A Novel, Synthetic DNA Alternative for mRNA Manufacturing

Gustavo Lou, David Wilson, Ivana Pastierikova, Luca Distefano, Marco Guarrera, Alexander Pekarsky, J. Omar Yáñez-Cuna, Joel de Beer
Anjarium Biosciences AG, Wagistrasse 23, 8952 Schlieren, Switzerland

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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.

The emergence of messenger RNA (mRNA) as a transformative platform for gene therapy and vaccine applications, especially in the wake of the COVID-19 pandemic, has ignited a quest for innovations in mRNA production.

A pivotal challenge in this space revolves around the choice of a DNA template for *in vitro* transcription (IVT) to generate mRNA. While bacterial-derived plasmid DNA has been used traditionally as a template, its utilization has presented a significant bottleneck in mRNA manufacturing due to issues of cost, turnaround time, and purity.

Here we show that ANJ-DNA, with a 140+ polyA tail, is a superior starting material for *in vitro* transcription and our off-the-shelf product can produce mRNA with higher potency than commercial mRNA from leaders in the field.

Schema of ANJ-DNA Designed for mRNA Production

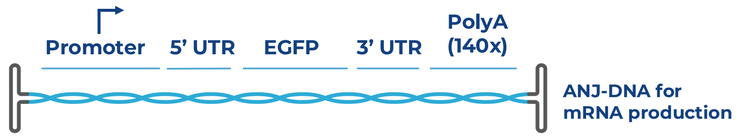

Figure 1: ANJ-DNA offering for the production of mRNA. ANJ-DNA was designed to encode a template construct with 140+ polyA for *in vitro* transcription. Each of the elements annotated in the schema can be customized.

ANJ-DNA Produces Higher mRNA Yields than Plasmid

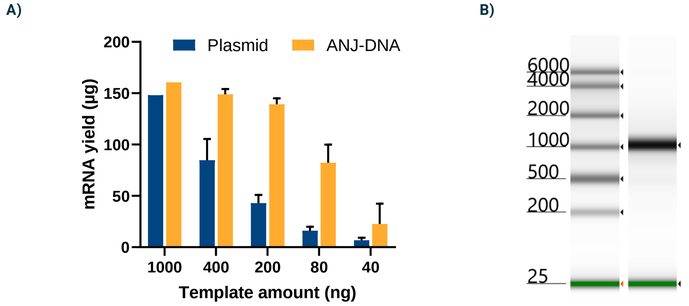

Figure 2: *In vitro* transcription (IVT) from ANJ-DNA resulted in higher mRNA yields compared to plasmid as observed by measuring total mRNA yield (in µg) after a 2 hours long IVT at 37°C using HiScribe® T7 High Yield RNA Synthesis Kit, and CleanCap® Reagent AG with increasing amount of template followed by LiCl precipitation **(A)**. The resulting mRNA is highly pure as shown in a TapeStation (RNA ScreenTape) **(B)**

ANJ-DNA Enables mRNA with Higher Potency than Commercial mRNA

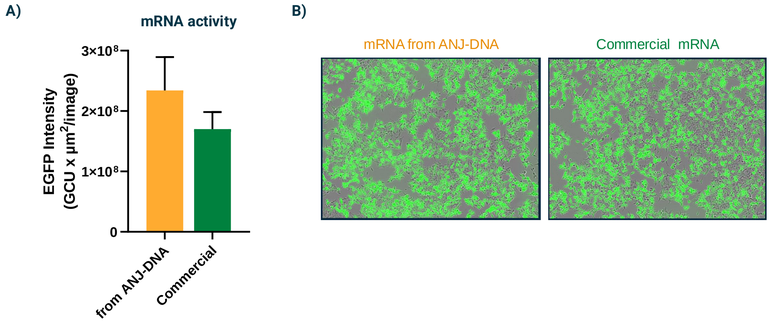

Figure 3: GFP mRNA produced from off-the-shelf ANJ-DNA shows higher potency than commercial mRNA from leaders in the field. 100ng/well of mRNA were transfected into HEK293T (N=3 independent IVT reactions and transfections). At 24 hours after transfection, the EGFP intensity was measured by cell imaging in an Incucyte SX5 **(A)**. Representative images of cells transfected with mRNA from synthetic DNA or commercial mRNA **(B)**.

ANJ-DNA Derived RNA has less dsRNA than Commercial mRNA

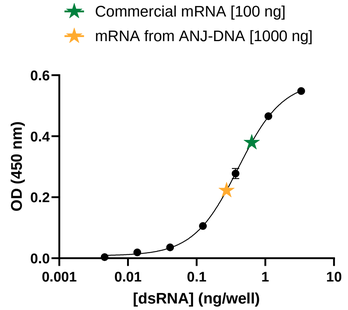

Figure 4: ANJ-DNA produced mRNA (yellow star) contain less dsRNA than commercial mRNA from leaders in the field (green star). Shown is an ELISA assay using the K1 monoclonal antibody. The standard curve was generated by measuring 1:3 serial dilutions of a dsRNA standard

mRNA Potency from ANJ-DNA is Comparable to mRNA from Plasmid

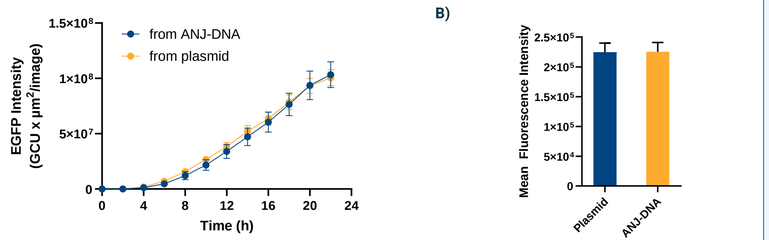

Figure 5: ANJ-DNA produced mRNA with potency comparable to one produced from the respective plasmid. 100 ng/well of mRNA were transfected into HEK293T (N=3). Incucyte SX5 was used to track EGFP intensity for 24 hours after transfection **(A)**, and flow cytometry to measure EGFP intensity at the 24 hours time point **(B)**.

Conclusions

- ANJ-DNA is a superior material for *in vitro* transcription compared to conventional plasmids
- It results in higher mRNA yields while producing functionally equivalent mRNA
- Our off-the-shelf synthetic DNA produce mRNA
  - with higher potency than commercially available mRNA from leaders in the field
  - with lower dsRNA than commercially available

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