rAAV Manufacturing Solutions: Strategic Designs of Engineered rAAV Two Plasmid Systems for Cost Effective Scaling and Product Safety (Abs 962)

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Introduction

Recombinant adeno-associated virus (rAAV) is becoming an increasingly common delivery system for a variety of gene therapy applications. Advances in rAAV engineering have led to the development of engineered AAV capsids with significantly improved target tissue tropism, higher transduction efficiency, and lower immune toxicity, enabling safer, more effective gene therapies. Manufacturing solutions with better scalability and reduced cost of critical raw materials are essential to meet supply demands.[1] We previously developed a scalable two plasmid rAAV transfection system that showed higher productivity and packaging efficiency than our three plasmid approach. Building on that success, we strategically redesigned the system to lower the risk of replication competent rAAV while maintaining productivity and vector quality.

Strategic goals for the redesigned two plasmid system:

- Revise two plasmid designs to minimize the risk of replication competent rAAV
- Maintain or increase rAAV productivity and packaging efficiency
- Successful scalability of the new configuration to stir tank reactors

Methods

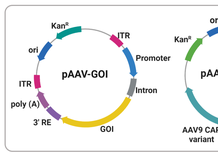

Figure 1. Schematic of standard three plasmid system

Triple transient transfection utilizing a GOI cargo plasmid, a RepCap plasmid with an engineered capsid variant, and an Ad-Helper plasmid is the standard for the HEK293 suspension-based rAAV production platform.[2]

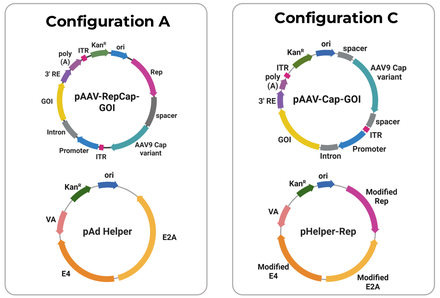

Figure 2. Schematic of two plasmid system configurations

- **Configuration A** combines the GOI and RepCap components into a single plasmid to be used with the standard pAd-Helper plasmid.
- **Configuration C** combines a modified version of Rep with Ad Helper components, splitting the Rep ORFs from the Cap variant. This arrangement reduces the risk of producing replication competent rAAV relative to configuration A where AAV Rep, Cap and ITR sequences are all located on one plasmid. **Note:** Configuration C is presented as a general design for the modified pHelper-Rep. Several versions of this configuration have been designed, developed and evaluated for optimal performance.[2]

Methods

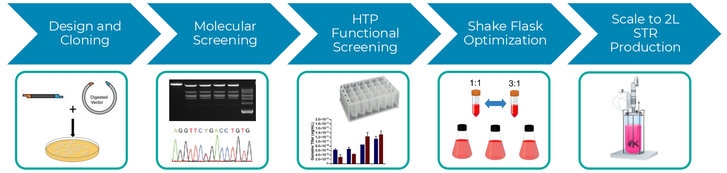

Figure 3. Plasmid Development and Screening Workflow

Plasmid modifications are designed, cloned and screened at the molecular level. New constructs are then assessed functionally using our high throughput (HTP) HEK293 suspension transfection process in 24 deep well plates. Top performing constructs are evaluated in shake flasks for further optimization before scaling to 2L STR.

Results and Discussion

Two Plasmid System Characterization with Configuration A

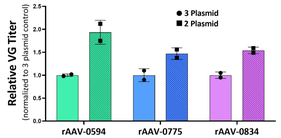

Figure 4. Config A Across Unique rAAV vectors

The two plasmid config A consistently demonstrates a 1.5 to 2-fold improvement in VG yield for a variety of rAAV vectors. Each rAAV ID correspond to a unique cargo-capsid combination.

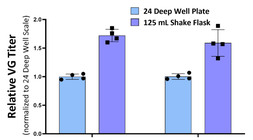

Figure 5. High Throughput Method for Functional Screening

Two rAAVs produced by the 24 deep well suspension transfection method exhibit consistency across wells. Scalability trends as expected from plate to shake flask for both vectors.

Rep Modifications to Improve rAAV Yield and Packaging Efficiency

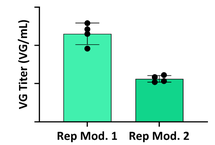

Figure 6. Modified Rep ORFs

Evaluation of two alternative Rep constructs differing from the platform Rep. Rep Mod. 1 produces 2-fold higher VG yield than Mod. 2.

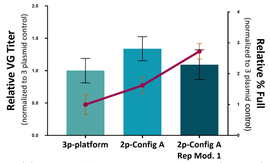

Figure 7. Modified Rep ORFs Improve Packaging Efficiency

Two plasmid config A produced 30% higher VG yield with a 1.6-fold improvement on percent full particles than the three plasmid control. Config A with Rep Mod. 1 produced comparable VG yield to the platform control with 2.6-fold improvement on percent full particles.

Results and Discussion

Helper Modifications for Configuration C Optimization

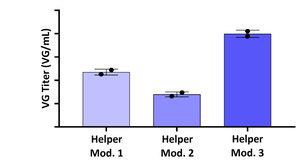

Figure 8. Alternative Ad Helper ORFs

Various combinations of modified Ad Helper E2A and E4 were evaluated. Helper Mod. 3 produced about 2-fold higher than Mod. 1 and 3-fold higher than Mod. 2.

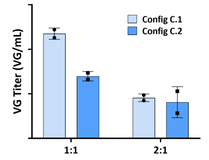

Figure 9. Config C Molar Ratio Optimization

An equimolar ratio shows a 70-150% increase in VG yield compared to a 2:1 molar ratio of pCap-GOI to pHelper-Rep across multiple versions of config C.

Two Plasmid System Configuration C Scalability

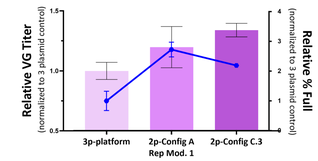

Figure 10. Config C.3 Improves Packaging Efficiency

Combining Rep Mod. 1 and Helper Mod. 3 components in config C.3 results in a 1.3-fold increase in VG yield and a 2-fold increase in percent full particles relative to the platform control.

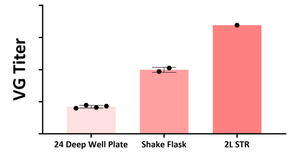

Figure 11. Configuration C.3 Scalability

Configuration C.3 displays linear scalability across deep well plates, shake flasks (SF) and stir tank reactors (STR). This trend is consistent with our three plasmid platform process.

Conclusions

- The new two plasmid configuration C design maintains improved productivity and packaging efficiency while controlling for safety risks (replication competence)
- Multiple Rep and Ad Helper sequence modifications were tested, identifying a configuration which boosts VG yield by 30% and doubles the percentage of full particles compared to the standard three plasmid system
- The new two plasmid design improves rAAV production and packaging, reduces GMP-associated costs, and strengthens our manufacturing capabilities to meet the rising clinical demands of both upcoming programs and future therapeutic needs
- 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

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Check out Capsida's other ASGCT presentations!

Presented on WEDNESDAY

- Systemic AAV Gene Therapy with Next Generation Engineered Capsid Demonstrates Expression Levels Supporting Potential Therapeutic Benefit for CNS, Cardiac, and Sensory Symptoms in Friedreich's Ataxia, 1:45-2:00 PM CT, Abstract Number: 75, Presenter: Celeste Stephany, Ph.D., Director of CNS and Ophthalmology Preclinical Research, Capsida
- Identification of Multiple Novel Blood-Brain-Barrier Receptors for CNS Gene Therapy and Other Drug Modalities via an Integrated AAV Capsid Engineering Platform, 2:45 – 3:00 PM CT, Abstract Number: 93, Presenter: Nick Goeden, Ph.D., Founder, Chief Technology Officer, Capsida
- Systemic Gene Therapy CAP-002 Demonstrates Potential for Disease-Modifying Treatment of Seizures and Motor and Cognitive Deficits of STXBP1-DEE Using an Engineered, CNS-Targeted AAV, 3:45-4:00 PM CT, Abstract Number: 123, Presenter: Nick Flytzanis, Ph.D., Founder, Chief Research and Innovation Officer, Capsida
- CAP-003, a CNS-Targeted IV-delivered AAV Gene Therapy, Safely Increases Brain GCase in NHPs to Level Supporting Potential Normalization of Activity in PD-GBA Patients, 5:30 – 7:00 PM CT, Abstract Number: 1435, Presenter: Kim McDowell, Ph.D., Director, Preclinical Research, Capsida
- Dual-Platform NGS for Comprehensive Characterization of Engineered rAAV Vector Integrity,5:30 – 7:00 PM CT, Abstract Number: 1326, Presenter: Zach Mason, Associate Scientist, Capsida

Presented on THURSDAY

- Development of a Novel Automated Loading Approach Which Significantly Reduces Processing Time for Enriching Full AAV Capsids Using Ultracentrifugation, 5:30 – 7:00 PM CT, Abstract Number: 1833, Presenter: Varun Gejji, Ph.D., Senior Scientist, Capsida

Acknowledgements

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References

1. Destro, F., et al. (2024). The state of technological advancement to address challenges in the manufacture of rAAV gene therapies. Biotechnology advances, 76, 108433. PMID: 39168354.
2. Plasmid maps and workflow diagrams created with BioRender.com