Increasing Quality and Productivity with Dual Transfection (DT) for AAV Production

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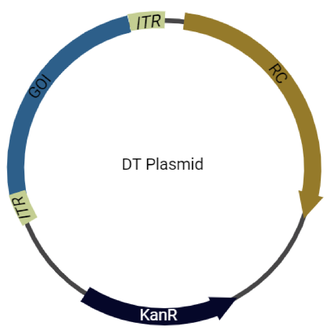
Introduction

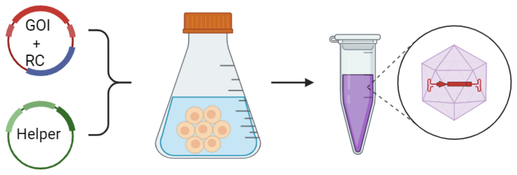
- Adeno-associated virus (AAV) is a common vector used in gene therapy. Developing a robust and high-yielding manufacturing process is important to meet clinical demands and reduce cost of goods.
- Traditionally in AAV manufacturing, a triple transfection (TT) system, using 3 plasmids: (1) the gene of interest (GOI), (2) AAV replication proteins and capsid (RepCap or RC) genes and (3) helper genes, is utilized to transiently transfect mammalian cells and produce AAV. A potential challenge using the TT system is large quantities of plasmid required to transfect at higher scales (>50L) for commercial supply while maintaining high yields and product quality.
- Here, we evaluate a dual transfection (DT) system (**Figure 1B**), which combined GOI and RepCap genes into a single plasmid (**Figure 1A**) decreasing number of plasmids from 3 to 2.
- We report similar or improved AAV analytics produced from the DT system, including AAV yield, genomic integrity, full:empty ratio and mispackaging of plasmid DNA, compared to TT system across a variety of production scales.

Plasmid Design - DT

1A

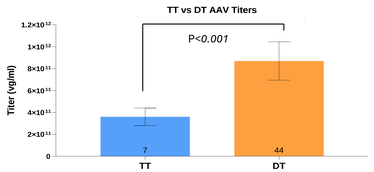


1B



Increased AAV Titers with DT

2A



2B

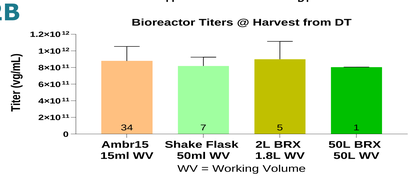


Figure 2A. More than a 2-fold increase in AAV harvest titers was observed with the (DT) system when compared to (TT) system with our AAV-SLB101 capsid and GOI1.

Figure 2B. Similar harvest titers were maintained for DT at scales up to a 50L bioreactor.*

*Additional 50L scale studies ongoing, preliminary result suggests similar yields to shaker flask and 2L bioreactor.

Numbers within each bar represent the number of experimental replicates.

NGS Long Read Sequencing Data DT vs TT

4

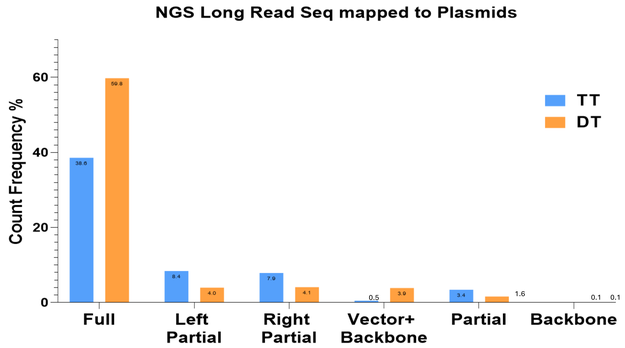
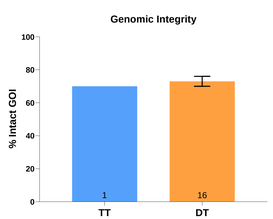


Figure 4. Population of single-stranded mapped reads in TT (affinity eluate then UC enriched for full capsids) versus DT (affinity eluate) AAV by PacBio long read sequencing show more full particles from DT produced material, despite less enrichment for full particles during purification. Numbers on each bar represent the frequency of those reads in %.

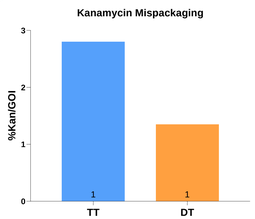
Full: Reads mapping the whole GOI from ITR to ITR
Left Partial: Reads containing Left ITR with some portions of GOI
Right Partial: Reads Containing Right ITR with some portions of GOI
Vector+ Backbone: Reads Containing GOI DNA and extending into backbone plasmid DNA
Partial: Reads aligning to GOI but not containing ITRs
Backbone: Reads mapping to plasmid backbone

Quality Attributes - AAV Produced by DT vs TT

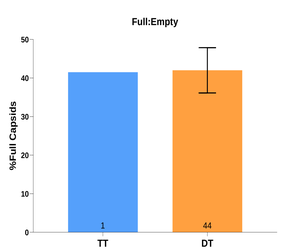
3A



3B



3C



3D

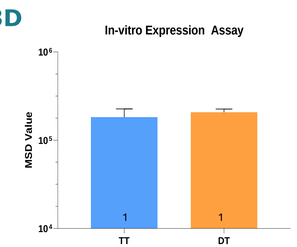
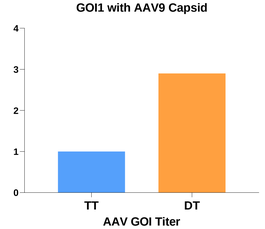


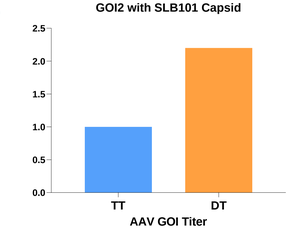
Figure 3. Representative data from relevant AAV quality attributes. Similar A) genomic integrity (as measured by multiplex ddPCR across the length of the GOI); C) % full capsids at harvest (as determined by dividing GOI titer by AAV capsid titer; and D) protein expression (as measured by in vitro transduction of C2C12 cells) was observed across AAV manufactured by TT vs DT. Additionally, B) reduced mispackaging of the kanamycin gene into the AAV capsid was observed in the DT system in comparison to the TT system, as determined by dividing the amount of kanamycin measured by the amount of GOI measured. Measurements were performed via ddPCR. Numbers within each bar represent the number of AAV Preps tested.

Increased Titers for Various GOI and Capsid Combinations with DT

5A



5B



5C

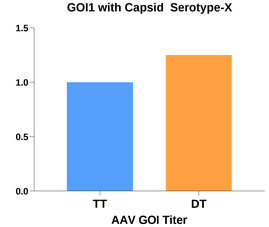


Figure 5. Fold increase of DT AAV titers across various capsid and GOI combinations in comparison to TT. A) DT produced AAV9-GOI1 resulted in a 3-fold increase compared to TT production system. B) DT produced AAV-SLB101-GOI2 resulted in a >2-fold increase compared to TT production system. C) DT produced AAVX-GOI1 resulted in a 1.25-fold increase compared to TT production system.

Conclusions

- Here we evaluated a dual transfection system that could potentially be used to manufacture AAV with similar or improved productivity and product quality to material produced by TT.
- AAV titers produced using DT system across multiple GOI and capsid combinations had 1.25-3 fold increases compared to TT.
- High AAV upstream titers were maintained up to a 50L bioreactor, implying a scalable platform for AAV production.
- DT produced material had similar or improved AAV quality attributes compared to TT produced material across multiple experiments.
- Further improvements and implementation of the DT system in clinical or commercial production settings may reduce COGs through decreased plasmid usage, increased productivity, and improved AAV quality.

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