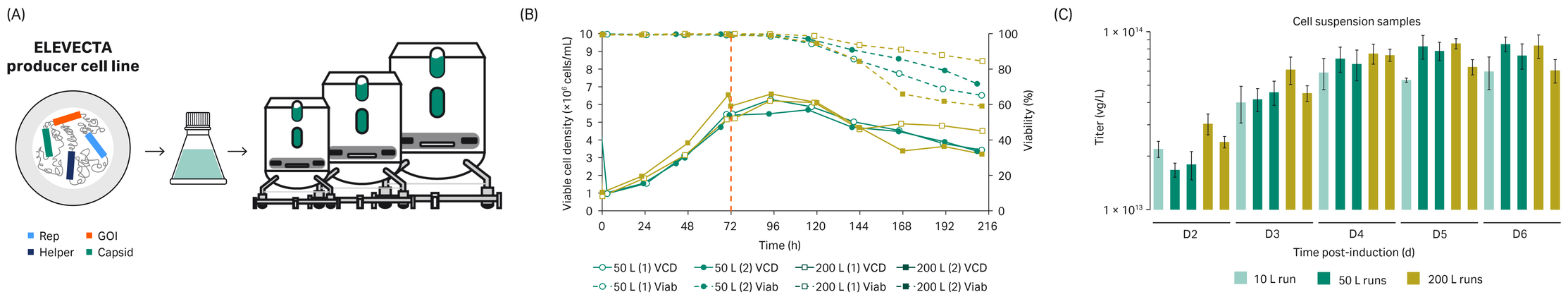
Introduction

- Recombinant adeno-associated virus (rAAV) is a widely used viral gene therapy vector. However, the delivery of the required amounts of vectors and the quality is still a challenge.
- The ELEVECTA™ AAV production platform is based on mammalian suspension cells. It has a stable integration of all components necessary for AAVproduction: adenovirus helper functions, AAV replicase, AAV capsid, and gene of interest (GOI; transgene).
- Here we describe the upstream process development and optimization of an AAV8-GFP proof-of-concept (PoC) producer single cell clone (SCC) in batch and perfusion modes.

ELEVECTA cell line development

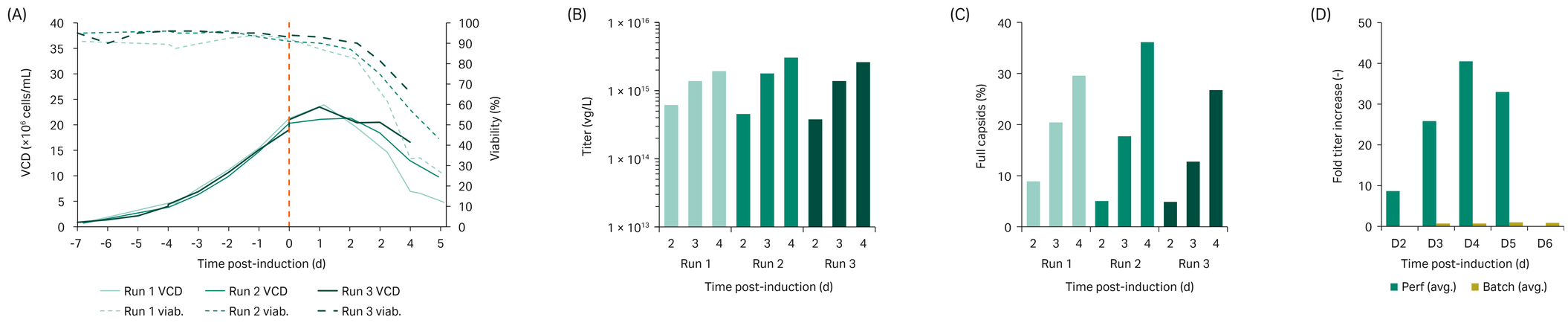


High-performance AAV vector production in a fully scalable and robust format



Scale-up of proof-of-concept producer single cell clone. (A) A PoC producer cell line for rAAV8-GFP was successfully scaled up from 10 L to 50 L up to 200 L. (B) Viable cell densities and viabilities of PoC producer cell line in 50 L and 200 L bioreactor runs highlight the robust and scalable production process. Cells were seeded at 1 ×10^6 cells/mL.AAV production was induced by the addition of doxycycline 3 d post-seeding. (C) qPCR analysis of viral genome titers (vg/L) from cell suspension samples day 2 to day 6 post-induction show constant productivity throughout scale-up.

Perfusion further increases quality and quantity of AAV production



Perfusion increases AAV production performance of stable ELEVECTA producer cells. (A) Perfusion with daily medium exchange was started after 3 d of batch mode until the end of production. AAV production was induced at a cell density of ~ 2 ×10^7 cells/mL by the addition of doxycycline. (B) Viral genome titer was measured on day 2 to 4 post-induction in cell suspension samples by qPCR. (C) Percentage of full capsids was calculated as the ratio of genome to capsid titer and measured by ELISA. (D) Fold titer increase of perfusion compared to batch mode. Data from three independent perfusion runs are shown.

Summary

- ELEVECTA stable AAV production platform eliminates the need for helper viruses, expensive transfection reagents, or cGMP-grade plasmids.
- ELEVECTA technology enables a robust and scalable AAV production, with excellent productivity throughout the whole the process.
- Use of perfusion systems further improves the quality and quantity of stable AAV production.

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