Optimization of a Vector Backbone to Enhance Plasmid Yields and Reduce AAV Manufacturing Costs

Airam S. Mitchell, Ding Li, Anne Minner and Kristin N. Heller

Andelyn BIOSCIENCES | Advancing Discoveries. Manufacturing Cures.

Introduction

The standard Adeno-Associated Virus (AAV) manufacturing platform relies on the triple-plasmid transfection method. As the industry shifts toward larger production scales to meet clinical and commercial demand, the yield requirements for high-quality plasmid DNA has increased proportionally, often becoming a significant driver of the Cost of Goods (COGS). To address this bottleneck, Andelyn Biosciences investigated the impact of key sequences within the traditional plasmid on overall plasmid yields.

Methods

The elements currently found in traditional vector plasmid were systematically evaluated. Several iterations of a proprietary optimized backbone were developed and tested across multiple scales. Performance was assessed in both shake flask and fermenter platforms, measuring specific plasmid yields, quality, ITR stability, and percent loss/recovery.

Discussion

Optimized constructs (1, 3, and 5) demonstrated a significant performance leap, **doubling initial harvest yields** compared to standard controls. In final product form, these constructs reached **10–14 mg/L**, a nearly **4x improvement** over the standard plasmid (~3 mg/L). Performance trends remained consistent from 250mL shake flasks to 2.5L scales, indicating a robust and scalable backbone.

Process efficiency was further validated by a **60% total recovery rate**, substantially outperforming the ~40% recovery seen with standard vector plasmids. While fermentation trends in Ambr® 250 vessels mirrored shake flask results, recovery deltas were less pronounced due to lower starting volumes. Ongoing studies are currently focused on scaling these results in larger fermentation vessels and across diverse transgene payloads.

Ultimately, this optimized platform significantly enhances plasmid yield and maintains ITR stability. This backbone provides a clear pathway to **reduce production timelines and material costs**, supporting the global accessibility of large-scale AAV gene therapies.

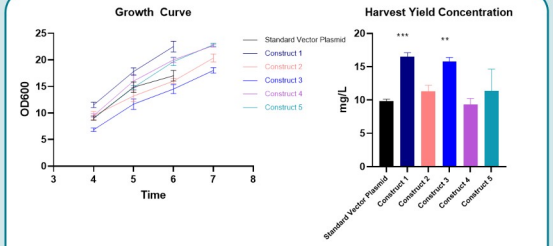

Figure 1. Effect of Vector Plasmid Construct Design on E. coli Growth.
Left: Growth curves of engineered constructs compared to a standard vector plasmid control. Optical density at 600 nm (OD600) was measured over time to assess cell growth for cultures carrying the standard vector plasmid or Constructs 1–5.
Right: Comparison of harvest yields across engineered constructs and standard vector plasmid. Product concentration (mg/L) was measured for the standard vector plasmid and Constructs 1–5 following harvest. Construct 1 and Construct 3 demonstrate the highest yields, both exceeding 15 mg/L, significantly outperforming the standard vector control (approximately 10 mg/L). (***p=<0.0005, **p=<0.005)

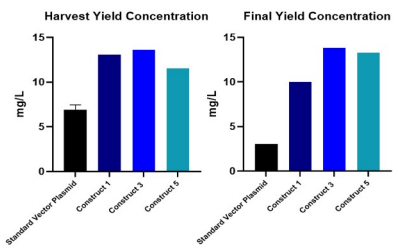

Figure 2. Increase in Harvest and Final Yields of the top 3 constructs compared the standard vector plasmid.
Left: Harvest Yield from the top 3 constructs compared to the standard vector plasmid during 2.5L Shake Flask production.
Right: The final yield from the top 3 constructs compared to the standard vector plasmid during 2.5L Shake Flask production.

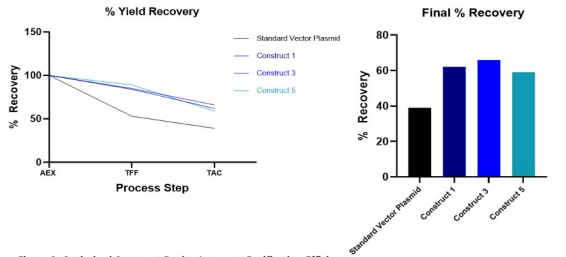

Figure 3. Optimized Construct Design Improves Purification Efficiency:
Left: Yield recoveries for all process steps for three constructs were compared to the original vector plasmid construct. The % recovery was determined for the individual processing steps: Anion Exchange Chromatography (AEX), Tangential Flow Filtration (TFF), and Thiophilic Aromatic Chromatography (TAC). The yield was divided by the yield from process step to AEX.
Right: Final yield recovery for the three constructs compared to the original vector plasmid. The % recovery was determined for the yield concentration at final fill compared to the yield to AEX.

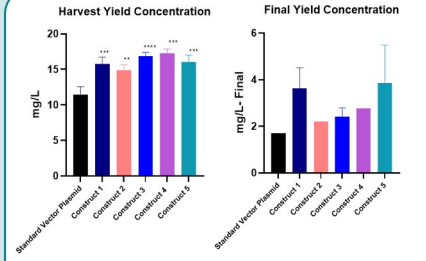

Figure 4. Optimized Construct Show Similar Trends in Fermenter for Yield Concentration.
Left: Harvest Yield Concentration from the 5 constructs compared to the standard vector plasmid during fermentation in the Ambr® 250.
Right: The Final Yield Concentration from the 5 constructs compared to the standard vector plasmid. (**** p= <0.0001, ***p=<0.0005, **p=<0.005)

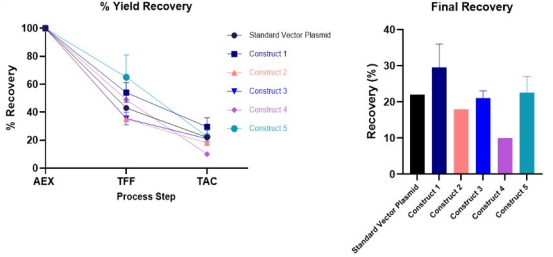

Figure 5. Optimized Construct Design shows Trend of Improvement Purification Efficiency:
Left: Yield recoveries for all process steps for five constructs were compared to the original vector plasmid construct. The % recovery was determined for the individual processing steps: Anion Exchange Chromatography (AEX), Tangential Flow Filtration (TFF), and Thiophilic Aromatic Chromatography (TAC). The yield was divided by the yield from process step to AEX.
Right: Final yield recovery for the five constructs compared to the original vector plasmid. The % recovery was determined for the yield at final fill compared to the yield at AEX. Due to the low overall volume processed (200mL), results are not as dramatic as the Shake Flask data.

	% Supercoiled Prior to Enrichment
Original	82
Construct 1	80
Construct 3	78
Construct 5	92

	% Supercoiled Prior to Enrichment
Original	95
Construct 1	96
Construct 2	96
Construct 3	97
Construct 4	99
Construct 5	99

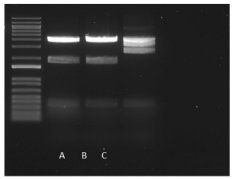

Figure 6. Supercoiled plasmid levels after AEX and ITR Stability.
Top Left: Chart shows the percentage of supercoiled plasmid for the 2.5L Shake Flask Production.
Bottom Left: Chart shows the percentage of supercoiled plasmid for the 200mL Ambr® 250.
Right: Agarose gel digesting Construct 1 (A), Construct 3 (B) and Construct (C) with Xmal to determine ITR stability. Image is a representative Image of the three constructs.

For inquires, please contact:
andelynbusiness@andelynbiosciences.com

Andelyn BIOSCIENCES