ELEGEN

ENFINIA™ IVT Ready DNA:

High-Quality Templates for Improved mRNA Yield and Purity

INTRODUCTION

ENFINIA IVT Ready DNA is a linear, double-stranded DNA template with an encoded poly(A) tail for fast, reliable mRNA production via *in vitro* transcription (IVT). Manufactured using Elegen's proprietary cell-free technology, it supports complex sequences and eliminates the cloning, purification, and linearization or amplification steps associated with plasmid-based workflows. Early customer testing has demonstrated yield, purity, and integrity results equivalent to or better than plasmid-derived templates, making ENFINIA templates a robust and scalable alternative.

ENFINIA templates are free from endotoxins, bacterial DNA, and antibiotic residues, and are available with customizable poly(A) tails (A70–A130 or segmented). ENFINIA templates ship NGS-verified in as few as 10 business days, enabling faster design and testing cycles.

Proof of Concept:

Linear- vs. Plasmid-Derived Templates

OVERVIEW

ENFINIA IVT Ready DNA templates were produced and evaluated by Elegen in comparison to templates produced by a market-leading gene synthesis supplier.

An ENFINIA template encoding the mCherry reporter gene (708 bp ORF), was synthesized with both A90 and A130 poly(A) tails. For comparison, a template for the same gene was synthesized by Supplier A using traditional plasmid cloning and was linearized before use. Both templates were transcribed using the **MEGAscript™ T7 Transcription Kit** (Thermo Fisher). The template's poly(A) tail length and distribution, mRNA yield, and mRNA size and distribution were measured using PacBio sequencing, Nanodrop spectrophotometer, and Agilent Bioanalyzer, respectively.

RESULTS

Higher mRNA Yield and Purity Using mCherry IVT Ready DNA

PacBio sequencing results of ENFINIA IVT Ready and linearized plasmid DNA templates demonstrated comparable poly(A) tail length and size distribution. However, a higher frequency of molecules with shorter poly(A) tails was observed from linearized plasmids, particularly in the A130 sample ***(Figure 1)***.

Transcription of ENFINIA IVT Ready DNA templates resulted in higher mRNA yield in comparison to linearized plasmid templates using the same starting mass ***(Figure 2)***. This outcome likely results from a higher molarity of the ENFINIA template, which includes only the gene of interest and the necessary elements for transcription.

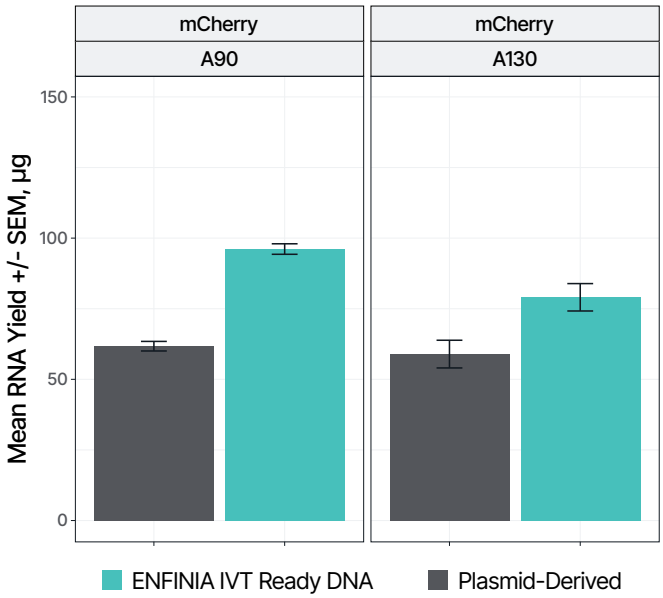


Figure 2. IVT reactions using ENFINIA IVT Ready DNA yielded higher mRNA levels compared to linearized plasmid templates at an input of 500 ng DNA. Bars represent mean ± SEM, n = 6.

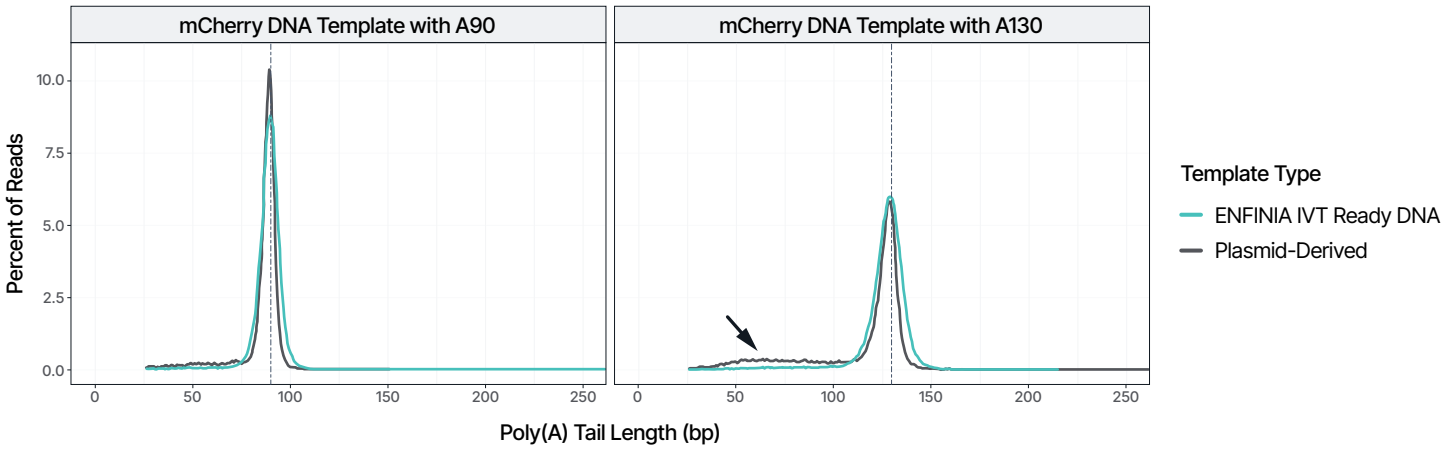


Figure 1. Poly(A) Tail Length Distribution in ENFINIA IVT Ready DNA vs. Linearized Plasmid DNA. PacBio sequencing revealed that both template types showed poly(A) tail length distributions centered around the expected size (dashed line), with comparable polydispersity. However, linearized plasmid templates, particularly the A130 sample, exhibited a higher proportion of molecules with truncated (shorter) poly(A) tails.

Transcribed ENFINIA IVT Ready DNA templates resulted in mRNA with both poly(A) tails (90 and 130 nts), with comparable or higher integrity than mRNA transcribed from linearized plasmid templates ***(Figure 3)***. The purity of the mRNA (A260/A280, A260/A230 ratios) was within the expected range.

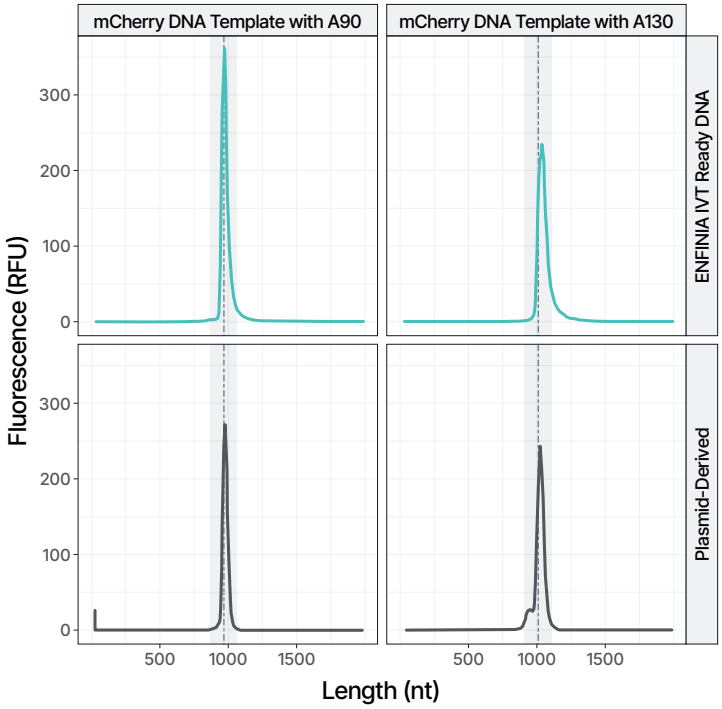


Figure 3. Representative Agilent Bioanalyzer traces of mRNA from IVT reactions using mCherry templates with 90 bp and 130 bp poly(A) tails. Traces show expected product size (dashed line; shaded box ±10%) and distribution. ENFINIA IVT Ready DNA integrity is comparable to or exceeds those from linearized plasmids.

Customer Validation:
ENFINIA IVT Ready DNA

OVERVIEW

Collaborator-designed templates, ranging from 1 to 5.1 kb with varying degrees of complexity, were synthesized as part of the ENFINIA IVT Ready DNA Early Access Program. All sequences included regulatory elements and poly(A) tails (continuous or segmented) and passed QC tests performed by Elegen for yield, sequence fidelity, DNA side products, and poly(A) tail length and distribution. Final samples were dried before shipment.

Collaborators performed IVT reactions using their own reagents and protocols, often including linearized or PCR-amplified plasmids as controls. mRNA products were evaluated for yield, integrity, and purity.

RESULTS

Collaborator Data Demonstrate High-Quality mRNA Products

The example results below were reported by Collaborator A using ENFINIA templates to encode a base editor for a gene editing therapeutic application. ENFINIA templates with gene sizes ranging from 4.9 to 5.1 kb, featuring continuous poly(A) tails of 130 nucleotides were used in IVT reactions and compared to mRNA produced from the collaborator's standard method.

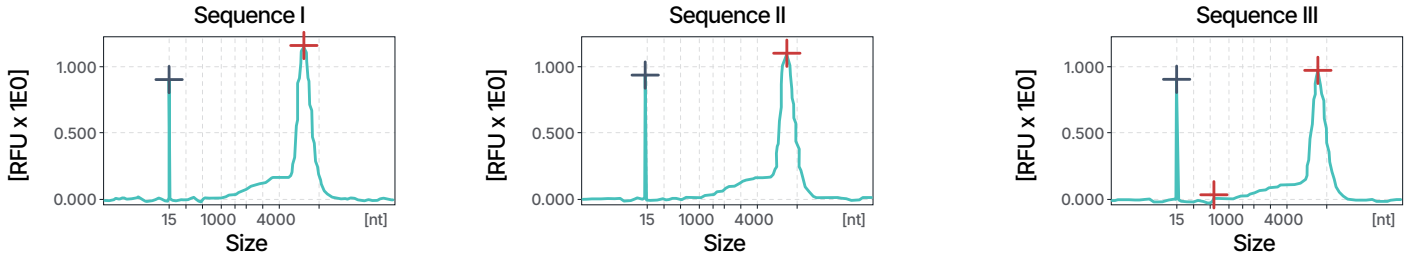
Table 1 summarizes the observations of Collaborator A.

Capillary electrophoresis analysis of mRNA from ENFINIA IVT Ready template and plasmid-derived templates (amplification and poly(A) tailing) indicated more side products from the plasmid-derived templates ***(Figure 4)***.

Table 1: DNA and mRNA Quality Assessments from Collaborator A

Criteria	Outcome
DNA template yield is a minimum of 5 µg	✓
DNA template purity is confirmed by spectrophotometric analysis (A260/A280 and A260/A230 ratios)	✓
Post-IVT mRNA concentration is comparable to or better than plasmid-derived templates	✓
Post-IVT mRNA is pure as measured by spectrophotometer (A260/A280 and A260/A230 ratios)	✓
Post-IVT mRNA has high integrity as measured by capillary electrophoresis or on an agarose gel	✓

ENFINIA™ IVT Ready DNA



Collaborator's Control: Amplified Plasmid Template

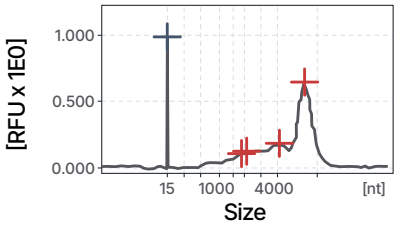


Figure 4. Higher mRNA integrity observed with ENFINIA IVT Ready DNA templates (characterization by Collaborator A). IVT products were analyzed using QIAxcel RNA QC capillary gel. ENFINIA template sequences 1 and 2 produced clean mRNA profiles with no notable secondary products. Sequence 3 shows a minor secondary product (~600 nts). In contrast, the amplified plasmid control displays multiple secondary products. For all studies: n = 3, representative traces shown, gray "+" indicates size marker, red "+" indicates detected peak.

An *ex vivo* study was performed to evaluate the functionality of mRNA encoding an adenine base editor in primary human fibroblasts, targeting a disease-associated SNP. Using the same guide RNA, the editing efficiency of base editors synthesized from ENFINIA IVT Ready DNA was compared to that of plasmid-derived templates. Base editors from ENFINIA templates achieved ~74% on-target editing, about ~8% higher than the plasmid-derived template, without sorting or selection *(Figure 5)*.

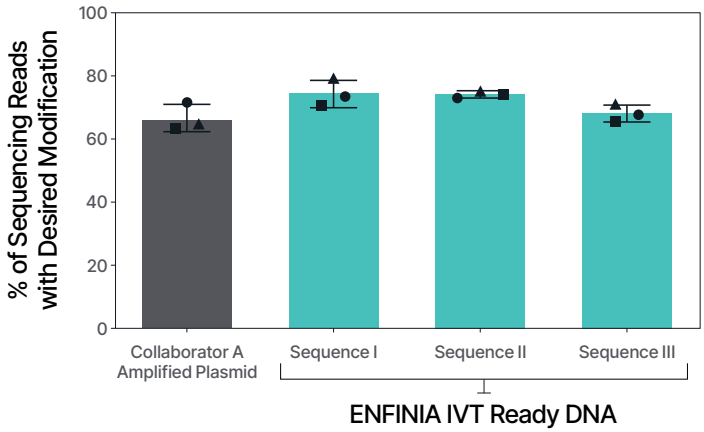


Figure 5. mRNA derived from ENFINIA IVT Ready DNA templates demonstrated high potency and slightly higher functionality than plasmid-derived templates.

All ENFINIA IVT ready DNA templates had the same ORF sequence, but had different UTRs. Collaborator sequence and ENFINIA Sequence I had identical UTRs.

For all experiments n = 3, cell type: human fibroblasts, editing strategy: ABE8 Adenine base editor and SpRY nuclease, editing analysis: 72h post-electroporation through sequencing using Illumina Miseq.

SUMMARY

A range of sequence designs—spanning various lengths, complexities, and poly(A) tail configurations—was evaluated using ENFINIA™ IVT Ready DNA templates. Both in-house and early collaborator data show that ENFINIA templates deliver comparable or superior IVT performance relative to traditional plasmid-based workflows, yielding high-quality mRNA and functional protein.

Across IVT methods, ENFINIA consistently produced equal or higher mRNA yields per template mass, likely due to its design, which encodes only the gene of interest and essential regulatory elements. The resulting mRNA also showed improved or comparable integrity and purity. An initial *ex vivo* gene editing experiment demonstrated that mRNA from ENFINIA templates is functionally active, with potency comparable to or superior to mRNA produced from plasmid-amplified templates.

Overall, initial findings support the use of ENFINIA IVT Ready DNA as a high-quality, reliable, and faster alternative to plasmid-derived templates—accelerating mRNA discovery, optimization, and scale-up.