A Novel, Synthetic DNA Alternative for Lentiviral Vectors Manufacturing

Andreia M Silva, Fabian Trick, David Wilson, Ivana Pastierikova, Luca Distefano, Marco Guarrera, Martin Cusack, Alexander Pekarsky, J. Omar Yáñez-Cuna, Joel de Beer

Anjarium Biosciences AG, Wagistrasse 23, 8952 Schlieren, Switzerland

Scan to visit our website

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.

Lentivirus vectors (LVV) are viral vehicles very effective in delivering transgenes up to 8-9 kb to target cells. They are key tools for the *ex vivo* generation of engineered cells for cell therapy applications, being currently the benchmark delivery vehicle for the generation of Chimeric Antigen Receptor (CAR) T-cells.

Here we show our synthetic DNA as a functional, cost- and time-effective alternative to plasmid DNA for LVV production.

Schema of ANJ-DNA designed for LVV production

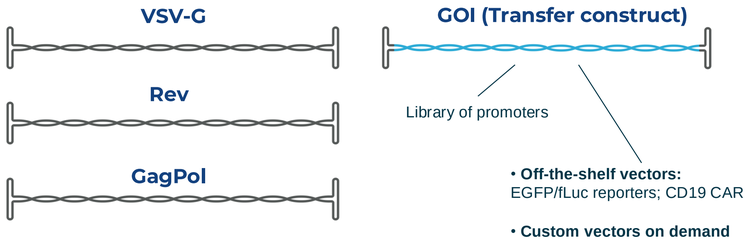
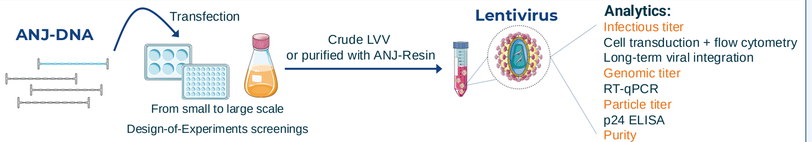


Figure 1: ANJ-DNA offering for the production of 3rd generation LVV. ANJ-DNA was designed to encode the three helper elements required for LVV production (Gag/Pol, Rev and VSV-G) as well as the transgene transfer vector encoding the required Gene-of-Interest (GOI). The four constructs can be customized 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 LVV production.

Pipeline for Production and Analysis of LVV



ANJ-Resin: see poster #1858

ANJ-DNA Produces High Functional Titer of LVV

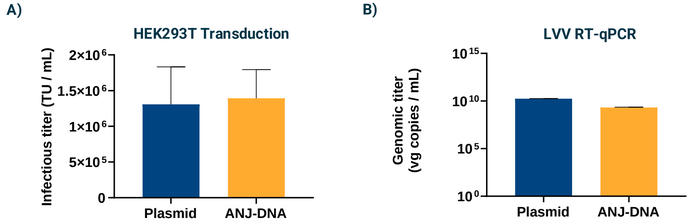


Figure 2: LVV produced with ANJ-DNA show a similar infectious titer to plasmid, while reducing the generation of non-functional virus. Infectious titer estimated by flow cytometry at 72 h after HEK293T cells transduction with a serial dilution of the LVV produced **(A)**. Quantification of LVV genome copies by RT-qPCR after viral RNA extraction **(B)**.

Platform Optimization Increases Titer of LVV Produced using ANJ-DNA

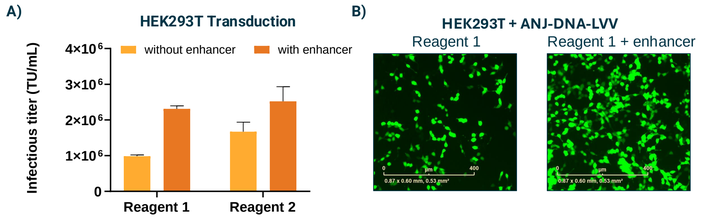
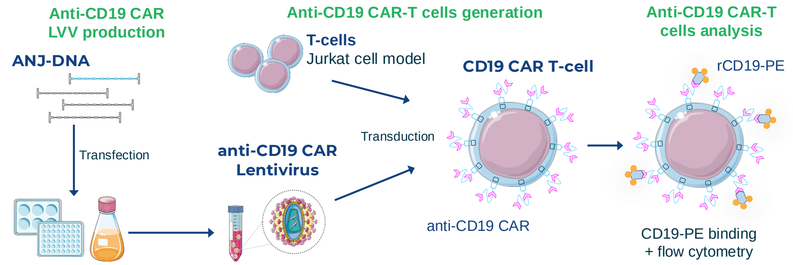


Figure 3. Infectious titer of LVV produced using ANJ-DNA is increased by optimizing transfection conditions that leverage its unique biochemical features. Exemplary infectious titer of EGFP-LVV produced by HEK293T cells transient transfection using commercially available transfection reagents 1 (GMP-compatible) and 2, with or without LVV production enhancers **(A)**. Microscopy images depicting EGFP expression in HEK293T cells transduced with EGFP-LVV produced using transfection reagent 1, in the presence/absence of LVV production enhancers. Scale bar: 400 µm.

ANJ-DNA Application for Production of CAR-T Cells by LVV Transduction



ANJ-DNA Produces LVV Encoding CD19 CAR which Efficiently Transduce Jurkat T-Cells

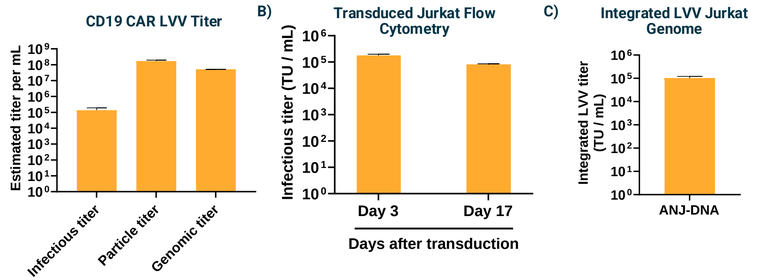


Figure 4: Stable CD19-CAR T cells can be generated from LVV produced from ANJ-DNA. LVV were produced from ANJ-DNA, and then used for transduction of Jurkat T-cell model cell line. LVV titer per mL were estimated as "Infectious titer" (TU/mL), "Particle titer" (LVV particle number/mL) assessed by p24 ELISA in LVV isolates, and "Genomic titer" by RT-qPCR in LVV isolates **(A)**. 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)**, and integrated LVV into the Jurkat genome was quantified by qPCR **(C)**. LVV produced with ANJ-DNA are fully functional, being capable of stably integrating in the genome of host cells.

Conclusions

- ANJ-DNA can be used for Lentiviral vector production at a functional infectious titer in the same range as plasmid. The advantages on high purity, scalability, speed, and flexibility, makes ANJ-DNA a superior material for LVV production
- ANJ-DNA can be used for the production of LVV encoding CD19 CAR, for the generation of CAR T-cells
- Optimization of the ANJ-DNA, and production pipeline, deliver DNA vectors that produce LVV with increasing functionality.

DNA to Catalyze Your Advanced Therapies

anjarium.com

Visit us at ASGCT Booth #312

ANJARIUM BIOSCIENCES