

A Novel, Synthetic DNA for Cell and Gene Therapy Application

Alexander Pekarsky, Luca Distefano, Marco Guarrera, Ivana Pastierikova, David Wilson, Martin Cusack, Anna Krutyhołowa, Roxanne Lourman, Fabian Trick, Gustavo Lou, Andreia M Silva, Ileana Guerrini, Nicolas Meier, J. Omar Yáñez-Cuna, Joel de Beer Anjarium Biosciences AG, Wagistrasse 23, 8952 Schlieren, Switzerland

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 the broad versatility of our synthetic DNA demonstrating its use as input for mRNA, rAAV, and lentivirus vector production, as well as for use in transgene expression in vivo.

Our synthetic DNA is well-positioned to replace plasmid DNA as key starting material for cell and gene therapy modalities.

ANJ-DNA allows the use of different nature-inspired ITRs



Figure 1: ANJ-DNA can leverage from different natural hairpin-ended structures and synthetic ITRs inspired by nature. Shown is a schema of an ANJ-DNA with the gene expression cassette (in blue) flanked by hairpin-ended structures (top). The hairpin ended structures can be customized to mimic ITRs from different viruses.

DNA to Catalyze Your Advanced Therapies

Anjarium's Process Results in High-purity and High-fidelity DNA Product



Figure 2: Our one-pot, enzymatic and cell-free production process results in high-purity and high-fidelity product. Agarose gel electrophoresis of pxDNA ranging on different sizes, from 1.6 Kb (1) to 8.6 Kb (8). Ladder (L) is shown in the first well (A). Fidelity of our ANJ-DNA compared to plasmid as determined by PacBio-seq, where mismatch errors as rare as 1:100,000,000 (10⁻⁸) could be detected. The error rate expected from the plasmid is based in the literature (B).

ANJ-DNA is a Superior Alternative for mRNA Production



Figure 3: ANJ-DNA is a superior alternative for mRNA production compared to conventional plasmids. It results in higher mRNA yields as observed by measuring total mRNA yield after a 2 hours long IVT at 37°C with increasing amount of template followed by LiCI precipitation (A). Our off-the-shelf EGFP ANJ-DNA produce mRNA with a superior potency to commercial mRNA from leaders in the field. 100 ng/well of mRNA were transfected into HEK293T (N=3). mRNA potency was measured by flow cytometry at 24 hours time point (B)

ANJ-DNA Outperforms Plasmid for rAAV Manufacturing





Figure 4: ANJ-DNA is a superior alternative for rAAV production compared to conventional plasmids. Higher viral titer (as total viral genomes per mL) is produced from ANJ-DNA, as compared from plasmid for different serotypes (A). A single band is observed from the ANJ-DNA AAV prep compared to two bands from the plasmid AAV prep from viral genome, as shown in an agarose gel electrophoresis for AAV9 prep (0.5 L scale) suggesting the absence of packed backbone (B).

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

A) 2×10⁶ ₽^{1.5×10^{6.}}

A)

1000

Conclusions

- Our synthetic DNA proves to be a versatile input material for cell and gene therapy
- ANJ-DNA is a superior alternative as a starting material for AAV and mRNA production compared to conventional plasmid
- Our off-the-shelf ANJ-DNA produce mRNA with potency higher than commercial mRNA from leaders in the field
- Absence of packed backbone in the AAV produced from ANJ-DNA
- ANJ-DNA can be used for LVV production at a functional infectious titer comparable to plasmid

anjarium.com Visit us at ASGCT Booth #312



ANJ-DNA Produces High Functional Titer of Lentivirus Virus Vector



Figure 5: ANJ-DNA produces high functional titer of LVV. Infectious titer estimated by flow cytometry at 72 h after HEK293T cells transduction with a serial dilution of the LVV produced (A) Jurkat T-cells are stably transduced by LVV made from ANJ-DNA. To verify LVV genome integration in the host genome, transduced Jurkat cells were then cultured long-term and the estimation of LVV infectious titer was performed at day 17 post-transduction by flow cytometry **(B)**.

ANJ-DNA Drives Transgene Expression In Vivo



