

# Scalable and flexible rAAV production using a fully characterized novel HEK293 cell line



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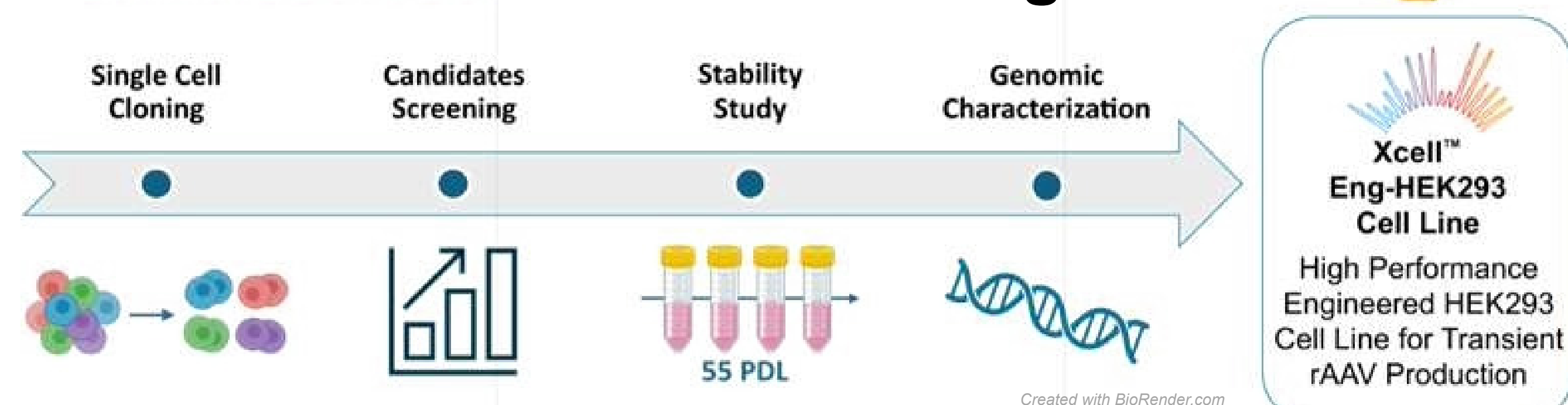
## Introduction

- HEK293 cells are the most commonly used rAAV production cell type.
- NewBiologix has developed its proprietary Xcell™ Eng-HEK293 cell line optimized for efficient plasmid transfection and rAAV production.
- Extensive genomic and phenotypic characterization have previously demonstrated the stability of Xcell™ Eng-HEK293.
- Previous results have also demonstrated the performance of Xcell™ Eng-HEK293 in rAAV in a variety of capsids and transgenes compared with other commercially available cell lines.

## Objective

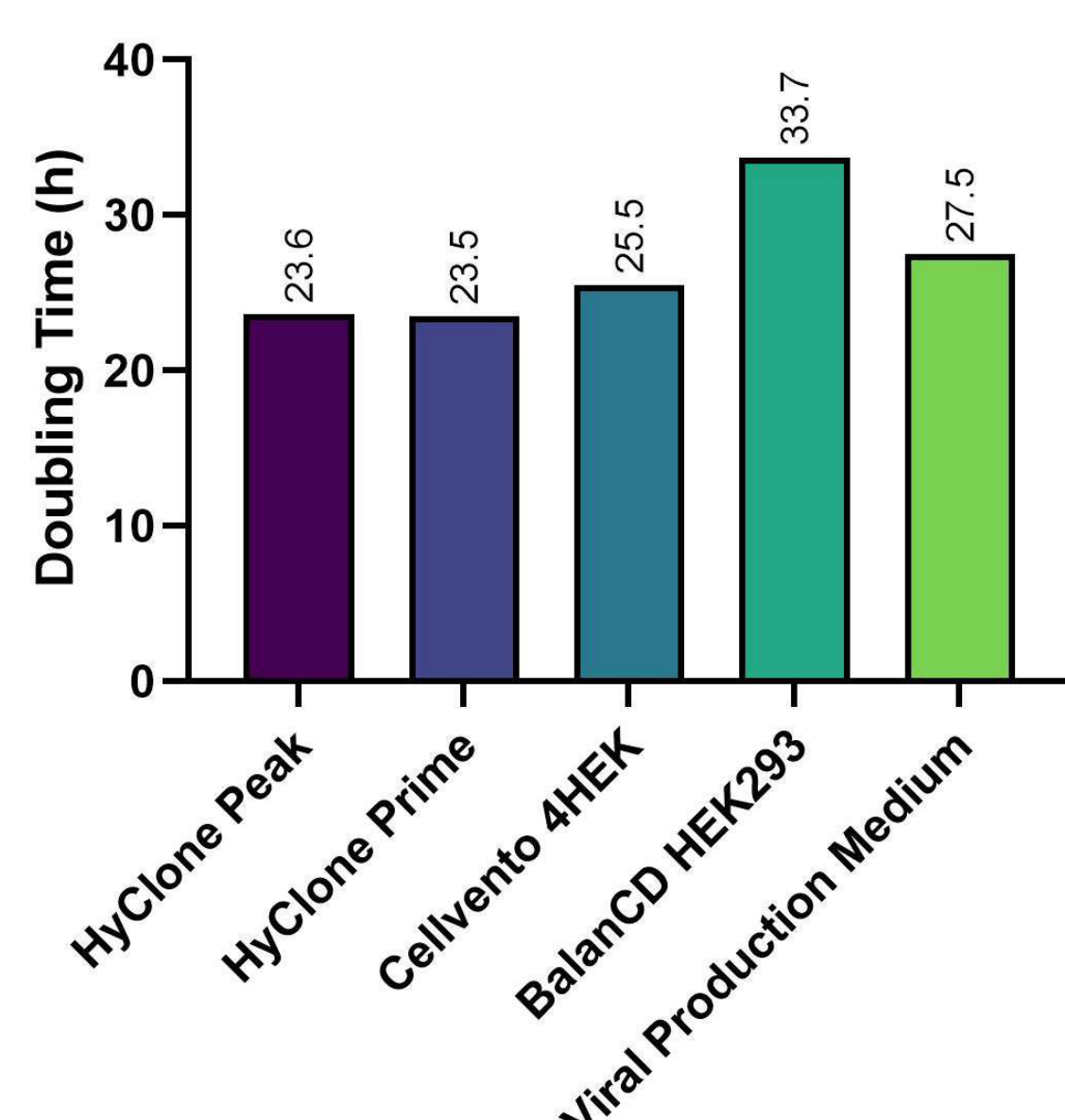
To demonstrate the scalability and flexibility of the Xcell™ Eng-HEK293 cell line for rAAV manufacturing in various commercial media formulations, culture volumes and bioreactor systems.

## Generation of Xcell™ Eng-HEK293



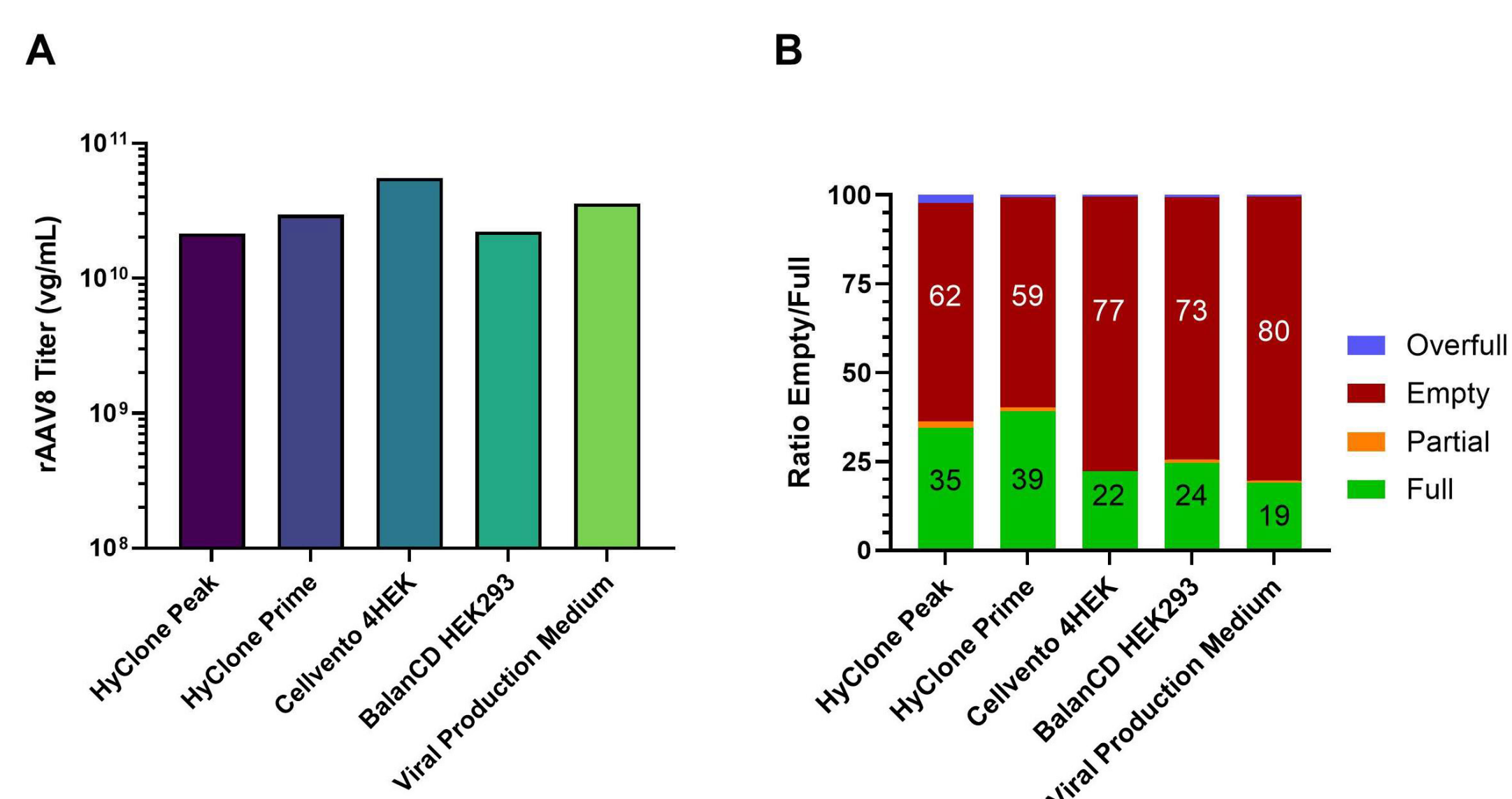
**Figure 1.** NewBiologix Xcell™ Eng-HEK293 cells were obtained following clonal selection and deep genotypic and phenotypic characterization.

## Xcell™ Eng-HEK293 is adaptable to various commercial media formulations



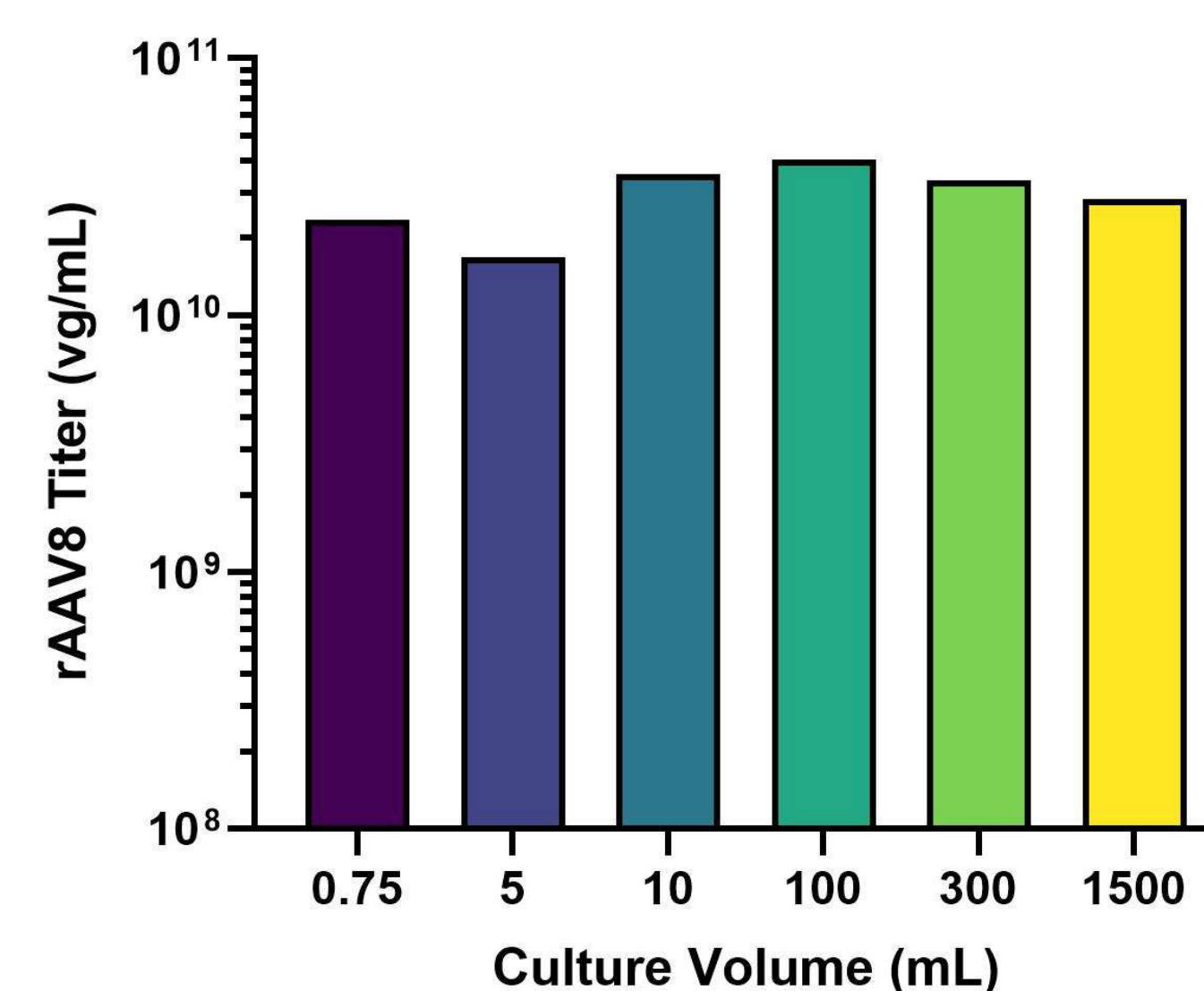
**Figure 2.** Doubling time of Xcell™ Eng-HEK293 cells in various commercial media formulations: HyClone Peak and Prime expression media (Cytiva), Cellvento 4HEK (Sigma-Aldrich), BalanCD HEK293 (Irvine Scientific) and Viral Production Medium (VPM, Gibco).

## Consistent rAAV8 production in Xcell™ Eng-HEK293 cultured in various commercial media



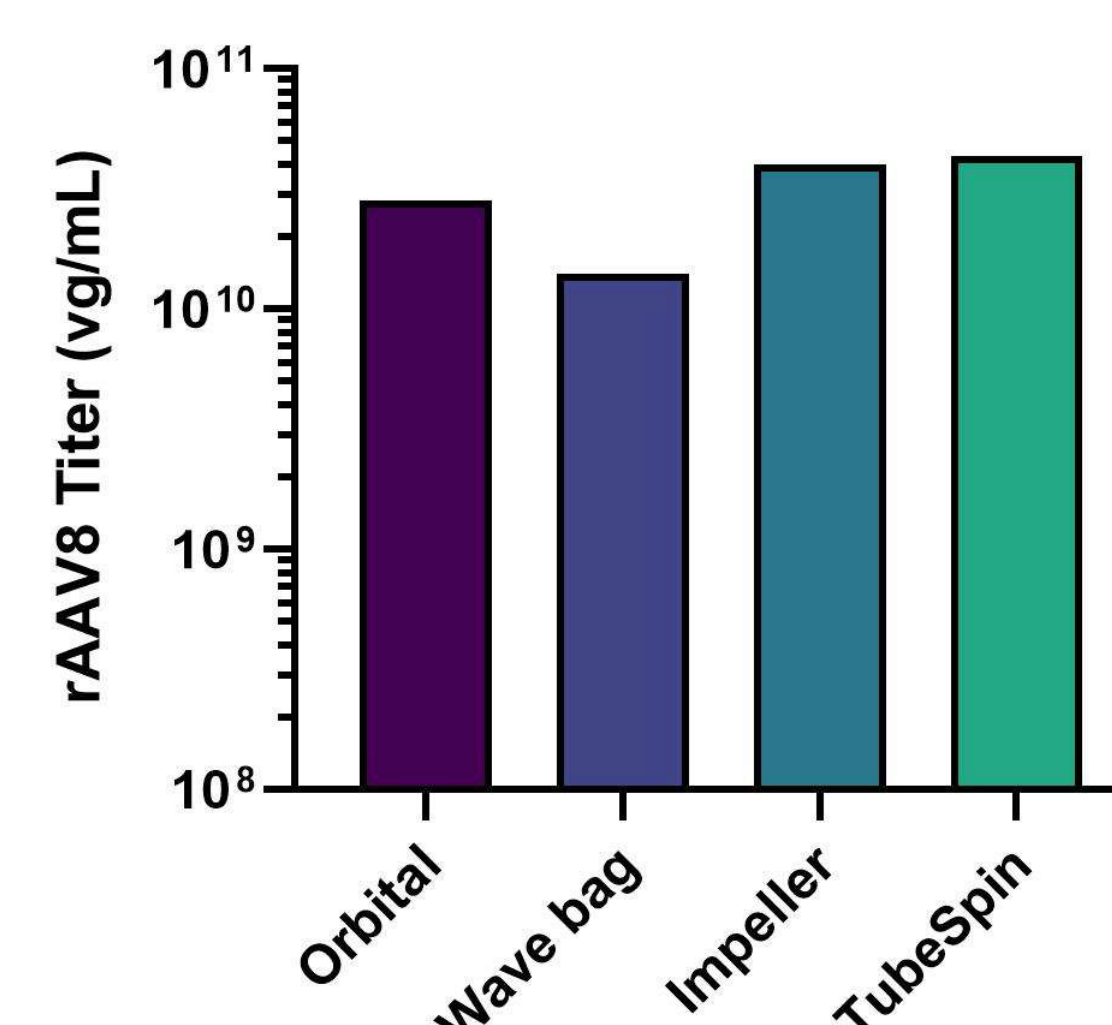
**Figure 3.** Consistent production of rAAV8 in Xcell™ Eng-HEK293 cultivated in various commercial media formulations. **A)** Crude rAAV8-GFP vector titers were determined by dPCR and expressed as the viral genome copy number per milliliter of cell culture and **B)** empty/full ratios of concentrated vectors produced in various media formulations assessed by mass photometry.

## Scalable rAAV production in Xcell™ Eng-HEK293



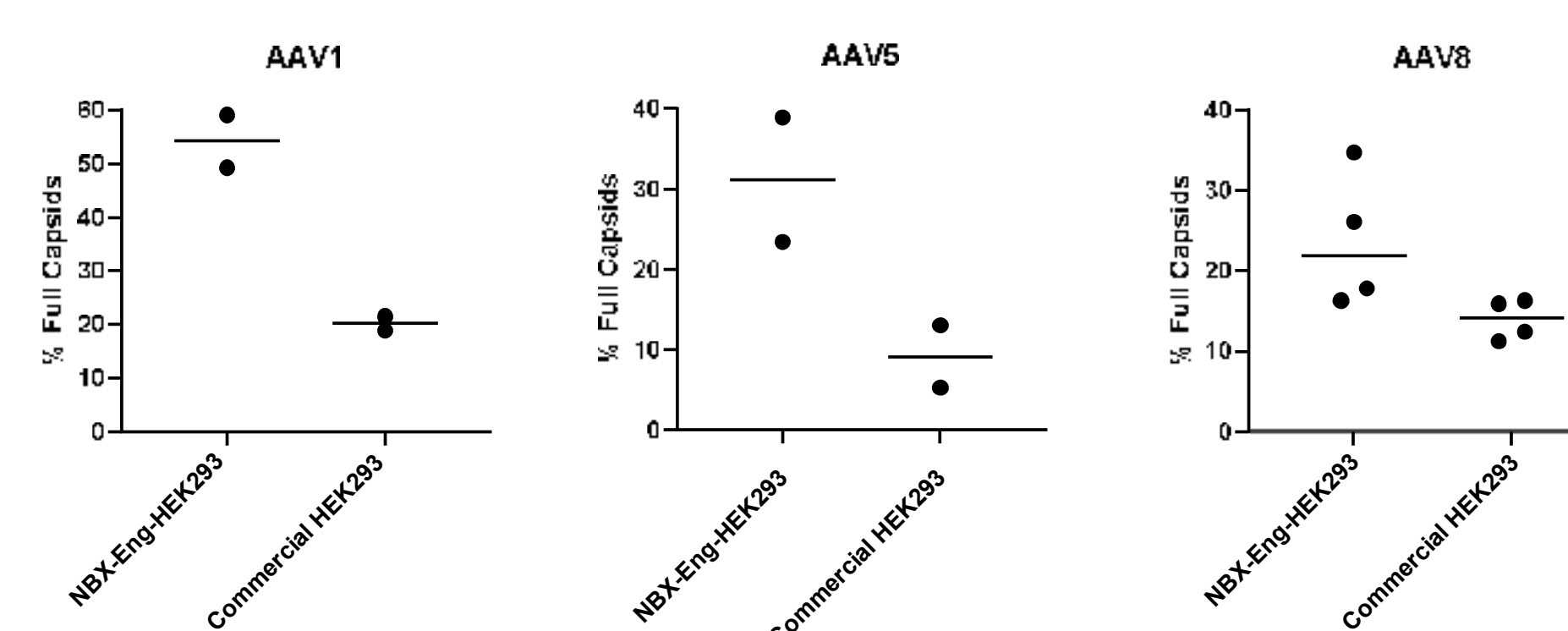
**Figure 4.** Consistent production of rAAV8-GFP production in Xcell™ Eng-HEK293 cells in various culture volumes. Cells were cultured in 24-well plates (0.75 mL), TubeSpins (5 and 10 mL) or shaker flasks (100, 300 and 1500 mL) using VPM (Gibco) and rAAV8-GFP was produced by the triple-plasmid transfection method. Vectors were harvested three days post-transfection and crude viral genome titers were quantified by dPCR.

## rAAV production in Xcell™ Eng-HEK293 is adaptable to various bioreactor formats



**Figure 5.** Consistent production of rAAV8-GFP production in Xcell™ Eng-HEK293 cells grown in Gibco's VPM using different bioreactor systems. Kuhner Orbital Shaker, Cytiva rocker/wave and Applikon stirred-tank systems were used to culture Eng-HEK293 cells in 1.5 L volumes and compared with 10 mL cultures. Transfections and vector productions were carried out in the same vessels. Vectors were harvested three days post-transfection and crude titers were quantified by dPCR.

## Xcell™ Eng-HEK293 consistently produce high amounts of full rAAV capsids



**Figure 6.** Xcell™ Eng-HEK293 consistently produced more full capsids compared to a leading commercial HEK293 cell line. Full capsids in affinity elutions were quantified by mass photometry.

## Conclusions

- NewBiologix has generated Xcell™ Eng-HEK293, a fully-characterized, clonal HEK293 cell line specifically for the production of viral vectors.
- We demonstrate that Xcell™ Eng-HEK293 is adaptable for growth in various commercial media formulations.
- rAAV production was evaluated in various media and bioreactor systems, demonstrating the flexibility of the cells.
- Xcell™ Eng-HEK293 consistently produced more full capsids compared with a leading commercial HEK293 cell line.
- Xcell™ Eng-HEK293 is available in both research-grade and GMP Master Cell Bank formats.

