High-Yield, Pan-Serotype Plasmid System for Manufacturing Adeno-Associated Virus Gene Therapies: Cost and Efficiency Benefits for R&D and Commercial Processes

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Abstract

A major challenge in development of adeno-associated virus (AAV)-based gene therapies is the low manufacturing yield and poor packaging efficiency of AAV capsids. While the enhanced tropism of novel capsids has reduced the total required dose, capsid modifications can have negative effects on manufacturability. Therefore, we design and screen for high yielding capsids during the capsid discovery phase. Even after discovering novel capsids with good manufacturability, a high-yield, capsid-agnostic manufacturing method drastically decreases the cost and difficulty of screening and production of these capsids and unlocks low-yielding capsids, such as AAV2, as viable delivery vehicles.

Affinia Therapeutics has developed a plasmid system which leads to an increased yield for all tested wild-type and novel capsids (Fig 3). Directed optimizations can increase vector genome yield more than 10-fold relative to traditional plasmid systems. Packaging efficiency increases of 4-fold are commonly achieved in poorly packaging serotypes and the transfection conditions can be tuned to prioritize vector genome yield or packaging efficiency. This system has shown consistent effectiveness across all tested wildtype and novel capsids, all evaluated commercially available helper plasmids, all tested transfection reagents, and in all evaluated HEK293-based cell lines (Fig 4).

These manufacturability enhancements decrease total upstream batch size requirements and increase capacity on the downstream unit operations by minimizing the presence of empty capsid contaminants. In 2024 Affinia Therapeutics implemented this system. In R&D the Affinia Therapeutics Vector Core when compared to 2023 productions, decreasing average batch size and increasing production throughput (Fig 1), thereby significantly reducing cost of goods per experiment (Fig 2).

Since initial rollout of this plasmid system, plasmid constructs have been optimized to increase yield 1.2x and packaging 1.5x for AAV9 in both bioreactors and shake flasks (Fig 4). Additional process development efforts have resulted in an intensified production process which has increased upstream titers for a lead novel capsid to 5.5e15 vg/L (1.8x the 2024 conditions) using the Affinia plasmid system in bioreactors.

In summary, this innovative plasmid system addresses critical manufacturing challenges, enabling more efficient production of AAV gene therapies and unlocking the potential of diverse capsid platforms for development, clinical, and commercial use.

Effect of System on Production Processes

Fig 1 – Affinia Plasmid Design Allows Smaller Production Size and More Efficient Purification

Increased harvest titers provided by the Affinia plasmid designs enable smaller-scale productions, reducing capital cost associated with large-scale material generation.

The high harvest titer allows for an initial concentration step to be bypassed completely, avoiding the yield loss and time / capital costs associated with this step. This decreased hands-on time allows for more conditions to be simultaneously evaluated, increasing process development throughput.

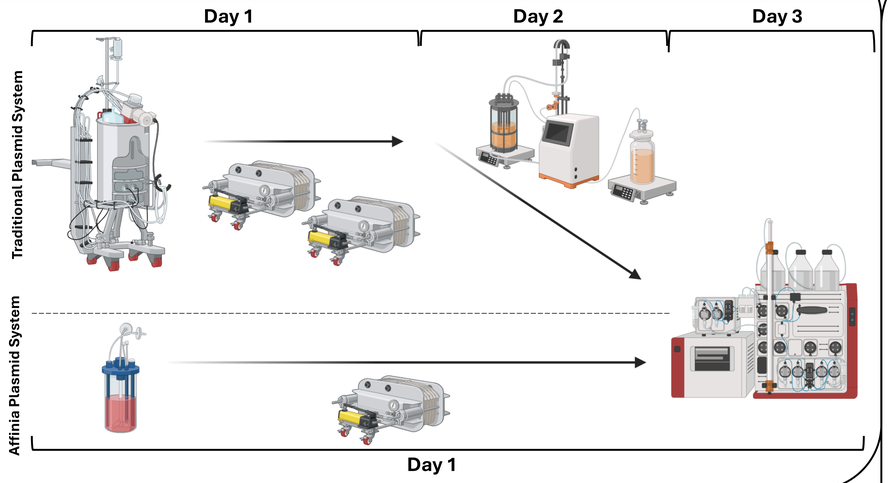
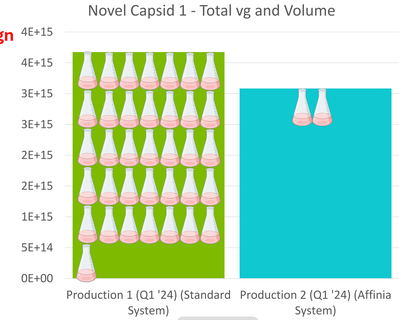


Fig 2 – Production Case Studies (R&D Grade)

A variety of capsids were produced in the Affinia vector core for various studies using both the traditional plasmid method and Affinia plasmid system. Since switching to the Affinia plasmid system production sizes have vastly decreased, leading to higher throughput, lower turnaround time, and lower costs.

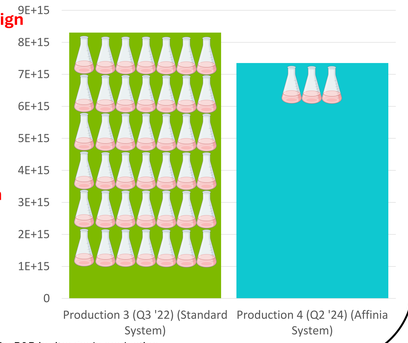
Production 1:
Standard Plasmid Design
3.7e15 vg @ Harvest
Q1 2024
Est. Cost[1]: **$21,700**
50L at Harvest
Novel Capsid 1

Production 2:
Affinia Plasmid Design
3.1e15 vg @ Harvest
Q2 2024
Est Cost[1]: **$1,200**
2.8L at Harvest
Novel Capsid 1



Production 3:
Standard Plasmid Design
8.3e15 vg @ Harvest
Q3 2022
Est Cost[1]: **$25,300**
69L at Harvest
Novel Capsid 2

Production 4:
Affinia Plasmid Design
7.4e15 vg @ Harvest
Q2 2024
Est Cost[1]: **$1,800**
4.2L at Harvest
Novel Capsid 2



[1] Estimated consumable cost for R&D in vitro grade production

Versatility of Plasmid System

Fig 3 – Affinia Plasmid System with Various Serotypes

Conditions were chosen for various serotypes to maximize yield while maintaining similar or better packaging efficiency to traditional 3-plasmid transfections. Affinia plasmid system shows substantial transfection performance across all tested serotypes.

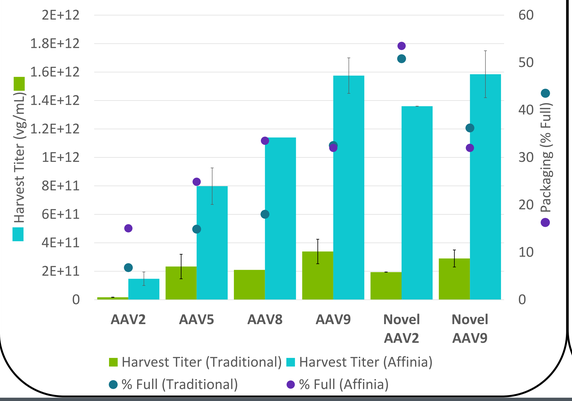
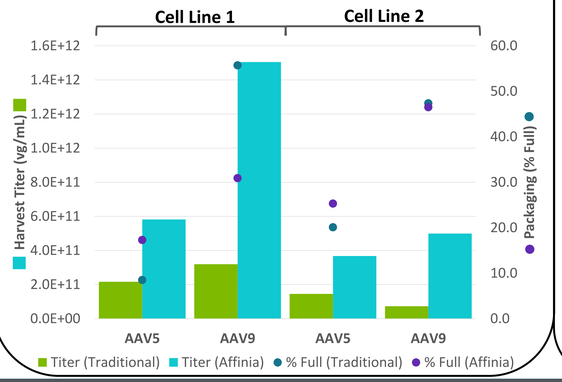


Fig 4 – Transferability to Other Cell Lines

The same transfection mixes were added to multiple cell lines at conditions targeted to maintain packaging while increasing yield relative to traditional transfection system. The Affinia plasmid system's large yield increases were maintained across cell lines. The high packaging of AAV9 in Cell Line 1 is unique to this transgene and not repeatable in bioreactor experiments.



Bioreactor Data - 2024 to 2025 System Improvements

Fig 5 - 2025 Plasmid Modifications

Plasmid designs have been optimized over the 2024 designs. 2025 designs provide 1.2x yield and 1.5x packaging efficiency of the 2024 plasmid designs in both shake flasks and in bioreactors. Data shown is AAV9 with a therapeutic transgene. Other serotypes / transgenes are being evaluated and similar increases are expected.

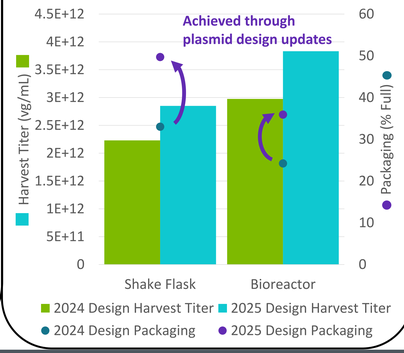
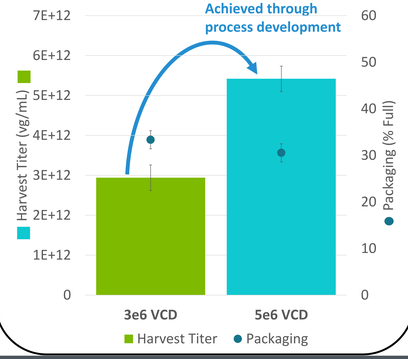


Fig 6 - 2025 Process Development

Process optimization over the past year has shown the Affinia plasmid can lead to higher yields with additional process development. Bioreactor seed density adjustments have shown yield of almost **6e12** vg/mL with no hit to packaging quality. Data shown is novel capsid with therapeutic transgene in 3L bioreactors.



Conclusions

- Affinia Therapeutics has developed a plasmid system which improves vector productivity across multiple serotypes, cell lines and production scales.
- Increased production efficiency from the novel plasmid system allows faster production times, higher throughput, and lower costs.
- Further optimization of Affinia's plasmid system has led to ever higher titer and packaging efficiency.

Contacts

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Affinia Plasmid System is available for licensing. Reach out for details.

See oral presentation (Abstract #1147) Friday at 4:45 for more details on a production process using this production system!

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