Enzymatically Produced z-dbDNA™ Templates Enable Efficient In Vitro Transcription with Reduced DNA input and Lower Double-Stranded RNA Impurity Compared to pDNA Templates

TOUCHLIGHT
DNA is our DNA™

Bhavana Kayvar PhD , Lisa Caproni PhD, Mitchell Brun PhD, Peter Archibald PhD, Milica Vukovic PhD

INTRODUCTION

Advancements in synthetic DNA technologies are transforming the landscape of cell and gene therapies. Plasmid DNA (pDNA) is widely used as a template for in vitro transcription (IVT) in RNA manufacturing; however, conventional pDNA production can limit manufacturing speed, scalability, and flexibility. Enzymatically produced z-dbDNA™ is a linear, double-stranded DNA molecule that lacks bacterial backbone sequences and represents an alternative DNA template for IVT applications. Here, we evaluate z-dbDNA templates and compare their performance against traditional linearised pDNA.

Plasmid DNA Challenges

- Transition from R&D to GMP is often slow and complex
- Common Bottlenecks
  - Inefficient workflows
  - Regulatory burdens
  - Scalability issues
  - Reproducibility

Synthetic DNA Solutions

- **Eliminates Complex Workflows**
- **Scalability without Bottlenecks**
- **Accelerates Time-to-GMP**
- **Higher Purity, Lower Risk**
- **Cost-Effective and Sustainable**

RESULTS

z-dbDNA templates reduced DNA input requirements while maintaining equivalent or higher IVT yields. Across multiple sequences, RNA integrity was comparable to pDNA-derived RNA. Poly(A) tail length and uniformity were preserved. z-dbDNA also offers the potential for reduced dsRNA and other process-related impurities across mRNA and saRNA modalities, enabling greater design flexibility than plasmid templates.

CONCLUSION

z-dbDNA serves as an effective and scalable IVT template that reduces DNA input and dsRNA impurity while enabling transcription of ***multiple RNA modalities including mRNA, saRNA and circRNA,*** addressing key limitations of plasmid-derived DNA in RNA manufacturing.

DNA quality: pA tail preserved in z-dbDNA

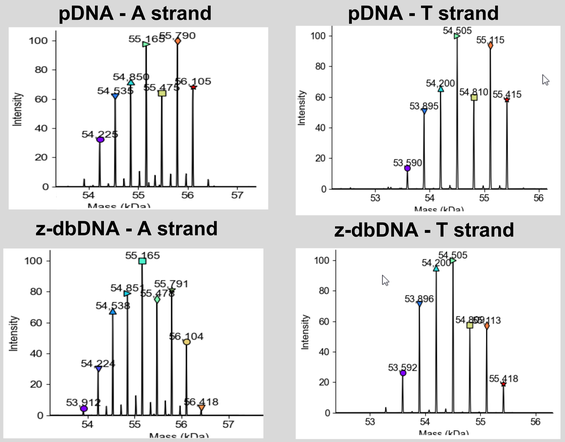


Figure 1: Mass spectrograms *of pA tail in DNA template: A strand (pA tail, left) and T-strand (right) from pDNA and z-dbDNA. pA tail size and distribution preserved from plasmid to z-dbDNA.*

RNA quality: High purity and integrity

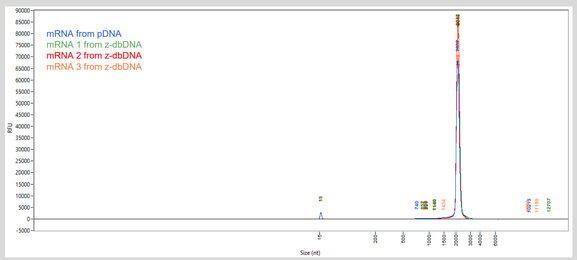


Figure 2: *Overlaid* ***capillary electrophoresis*** *of mRNA from pDNA or z-dbDNA. Aligned peaks at expected size with comparable distribution and no extra fragments confirm RNA integrity.*

RNA quality: Intact polyA

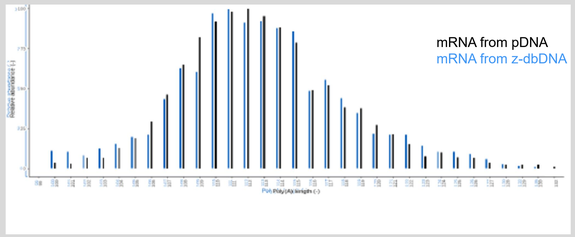


Figure 3: *Overlayed* ***mass spectrometry*** *profiles of poly(A) tails from in vitro–transcribed mRNA generated using pDNA (black) or z-dbDNA (blue) templates. The distributions show highly similar poly(A) tail lengths and overall abundance profiles across the measured range.*

RNA quality: Reduced dsRNA profile

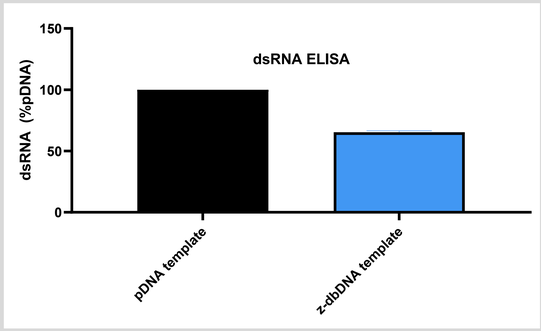


Figure 4*: dsRNA levels quantified by sandwich ELISA, shown relative to pDNA-templated reactions. IVT used wild-type T7 RNAP with unmodified NTPs (manufacturer conditions). z-dbDNA-templated mRNA trended toward lower dsRNA levels than pDNA-templated mRNA.*

RNA potency: Comparable translation

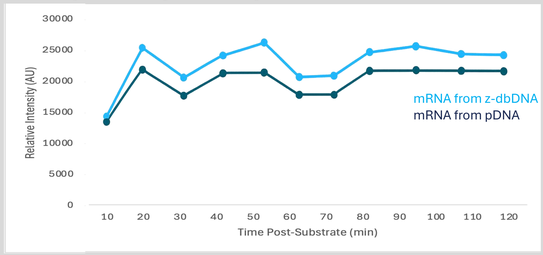
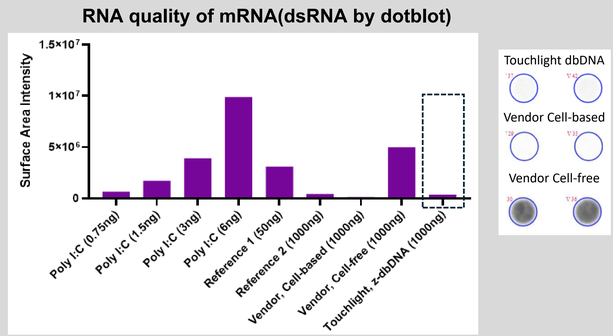


Figure 5: *In vitro translation of mRNA from pDNA (midnight blue) vs z-dbDNA (light blue) templates. Protein expression tracked over time as relative band intensity. z-dbDNA-derived mRNA yielded higher expression than pDNA.*

Client testimonials

Reduced dsRNA vs alternative cell-free DNA

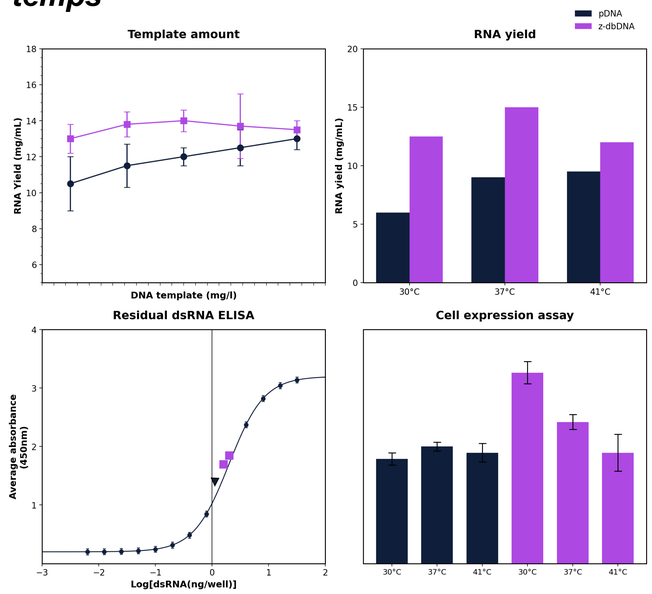


Client case study 1*: mRNA from Touchlight's z-dbDNA showed low dsRNA, substantially below another vendor's cell-free DNA template. Dot-blot images (right) confirm the quantitative data.*

High quality mRNA at 70% reduced template input

Client case study 2*: mRNA from z-dbDNA (using 70% less template), PCR amplicon, or pDNA assessed for integrity and dsRNA. Gel showed intact mRNA from z-dbDNA and pDNA; slightly reduced from PCR. Dot-blot (right): no detectable dsRNA from z-dbDNA, lower than PCR amplicon.*

Increased saRNA yield across three IVT temps



Client case study 3: *saRNA IVT optimisations with pDNA and z-dbDNA — template titrations (25–125 mg/L) at 30, 37, 41 °C. z-dbDNA yielded 1.5 × higher RNA; minimal dsRNA from either template. z-dbDNA-derived saRNA potency exceeded pDNA-templated IVT, dependent on reaction temperature.*

ACKNOWLEDGEMENTS: *Mass spectrometry data generated by Prof Mark Dickman, School of Chemical, Materials and Biological Engineering, University of Sheffield*
Capillary electrophoresis (Figure 2) and in vitro protein translation data (Figure 5) generated by RNA Innovation Centre, Johns Hopkins University