

# Scalable TESSA<sup>®</sup> vector manufacture to support AAV gene therapy

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## 1) Introduction

The scalable and cost-effective production of AAV vectors for gene therapies remains a challenge. We have developed TESSA<sup>®</sup>, a novel self-silencing helper adenoviral vector system designed for high-yield, contaminant-free AAV manufacturing in serum-free suspension HEK293 cells.

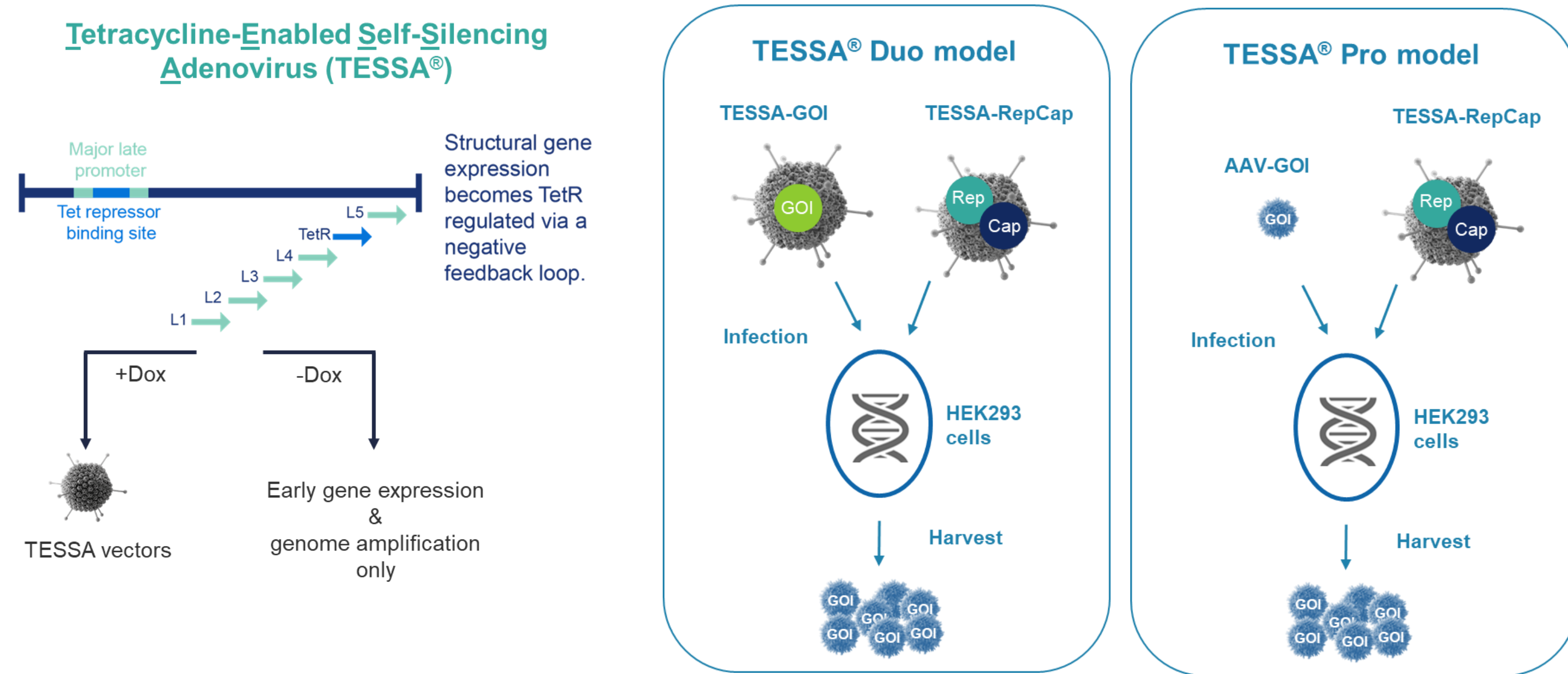
TESSA<sup>®</sup> is an E1/E3-deleted adenoviral vector with a modified Major Late Promoter (MLP) containing tetracycline repressor (TetR) binding sites and encoding the TetR gene within its late region. This configuration establishes a negative feedback loop, suppressing adenoviral structural protein expression in the absence of doxycycline. Consequently, adenoviral particle formation is inhibited during AAV production, minimizing contamination risks and improving safety.

To produce AAV with TESSA<sup>®</sup>, researchers can use one of two methods:

- **TESSA<sup>®</sup> Duo** – A TESSA<sup>®</sup>-RepCap combined with a TESSA<sup>®</sup> containing the AAV-GOI transfer genome (TESSA<sup>®</sup>-AAV-GOI) for co-infecting HEK293 cells
- **TESSA<sup>®</sup> Pro** – A TESSA<sup>®</sup>-RepCap combined with an AAV-GOI particle for co-infecting HEK293 cells

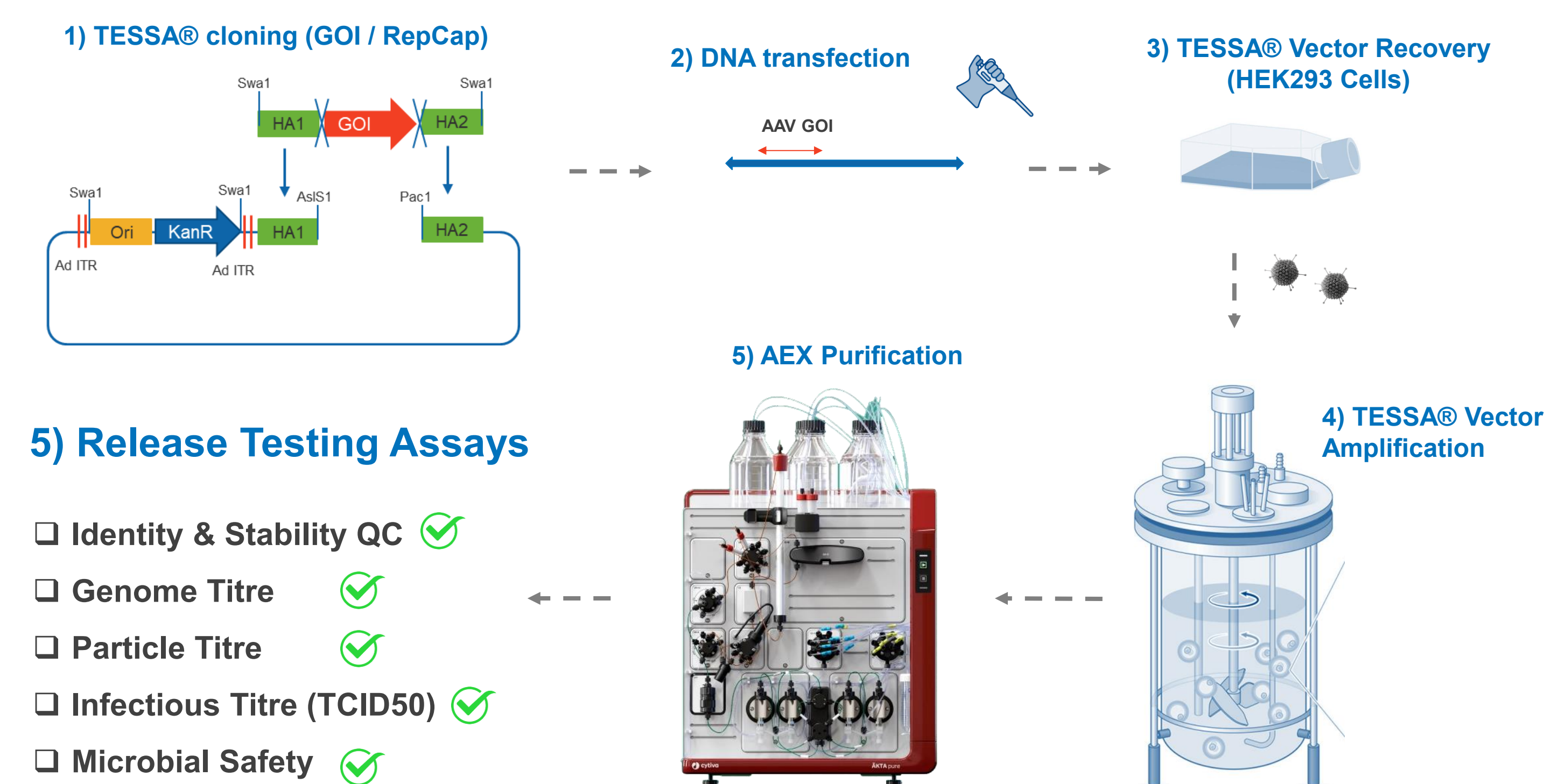
The TESSA<sup>®</sup> platform supports efficient and scalable rAAV manufacturing with productivities exceeding 1 × 10<sup>6</sup> vector genome copies (GC) per cell and total outputs greater than 1 × 10<sup>17</sup> GC from a 200-liter bioreactor.

TESSA<sup>®</sup> vectors are efficiently produced in HEK293 cells, purified by ion-exchange chromatography, and coupled with extensive release testing that includes genome and infection titration, stability, and purity. A **single 200L** batch of TESSA<sup>®</sup> is able to support **>80x 2000L AAV Mfg runs**



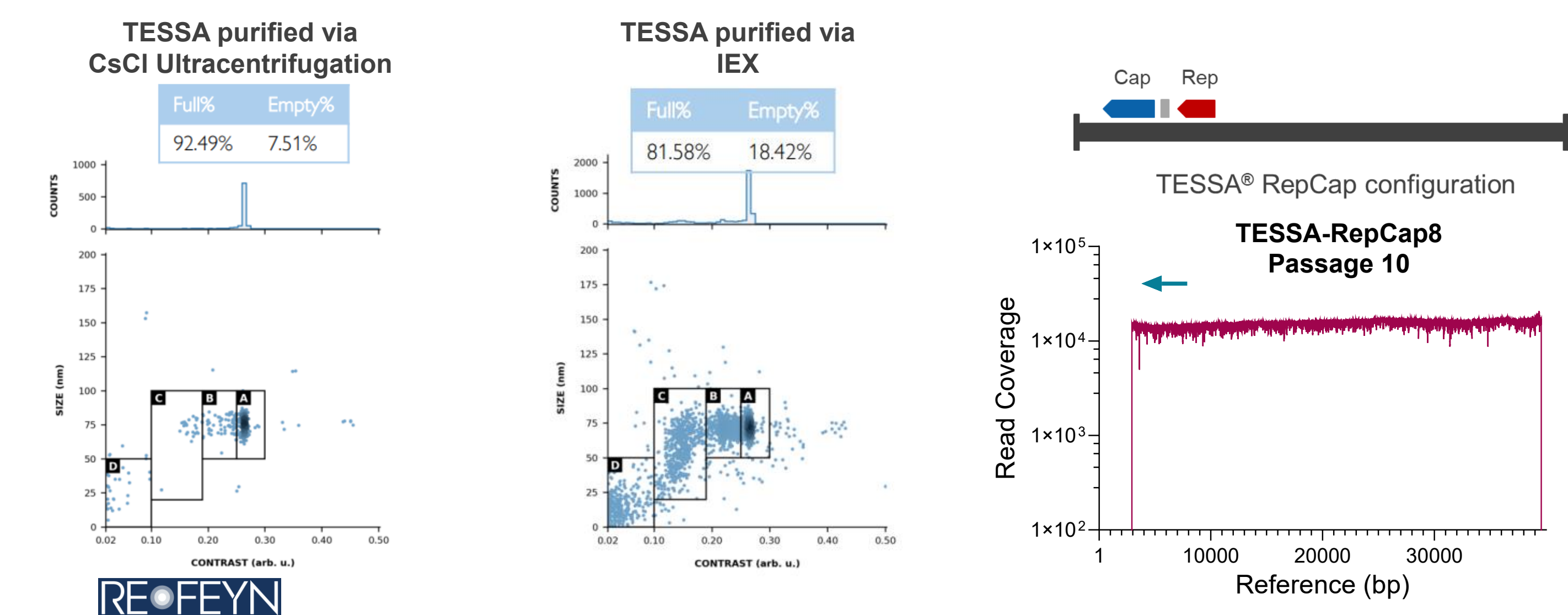
## 3) Scalable platform for the manufacture of TESSA<sup>®</sup> vectors

- One-step cloning, via Gibson Assembly, is required for the generation of TESSA<sup>®</sup> RepCap and TESSA<sup>®</sup> GOI AdV
- TESSA<sup>®</sup> vectors are manufactured in GMP-compliant serum-free suspension HEK293 cells and purified by anion-exchange chromatography (AEX)
- Release testing for TESSA<sup>®</sup> vectors includes assessment of genome vector, particle, and infectious titre, and genetic and particle stability



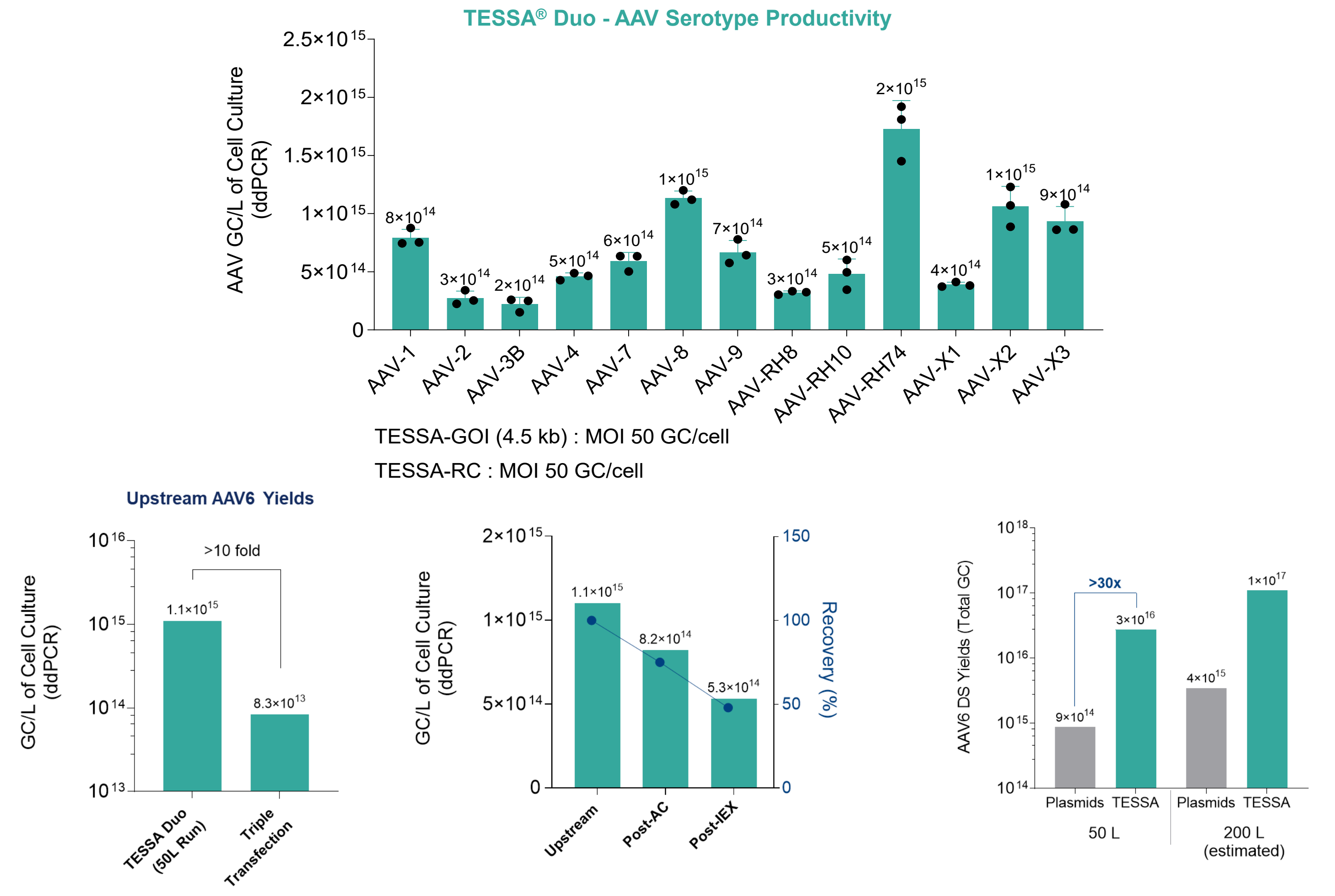
## 5) TESSA<sup>®</sup> characterization and genome stability

- Analysis of TESSA<sup>®</sup> vectors via Macro Mass Photometry (KartroMP) in collaboration with Refeyn, UK
- High ~80% full particles from IEX platform process, comparable to CsCl ultracentrifugation
- TESSA<sup>®</sup> vectors are sequentially passaged, purified, and analysed via Nanopore NGS to ensure viral genome stability



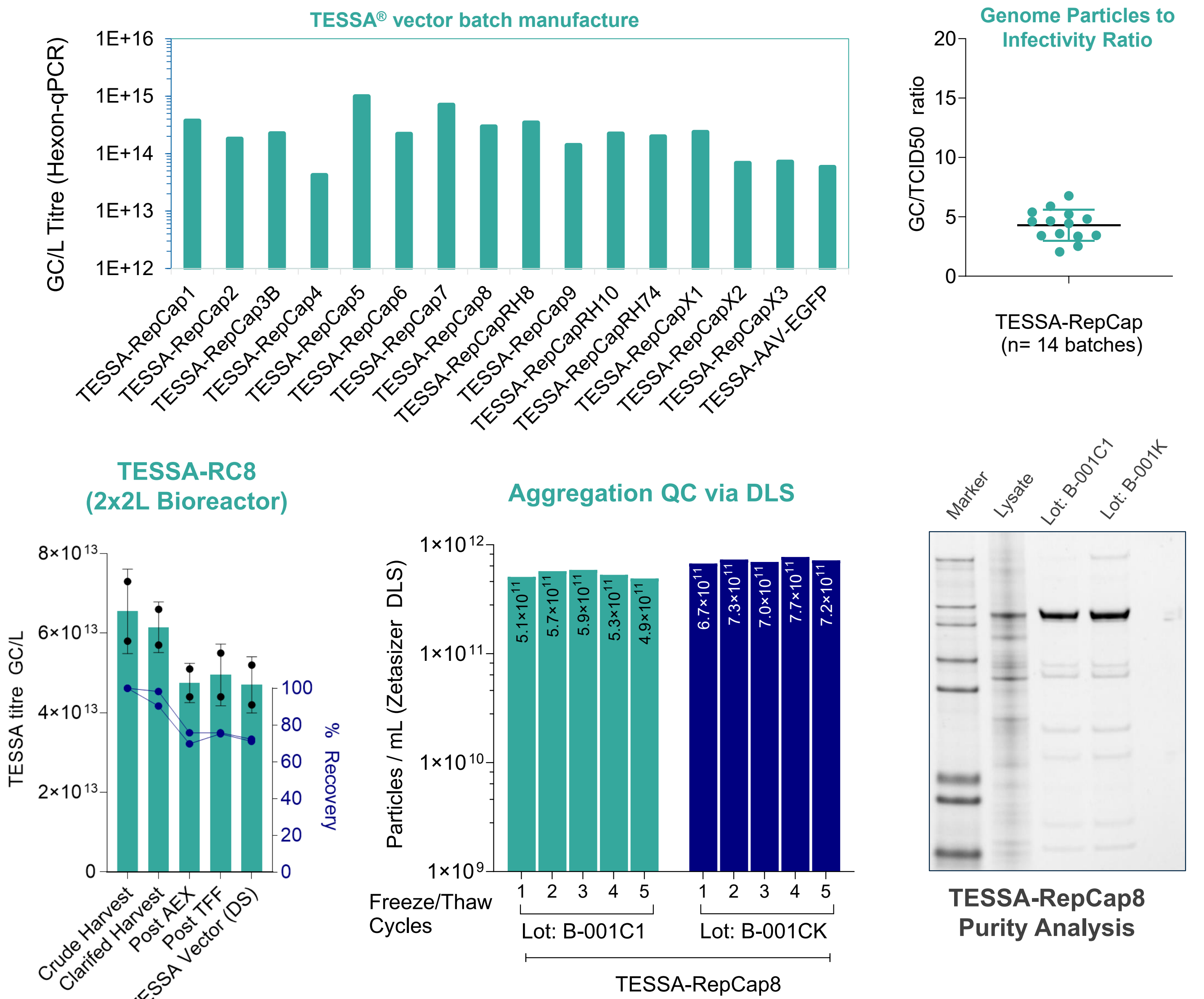
## 2) TESSA<sup>®</sup> improves AAV productivity for multiple serotypes

- High AAV productivity across all AAV natural and engineered (AAVX) capsids tested in suspension HEK293 cells
- Easy to scale to 200L and beyond - high proportion of 'full' capsids and very low levels of 'Partials'
- High AAV recovery (%) from AC and IEX purification processes

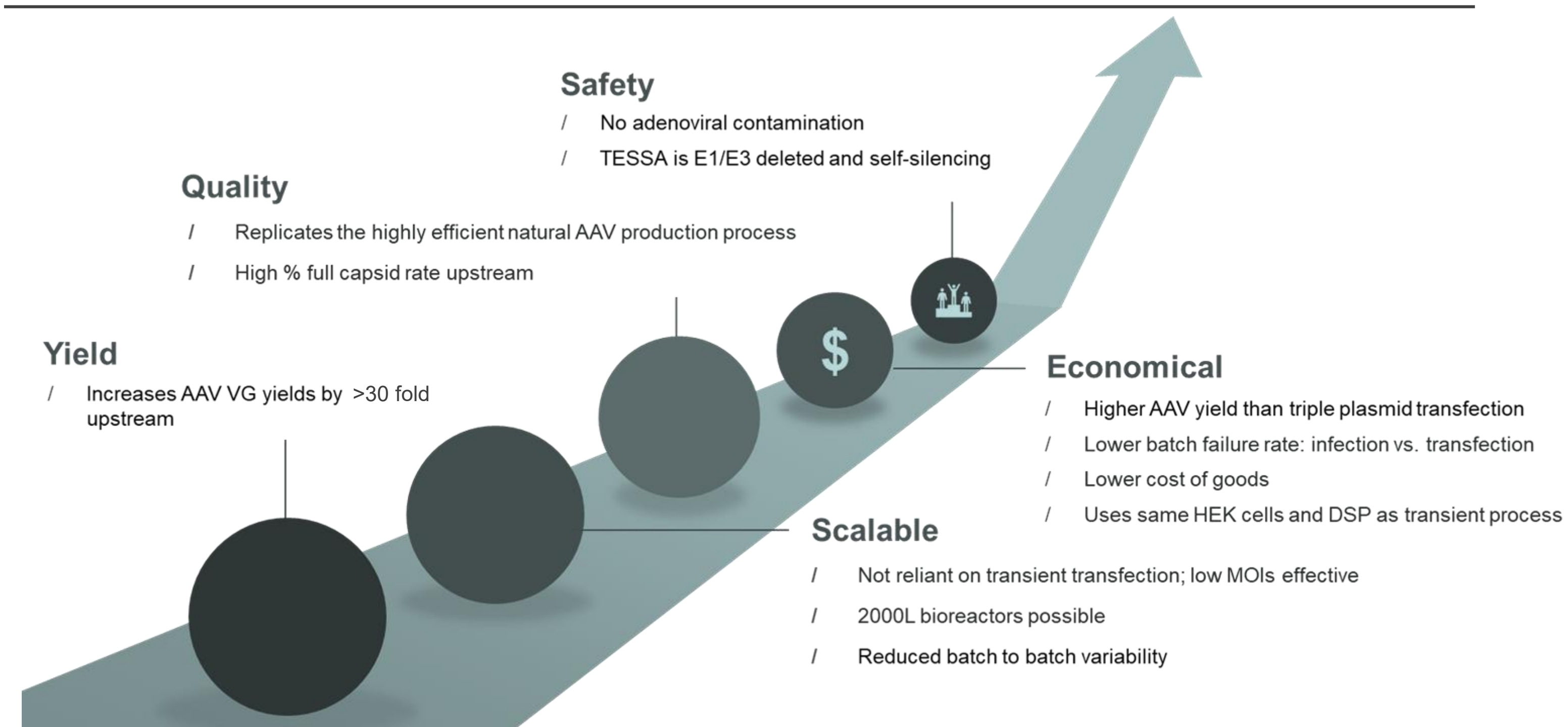


## 4) TESSA<sup>®</sup> Batch Manufacture & Quality control

- TESSA<sup>®</sup> RepCap shows variable productivity (capsid-dependent) but yields high-titre 4E+13 - 1E+15 GC/L
- TESSA<sup>®</sup> vectors exhibit a high genome particle (via qPCR) to infectivity ratio (via TCID50) of ~5
- QC release testing of TESSA<sup>®</sup> RepCap is accompanied by particle stability by Zetasizer DLS and purity
- Highly efficient platform process for purification of TESSA<sup>®</sup> vectors (>60% recovery)
- 1L production batch of TESSA<sup>®</sup> RepCap (1E+13 GC) is sufficient for manufacture of AAV at >150L scale



## 6) Conclusion



Enquire about our TESSA<sup>®</sup> Platform at [www.minaris.com](http://www.minaris.com)