

# TESSA® Platform Optimization for AAV Manufacture Efficiency

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## 1) TESSA® Technology Overview

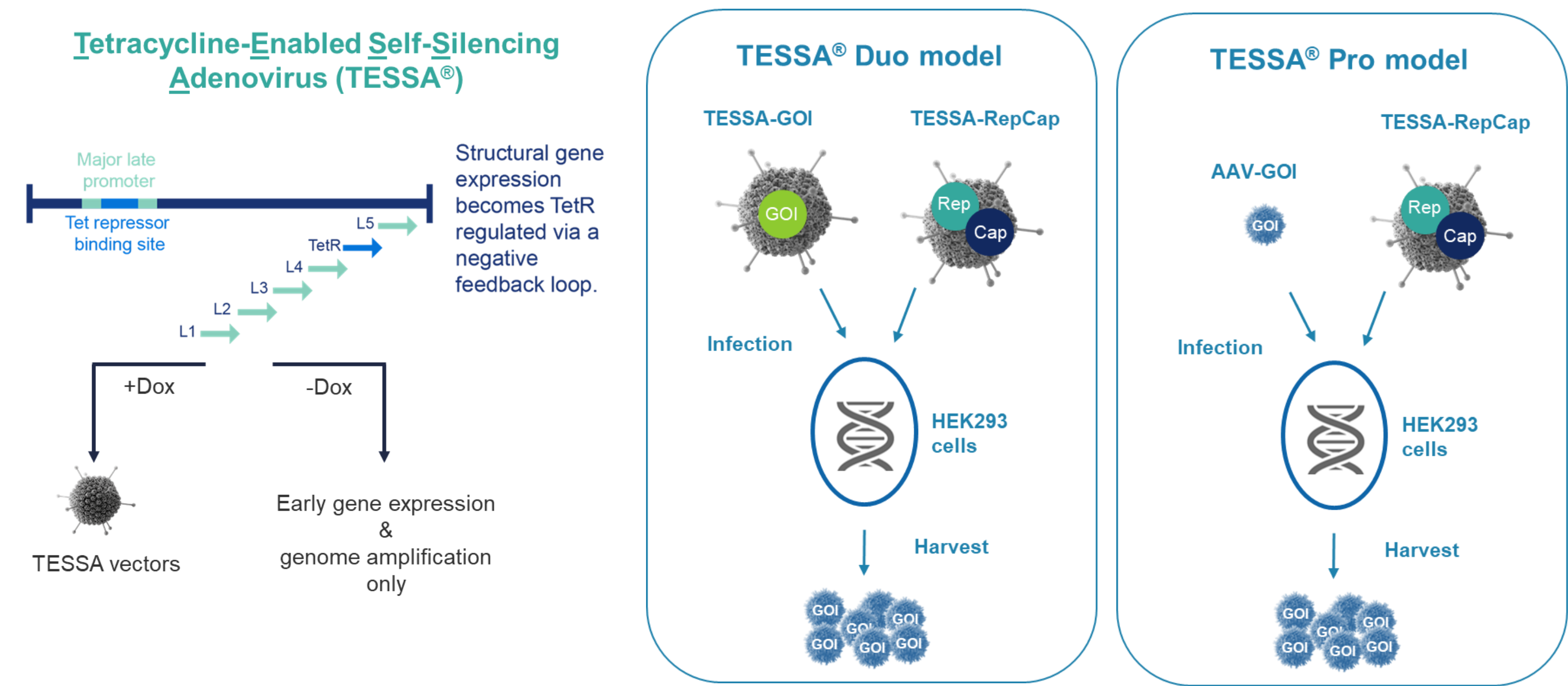
To overcome challenges in scalable and cost-effective production of recombinant adeno-associated virus (AAV) vectors, we developed the TESSA® platform — a self-silencing helper adenoviral vector system that inhibits the expression of adenoviral late structural proteins, providing a clean AAV manufacturing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

TESSA® platform supports efficient and scalable rAAV manufacturing with productivities exceeding  $1 \times 10^6$  vector genome copies (GC) per cell and total outputs greater than  $1 \times 10^{17}$  GC from a 200-liter bioreactor.

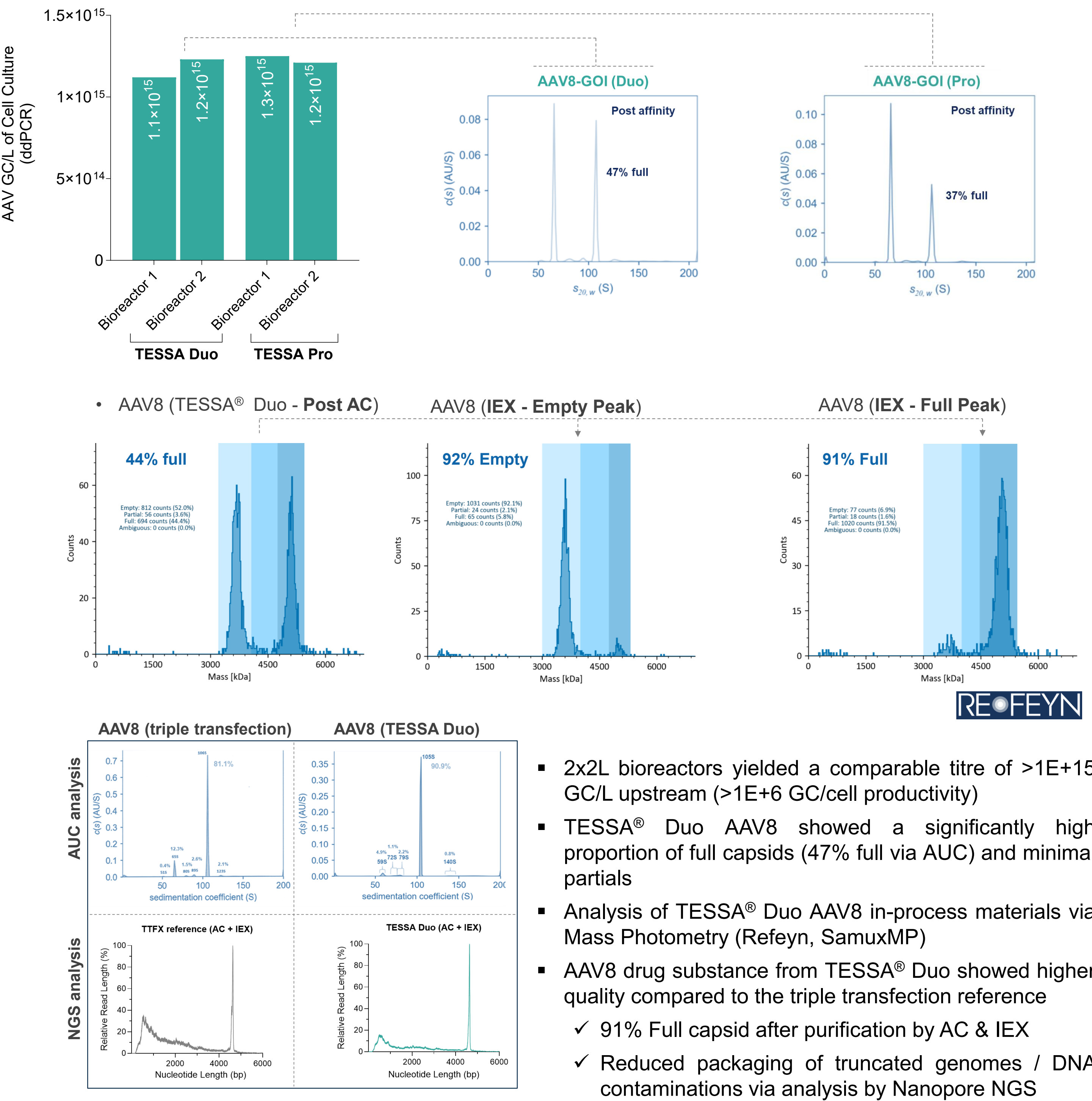
How to produce AAV using TESSA® in suspension HEK293 cells:

- **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.

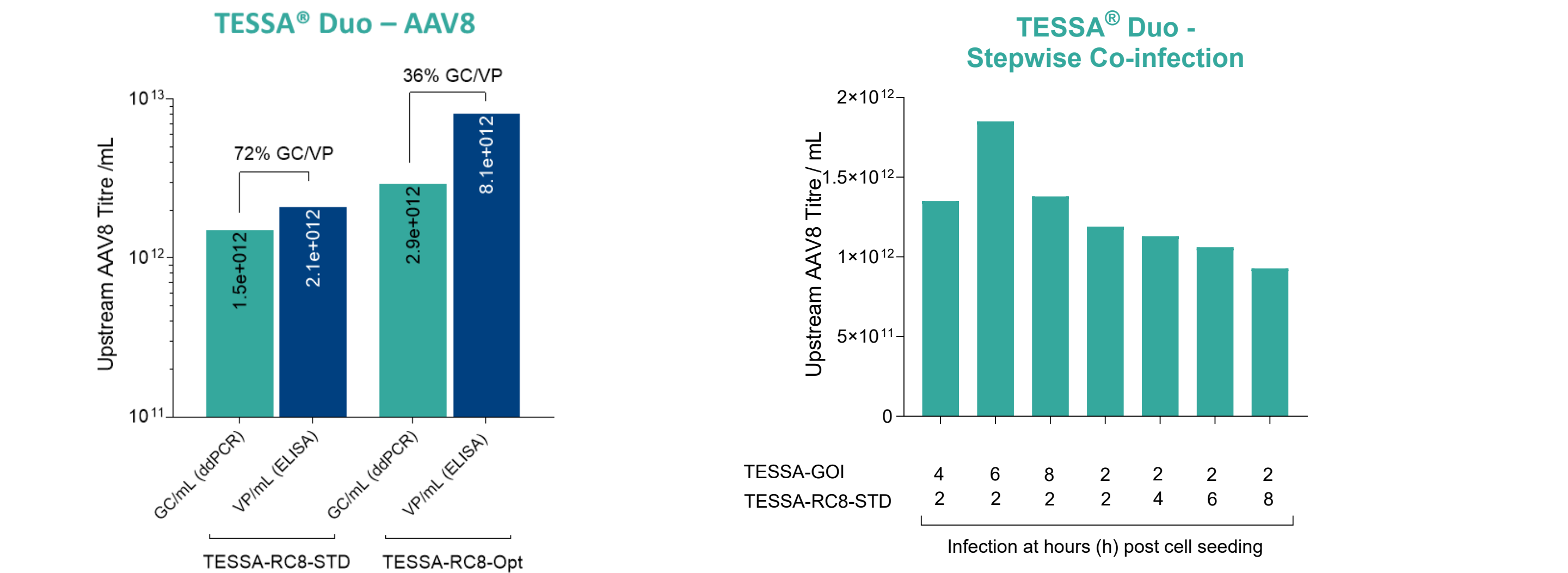
Here, we employed a rational approach to further optimize both AAV yields and product quality from the TESSA® platform by enhancing AAV Cap expression through promoter screening and increasing Rep in the packaging cells. We achieved vector genome titers exceeding  $2\text{--}3 \times 10^{12}$  GC/mL of cell culture, representing approximately up to a 5-fold increase over first-generation TESSA® RepCap vectors and more than a 30-fold improvement compared to the traditional triple-transfection method.



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

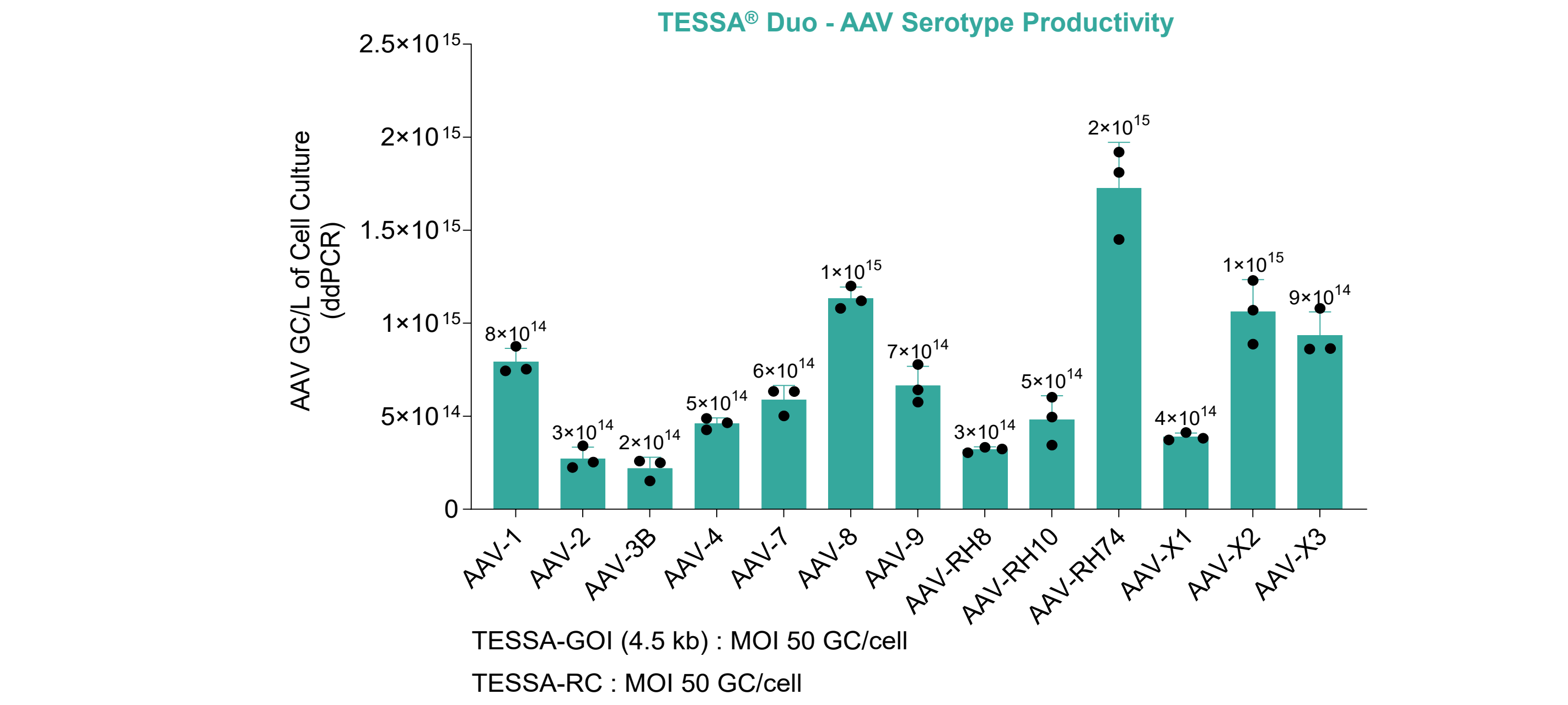
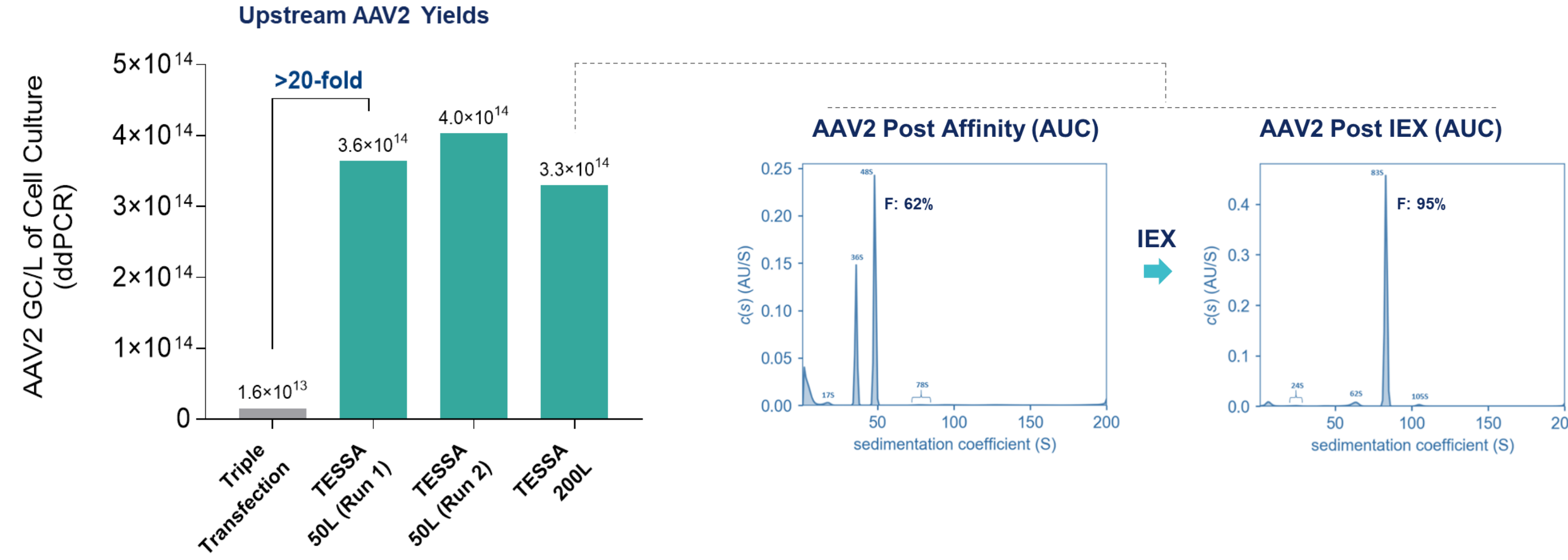


## 5) TESSA® Duo – Stepwise co-infection increases AAV production



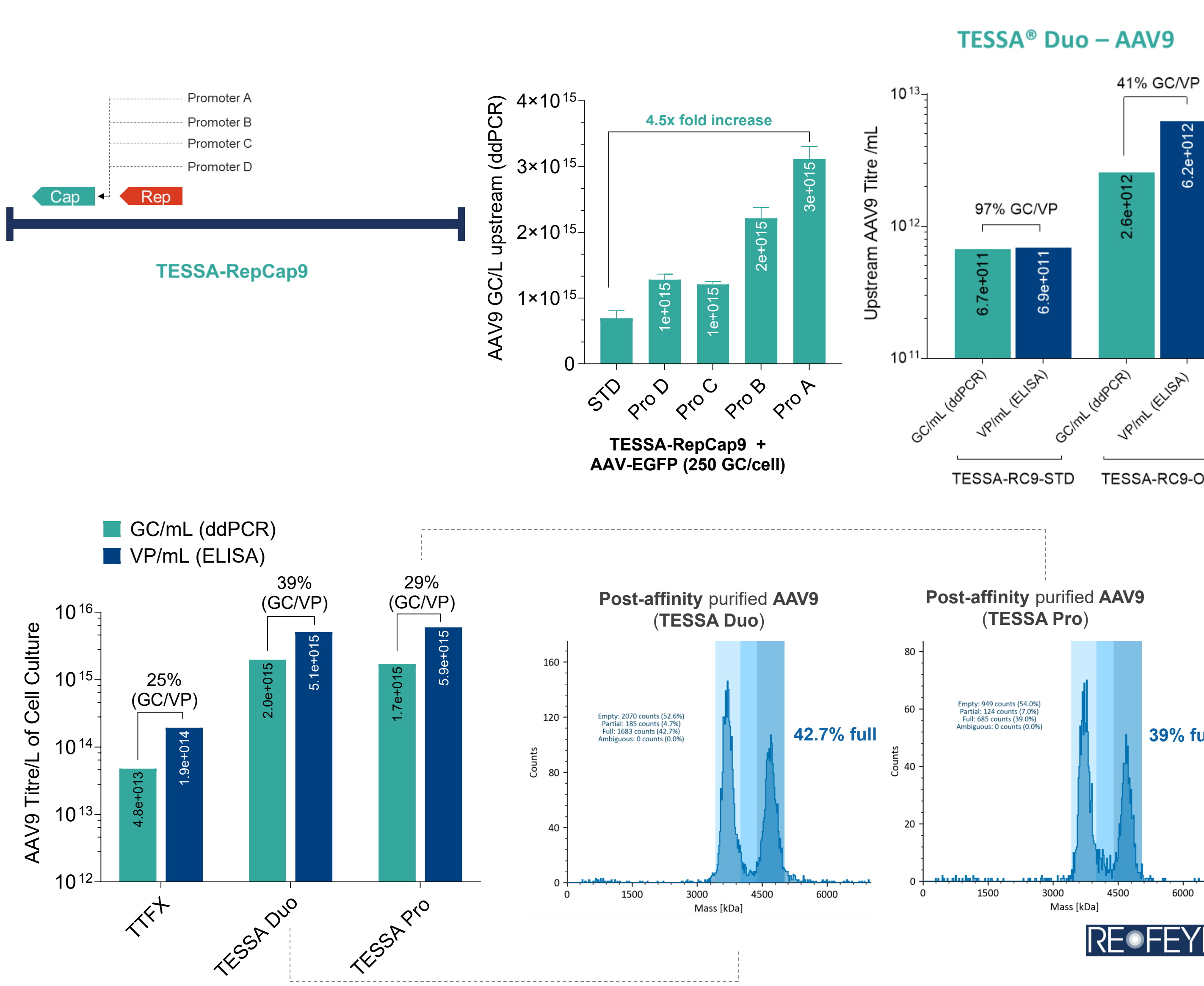
- TESSA-RepCap8 (Opt Cap promoter) generated higher GC and VP titre -  $\sim 2\text{x}$  increase in AAV8 production
- AAV productivity can be further increased via a stepwise/staggered infection

## 2) TESSA® Duo Platform - Upscale AAV Manufacturing



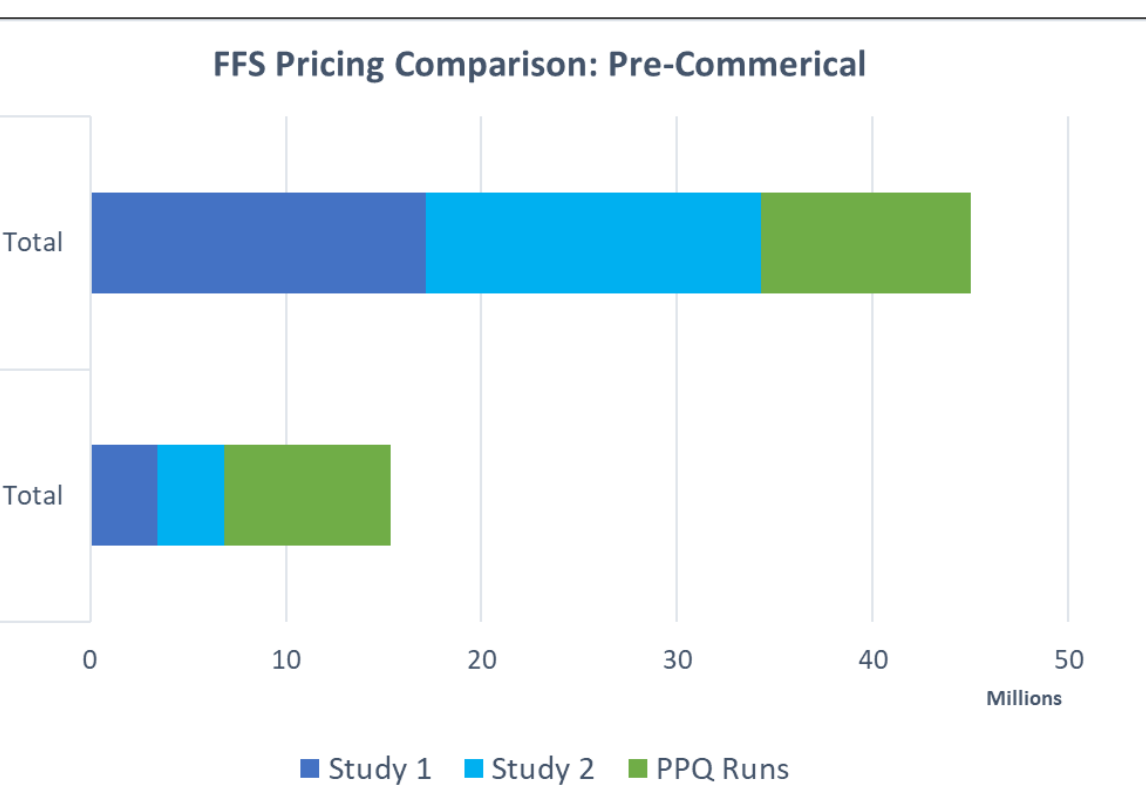
- 20x-fold increase in AAV2 yields compared to triple-transfection.  $>60\%$  full before IEX &  $95\%$  full after polishing
- High AAV productivity across all AAV natural and engineered (AAVX) capsids tested in suspension HEK293 cells
- Easy to scale to 200L and beyond. Qualified release tests developed 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  $>1.5\text{E}+6$  GC per cell
- TESSA-RepCap9 (Opt Cap promoter) generates higher GC and VP titre.  $\sim 40\%$  full capsids after affinity chromatography via Mass Photometry (Refeyn, SamuxMP)
- Reduce VP/VC 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
- **Contamination-free:** AAV is free of adenoviral vector and small-molecule contaminants
- **Completely scalable:** 1x 200L batch of TESSA® is able to support  $>80\text{x}$  2000L AAV Mfg runs
- **Significant COGS reduction** by  $>85\%$  at commercial scale