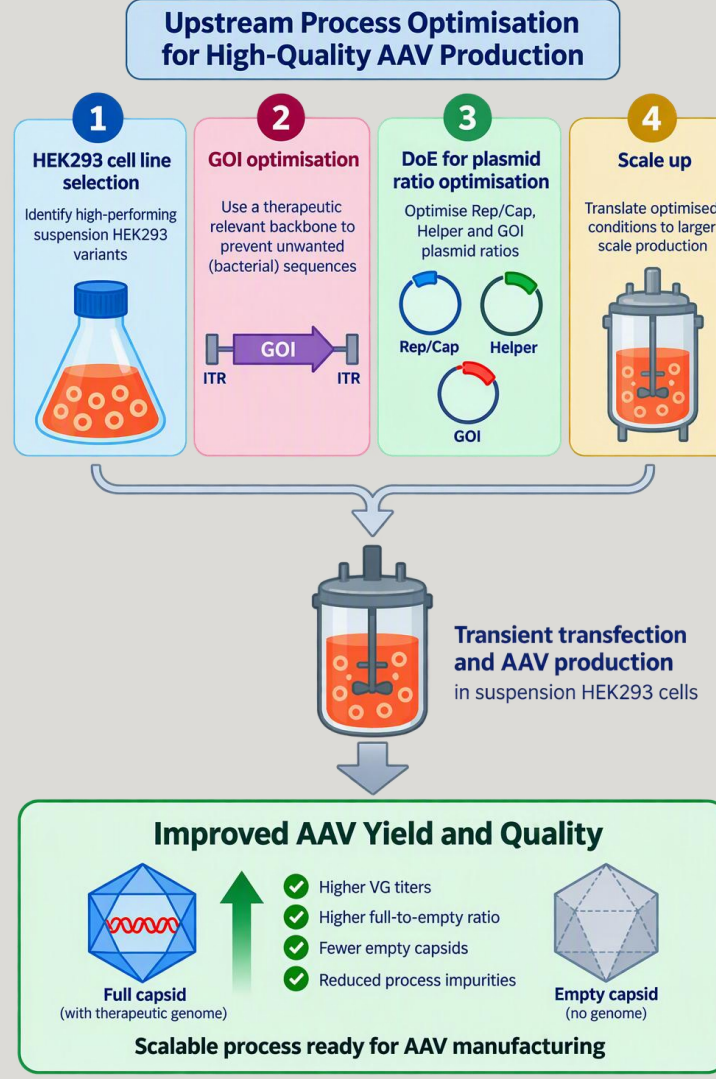


Multi-Parametric Optimization of Upstream AAV Production for Enhanced Titers and Vector Quality

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The rapid advancement of gene therapy has intensified the need for **robust and scalable AAV manufacturing processes** capable of delivering high vector productivity while maintaining critical product quality attributes. This study presents a systematic upstream process development strategy focused on **enhancing vector genome (VG) titers and improving genome packaging efficiency**, as reflected by the **full-to-empty capsid ratio (MP)**. Key upstream parameters were evaluated to identify conditions that support consistent cellular performance, efficient transfection, and reproducible AAV production while minimizing empty capsids and process-related impurities. These attributes emphasizes by constant optimisation process like 1. Suspension HEK293 cell line selection 2. GOI optimisation 3. Design of Experiment's (DoE) for plasmids ratio titration and 4. Transfection conditions confirmation in scale up.



1 Cell line selection shapes AAV yield & quality

Cell Lines as Production Platforms for Viral Vectors

Two suspension HEK293 cell lines were evaluated for AAV9 production under comparable culture conditions. Both cell lines exhibited similar viable cell density (VCD) profiles, growth kinetics and population doubling times, with viability exceeding 99% following initial recovery (Figure 1A). At the target cell density, cells were triple-transfected with GOI, Rep/Cap9 and adenoviral helper plasmids at equimolar ratios. At 70 hours post-transfection, cells were lysed and the harvests clarified for vector genome (VG) titer analysis.

Cell line 2 achieved slightly higher VG titers and VG productivity per cell (Table 1). However, mass photometry following affinity capture revealed a higher full-particle fraction in cell line 1, comprising 45.1% full and 29.2% partial particles. In comparison, cell line 2 contained 24.2% full and 13.7% partial particles, approximately half the partial-particle fraction observed in cell line 1 (Figure 1B–C). Cell line 2 achieved slightly higher VG productivity, whereas cell line 1 produced a higher proportion of full AAV9 particles, highlighting a trade-off between productivity and particle quality.

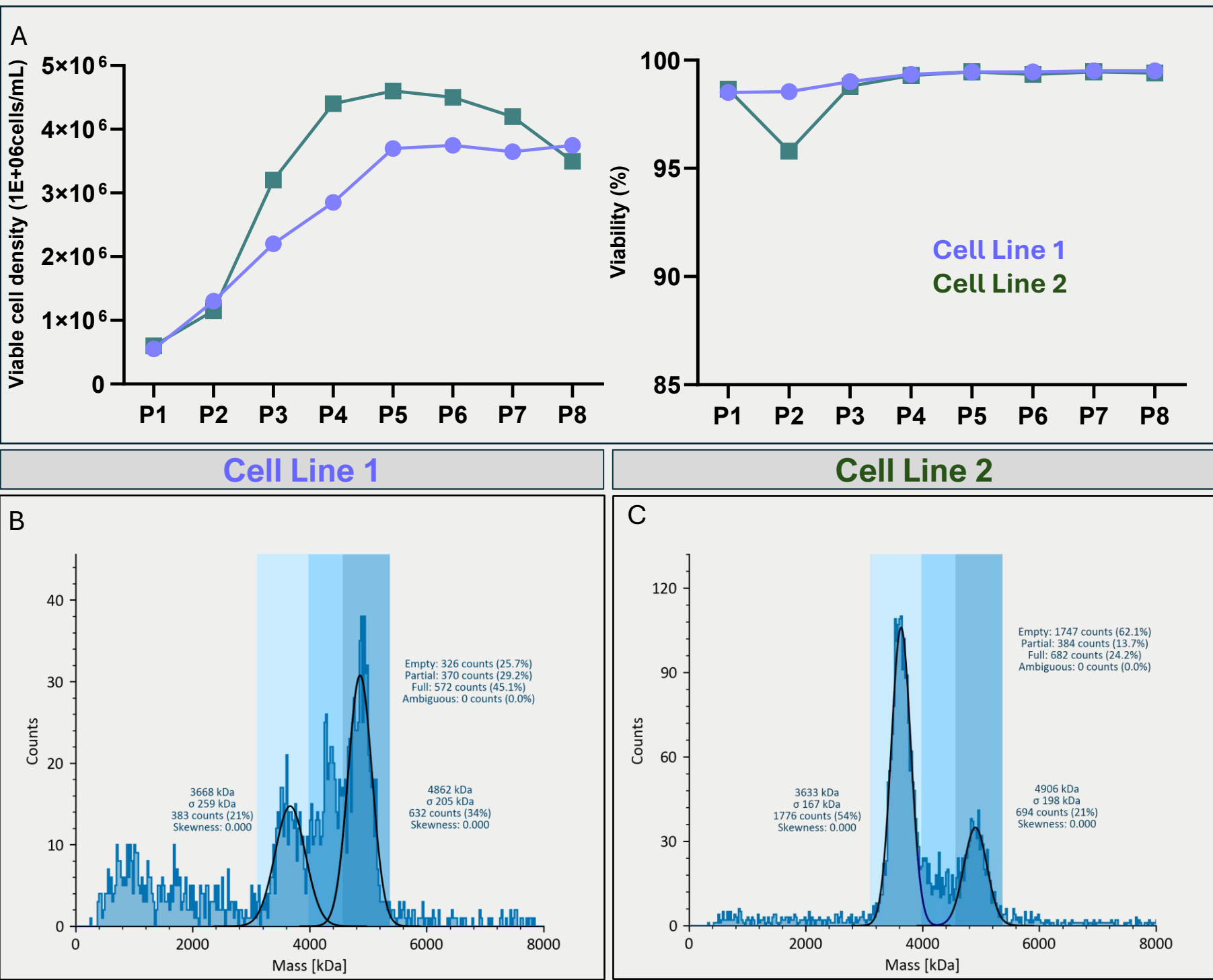


Table 1: AAV9 clarified harvest VG titers & cell-specific productivity comparison

susHEK293	Vg/mL	Vg/cell	% Empty	% Partial	% Full
Cell Line 1	2.2e+11	6.1e+4	25.7	29.2	45.1
Cell Line 2	3.2e+11	1.0e+5	62.1	13.7	24.2

Figure 1: Comparison of cell growth, viability and AAV9 particle composition in two suspension HEK293 cell lines. (A) Viable cell density (VCD) and viability profiles of cell line 1 (purple circles) and cell line 2 (green squares). (B–C) Mass photometry distributions of purified AAV9 from cell line 1 (B) and cell line 2 (C). Empty, partial and full particles represented 25.7%, 29.2% and 45.1% for cell line 1, and 62.1%, 13.7% and 24.2% for cell line 2, respectively. Shaded regions indicate particle classification ranges.

Table 1 summarises clarified-harvest VG titers, cell-specific productivity and full-to-empty capsid ratios.

2 Upstream DoE boosts CNS specific Apertura GTx engineered BI-TfR1 CapX titers and full AAV particles

CapX Engineered to Cross the BBB for CNS Gene Delivery

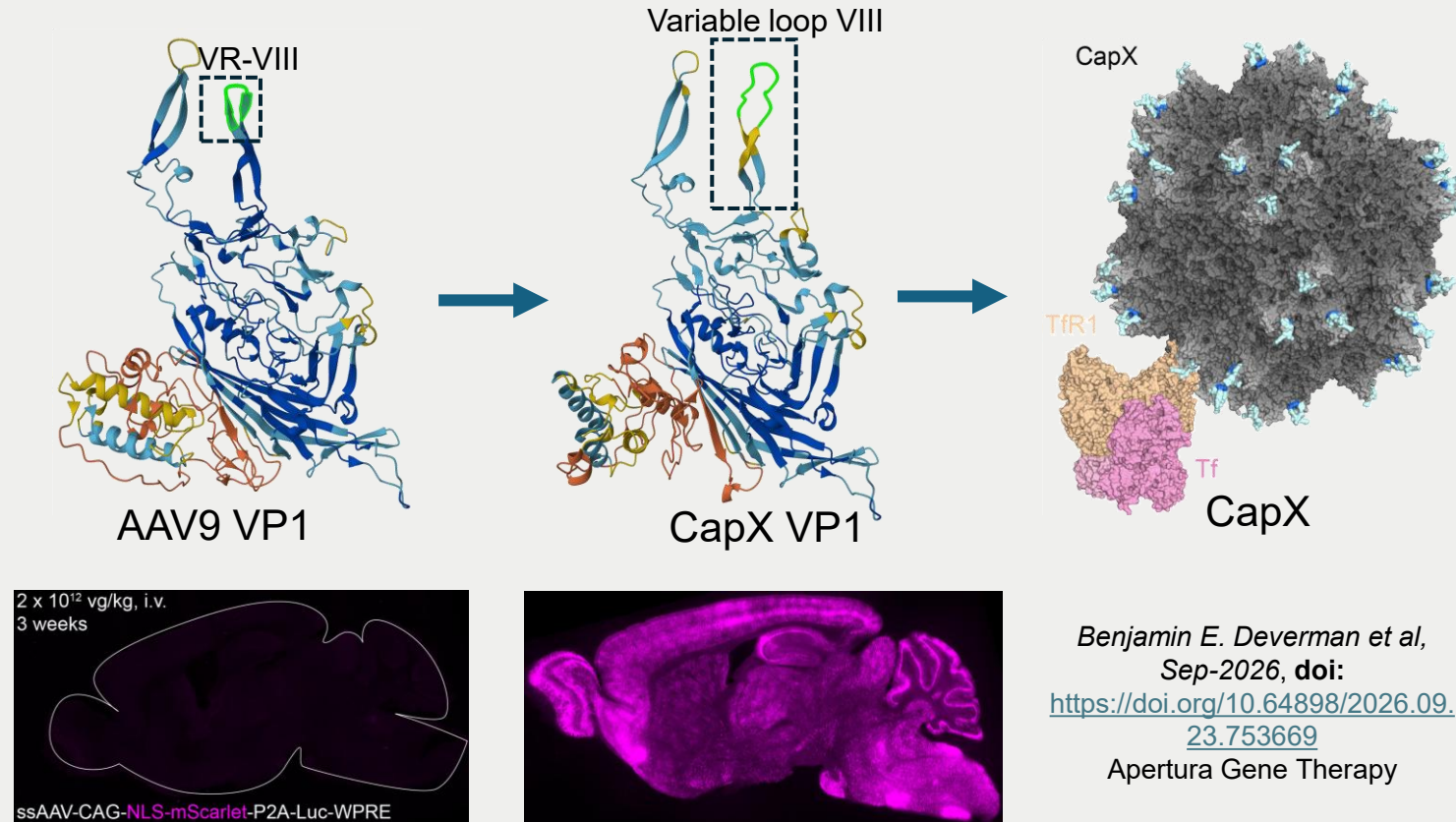


Figure 2. Boltz-2-predicted VP1 structures of AAV9 and CapX, alongside a 3D representation of CapX binding to human transferrin receptor 1 (TfR1). TFR1 targeting enables blood-brain barrier crossing and enhanced CNS delivery.

USP feasibility identify CapX process development

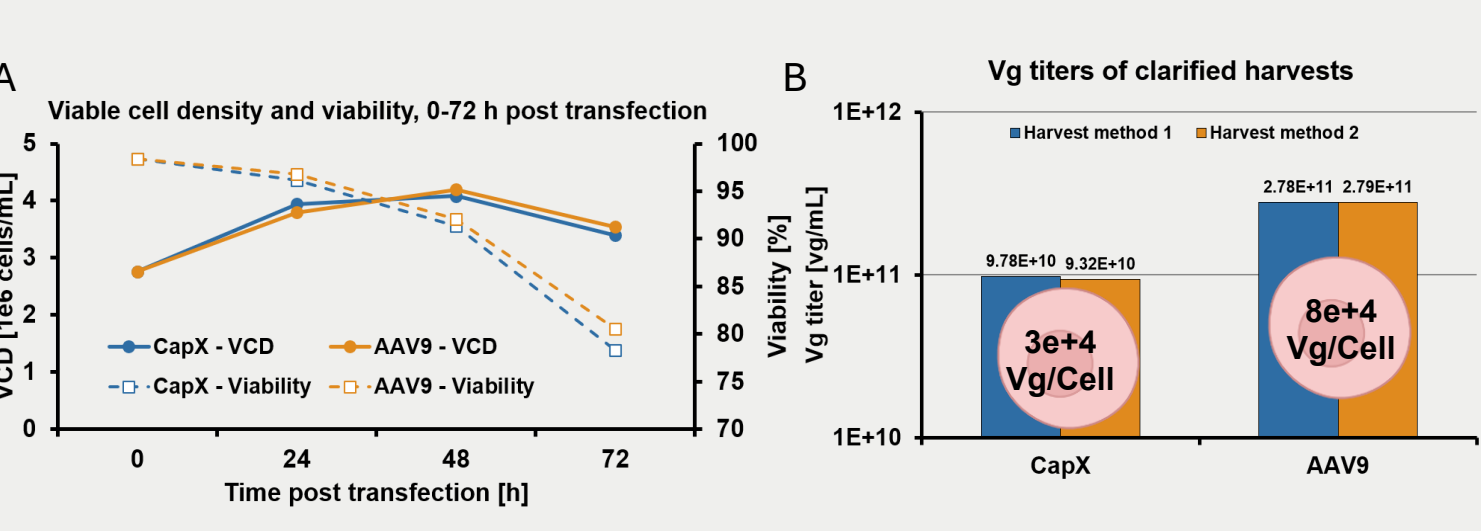


Figure 3. CapX production feasibility on the standard DINAMIQS platform. (A) CapX production on the standard DINAMIQS suspension HEK293 platform showed VCD and viability profiles comparable to the AAV9 control. (B) Both harvesting methods, including cleanroom-tested Process 1, achieved comparable vg recovery. These findings establish platform feasibility and a baseline for further optimization to increase titers.

3 GOI optimization minimizes DNA impurities

Safety by design: minimise backbone packaging through GOI plasmid optimisation

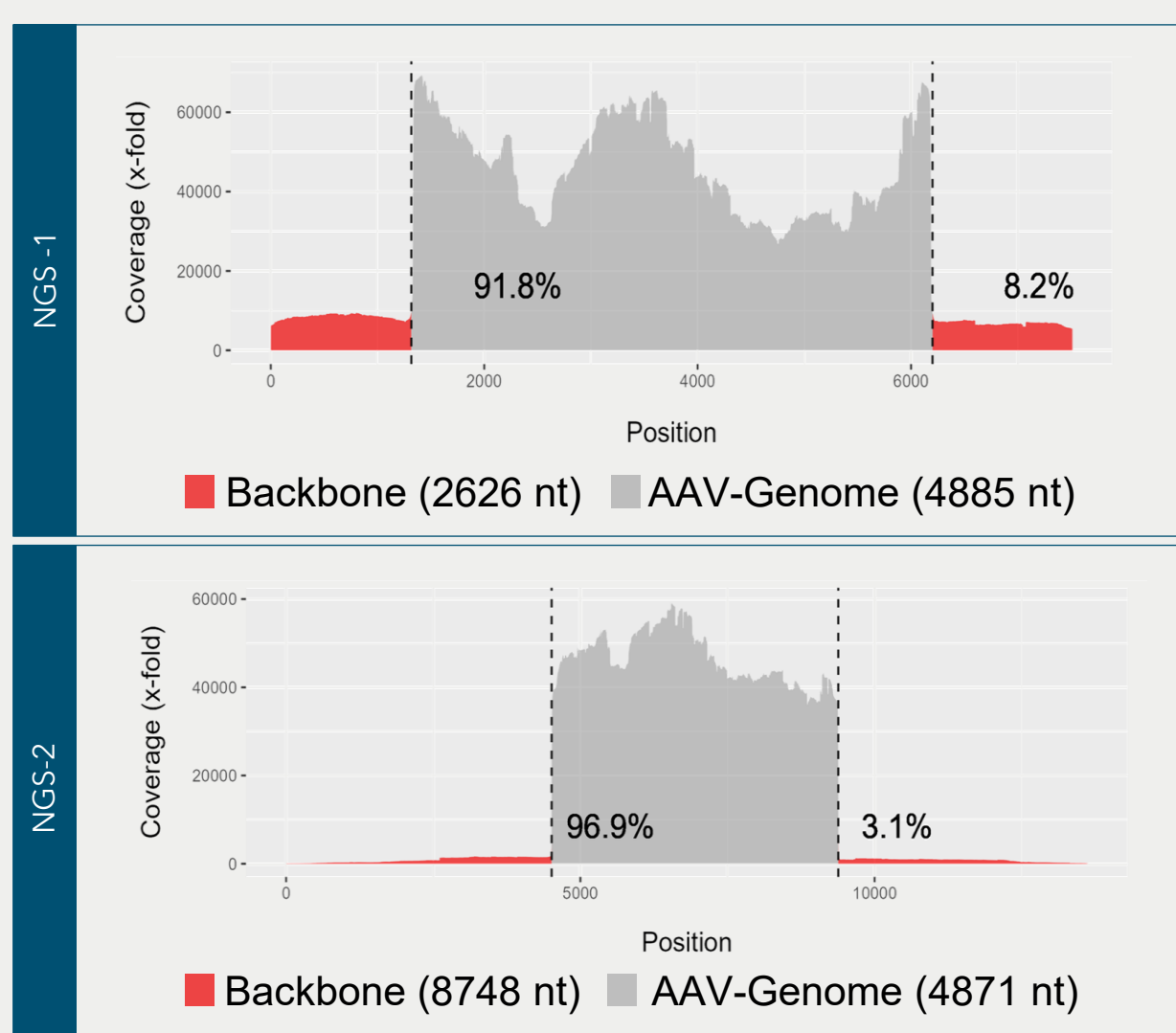
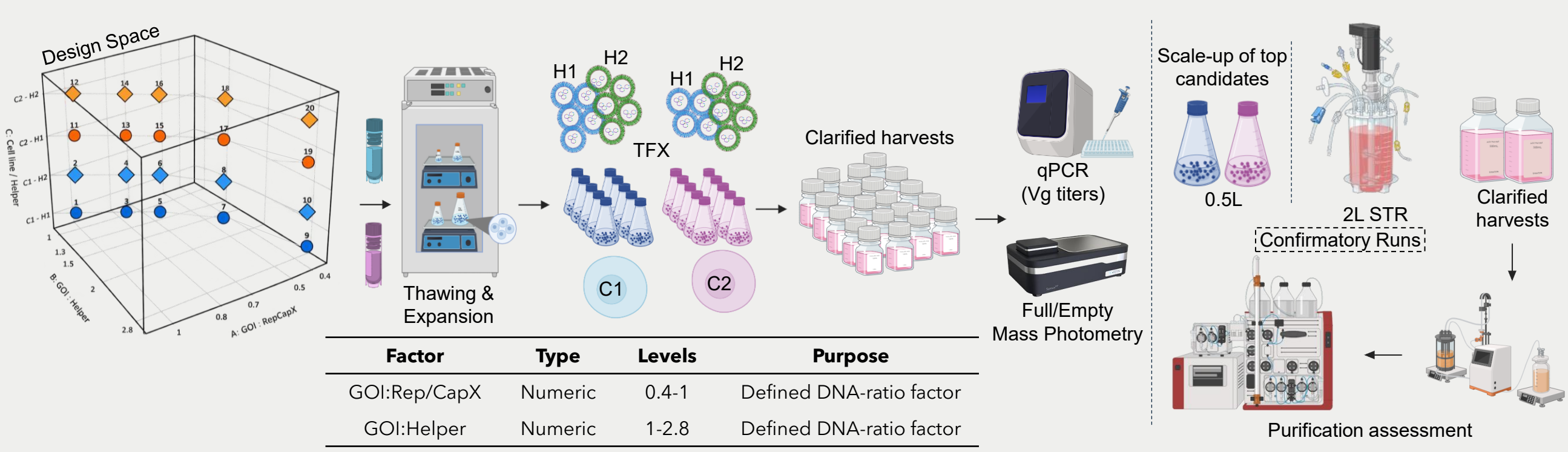


Figure 5. GOI plasmid optimisation reduces backbone coverage AAV viral genome isolated from the final drug products was analysed by nanopore long-read next-generation sequencing and mapped to the corresponding GOI plasmid references. Coverage profiles are shown for NGS-1 (top) and NGS-2 (bottom). Grey indicates the ITR-flanked therapeutic gene expression cassette; red indicates backbone regions outside the ITRs. Dashed lines mark cassette boundaries. Cassette/backbone coverage proportions were 91.8%/8.2% for NGS-1 and 96.9%/3.1% for NGS-2.

Upstream DoE-driven CapX gains 19-fold higher Vg titers with 40% full capsids



A DoE-driven screening strategy to identify high-performing cell lines and transfection conditions that enhanced vg productivity and full AAV particle content at 30mL. Selected candidates will be scaled to 0.5 L and / or 2 L bioreactors for upstream DoE confirmation and purification assessment by affinity and AEX chromatography.

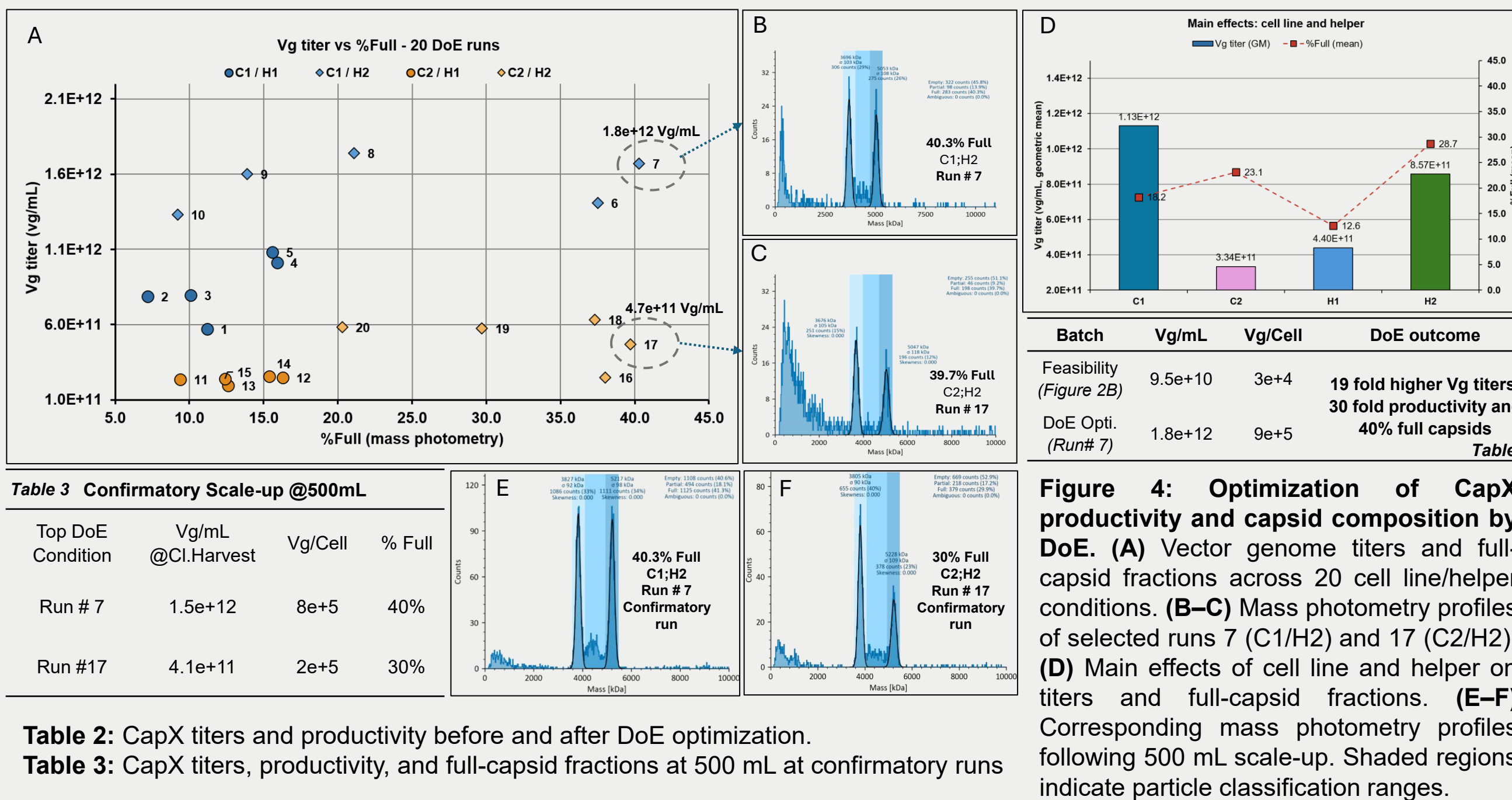


Table 2: CapX titers and productivity before and after DoE optimization.

Table 3: CapX titers, productivity, and full-capsid fractions at 500 mL at confirmatory runs

4 Upstream scalability of AAV8

Optimize early. Scale predictably. Manufacture with confidence.

- CMC Challenges for Manufacturability and Scalability
- High-titer, scalable vector production
- Cell line and transfection process optimization
- Capsid quality and genome packaging efficiency
- Efficient vector recovery while maintaining product quality

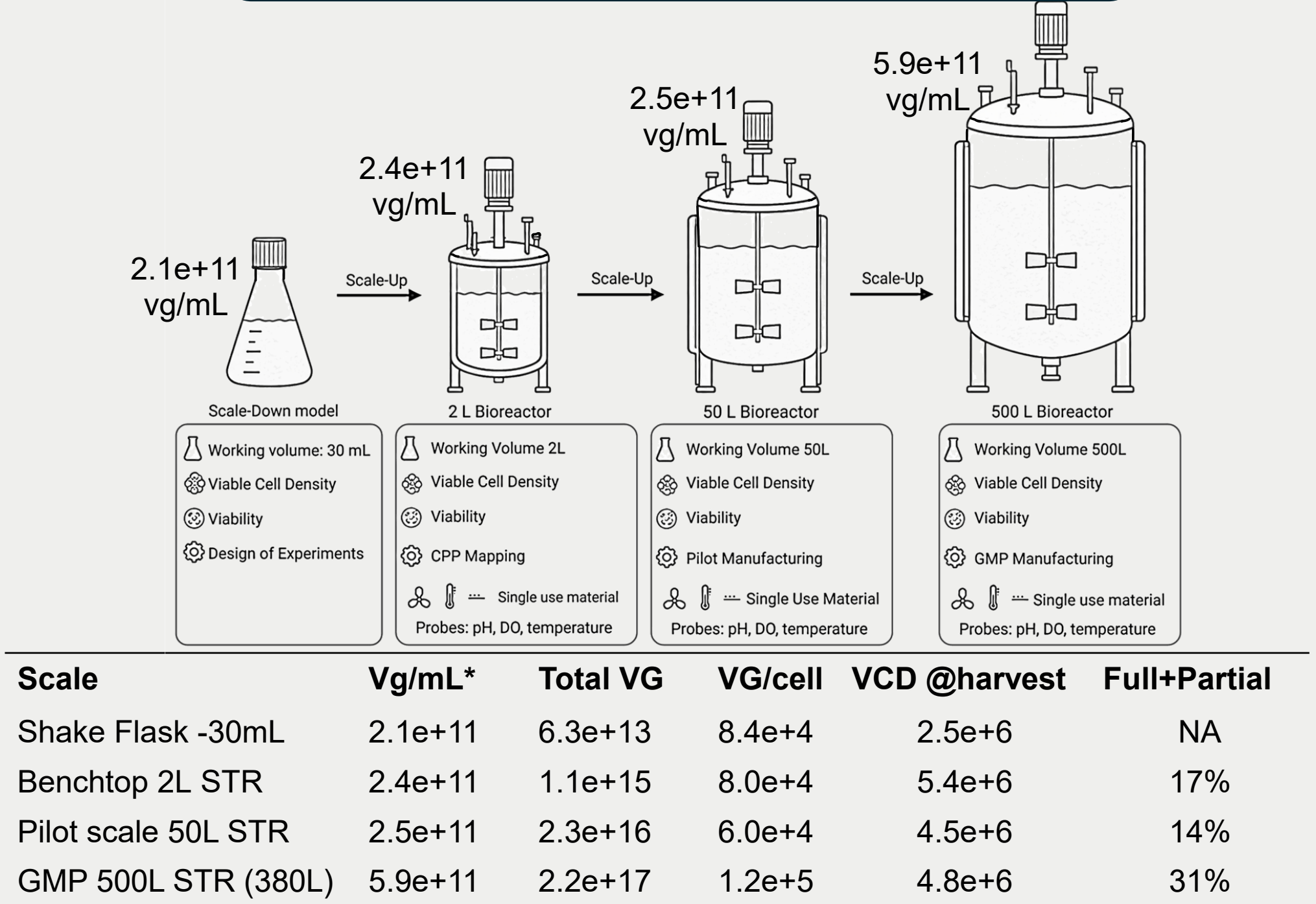


Figure 6. Scale-up strategy for AAV8 manufacturing from process optimisation to GMP production. A staged scale-up approach of therapeutic gene from process optimisation from 30 mL to 2 L process characterization and 50 L pilot manufacturing enabled seamless transfer into GMP production. Comparable *clarified harvest titers and cell-specific productivity across all scales demonstrate a robust, predictive, and linear scale-up strategy, significantly reducing manufacturing risk.

Conclusions

- Optimize early:** Cell line and plasmid selection establish the foundation for AAV productivity and product quality.
- Combine platform expertise with DoE:** The optimized DINAMIQS USP platform identifies key process parameters to improve vector titers and yields.
- Demonstrate the benefit:** DINAMIQS DoE approach delivered substantial CapX productivity gains, supporting early optimization to de-risk scale-up and potentially reduce development and manufacturing costs.
- De-risk development:** Early optimization can improve manufacturing success by reducing scale-up risks, development time, and costs.

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