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 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.

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.

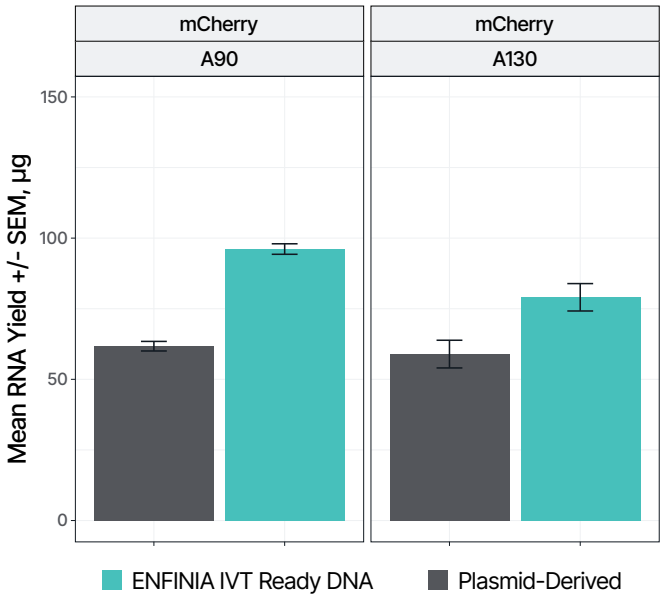

Figure 2. IVT reactions using ENFINIA IVT Ready DNA yielded higher mRNA levels compared to linearized plasmid templates at an input of 500 ng DNA. Bars represent mean ± SEM, n = 6.

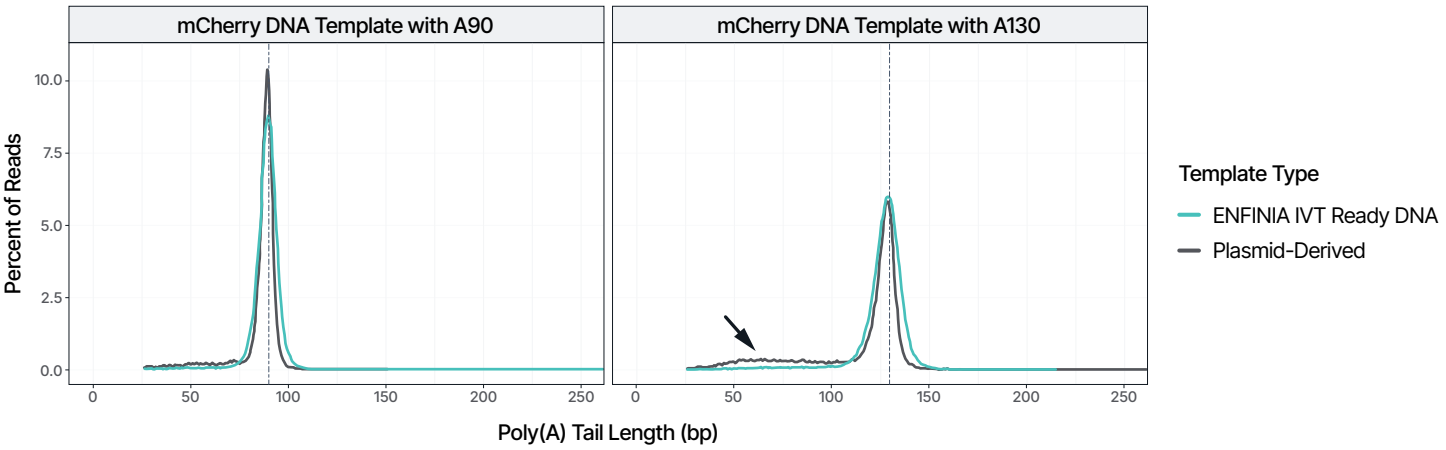

Figure 1. Poly(A) Tail Length Distribution in ENFINIA IVT Ready DNA vs. Linearized Plasmid DNA. PacBio sequencing revealed that both template types showed poly(A) tail length distributions centered around the expected size (dashed line), with comparable polydispersity. However, linearized plasmid templates, 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.

SUMMARY

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

Across IVT methods, ENFINIA Templates 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.

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.

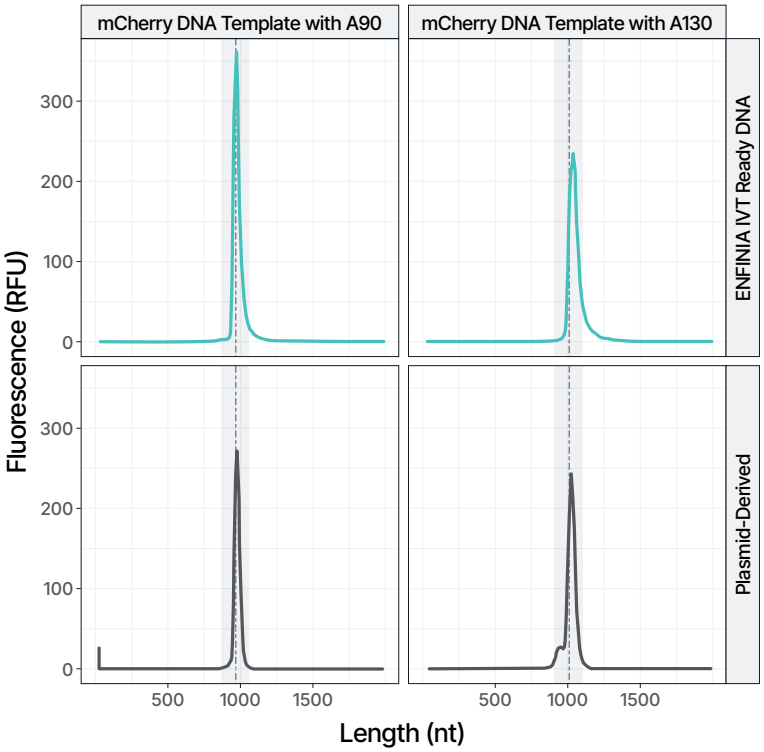

Figure 3. Representative Agilent Bioanalyzer traces of mRNA from IVT reactions using mCherry templates with 90 bp and 130 bp poly(A) tails. Traces show expected product size (dashed line; shaded box ±10%) and distribution. ENFINIA IVT Ready DNA integrity is comparable to or exceeds those from linearized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