Navigating AAV Manufacturing Complexity: A Comprehensive Comparison Of Commercial And Proprietary HEK293 Platforms For Optimized Vector Quality.

M Boscher, B L Carnio, J Wagner, T Fischer, B Hager, R Feiner, N Spada, A Youssef

Ascend Advanced Therapies GmbH, Process Development, Munich, Germany

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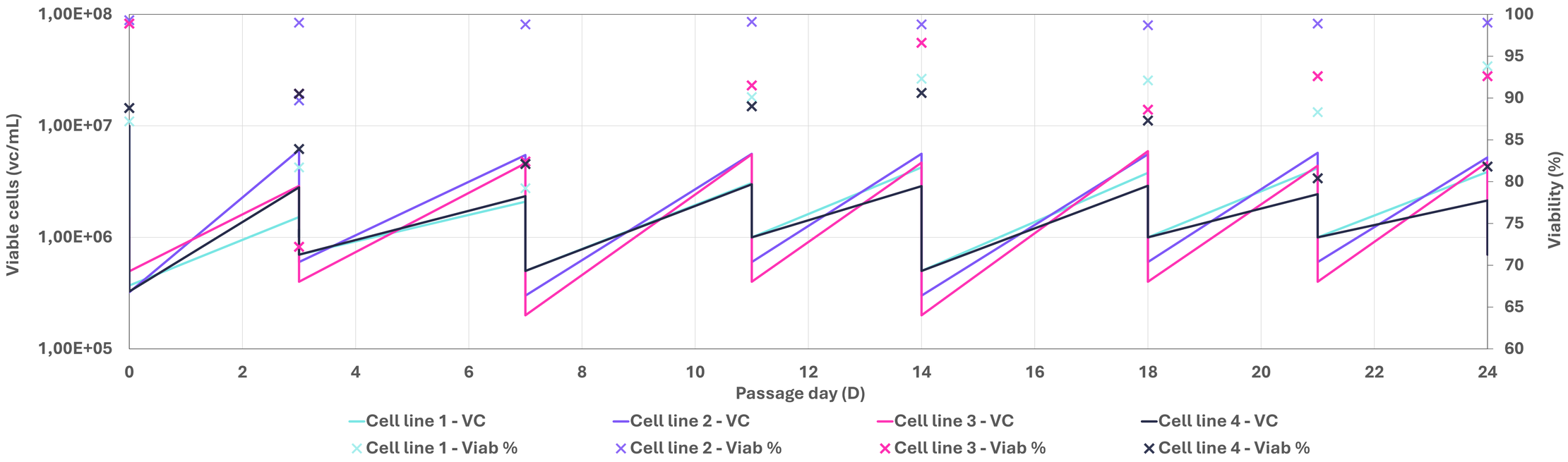
Introduction

Recombinant adeno-associated viruses (rAAVs) are leading vehicles for gene therapy, yet clinical viability hinges on manufacturing processes that balance efficient production with stringent quality standards. While mammalian HEK293 cells are the industry standard, the specific lineage significantly impacts cell kinetics, volumetric productivity, and critical quality attributes (CQAs) such as full/empty capsid ratios and DNA impurity levels.

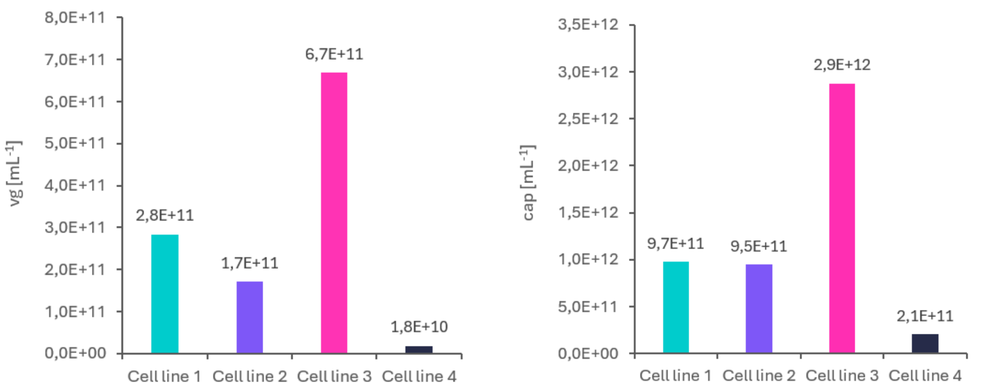
This study evaluated three commercially available suspension HEK293 lines against our proprietary in-house animal-derived component-free (ADCF) suspension-adapted HEK293 platform. All lines were cultured in shake flasks according to their supplier's recommendation, and AAV production was carried out in stirred-tank bioreactors at a fixed seeding density. Production utilized our proprietary EpyQ® two-plasmid system and PEI-mediated transfection to produce an AAV9 vector encoding a 3-kb reporter gene. We assessed growth kinetics, vector genome titers (ddPCR), total capsids (immunoassay), and impurities, including host-cell and plasmid-derived DNA (ddPCR and LR-NGS).

Cell growth kinetics



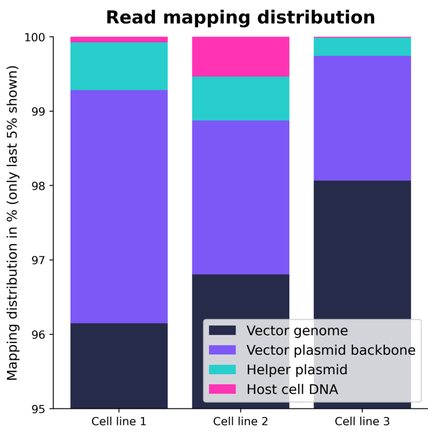
- Highest growth rate by cell line 3 followed by cell line 2
- Highest viability by cell line 2
- Comparable growth rate and viability by cell line 1 and cell line 4 with an advantage for cell line 1 in the later passages

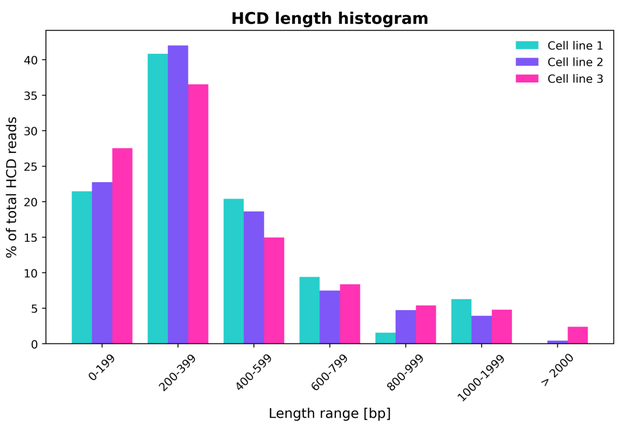
Volumetric productivity (vg and capsid titer)



- Highest volumetric productivity by cell line 3, followed by cell line 1 and cell line 2
- Lowest productivity for volumetric vg and cap titers for cell line 4

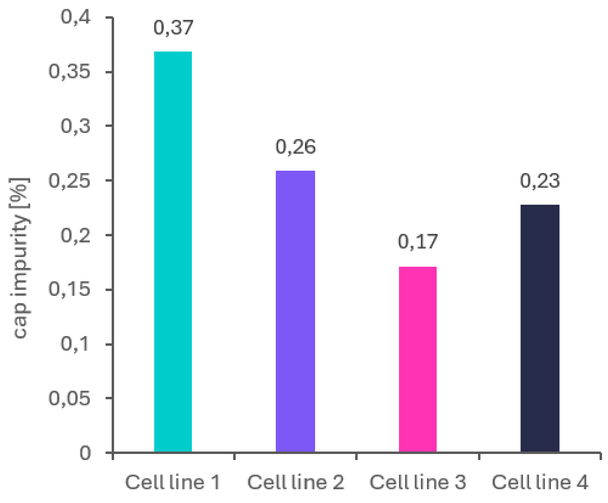
Nanopore sequencing

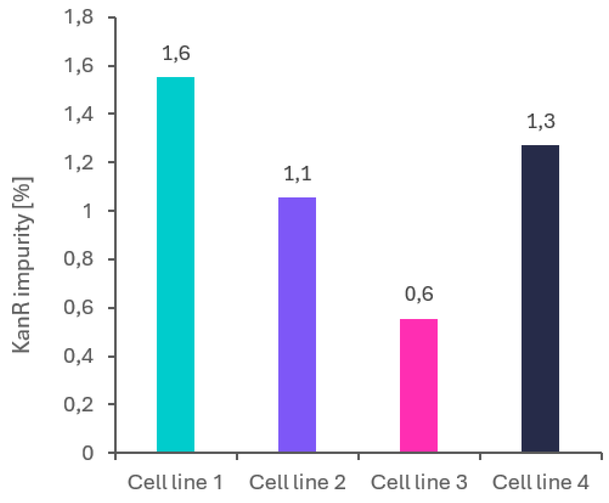


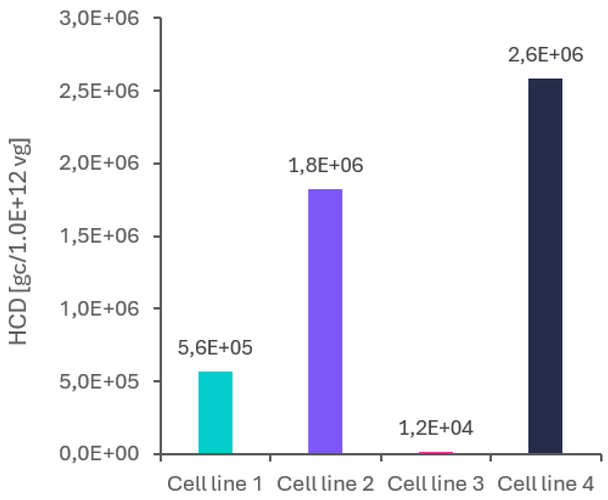


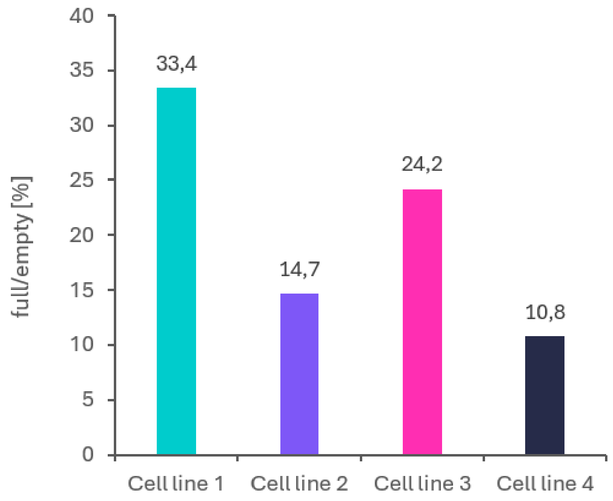
- > 95 % of all reads matched the expected vector genome, with the vector plasmid backbone as main impurity
- Lowest overall product-related impurity content for cell line 3
- Higher host cell DNA (HCD) impurities for cell line 2
- Most HCD reads were short, but slightly more long (> 2kb) HCD reads for cell line 3
- No NGS data available for cell line 4 because of low yield

Critical quality attributes (full/empty capsid ratio by Mass Photometry and DNA impurity levels by ddPCR)









- Best impurity profile by cell line 3
- Highest plasmid derived impurities by cell line 1 followed by cell line 2 for cap and kanR by cell line 4
- Highest host cell DNA impurity per vg by cell line 4, followed by cell line 2 and cell line 1
- Highest full/empty ratio by cell line 1, followed by cell line 3, cell line 2 and cell line 4

Summary

Comparative evaluation reveals that higher peak titers often present distinct product quality trade-offs, demonstrating that cell line selection must be tailored to each program's specific Quality Target Product Profile (QTPP). As a CDMO, leveraging our pre-characterized cell line portfolio within a Quality by Design (QbD) framework eliminates the need for extensive process development, saving critical project timelines and accelerating clinical translation while maintaining flexibility for lineage-specific fine-tuning.

www.ascend-adv.com
business@ascend-adv.com

Ascend Advanced Therapies, 9800 Medical Center Dr D, Rockville, MD 20850

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