Alternative Plasmid Designs Including Two Plasmid Transfection Systems for Improved Production and Packaging of Engineered AAV Capsids (Abstract 530)

Lysa-Anne Volpe, Jenna Rodden, Heidy Morales, Garrett Garrido, Hari Acharya | Capsida Biotherapeutics, Thousand Oaks, CA

Introduction and Objectives

Recombinant adeno-associated virus (rAAV) mediated gene therapy has progressed significantly over the past few decades proving clinical efficacy for treating rare diseases. Despite such progress, numerous challenges have limited its broader use, including inefficient crossing of the blood–brain barrier, suboptimal tissue specificity and cost-effective scalability to meet the material and quality demands for commercial manufacturing.[1,2] To address these issues, Capsida Biotherapeutics, leveraging its fully integrated, end-to-end gene therapy solutions from research to in-house manufacturing, is engineering novel AAV capsids with improved in vivo gene delivery and reduced toxicity. These engineered capsids create opportunities to treat rare and common diseases across all age groups.

Building on Capsida's gene therapy platform, we sought to develop a simplified two-plasmid rAAV production system to achieve the following goals:

- Higher rAAV productivity
- Improved packaging efficiency by increasing % full capsids
- Reduction in packaged residual DNA impurities
- Successful scale-up to stirred-tank bioreactor

Methods

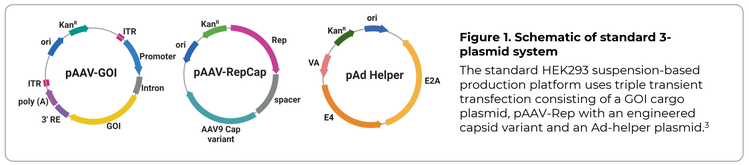

Figure 1. Schematic of standard 3-plasmid system

The standard HEK293 suspension-based production platform uses triple transient transfection consisting of a GOI cargo plasmid, pAAV-Rep with an engineered capsid variant and an Ad-helper plasmid.[3]

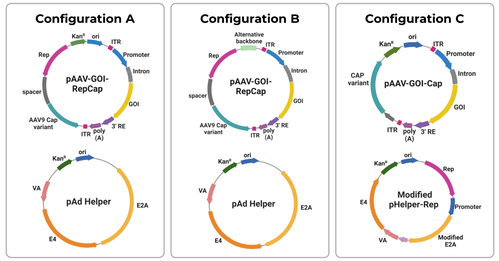

Figure 2. Schematic of 2-plasmid configurations

- **Configuration A** utilizes the pAd-helper combined with a single pGOI, engineered capsid variant and Rep.
- **Configuration B** is similar in arrangement to A, with modifications to the backbone including reduced size and alternative selection markers.
- **Configuration C** splits the Rep ORF from the Cap variant to create a versatile single pGOI-CAP and is combined with a modified pHelper-Rep bringing both plasmids to similar mass that is within 250 bp in size.[3]

Methods

Design & Cloning » Molecular Screen » Functional Screen » Scale to 2L STR Upstream Production » Downstream Purification & R&R readouts » Bio-functional Assessment

Figure 3. Plasmid Development Workflow

Plasmid modifications are carefully designed and screened at the molecular and functional levels using our HEK293 suspension platform process in shaker flasks. Selected constructs are scaled to 2L STR and purified to drug substance. Recovery and residual (R&R) readouts are measured, then assessed for transduction efficiency and transgene expression.

Results

Productivity Assessment & 2L STR Scale-up and Recovery

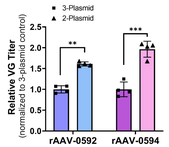

Figure 4. Productivity Assessment

Independent rAAV vectors display similar trend of 40-50% higher vg titer compared to the 3-plasmid system. The rAAV ID correspond to a unique cargo/capsid combination.

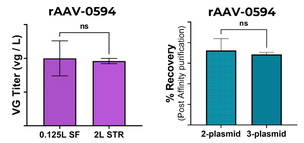

Figure 5. Scaling to 2L STR and Affinity Recovery

rAAV-0594 2-plasmid system displays linear scalability from shake flask (SF) to 2L Stir Tank bioreactor (STR) with similar % recovery to the 3-plasmid system.

Transfection Optimization

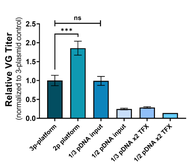

Figure 6. pDNA Input & Transfection Reagent Ratios

Platform pDNA input with the 2-plasmid system exhibits a 2-fold increase in vg titer. Reduction of pDNA input by 1/3 of the platform process results in comparable vg titer to the 3-plasmid system. Further decrease in DNA input with double the transfection reagent drastically lowers production yields.

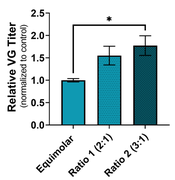

Figure 7. Molar Ratio Optimization

Molar ratio optimization was performed with the 2-plasmid system at 2:1 and 3:1 compared to the equimolar control. Both ratios yield >30% increase in vg titer.

Results

Production and Packaging Efficiency

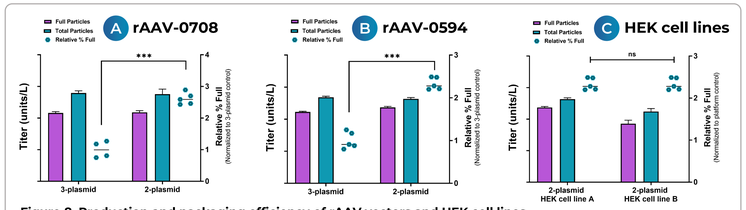

Figure 8. Production and packaging efficiency of rAAV vectors and HEK cell lines

Figures **A** and **B** are reporting titer yield and estimated % full particles based on ddPCR and ELISA from independent rAAV vectors (rAAV ID correspond to a unique cargo/capsid combination). Estimated % full particles display similar trend of 2 to 3-fold higher packaging with the 2-plasmid vs the 3-plasmid system. Figure **C** is reporting titer yield and % full particles from different HEK293 cell lines exhibiting similar packaging efficiency.

Residual DNA Impurity Levels from Affinity Purified rAAV Vectors

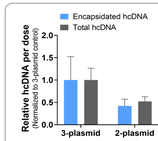

Figure 9. Residual hcDNA levels

Total and encapsidated hcDNA levels were measured post affinity purification. The 2-plasmid system displays at least 2-fold less packaged and total hcDNA relative to 3-plasmid control.

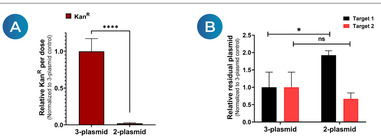

Figure 10. Encapsidated residual pDNA levels

Figure **A** is reporting relative Kan^R levels measured by ddPCR. The 2-plasmid system exhibits ~3% of packaged Kan^R relative to the 3-plasmid control. Figure **B** is reporting relative packaged residual pDNA. Target 1 is reporting higher levels in 2-plasmid system and target 2 exhibits comparable levels between both systems.

Conclusions

- Capsida's two plasmid transfection system demonstrates design benefits that improve productivity, higher % full particles and reduction of packaged DNA impurities
- This increase in rAAV packaging efficiency alleviates pressure on downstream purification steps while enhancing product quality and safety
- The two-plasmid process requires less DNA input reducing manufacturing plasmids cost by up to 60%
- This system will support Capsida's gene therapy platform by expanding the range and accessibility of rAAV-based treatments for rare and common diseases across all ages

Lysa-Anne Volpe
Capsida Biotherapeutics - Principal Scientist, Cell Line Development Lead
Lysa.volpe@capsida.com

Check out Capsida's other ASGCT presentations!

PRESENTED ON TUESDAY
- Chen et al, AAV Gene Therapy Corrects Neurological Phenotypes with Clinically Relevant Doses in a Mouse Model of STXBP1-Related Developmental and Epileptic Encephalopathy (Oral Abs 38)

PRESENTED ON WEDNESDAY
- Knoll et al, CAP-002: Systemic AAV Gene Therapy with Next Generation Capsids for Treatment of STXBP1 Encephalopathy (Abs 504)
- Morales et al, Characterization of engineered AAV capsids from different HEK293 cell culture fractions, crude lysate versus cell pellet material (Abs 529)

PRESENTED ON THURSDAY
- Shi et al, Directed Evolution of AAV2 Libraries Yields Capsids with Improved Performance in the Central Nervous System and Cross-species Translatability (Abs 992)
- Gejji et al, Separation of Empty and Full Engineered Adeno-Associated Virus Capsids Using a Weak Anion Exchange (Abs 1038)

PRESENTED ON FRIDAY
- McDowell et al, Systemic AAV Gene Therapy with CNS-Targeted Engineered Capsids Achieves Significant GCase Activity Increases in the Primate Brain to Support the Potential Treatment of GBA-PD (Oral Abs 274)

References

1. Wang, JH., et al. (2024). Adeno-associated virus as a delivery vector for gene therapy of human diseases. Sig Transduct Target Ther, 9, 78-111. doi.org/10.1038/s41392-024-01780-w
2. Fu, Q., et al. (2023). Critical challenges and advances in recombinant adeno-associated virus (rAAV) biomanufacturing. Biotechnology and Bioengineering, 120, 2601–2621. PMID: 37126355
3. Plasmid maps created with BioRender.com

Acknowledgements

This study was supported by the following members of the Analytical Development team at Capsida Biotherapeutics: Jesse Granados, Leandro Fernandez, Regan Sobaje, Tiffany Morrison, Sara Jabalameli, Vernon Benedicto