Comparison of modified T7 RNAPs to develop a simplified, cost effective, robust mRNA production process

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INTRODUCTION

In vitro transcription (IVT) reaction for synthesis of mRNA is commonly performed by adding T7 RNA polymerase (RNAP) and a DNA template. Although the reaction is very efficient, T7 RNAP generates additional immunostimulatory dsRNA impurities – abortive, antisense, and loop-back dsRNAs during the transcription initiation and termination.

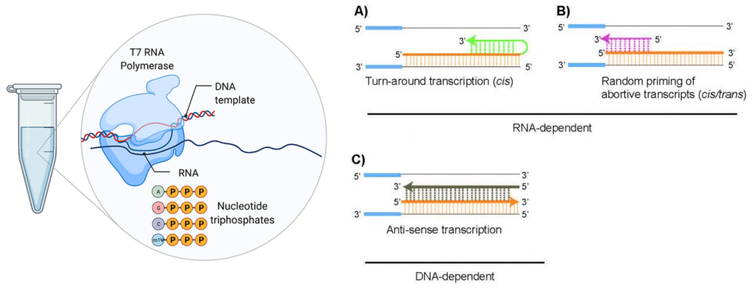
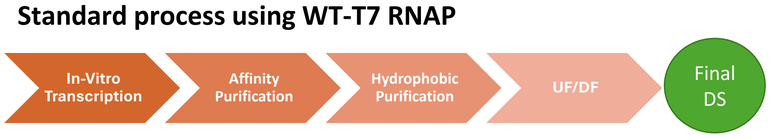


Figure 1. Mechanism of in vitro transcription and dsRNA byproduct formation [1,2].

Several downstream processes such as cellulose-based purification and reverse-phase column chromatography have been developed for removal of dsRNA [3]; however, they are costly, time-consuming, or require special operational environments. To address this challenge, we evaluated different components and conditions of the IVT reaction including the T7 polymerase to gain insights on how to produce mRNA with low dsRNAs.

Standard process using WT-T7 RNAP



Simplified process using modified T7 RNAP

Figure 2. Standard vs simplified purification methods

The standard process uses wildtype (WT)-T7, however the simplified process with the modified T7 RNAP does not require the polishing step for removal of dsRNA. The simplified process is advantageous as the mRNA can be made quicker, does not use organic solvents, and the mRNA contains fewer impurities.

REFERENCES

1. Nance KD. Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. ACS Cent Sci. 2021.
2. New England Biolabs. Understanding and Overcoming the Immune Response from Synthetic mRNAs. Genetic 2. Engineering and Biotechnology News. 2019.
3. Zhang J. Recent Advances and Innovations in the Preparation and Purification of In Vitro-Transcribed-mRNA-Based Molecules. Pharmaceutics. 2023.

RESULTS

Evaluation of mRNAs produced using four T7 RNAPs and different DNA templates

Target mRNAs of varying sizes were produced in individual IVT mixtures with four different T7 RNAPs (WT-T7 and three modified variants: M1-T7, M2-T7, and M3-T7). The mRNAs were purified using the simplified process which omitted the dsRNA polishing step. HPLC was employed to assess the yield of the synthesized mRNAs, and CGE was used to evaluate the integrity of the fully purified mRNAs. The results demonstrated that all four T7 RNAPs produced mRNA with high integrity. Among the different polymerases, M2-T7 produced moderate yields, M3-T7 achieved high yields comparable to those obtained with WT-T7 RNAP, and M1-T7 yielded significantly lower amounts of mRNA for Construct 1.

Table 1. Yield and integrity of mRNAs produced using different T7 RNAP

mRNA	Construct 1 (~700 nt)				Construct 2 (~2000 nt)				Construct 3 (~4000 nt)			
RNAP	WT-T7	M1-T7	M2-T7	M3-T7	WT-T7	M1-T7	M2-T7	M3-T7	WT-T7	M1-T7	M2-T7	M3-T7
Yield%	84%	19%	69%	99%	89%	88%	77%	114%	105%	95%	56%	90%
Integrity	100%	100%	100%	99%	99%	98.8	96%	100%	93%	96%	87%	93%

Figure 3. CGE results for Construct 2 mRNA produced using WT-T7 and modified T7 RNAPs. All T7 RNAPs produced mRNAs for Construct 2 with high integrity (all about 2000 nt in size).

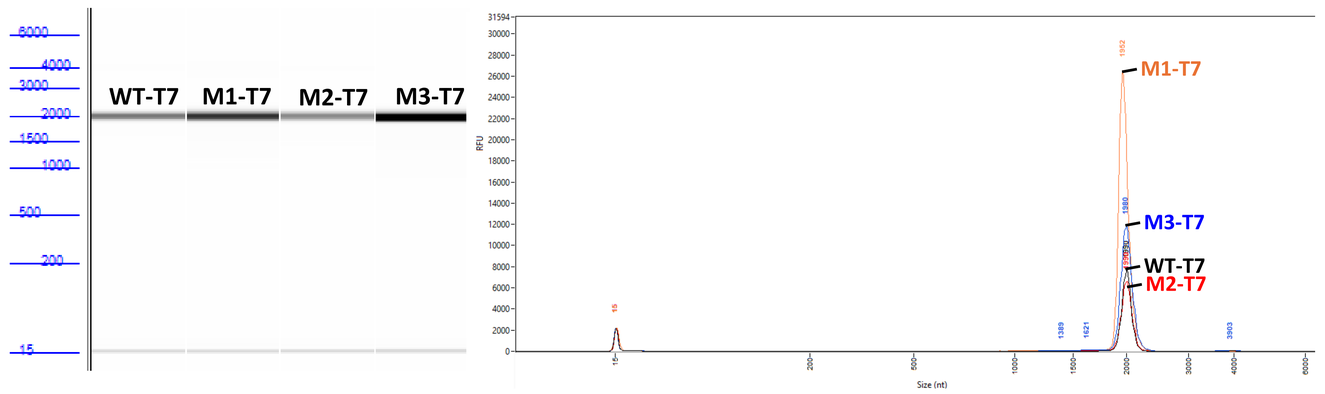
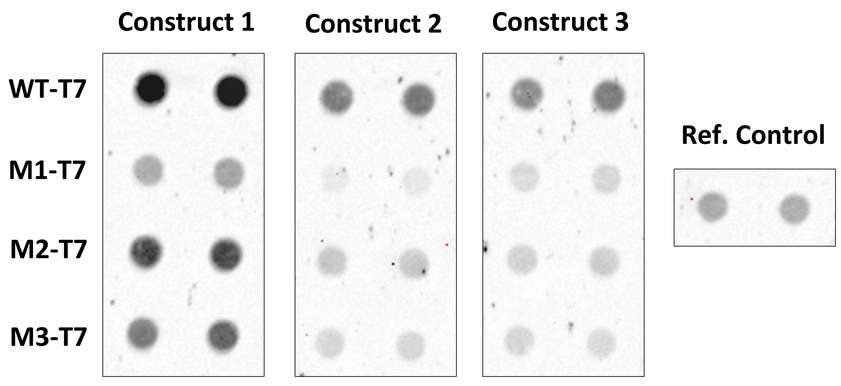


Figure 4. dsRNA impurities in mRNAs produced using different T7 RNAPs

The mRNAs produced using different RNAPs were purified using the simplified purification process without the dsRNA removal polishing step. A representative dot-blot, using the J2 antibody, was performed to detect residual dsRNA. Compared to the WT-T7, a significant reduction of residual dsRNA was observed in the mRNAs produced using the modified T7 RNAPs.



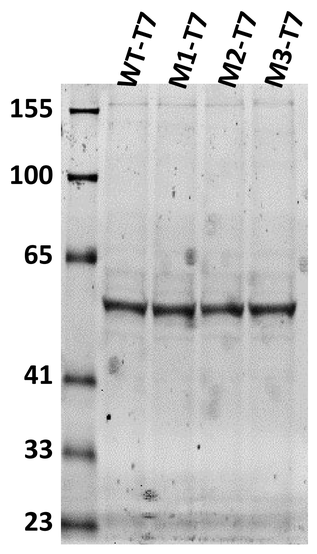


Figure 5. Functionality and expression analysis

Construct 2 mRNA synthesized with various T7 RNAPs were evaluated in an in vitro translation system and the resulting protein products run on SDS-PAGE. Similar expression and band intensities for all four translated mRNAs (~60kD) were observed for the different T7 RNAPs.

CONCLUSIONS

We have demonstrated that a simplified mRNA production process using modified T7 RNAPs can be used to reduce dsRNA in the final mRNA products. Modified T7 RNAPs can be used to enhance the purity of the mRNA while maintaining high functional quality, making them a valuable alternative to WT-T7 RNAP for mRNA synthesis.