A Novel, Synthetic DNA Alternative for rAAV Manufacturing

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Introduction

Demand for DNA as a critical starting material for viral vector manufacturing, mRNA production, and gene therapy delivery applications continues to rise, increasing the need for efficient, timely, and scalable DNA manufacturing.

Our One-pot Enzymatic DNA Synthesis

Anjarium's novel, cell-free enzymatic approach for producing linear, double-stranded DNA enables a complete range of applications with significantly faster delivery times than traditional methods.

Our enzymatic DNA synthesis provides multiple benefits:

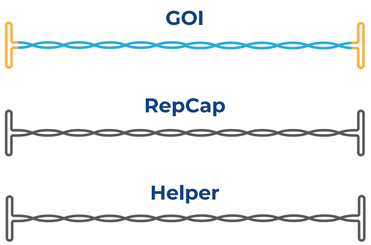
- **Purity**: Synthetic DNA is devoid of bacterial sequences.
- **Scale**: DNA batches ranging from microgram to multigram produced in small bioreactors with minimal reagents.
- **Speed**: Production time takes just weeks from circular DNA template to vial delivery.
- **Stability**: Hairpin-ended structures, inspired by nature, protect the integrity of the DNA and provide specific functionality in certain applications.
- **Flexibility**: Complex and customized transgene sequences can be produced.

Anjarium's Synthetic DNA (ANJ-DNA)

ANJ-DNA is designed to catalyze advanced therapy research and clinical development programs across AAV, mRNA, Lentivirus and other applications.

Here we show that our synthetic DNA is a superior starting material for the manufacturing of ssAAV and scAAV with a range of serotypes in a conventional triple transfection setting.

Schema of ANJ-DNA Designed for AAV production



Synthetic hairpin-ended structures functionally resemble AAV ITRs

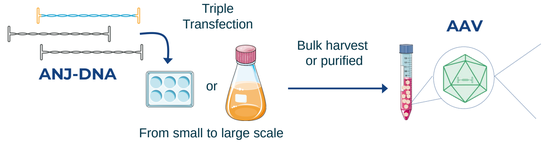
Replicated and packaged in producing cells without the need of extra flanking sequences

Synthetic hairpin-ended structures without Rep function

Avoid packaging of helper sequences

Figure 1: ANJ-DNA offering for the production of rAAV. ANJ-DNA was designed to encode the Helper construct and the RepCap required for rAAV production as well as the Gene-of-interest (GOI). Interestingly, our GOI is designed to have hairpin-structures that mimic AAV2 ITRs so it can replicate and be packaged into rAAV without the need for extra flanking sequences. The three constructs can be customized to encode any required GOI, or optimized helper sequences. ANJ-DNA can also be used in combination with other plasmids or packaging cell lines for AAV production.

Pipeline for the Production and Analysis of AAV



Analytics:

Genomic titer	Functionality
ITR or GOI-specific qPCR	HT1080 transduction + flow cytometry
Particle titer	Capsid integrity
AAV9 ELISA	VP1, 2, 3 Western Blot
Viral genome integrity	Viral genome analysis
Alkaline agarose gel	NGS

ANJ-DNA Yields Higher rAAV Titer than Plasmid

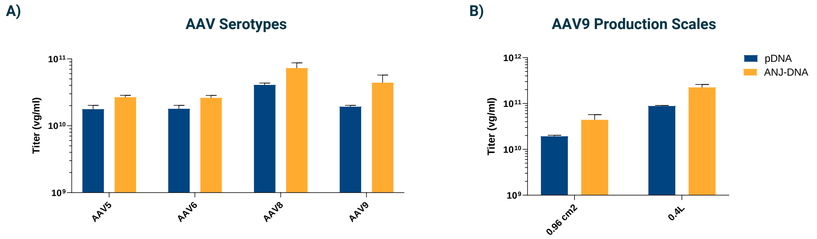


Figure 2: Higher viral titers (as total viral genomes per ml) are produced from ANJ-DNA, as compared to plasmid for different serotypes **(A)** or for different production scales **(B)**.

ANJ-DNA Outperforms Plasmid (rAAV9; 0.4 L Scale)

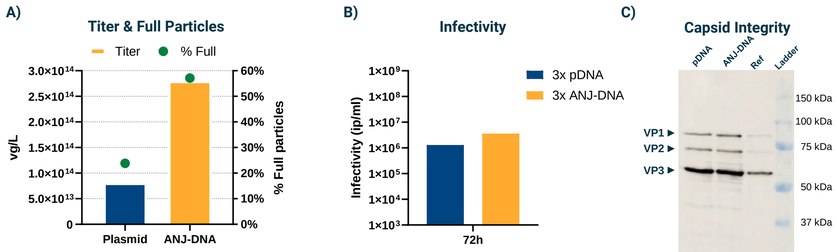


Figure 3: ANJ-DNA outperformed plasmid for rAAV9 production at 0.4 L scale as shown by higher titers based on viral genomes and higher percent of full particles based on the qPCR/ELISA titer **(A)**, and infectivity (ip/ml) **(B)**. Capsid integrity is comparable as shown by western blot **(C)**.

** We thank the TaRGeT Laboratory including the Viral Vector Manufacturing Center (ViVeM / Nantes Université, CHU Nantes, INSERM, TaRGeT, Nantes, France) for the vector production and the analytical characterization performed.*

No Packaging of Backbone Sequence in rAAV from ANJ-DNA

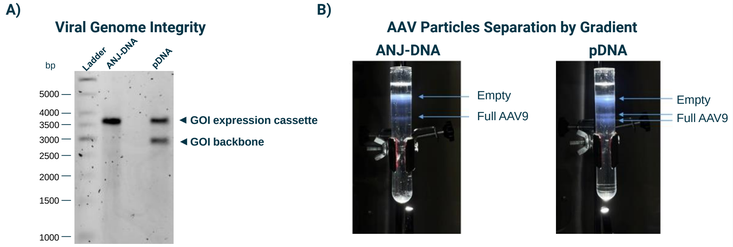


Figure 4: The viral genome is observed as a single band in the ANJ-DNA AAV prep compared to two bands in the plasmid DNA AAV prep, as shown in an agarose gel electrophoresis **(A)** and from full particles in an ultracentrifugation density gradient **(B).** This suggests the absence of packaged backbone.

** We thank DiNAMIQS AG for the AAV production and the analytical characterization perfomed.*

Functional scAAV Can Be Produced from ANJ-DNA

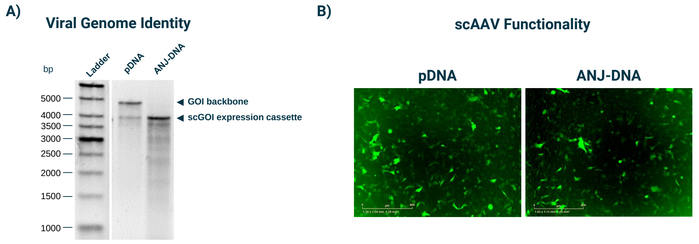


Figure 5: Functional self-complementary AAV (scAAV) can be produced from ANJ-DNA as observed by a denaturing agarose gel showing a band corresponding to the correct size of the scAAV **(A)**, and cell imaging at 96 hours after transduction **(B)**. scAAV prep from plasmid is shown as a comparison.

Conclusions

- ANJ-DNA is a superior starting material for AAV production compared to conventional plasmid DNA
- ANJ-DNA delivers superior rAAV titers, higher percentages of full particles, and avoids the packaging of backbone.
- Unwanted flanking sequences are avoided due to the innovative way in which ANJ-DNA hairpin ends mimic AAV2 ITRs for ssAAV and scAAV GOI.

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