

TESSA® Platform Optimization for AAV Manufacture Efficiency

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1) TESSA® Technology Overview

To overcome challenges in scalable and cost-effective production of recombinant adeno-associated virus (AAV) vectors, we developed the TESSA® platform — a self-silencing helper adenoviral vector system that inhibits the expression of adenoviral late structural proteins, providing a clean AAV manufacturing process without adenoviral contamination.

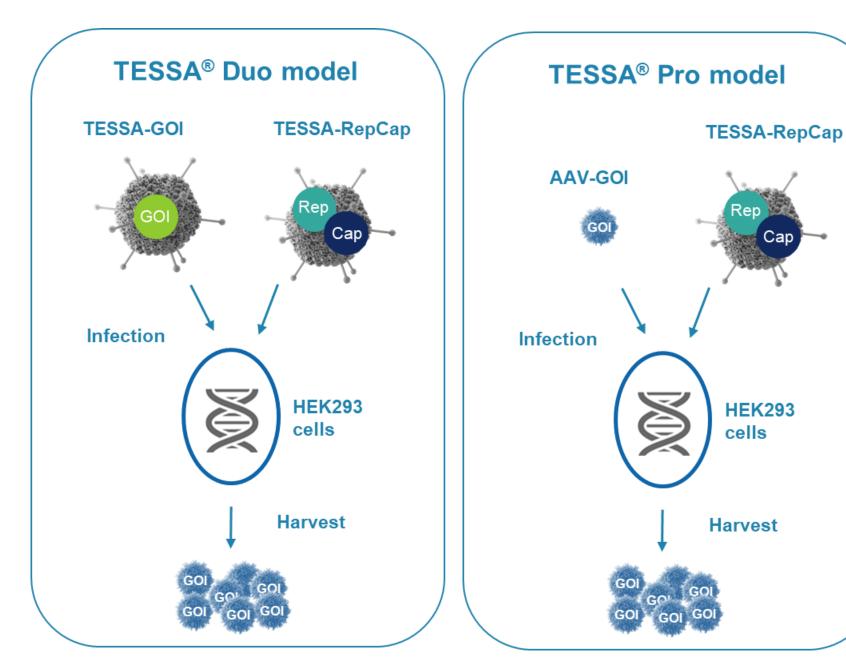
TESSA® platform supports efficient and scalable rAAV manufacturing with productivities exceeding 1 × 106 vector genome copies (GC) per cell and total outputs greater than 1 × 10¹⁷ GC from a 200-liter bioreactor.

How to produce AAV using **TESSA®** in **suspension HEK293 cells**:

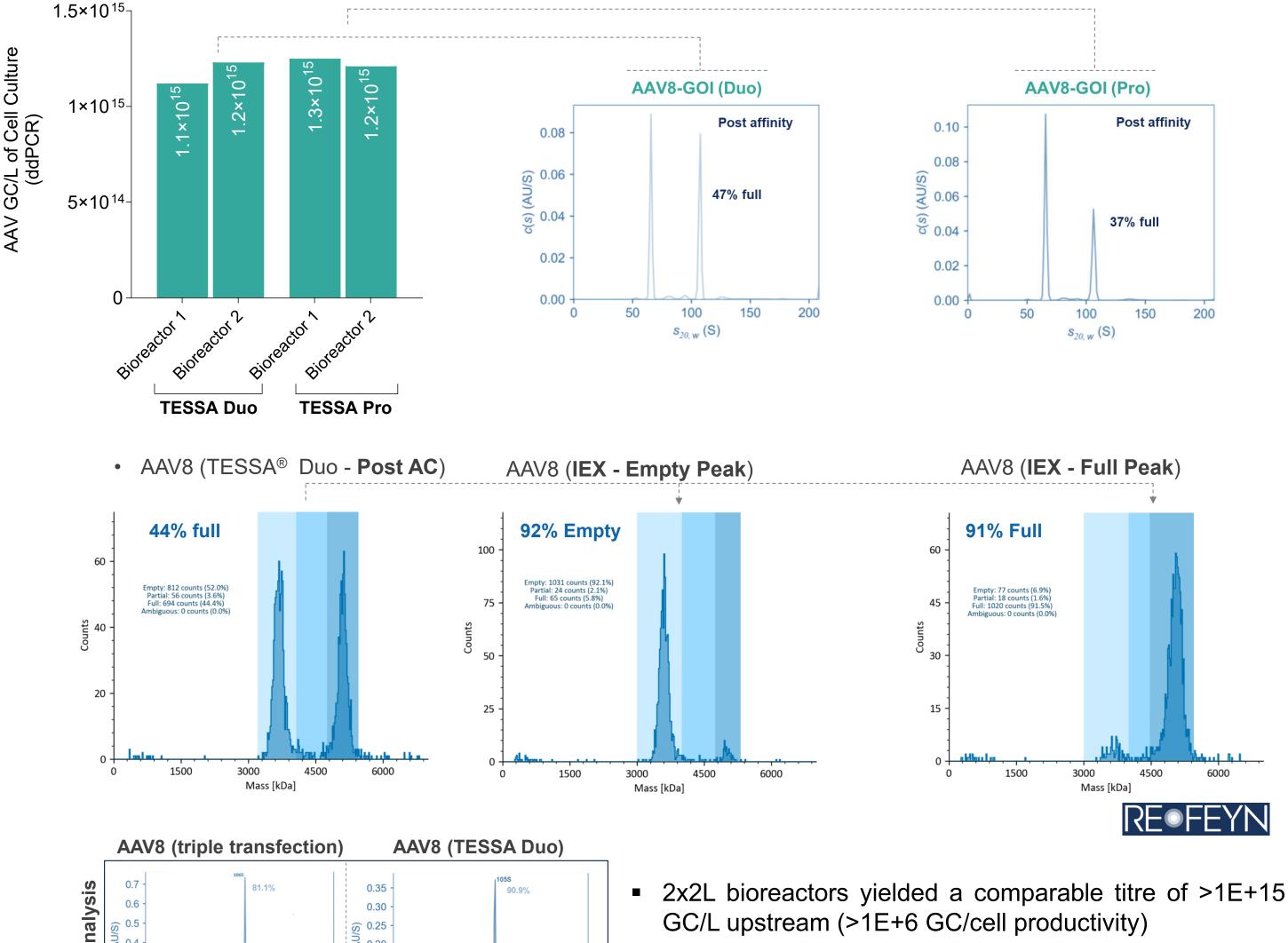
- TESSA® Duo: co-infection with a TESSA® RepCap vector and a TESSA® vector carrying the AAV GOI.
- TESSA® Pro: co-infection with a TESSA® RepCap vector and an AAV particle carrying the AAV GOI.

Here, we employed a rational approach to further optimize both AAV yields and product quality from the TESSA® platform by enhancing AAV Cap expression through promoter screening and increasing Rep in the packaging cells. We achieved vector genome titers exceeding 2–3 × 10¹² GC/mL of cell culture, representing approximately up to a 5fold increase over first-generation TESSA® RepCap vectors and more than a 30-fold improvement compared to the traditional triple-transfection method.

<u>Tetracycline-Enabled Self-Silencing</u> Adenovirus (TESSA®) Structural gene Major late expression becomes TetR regulated via a binding site feedback loop. +Dox -Dox Early gene expression genome amplification **TESSA** vectors



3) Production of AAV8-GOI (4.5kb) via TESSA® Duo & Pro



- TESSA® Duo AAV8 showed a significantly high proportion of full capsids (47% full via AUC) and minimal partials
- Analysis of TESSA® Duo AAV8 in-process materials via Mass Photometry (Refeyn, SamuxMP)
- AAV8 drug substance from TESSA® Duo showed higher quality compared to the triple transfection reference
 - ✓ 91% Full capsid after purification by AC & IEX
- ✓ Reduced packaging of truncated genomes / DNA contaminations via analysis by Nanopore NGS

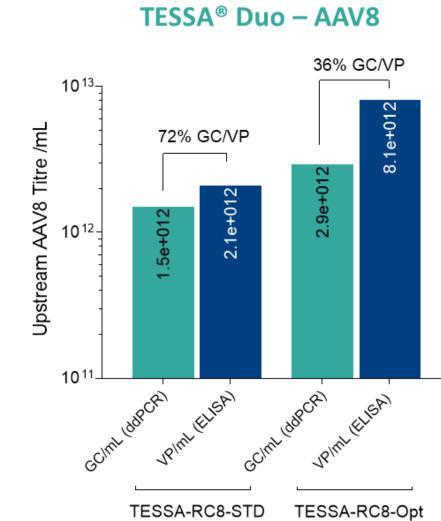
5) TESSA® Duo – Stepwise co-infection increases AAV production

sedimentation coefficient (S)

TESSA Duo (AC + IEX)

2000 4000 6000

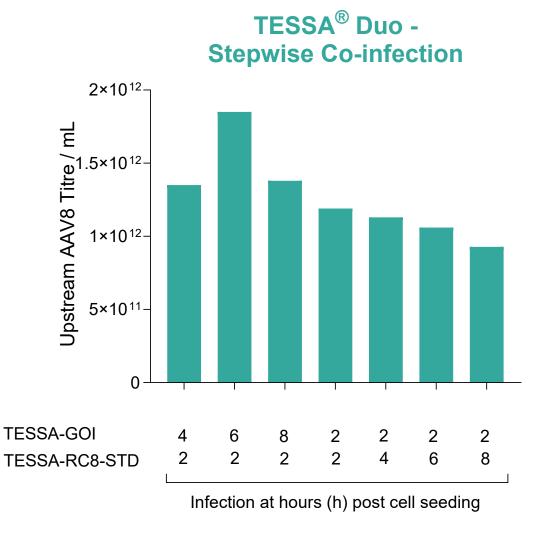
Nucleotide Length (bp)



sedimentation coefficient (S)

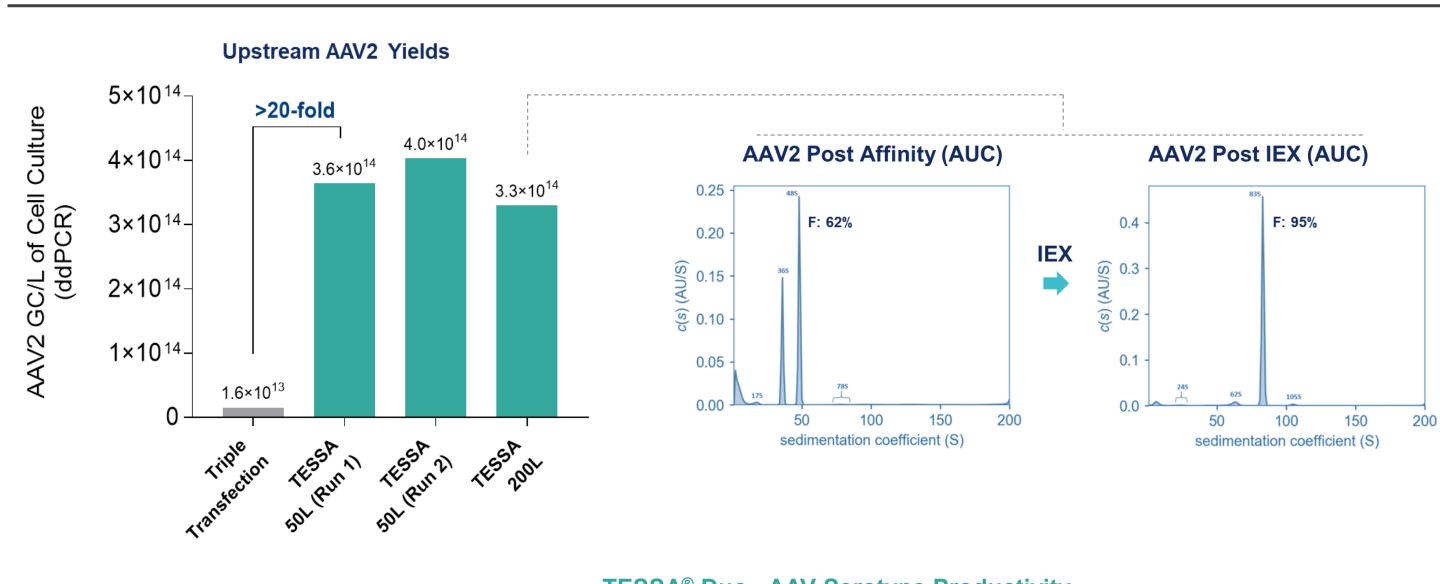
TTFX reference (AC + IEX)

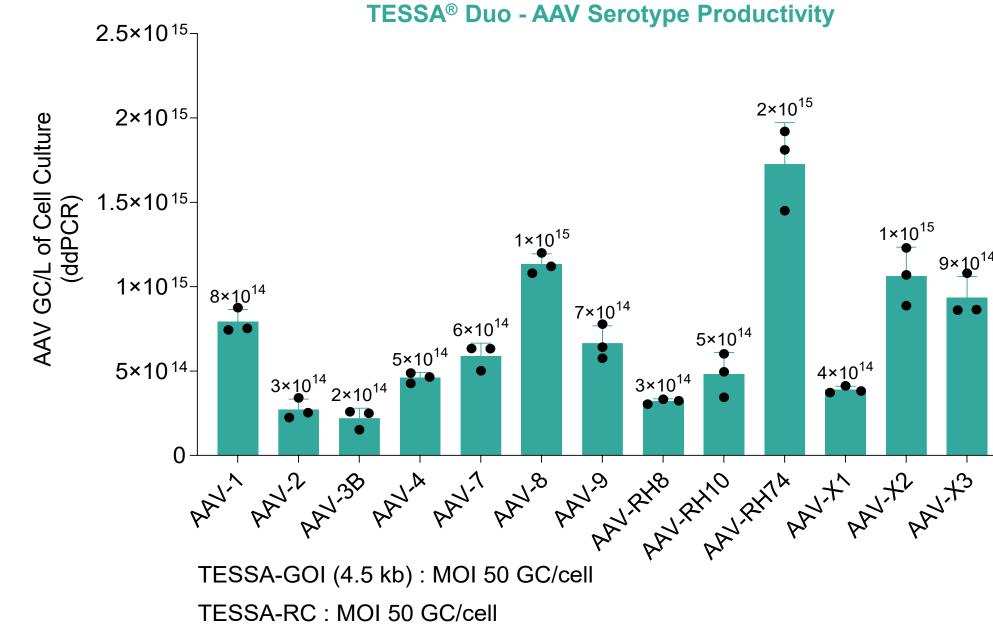
Nucleotide Length (bp)



- TESSA-RepCap8 (Opt Cap promoter) generated higher GC and VP titre ~2x increase in AAV8 production
- AAV productivity can be further increased via a stepwise/staggered infection

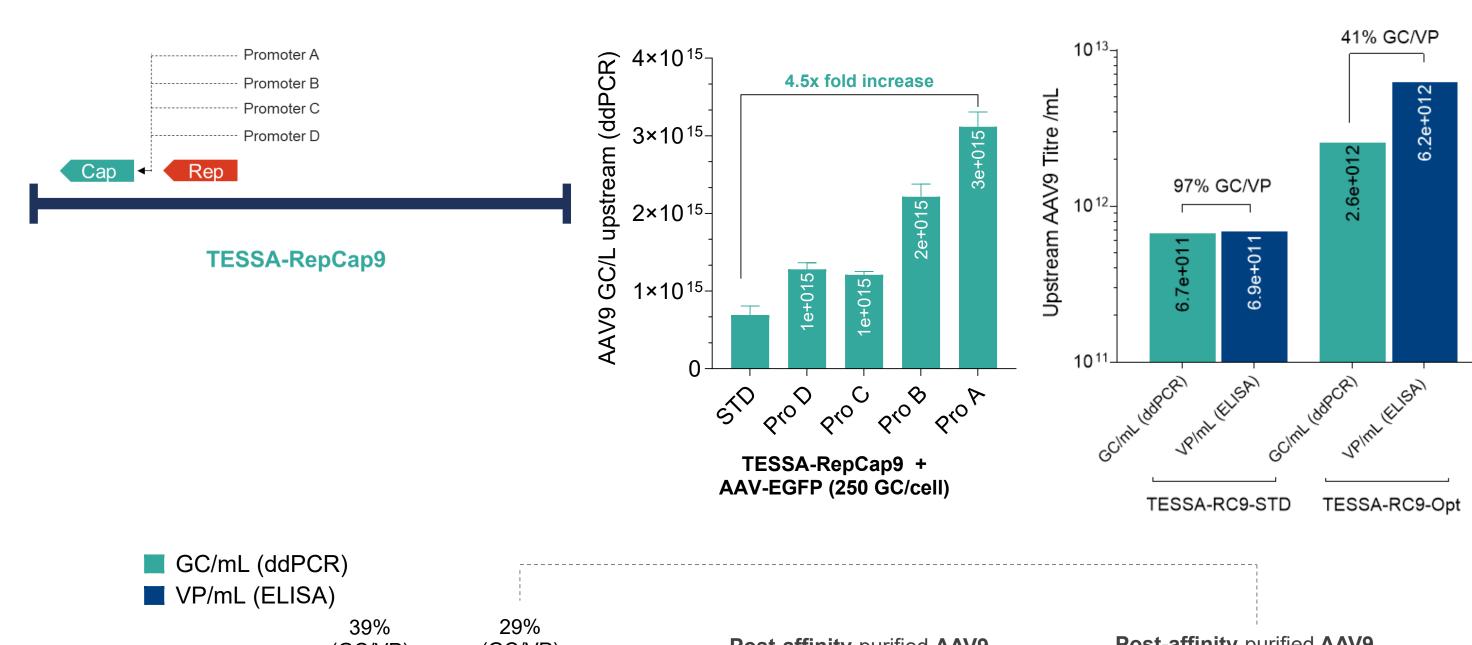
2) TESSA® Duo Platform - Upscale AAV Manufacturing

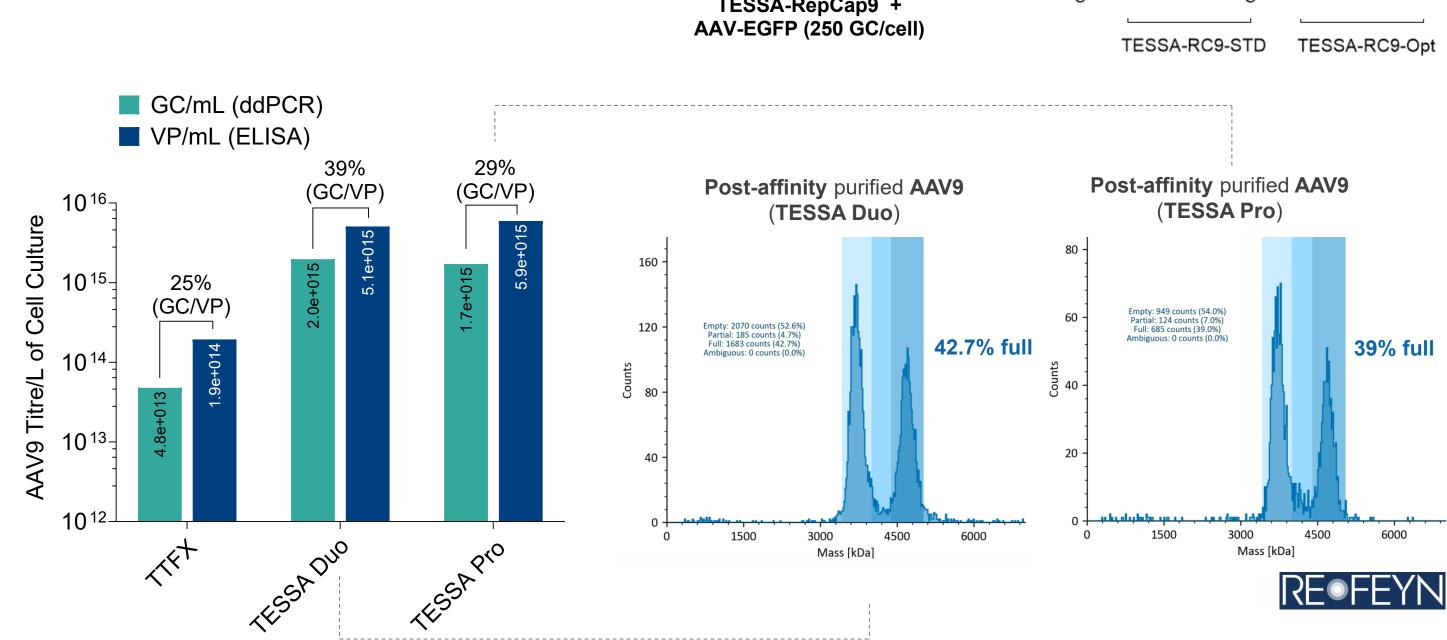




- 20x-fold increase in AAV2 yields compared to triple-transfection. >60% full before IEX & 95% full after polishing
- High AAV productivity across all AAV natural and engineered (AAVX) capsids tested in suspension HEK293 cells
- Easy to scale to 200L and beyond. Qualified release tests developed to complement the TESSA® platform

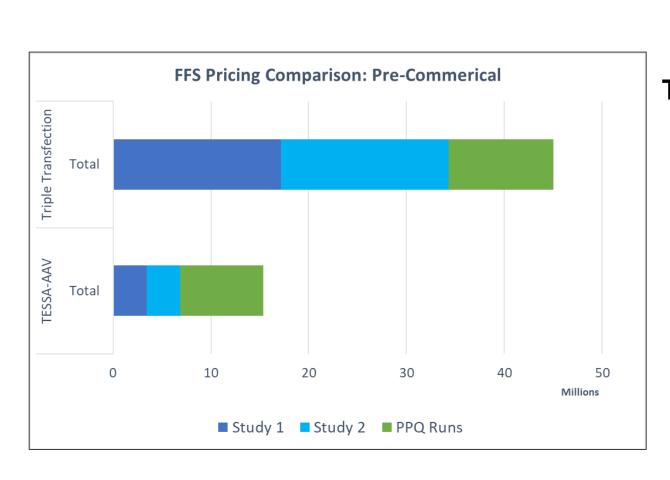
4) Optimization of TESSA® RepCap Vectors





- Promoter evaluation to increase Cap expression and AAV yields promoter A yielded a 4.5-fold increase in AAV9 compared to the previous configuration (STD). Significantly high productivity of >1.5E+6 GC per cell
- TESSA-RepCap9 (Opt Cap promoter) generates higher GC and VP titre. ~40% full capsids after affinity chromatography via Mass Photometry (Refeyn, SamuxMP)
- Reduce VP/VG ratio, compared to TESSA-RepCap (STD) 97% after affinity purification, but significantly higher than triple transfection

6) Conclusion



TESSA® is a next-generation AAV manufacturing platform

■ **High yielding:** up to 30x, and more, compared to transfection-based approaches

TESSA® Duo – AAV9

- Contamination-free: AAV is free of adenoviral vector and
- small-molecule contaminants
- Completely scalable: 1x 200L batch of TESSA® is able to support >80x 2000L AAV Mfg runs
- Significant COGs reduction by >85% at commercial scale
- Su, W., et al., Nat Comms. 2022. Hartweger, H., et al, J Vis Exp. 2023.
- Su, W. et al., Sci Rep. 2023. Roach, M.K., et al., Mol. Ther. Methods Clin. Dev. 2024.