

A multi-output Design of Experiment framework to simultaneously optimize rAAV yield and quality using a GMP-banked clonal HEK293 platform

Number : 3141

Type: Poster

Pelin Kolcak Yasli¹, Steffen Hinz¹, Luca Nanni¹, Jens Stolte¹, Marc-Antoine Perrenoud¹, Rachel Buchs¹, Ann-Kristin Hov¹, Efrain Guzman¹, Deborah Ley¹, Thierry Schuepbach¹, Igor Fisch¹, Nicolas Mermoud¹

¹ NewBiologix SA, Epalinges, Switzerland

Introduction

Efficient, scalable and reproducible manufacturing of recombinant adeno-associated virus (rAAV) vectors remains a central challenge for gene-therapy development. While HEK293-based transient triple-transfection is widely used, production performance is highly sensitive to multiple interdependent parameters, and conventional process-development strategies focus almost exclusively on maximizing vector titers—often at the expense of key quality attributes such as genome integrity and full-to-empty capsid ratios.

Methods

Here, we apply a multi-output Design of Experiment (DoE) strategy to optimize rAAV production in NBX-Eng-HEK293, a GMP-banked, clonal, suspension-adapted HEK293 cell line fully characterized at the genomic and functional levels. Using a Box-Behnken experimental design, we systematically evaluated the impact of culture media, plasmid DNA ratios, and seeding densities on both rAAV yield and critical quality attributes (CQAs). To enable this approach, we established an integrated analytical pipeline combining small-scale crude production, mass photometry for empty-to-full capsid quantification, multidimensional digital PCR for genome integrity, and long-read DNA sequencing to assess vector structure and to detect host-cell or plasmid-derived DNA.

Results

An initial screening of commercially available culture media identified optimal conditions for NBX-Eng-HEK293 growth and productivity, which were then incorporated into the DoE to refine additional process variables. The multi-output DoE identified parameter combinations resulted in substantial improvements in rAAV packaging efficiency and genome integrity, while maintaining high vector titers. Importantly, this approach enabled simultaneous optimization of productivity and quality, rather than sequential or yield-only tuning.

Conclusion

In conclusion, this multi-output, analytics-driven DoE framework enables rational fine-tuning and optimization of rAAV manufacturing conditions to deliver high-titer, high-quality vectors. Applied to a GMP-banked clonal HEK293 platform, it provides a scalable and quality-centric process-development strategy directly applicable to preclinical and clinical gene-therapy manufacturing.