AAV Vector Production is Driven by a Small Fraction of Transfected Cells

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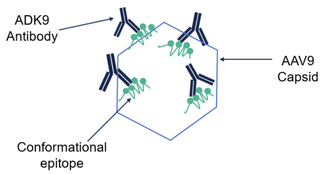
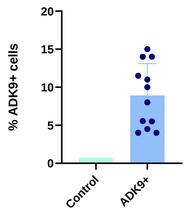
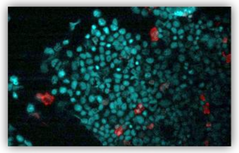
Introduction

A common method used to produce adeno-associated virus (AAV) vectors is transient transfection of cells using plasmid DNA. Transient transfection offers a versatile and scalable platform for delivering effective gene therapies. However, low yields present a bottleneck for cost effective gene therapies. While transfection efficiency typically trends much higher, our studies show that the actual population of cells producing AAV capsids was quite low, between only 5-15%. This was determined by staining transfected cells with ADK9, an antibody that binds to assembled AAV9 capsids, and quantified with flow cytometry and immunofluorescence microscopy.

Detection of Empty and Full Intact AAV9 Capsids Shows 5-15% of Cells Produce AAV



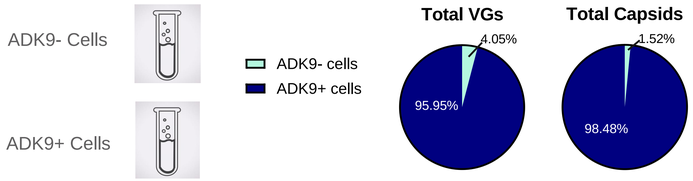




ADK9 + Cells (Red), DAPI nuclear stain (Cyan)

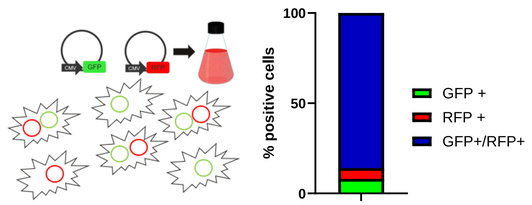
Multiple studies show that there is variability in the number of cells positive for ADK9 staining. This could be due to a number of different upstream process parameters used across data points. However, ADK9+ cell counts remains under 15% for all experiments.

Over 95% of VG and Capsid Titer are From ADK9+ Sorted Cells



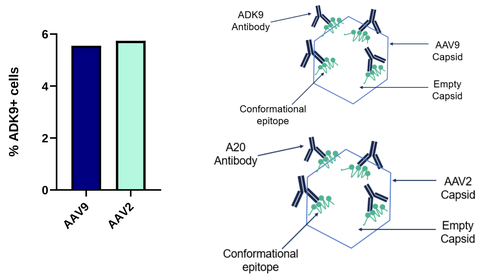
To confirm all AAV vector production was occurring in cells staining positive for assembled capsids, the cells were sorted into two populations: ADK9+ cells and ADK9- cells. These sub-populations were both titered to reveal which population contained the vector genomes (VG) and capsids.

>85% of Transfected Cells Receive Both Plasmids Transfected



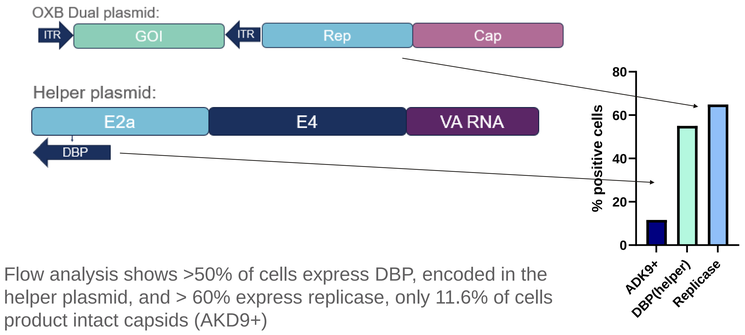
GFP and RFP expression plasmids were utilized to determine the proportion of cells receiving each plasmid using flow cytometry. The vast majority of cells are transfected with both plasmids.

Proportion of Cells Producing AAV is Not Serotype Specific



ADK9 staining for AAV9 was compared to A20 staining for AAV2 and it was found that the proportion of cells producing AAV was consistent across different serotypes.

Number of Cells Expressing DBP & Replicase is Significantly Higher than Cells Producing Intact Capsids



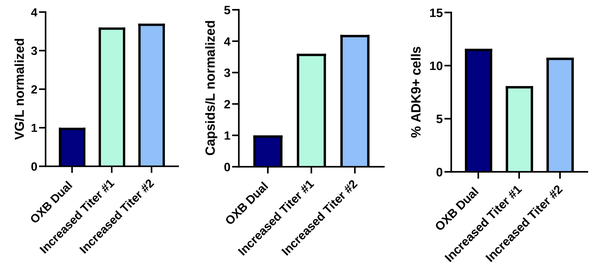
Flow analysis shows >50% of cells express DBP, encoded in the helper plasmid, and > 60% express replicase, only 11.6% of cells product intact capsids (AKD9+)

Methods

All experiments were completed using 125 mL shake flasks with a 50 mL working volume. VPC 2.0 cells (Thermo Fisher) were cultured at 2E6 cells/ mL in Expi293 media (Thermo Fisher) and transfected with 0.75 µg of DNA/ 1E6 cells at equal molar plasmid ratios using PEI. The OXB dual plasmid design (GOI+RepCap) bearing a CMV-Luciferase genome and AAV9 or AAV2 capsid was used for all experiments. Vector genome (VG) and capsid titers were analyzed by ddPCR and AAV9 ELISA (Progen) respectively.

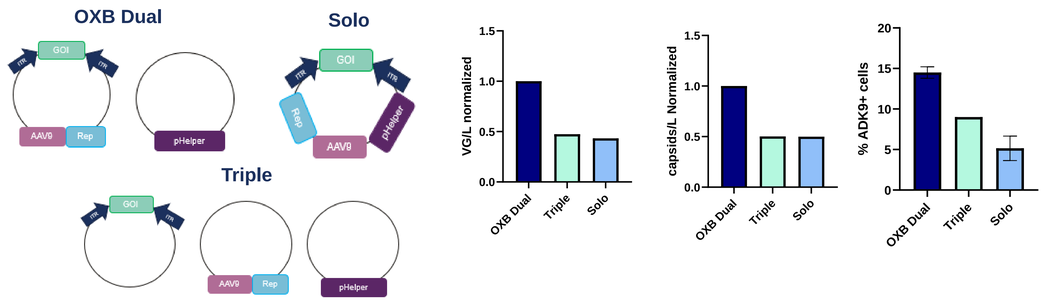
Results

Increasing VG and Capsid Productivity Does Not Increase Cells Producing AAV



Different plasmids were used that are known to increase VG titer were used to determine if increasing titers would correlate with the number of cells producing AAV. Increased Titer #1 utilized an alternative capsid expression cassette with a strong promoter. Increased Titer #2 used an engineered helper plasmid. In either case, increased VG titers did not increase the proportion of cells producing AAV vector.

Number of Plasmids Transfected Does Not Correlate with the Proportion Cells Producing AAV



The number of plasmids being transfected (three, two or one) did not directly correlate to proportion of cells producing AAV. Cell producing AAV remained consistent across multiple serotypes.

Conclusion

These findings further demonstrate that AAV is being produced by only a small fraction of transfected cells. With this confirmed, it is of high importance to understand the basis for this phenomenon to improve the proportion of total cells producing AAV to enable more cost-effective AAV manufacturing.

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