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

Patrik Engström, Malithi Jayasinghe, Barbara Frey, Nick Halloran, Jasmina Radoicic, Xavier Deogaygay, Ivan Sabath, Lindsey Jasch, Laila Halim, Chris Warner
Primrose Bio, San Diego, CA, USA

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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 SELECTION

Prima RNApols®

- Variants from UHTP screens and rational design
- All KPIs: yield, integrity, dsRNA, capping
- All KPIs across templates
- IVT condition optimization (DoE)
- Immunogenicity
- Translatability

PRIMA CAP AG & PRIMA 7 PRO

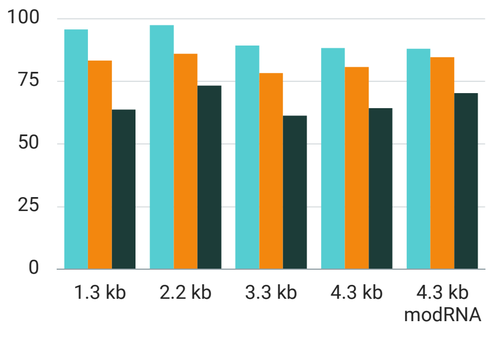
RESULTS PRIMA CAP AG & PRIMA 7 PRO

Promoter compatibility:

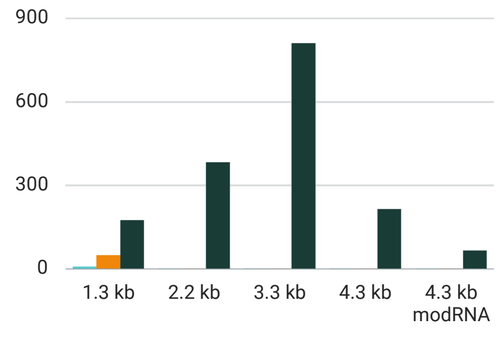
- Prima 7 Pro is compatible with the T7 promoter
- Prima Cap AG requires its own optimized promoter

Legend: Prima Cap AG · Prima 7 Pro · T7

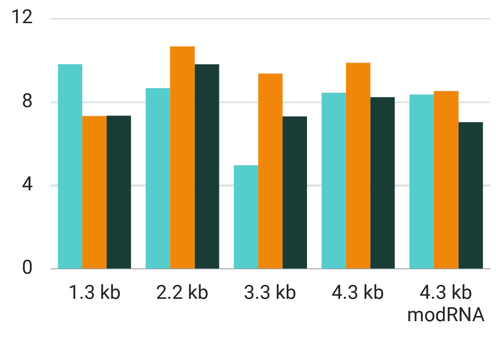
CAPPING EFFICIENCY
% capped, LC-MS



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

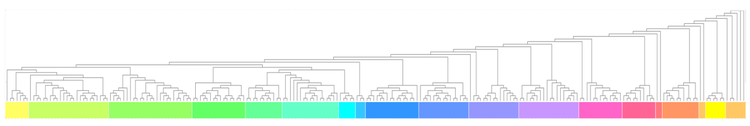


YIELD
mg/mL, A260

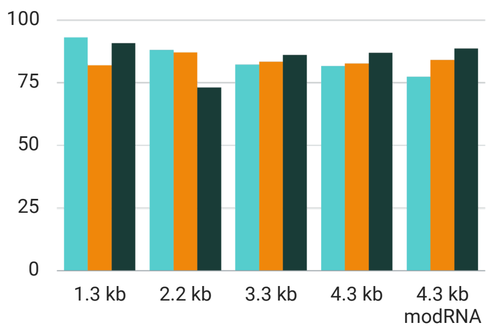


RNA POLYMERASES

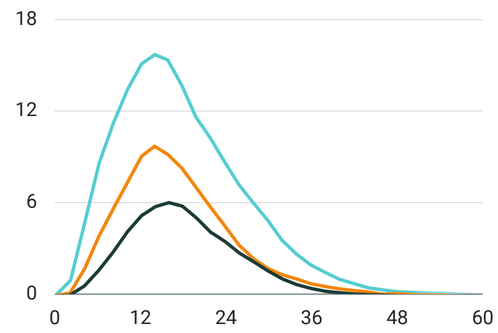
- 41 proprietary non-T7 RNA polymerases discovered.
- 25 to 90% amino acid sequence identity to T7 RNA polymerase.



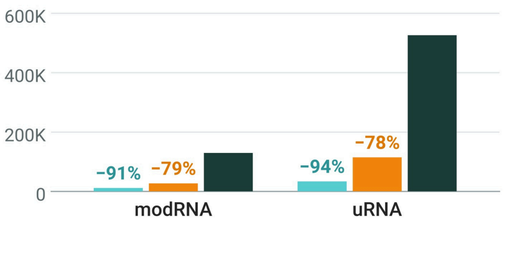
INTEGRITY
% full length, fragment analyzer



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



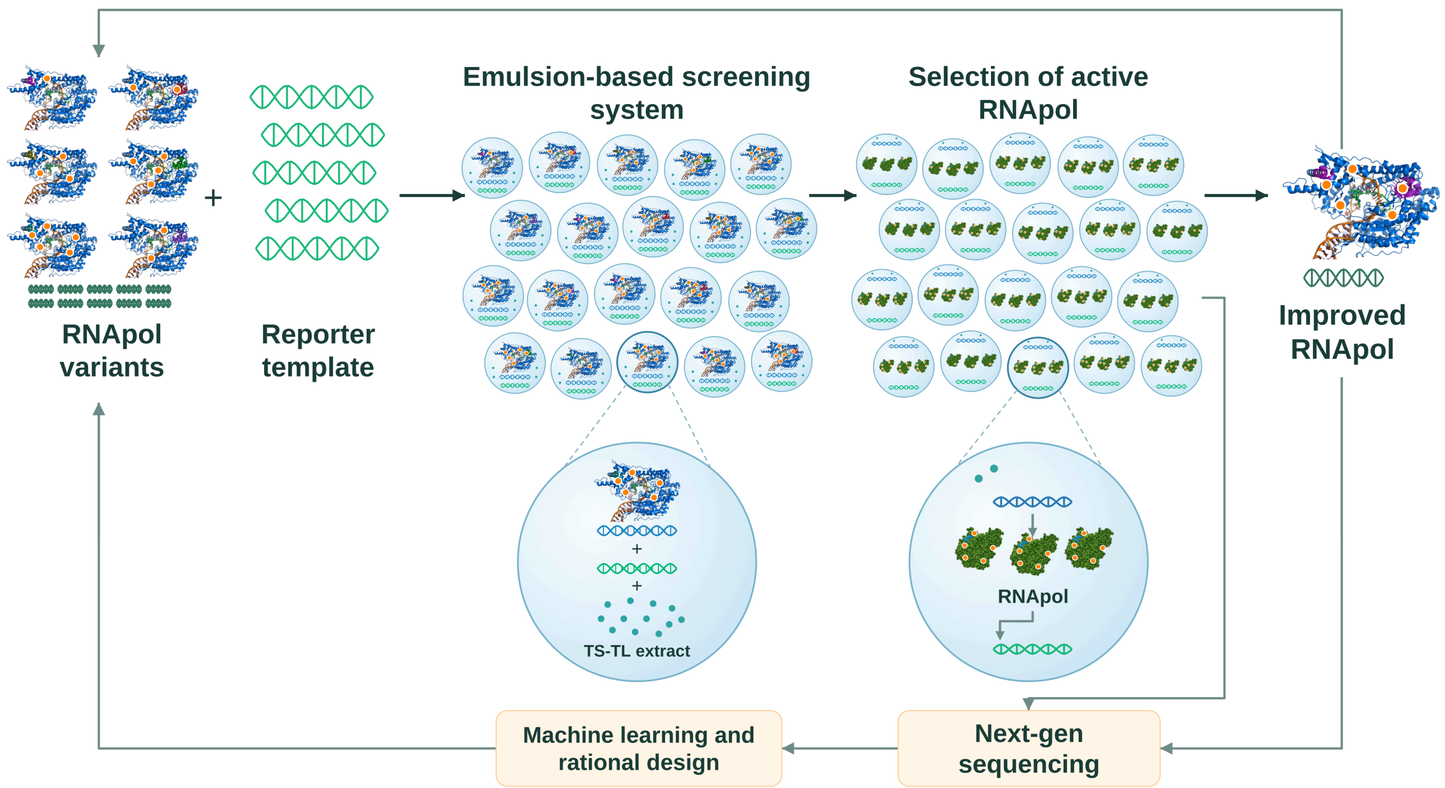
IMMUNOGENICITY
Cumulative reporter score; % change vs T7 above bars. Average data for Cap AG and 7 Pro lineage variants are shown.



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

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 × 10^9 enzyme variants from a 1 mL water-in-emulsion extract to select for RNApols with higher yield, integrity, capping efficiency, and lower dsRNA.

CONCLUSIONS

- Prima Cap AG and Prima 7 Pro are robust enzymes
- 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

Portions of this work were supported by NIH SBIR grants No. 1R43GM131548-01 (Phase I) and 1R44GM131548-02 (Phase II).

NIH National Institutes of Health
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