Engineered RNA Polymerases for Improved Yield, Integrity, Capping, and dsRNA Control in IVT

primrose bio®

Learn more about Prima RNApols®

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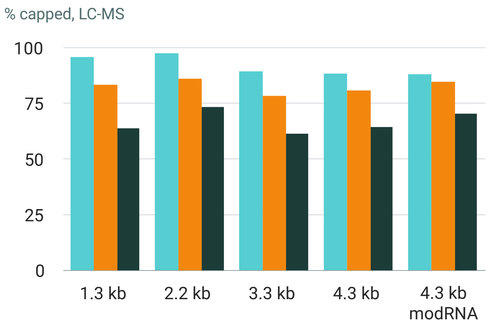
ABSTRACT

RNA therapeutics are rapidly expanding across a wide range of applications, including oncology, infectious diseases, rare diseases, cardiovascular disease, and autoimmune diseases. Central to RNA manufacturing is the *in vitro* transcription (IVT), a method that uses RNA polymerases to synthesize RNA. To reduce manufacturing costs and unwanted immunostimulatory products, Primrose Bio developed the Prima RNApols®, a portfolio of engineered RNA polymerases, designed to integrate effortlessly into early and late-stage mRNA workflows. The Prima RNA polymerases are designed to deliver on key performance indicators: yield, integrity, capping efficiency, and ultra-low double-stranded RNA (dsRNA), increasing translation in human cell lines and lowering immunostimulatory effects.

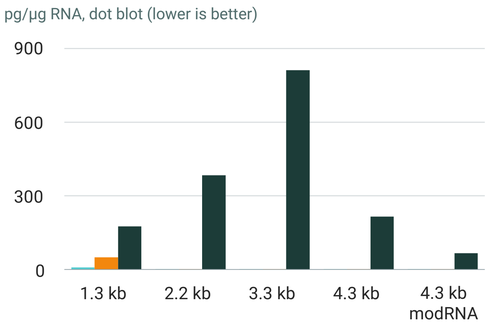
Primrose Bio's Prima RNApols® are built on an enzyme evolution platform based on ultra-high-throughput screens and protein-engineering approaches. The wealth of data generated by the evolution platform places our enzyme portfolio in a favorable position to address future manufacturing challenges and support the development of next generation of mRNA therapeutics.

PRODUCT COMPARISON

CAPPING EFFICIENCY
% capped, LC-MS



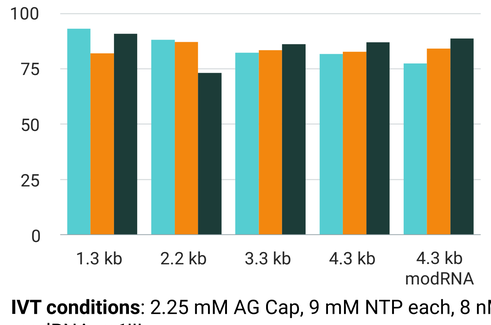
dsRNA
pg/µg RNA, dot blot (lower is better)



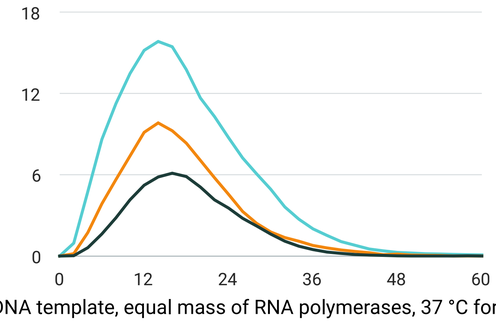
YIELD
mg/mL, A260



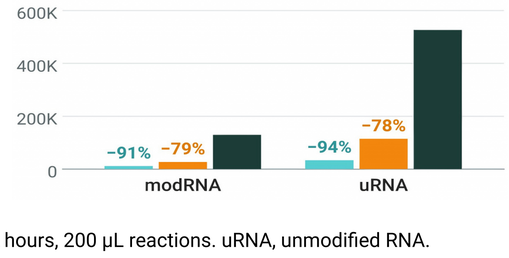
INTEGRITY
% full length, fragment analyzer



TRANSLATABILITY
Normalized GFP expression vs hours; up to 3x AUC vs T7



IMMUNOGENICITY
Cumulative reporter score; % change vs T7 above bars. Average data for Cap AG and 7 Pro lineage variants are shown.



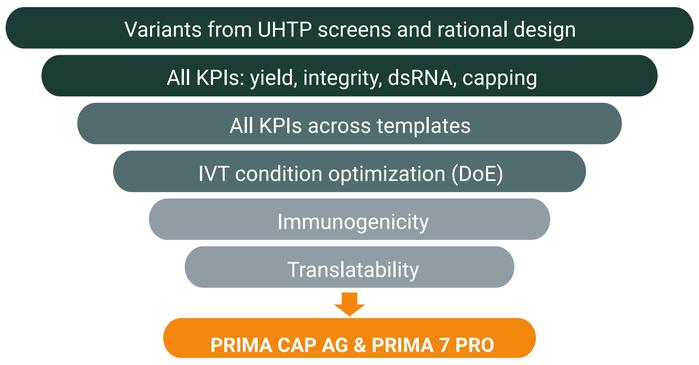
IVT conditions: 2.25 mM AG Cap, 9 mM NTP each, 8 nM DNA template, equal mass of RNA polymerases, 37 °C for 2 hours, 200 µL reactions. uRNA, unmodified RNA. modRNA, m1Ψ

Promoter compatibility:

- Prima 7 Pro is compatible with the T7 promoter
- Prima Cap AG requires its own optimized promoter

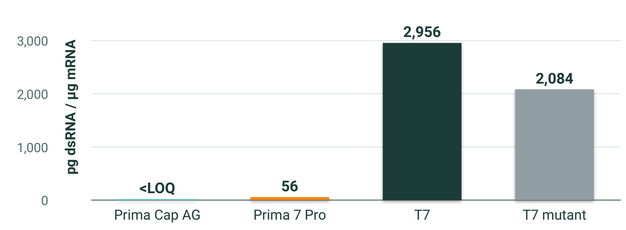
Legend: Prima Cap AG; Prima 7 Pro; T7

PRODUCT SELECTION

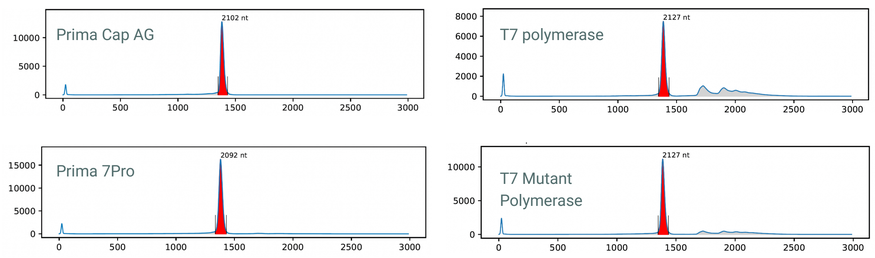


COMPARISON TO T7 MUTANT POLYMERASE

dsRNA
pg/µg RNA, dot blot (lower is better)



FRAGMENT ANALYZER
T7 and T7 mutant polymerase show peaks greater in size than the target mRNA.

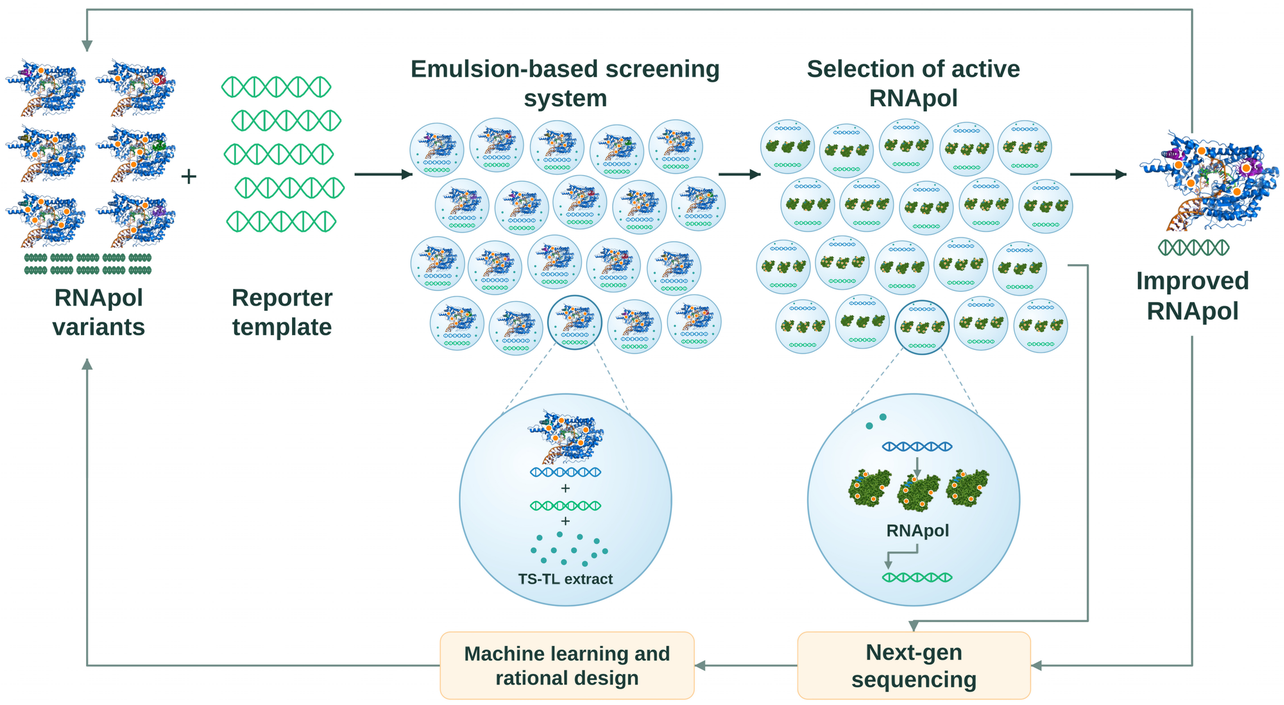


Legend: Prima Cap AG; Prima 7 Pro; T7; T7 mutant polymerase

IVT conditions: 2 kb template 3 mM AG Cap, 9 mM NTP each, 8 nM DNA template, equal mass of RNA polymerases, 37 °C for 3 hours, 200 µL reactions.

ULTRA-HIGH THROUGHPUT ENZYME EVOLUTION

- Our flexible screening platform uses an emulsion-based transcription-translation (TS-TL) extract to screen for RNApol performance improvements.



- The screen is capable of processing >2 × 10⁹ enzyme variants from a 1 mL water-in-emulsion extract to select for RNApols with higher yield, integrity, capping efficiency, and lower dsRNA.

CONCLUSIONS

- Robust RNA polymerases: more protein, less dsRNA, better capping
- Up to 3x increased translatability
- 97% capping efficiency with a low amount of Cap and a high concentration of NTPs
- Ultra-low dsRNA
- Depending on the proposed project's complexity, we can develop a custom enzyme for your workflow in 2-6 months

ACKNOWLEDGMENTS

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NIH National Institutes of Health
Turning Discovery Into Health

GMP-READY SUPPLY

Primrose Bio has established manufacturing capabilities to produce GMP-grade RNA polymerases, supplied through ISO 13485-certified quality systems, analytical capability and manufacturing infrastructure.

ISO 13485 MEDICAL DEVICES