Enabling CDMOs to Fast-Track mRNA Discovery with ENFINIA™ IVT Ready DNA

Cell-free, linear IVT ready DNA eliminates plasmid DNA to enable more design flexibility and and faster screening cycles

At-a-Glance

Vernal Biosciences, a CDMO for mRNA therapies, evaluated **ENFINIA™ IVT Ready DNA** as an alternative to plasmid templates. Manufacturing manager Max Mashrick assessed its quality for manufacturing workflows that involve screening and optimizing mRNA candidates.

The Challenge

Vernal's customers need rapid mRNA discovery for vaccines, protein therapies, and cancer treatments. But plasmid templates, reliant on bacterial cloning, can take a month to prepare, slowing high-throughput automation.

The Solution

Elegen's ENFINIA IVT Ready DNA, with poly(A) tail and sequence flexibility, matched plasmid templates in mRNA yield and quality but took just days to produce, automation-ready, to speed mRNA discovery.

"Time for getting DNA templates in a ready-to-use format is crucial for the discovery phase. Templates that can cover a wide range of sequences and arrive in less than 4 weeks in a 96-well format that is automation friendly will expedite the process."

- **Max Mashrick,** Manufacturing Manager, Vernal Biosciences

As Fast as 10 Business Days

Cell-Free, Reaction-Ready Format

Test Broad Sequence Complexities

INTRODUCTION

Vernal Biosciences (Vernal) is a fully integrated CDMO focused on delivering high-purity mRNA and LNP-formulated mRNA throughout the scaling of manufacturing processes. Vernal provides research, development, and phase-appropriate cGMP manufacturing, providing turnkey platform solutions for mRNA-based drug substance and drug product manufacturing.

For clients in the discovery stage, Vernal produces initial screening libraries and individual sequences for hit validation. The production of template DNA is often a bottleneck in IVT-mediated mRNA synthesis, causing customers to wait weeks or longer to validate hits.

To shorten the time from screen to validation of a lead candidate, Vernal evaluated Elegen's **ENFINIA IVT Ready DNA** templates. ENFINIA IVT Ready DNA is produced without ever touching cells and ships NGS-verified in a linear format with an encoded poly(A) tail from 70 - 130 bp in as few as 10 business days. Additionally, ENFINIA IVT Ready DNA is delivered in an automation-friendly microplate for immediate use on high-throughput robotic systems.

ACCELERATING DNA TEMPLATE SYNTHESIS

Conventional plasmid-derived templates present several challenges. Selection and maintenance of the appropriate bacterial strain for plasmid propagation is not trivial and its performance is often dependent on the target gene sequence. Maintaining a stable poly(A) tail requires rigorous QC to identify a correct, sequence-perfect clone. As projects advance toward GMP production, quality requirements become increasingly stringent, necessitating extensive purification and QC to remove residual plasmid DNA (pDNA), endotoxin, and other safety-related impurities to minimize bioburden.

ENFINIA IVT Ready DNA is a linear, double-stranded DNA template with an encoded poly(A) tail, designed for immediate and reliable IVT-mediated mRNA production. Produced with Elegen's proprietary cell-free technology, it accommodates complex sequences while eliminating the cloning, purification, linearization, and amplification steps required with plasmids. ENFINIA templates help avoid risks of endotoxins, bacterial DNA, and antibiotic residues.

The linear DNA templates are available with customizable poly(A) tails (A70–A130 or segmented). Each sequence is NGS-verified and can ship in as few as 10 business days, accelerating design and testing cycles.

Study Objectives

Vernal conducted a study aimed at making an initial assessment of Elegen's ENFINIA IVT Ready DNA as a potential alternative source of high-quality linear DNA templates. mRNAs produced using standard Vernal linearized pDNA or ENFINIA IVT Ready DNA were evaluated for process yield and for several critical quality attributes, allowing a direct comparison between the two template types. Additionally, this study validated the suitability of ENFINIA IVT Ready DNA for mRNA production using Vernal's established processes and platforms.

Study Design and Experimental Details

ENFINIA IVT Ready DNA templates were synthesized by Elegen per Vernal's design. The sequences encoded three standard complexity ORFs spanning lengths of 1 - 4.6 kb, each with an A130 poly(A) tail. For comparison, an identical Firefly luciferase (fLuc) gene was cloned into a plasmid by Vernal, and was used as a linearized plasmid template control. IVT-ready and linearized plasmid templates were used in Vernal's proprietary IVT and purification methods. The template concentrations were adjusted according to the capping procedure used ***(Table 1)***.

For each reaction, the mRNA yield was measured post-purification using UV spectrophotometry (A260). The resulting products were then tested for multiple quality attributes:

- mRNA integrity: assessed by capillary gel electrophoresis on the Agilent 5200 Fragment Analyzer using the Agilent DNF-471-55-SS Total RNA Kit with a 15 nt marker and 200-6000 nt ladder.
- dsRNA content: assessed by the Yeasen Biotechnology Double-stranded RNA ELISA kit with the included N1-Me-pUTP dsRNA standard.
- 5′ cap characterization: capping efficiency measured by IP-RP-UPLC with UV and MS detection.
- Poly(A) integrity: Poly(A) tail length measured by enzymatic digest and IP-RP-UPLC with MS detection.

Results

mRNA yield from **ENFINIA IVT Ready DNA** templates was comparable to that of linearized plasmid DNA templates. Results as calculated based on initial DNA template input and post-purification mRNA yield are presented in ***Table 1***. Yield variation between different genes is expected as of the different sequence attributes and and its length.

To ensure safety and efficacy, clinical translation of mRNA substances and products requires high purity, consistency and rigorous characterization of key quality attributes such as identity, integrity, and stability.[1,2] As shown in Table 1, all ENFINIA IVT Ready DNA templates show comparable performance to Vernal's plasmid-derived control.

Table 1. Summary of DNA templates performance and mRNA quality attributes

	Supplier			
	Elegen		Vernal	
Gene	**mCherry**	**Cas9**	**fLuc**	**fLuc**
ORF Size (bp)	1,056	4,565	1,989	1,989
Template type	Linear	Linear	Linear	Plasmid
Capping process	Co-transcriptional	Enzymatic	Enzymatic	Enzymatic
IVT Total Process Recovery (mole RNA/mole template)	238	82.6	190	196
% dsRNA	0.068	0.083	0.025	0.015
% Cap	98.4	92.2	100	100
Poly(A) Target Length (bp)	130	130	130	130
Average Tail Length* (nt)	136	138	138	129

(*) Based on intensity weighted average

Measurement of mRNA integrity displayed a singular peak at the expected size for all samples, representing over 85% of all measured molecules. These results demonstrate comparable mRNA integrity for ENFINIA IVT Ready DNA and linearized plasmid templates ***(Figure 1)***.

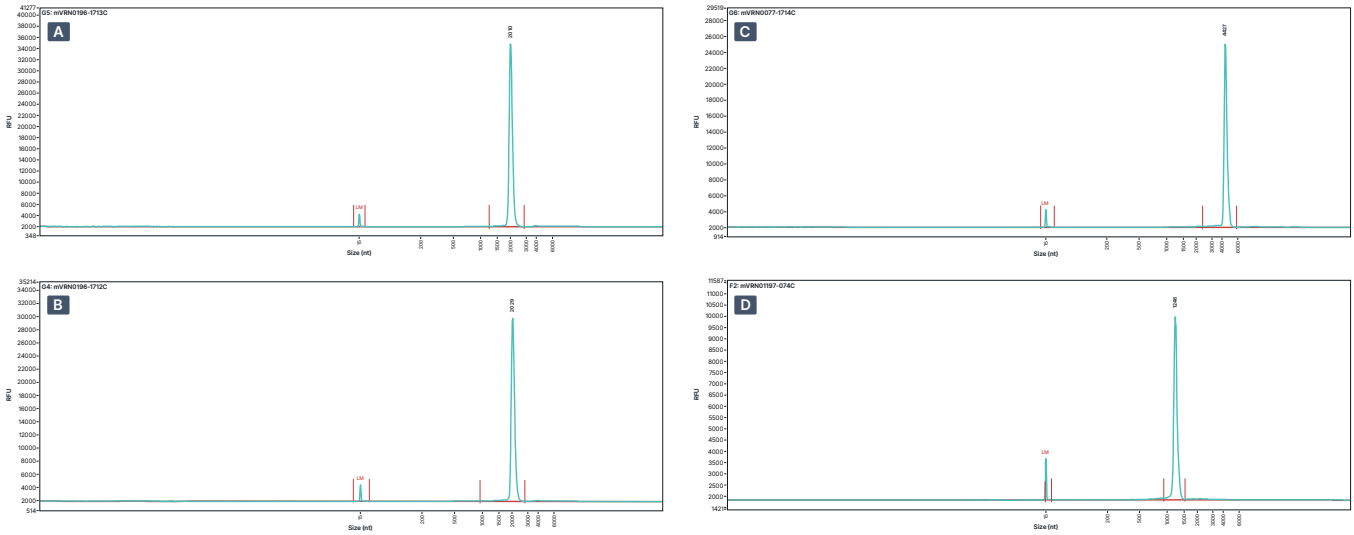

Figure 1. mRNA integrity of four IVT reaction-purified products as determined via capillary gel electrophoresis using the Agilent 5200 Fragment Analyzer and Agilent DNF-471-55-SS Total RNA Kit with a 15 nt marker (Low Marker, LM) and 200-6000 nt ladder.

A. mRNA encoding for fLuc protein, transcribed from a linearized plasmid template. **B.** mRNA encoding for fLuc protein, transcribed from an ENFINIA IVT Ready DNA template. Expected fLuc size: ~2 kb. **C.** mRNA encoding for Cas9, transcribed from an ENFINIA IVT Ready DNA template. Expected Cas9 size: ~4.6 kb. **D.** mRNA encoding for mCherry protein, transcribed from an ENFINIA IVT Ready DNA template. Expected size: ~1 kb. All DNA templates were designed with an A130 poly(A) tail.

Assessment of mRNA quality demonstrates comparable transcription of both ENFINIA IVT Ready DNA and linearized plasmid templates. The measurement of dsRNA content was below the 0.1% threshold for all samples. Capping efficiencies were also above the target threshold for all samples. Additionally, no major differences were observed between the different capping methods.

The mRNA 3′ poly(A) tail is vital for efficient translation and stability *in vivo*.[3] The poly(A) tail protects the mRNA from exonucleolytic degradation, enhancing transcript stability and thereby supporting higher protein expression.[4] Overall, the tails of all samples were comparable and within +/- 10% of the target length. The distribution bias towards higher length products rather than the encoded poly(A) length is presumably a result of the well-described transcription slippage of the T7 RNA polymerase.[5]

SUMMARY

Vernal Biosciences evaluated a range of sequence designs of varying lengths with encoded A130 poly(A) tails using **ENFINIA IVT Ready DNA**. Across designs, ENFINIA templates demonstrated IVT performance comparable to their traditional plasmid-based workflows, yielding high-quality mRNA.

To accelerate scale-out, ENFINIA IVT Ready DNA allows many unique sequences to be produced and delivered in parallel. This enables multiplexing of hundreds of constructs within a single order, all NGS-verified and ready in as few as 10 business days. Consistent yields across constructs also make it simple to normalize samples in 96- or 384-well plates, streamlining automated workflows.

With growing demand to scale and accelerate mRNA production for therapeutic applications, Vernal has successfully validated **ENFINIA IVT Ready DNA** as a rapid, high-performance alternative to their conventional plasmid-derived DNA. The range of sequence complexity, rapid turnaround, and automation-friendly format are well-suited for Vernal's high throughput manufacturing processes for mRNA discovery.

Acknowledgements

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